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Synthesis and Structure–Activity Relationships of Substituted Urea Derivatives on Mouse Melanocortin Receptors

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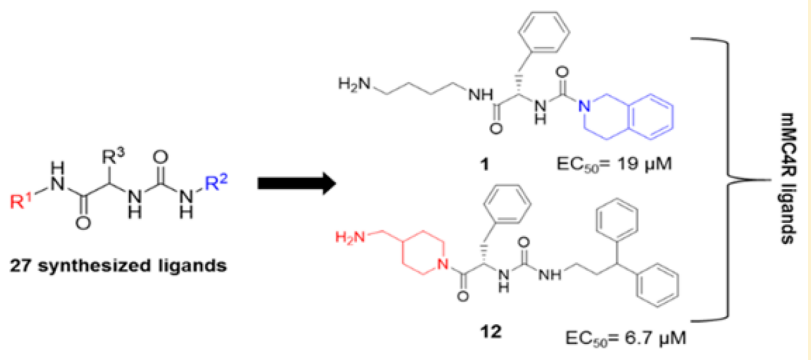
Supporting Information



ABSTRACT: The melanocortin system is involved in the regulation of several complex physiological functions. In particular, the melanocortin-3 and -4 receptors (MC3R/MC4R) have been demonstrated to regulate body weight, energy homeostasis, and feeding behavior. Synthetic and endogenous melanocortin agonists have been shown to be anorexigenic in rodent models. Herein, we report synthesis and structure–activity relationship (SAR) studies of 27 nonpeptide small molecule ligands based on an unsymmetrical substituted urea core. Three templates containing key residues from the lead compounds, showing diversity at three positions (R^1, R^2, R^3), were designed and synthesized. The syntheses were optimized for efficient microwave-assisted chemistry that significantly reduced total syntheses time compared to a previously reported room temperature method. The pharmacological characterization of the compounds on the mouse melanocortin receptors identified compounds **1** and **12** with full agonist activity at the mMC4R, but no activity was observed at the mMC3R when tested up to 100 μM concentrations. The SAR identified compounds possessing aliphatic or saturated cyclic amines at the R^1 position, bulky aromatic groups at the R^2 position, and benzyl group at the R^3 position resulted in mMC4R selectivity over the mMC3R. The small molecule template and SAR knowledge from this series may be helpful in further design of MC3R/MC4R selective small molecule ligands.

KEYWORDS: *Melanocortin receptors, melanocortin agonist, urea ligands, small molecules, melanocortin-3 receptor, melanocortin-4 receptor*

The melanocortin receptors (MCRs) belong to the family of seven transmembrane G protein-coupled receptors (GPCRs) and signal through the 3′,5′-cyclic adenosine monophosphate (cAMP) signal transduction pathway. The five known melanocortin receptor subtypes (MC1R-MC5R)[1−8] interact with the endogenous peptides to mediate several complex physiological functions including weight and energy homeostasis,[9] feeding behavior,[10] skin pigmentation,[11] steroidogenesis,[12] cardiovascular,[13] and erectile function.[14−16] The endogenous agonists for these receptors, α-, β-, and γ-melanocortin stimulating hormone (MSH) and adrenocorticotropic hormone (ACTH), are peptide hormones that are produced by post-translational processing of the pro-opiomelanocortin (POMC) gene.[17] These peptides contain a conserved core sequence His^6-Phe^7-Arg^8-Trp^9 (Figure 1; α-MSH numbering) postulated to be important for melanocortin ligand–receptor recognition and activation.[18] The melanocortin receptors respond to all POMC-derived melanocortin peptides, albeit to varying degrees of potencies. The only exception to this is the MC2R, which only responds to ACTH but not to other MSH agonists.[19]

The melanocortin-3 (MC3R) and -4 receptors (MC4R) are expressed in the brain and have been demonstrated to be involved in the regulation of feeding behavior, metabolism, and energy homeostasis, as well as, erectile and cardiovascular functions.[9,10,13,15,20−22] The role of the MC4R in feeding behavior is well documented through the use of nonselective and selective ligands and by genetic knockout studies.[9,10,23] Activation of the MC4R via endogenous or synthetic agonists decrease food intake.[10] The importance of this receptor in food

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ACTH (1-24)	SYSME**HFRW**GKPVGKKRRPVKVYP
α-MSH	Ac-SYSME**HFRW**GKPV-NH_2
β-MSH	AEKKDEGPYRME**HFRW**GSPPKD-OH
γ_2-MSH	YVMG**HFRW**DRFG-OH

Figure 1. Sequences of the endogenous melanocortin peptides. The core His, Phe, Arg, and Trp residues amino acids are conserved in all melanocortin peptides, and this is highlighted in the sequences.

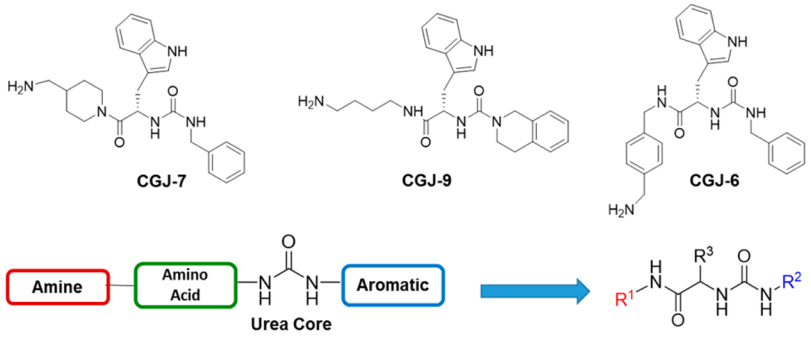

Figure 2. Example of common features of the substituted urea derivatives and structures of reported compounds CGJ-7, CGJ-9, and CGJ-6.[34]

intake and energy homeostasis is also highlighted by naturally occurring mutations identified in morbidly obese individuals[24] that result in partial or total dysfunction of the MC4Rs in vitro. It has been reported that single nucleotide polymorphisms (SNPs) in the MC4R may be responsible for morbid obesity in ~6% children and adults.[24] The clinical symptoms include lack of satiety, extreme hyperphagia, decrease energy expenditure, increased linear growth, and hyperinsulinemia. The MC