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Urea Small Molecule Agonists on Mouse Melanocortin Receptors

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Abstract—A series of urea compounds based on the tripeptide Phe-Trp-Lys were synthesized and pharmacologically characterized at the mouse melanocortin receptors. The results include identification of novel melanocortin receptor agonists with potencies ranging from nanomolar to micromolar.

The melanocortin natural peptide ligands, α-MSH, β-MSH, γ-MSH and ACTH are peptides derived by post-translational processing of the pro-opiomelanocortin (POMC) gene[1] and stimulate the melanocortin receptors to activate the adenylate cyclase signal transduction pathway resulting in adenosine 3′,5′-cyclic monophosphate (cAMP) accumulation.[2] Cloning and characterization of the receptors for these natural ligands led to the identification of five melanocortin receptor subtypes (MC1R-MC5R)[3–9] that have different ligand-binding characteristics and tissue expression distributions. All five melanocortin receptors belong to the superfamily of seven transmembrane-spanning receptors that are coupled to G-proteins. A number of potent melanocortin peptide agonist analogues, such as NDP-MSH (Ac-Ser-Tyr-Ser-Nle-Glu-His-DPhe-Arg-Trp-Gly-Lys-Pro-Val-NH_2) and MT-II (Ac-Nle-c[Asp-His-DPhe-Arg-Trp-Lys]NH_2) of the natural ligand, α-MSH (Ac- Ser-Tyr-Ser-Met-Glu-His-Phe-Arg-Trp-Gly-Lys-Pro-Val-NH_2) have been developed.[10,11] However, neither of these analogues is selective for any of the melanocortin receptors. Peptide analogues are potentially not very suitable as orally deliverable therapeutic agents, thus, there exists a need for non-peptide ligands. Some peptoids,[12] compounds prepared by reductive amination,[13] *N*-alkylaminoacids,[14] and β-turn related heterocycles[15,16] have been reported to show moderate activity on melanocortin receptors. MC4R potent and selective 4-substituted-4-cyclohexylpiperidine based compounds have been recently reported.[17] The study presented herein was undertaken to design small non-peptide molecules based on the tri-peptide, Phe-Trp-Lys-NH_2 that includes a urea linkage. Previous tripeptide based β-turn heterocyclic molecules[15] resulted in micromolar agonist activity at the melanocortin receptors that were less potent than the tetrapeptide template possessing nanomolar potency,[16] hence, a linear template was chosen for this project.

The solid-phase synthesis of the library compounds used in this study is illustrated in Scheme 1. The chemistry has been modified from previously published methods.[18,19] The diamine, R_1 subunit was coupled to 4-[4 - (hydroxymethyl) - 3 - methoxyphenoxy]butanoyl - p-methylbenzhydrylamine (HMPB-MBHA) resin through a carbamate linkage after activation of the resin with *p*-nitrophenylchloroformate. The Fmoc-protected amino acid, R_2 subunit was then added to the amine to form an amide bound with diisopropylcarbodiimide (DIC) and 3% *N*,*N*-(dimethylamino)-pyridine (DMAP) in *N*,*N*-dimethylformamide (DMF) as the coupling reagents. This was followed by Fmoc deprotection with 20% piperidine. The R_3, amine subunit was subsequently attached through a urea linkage after carbonylation of the free amine of the R_2 subunit. The product was cleaved from the resin with glacial acetic acid at 45 °C on an Advanced Chemtech 440MOS automated synthesizer. Compound synthesis was performed using a manual reaction vessel or by semi-automation on an Advanced Chemtech LabTech. The assayed compounds were >96% pure as determined by analytical RP-HPLC in two solvent systems, had the correct molecular mass as determined by mass spectrometry (University of Florida protein core facility). One dimensional ^{1}H NMR (Brucker Advance 500, d-MeOH) was used to verify that the correct chemical structures were obtained (University of Florida McKnight Brain Institute).

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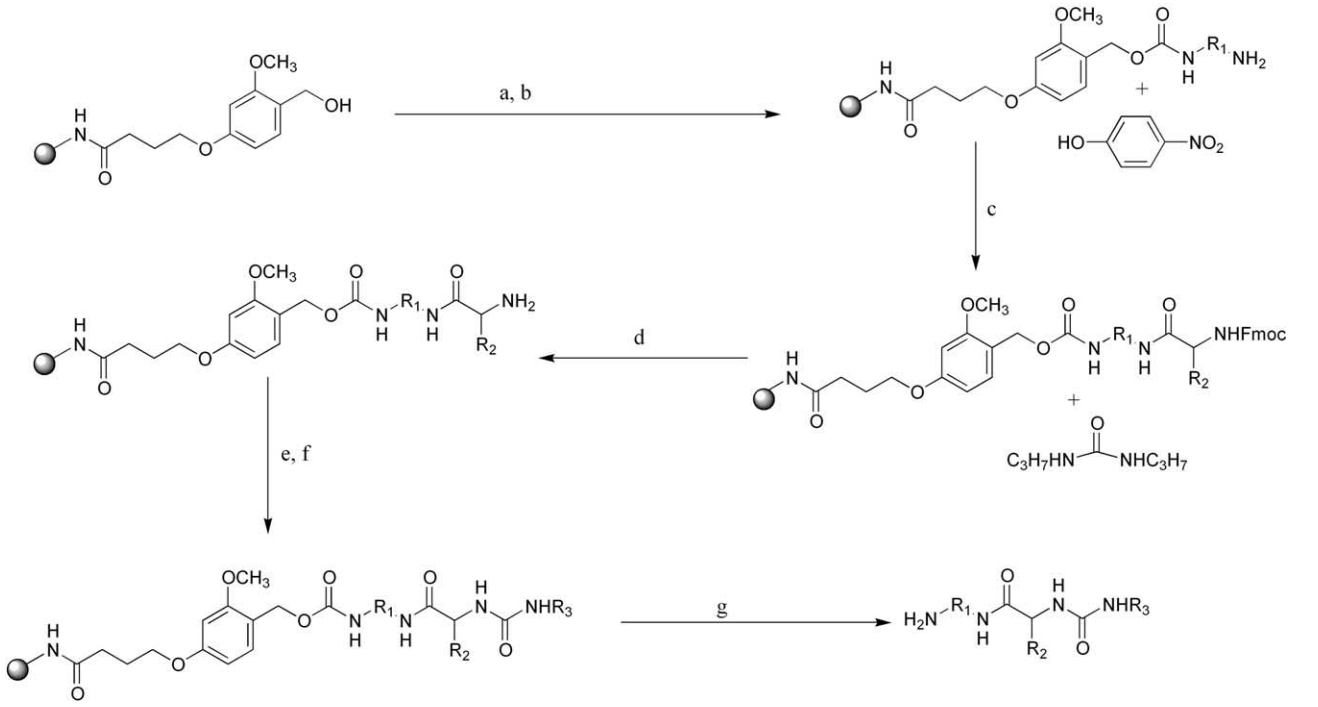


Scheme 1. Reagents and conditions: (a) *p*-nitrophenylchloroformate (18.7 equiv), diisopropylethylamine (DIEA, 18.7 equiv), THF/CH_2Cl_2 (1:1), overnight; (b) diamine, R_1 (12.5 equiv), DIEA (12.5 equiv), DMF, overnight; (c) Fmoc-amino acid, R_2 (7.2 equiv), DIC (7.2 equiv), DMAP (0.22 equiv), DMF, 2×3 h; (d) 20% piperidine in DMF, 30 min; (e) *p*-nitrophenylchloroformate (1.4 equiv), diisopropylethylamine (DIEA, 1.4 equiv), THF/CH_2Cl_2 (1:1), 1 h; (f) amine, R_3 (1.4 equiv), DIEA (1.4 equiv) DMF, overnight; (g) acetic acid, 45 °C, 24 h.

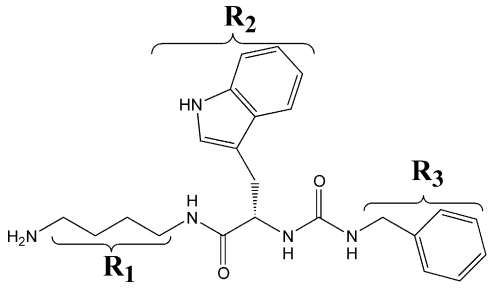
The agonist assays were performed using a reporter gene bioassay[20] and HEK-293 cells stably expressing the mouse melanocortin receptors (mMC1, mMC3, mMC4 and mMC5) as previously reported.[16,15] The compounds were tested for agonist activity and the EC_{50} values reported in Table 1 are the average of at least three independent experiments.

Compound **1** was synthesized to validate whether melanocortin receptor activity will be maintained going from a tripeptide to a urea based small organic molecule. Compound **1** possessed μM MC1R and MC4R agonist activity. A library of 14 compounds was subsequently prepared making changes at the R_1, R_2 and R_3 positions with the intention of creating more potent and selective compounds for the melanocortin receptor subtypes. Table 1 summarizes the agonist activity for this library at the melanocortin receptors.

Compounds **2**–**7** had modifications at the R_1 (diamine) position, in relation to the lead compound, **1**. When R_1 was changed to propyl or pentyl (compound **2** or **3**) groups MC4R activity was lost. Compound **2** was equipotent at the MC1 and MC5 receptors as compared to **1**. Compound **3** was equipotent at the MC5R and 4-fold more potent at the MC1R compared to **1**. When R_1 was hexyl (compound **4**), agonist activity was only observed at the MC1R ($EC_{50} = 22.3 \pm 8.8$ μM) which is within the 3-fold inherent experimental error, as compared to **1**. Compounds **5**–**7** contain cyclo-hexyl rings that were used to determine whether conformational constraints will have any effect at this position. Compound **5** with a benzyl aromatic ring and a methyl group at the 3-position resulted in activity at the MC1R equipotent to **1**. However, compound **6** with the methyl group at the 4-position resulted in activity at all four receptors. Compared to **1**, compound **6** was approximately 140-fold more potency at the MC1R, 60-fold at the MC4R and 6-fold at the MC5R and it possessed μM agonist activity at the MC3R. When the benzyl ring in **6** was modified to a piperidine ring, compound **7**, MC4R activity was abolished and decreased potency resulted at the MC1, MC3, and MC5 receptors.

Compounds **8**–**11** had modifications made at the R_3 (amine) group, while R_1 and R_2 were retained, as in compound **1**. When the benzyl group of **1** was changed to phenethyl, **8**, a complete loss of activity resulted at all four receptors tested at up to 100 μM concentrations. When R_3 was 1,2,3,4-tetraisoquinoline (compound **9**), 6- to 7-fold increased potency resulted at the MC1R, MC4R and MC5R respectively, compared to **1**. Compared to **6**, **9** was equipotent (within experimental error) at the MC5 receptor, was 20-, 6-, and 9-fold less potent at the MC1R, MC3R and MC4R, respectively. Compound **10** with a benzyl group attached to a piperidine ring at the 1-position, was an agonist at all four melanocortin receptors tested. Compound **10** was a high μM agonist at the MC3R ($EC_{50} = 4.4 \pm 2.8$ μM), 8-fold more potent at the MC4R compared to **1**, but was 11- and 4-fold more potent at MC1R and MC5R, respectively. Compound **11** with the benzyl group attach to the piperidine ring at the 4-position, however, had no agonist activity at the MC3 and MC4 receptors, but possessed equipotent agonist activity at the MC1 and MC5 receptors, as compared to **1**.

Table 1. Pharmacology of compounds characterized at the mouse melanocortin receptors



1

ID	R_1	R_2	R_3	EC_{50} μM			
				mMC1R	mMC3R	mMC4R	mMC5R
α-MSH				0.32±0.04	1.21±0.27	2.47±0.32	1.49±0.46
NDP-MSH				0.012±0.0036 nM	0.075±0.018 nM	0.12±0.013 nM	0.17±0.031 nM
1	Butyl	L-Trp	Benzyl	55.0±22.7	>100	23.7±6.4	30.5±15.1
2	Propyl	L-Trp	Benzyl	51.2±19.0	>100	>100	39.9±8.8
3	Pentyl	L-Trp	Benzyl	14.8±7.3	>100	>100	20.0±3.1
4	Hexyl	L-Trp	Benzyl	22.3±8.8	>100	>100	>100
5	3-Methylbenzyl	L-Trp	Benzyl	28.0±7.7	>100	>100	>100
6	4-Methylbenzyl	L-Trp	Benzyl	0.4±0.2	10.7±1.0	0.4±0.2	4.9±4.8
7	4-Methylpiperidinyl	L-Trp	Benzyl	5.2±2.7	4.4±2.8	>100	8.2±7.9
8	Butyl	L-Trp	Phenethyl	>100	>100	>100	>100
9	Butyl	L-Trp	1,2,3,4-Tetraisoquinoline	7.9±1.0	1.7±1.0	3.4±1.9	4.9±4.8
10	Butyl	L-Trp	1-Benzylpiperidinyl	5.0±2.0	4.4±2.8	2.8±2.5	8.2±7.9
11	Butyl	L-Trp	4-Benzylpiperidinyl	20.0±4.8	>100	>100	28.9±6.8
12	Butyl	L-homophe	Benzyl	>100	>100	>100	>100
13	Butyl	L-Tyr(Bzl)	Benzyl	20.5±10.1	>100	>100	14.4±3.6
14	Butyl	D-Trp	Benzyl	42.4±15.8	>100	23.7±7.4	32.8±19.1

Values are mean of at least three independent experiments with associated standard error.
>100 indicate that no agonist activity was observed at up to 100 μM concentrations. These compounds were not tested as antagonists.

Compounds **12**–**14** had modifications at the R_2 (amino acid) group, while retaining the R_1 and R_3 groups of compound **1**. None of the three compounds showed improved agonist activity at any of the receptors as compared to **1**. Compound **12** containing the R group of L-homophenylalanine lost activity at all receptors. MC4R activity was observed when R_2 was changed to D-tryptophan and was equipotent to **1** at this receptor.

This study was undertaken to prepare small organic molecules that possessed agonist activity at the melanocortin receptors. The MC3 and MC4 receptors are expressed in the brain[5,21] and are implicated in regulating weight and energy homeostasis.[22–24] Of the 14 compounds synthesized herein, six possessed either agonist activity at the MC3R, MC4R or both. Compound **6** being the most potent at the MC1, MC3 and MC4 receptors was 27-, and 12-fold more selective for the MC4R ($EC_{50}=400\pm200$ nM) versus MC3R and MC5R, respectively. However compound **7** with a piperidine ring replacing the benzyl group of compound **6** lost agonist activity at the MC4R but retained reduced agonist potency and selectivity at the MC1, MC3 and MC5 receptors. When a ring system was used at the R_1-position, MC4R agonist activity resulted when the aromatic ring had a modification at the 4-position (compounds **5**, **6** and **7**).

Comparison of the R_1 aliphatic diamines resulted in the identification that the optimal spacing between the amine groups consisted of four methylene groups in order for the ligand to possess agonist activity at the MC3 and MC4 receptors. Compounds **1**–**4** demonstrates that as the aliphatic chain length of the diamine (R_1) were lengthened or shortened from four methylene groups, all agonist activities at the MC4 receptor was abolished, but retained at the MC1R.

Compounds **12** and **13** lost agonist activity at the MC3 and MC4 receptors when the R_2 subunit was modified. The D-tryptophan R_2 subunit, (compound **14**) retained agonist activity at the MC4R but was equipotent (within experimental error of **1**) at this receptor. All of these compounds, with the exception of **12**, possessed agonist activity at the MC1 and MC5 receptors. Hence, these data supported the hypothesis that an indole ring is important for maintaining agonist activity at the MC3R and MC4R in this small molecule template.

Finally, changes made at the R_3 (amine) subunit produced varied results. When the CH_2 link was extended to two methylene groups, compound **8**, all agonist activity was abolished at all four melanocortin receptors tested. Removal of the methylene group completely, resulted in agonist activity once again at either all four melanocortin receptors or two of the four tested (compounds **9**–**11**). However, in the case of compounds **10** and **11** agonist activity at the MC3R and MC4R was dependent upon whether the benzyl ring was attached

directly to the nitrogen or at the 4-position of the piperidine ring. Attachment directly to the nitrogen in the piperidine ring (compound **10**) produce high μM EC_{50} values at the MC3 and MC4 receptors but no activity was observed at these receptors when the benzyl group was attached at the 4-position (compound **11**). Although **11** retained agonist activity at the MC1R and MC5R, **10** was 4-fold more potent at these receptors compared to **11**, respectively.

We have synthesized a focused small non-peptide melanocortin agonist library, via solid-phase methodology based upon a urea scaffold, displaying diversity at up to three positions. The compounds prepared herein resulted in micromolar to nanomolar agonist potencies at the mMC1R and mMC3-5R. Compound **7** was selective for the MC3R (4.4 ± 2.8 μM) versus the MC4R with no agonist activity observed up to 100 μM. These results may be useful in the further design of potent and selective non-peptide ligands for the melanocortin receptors.

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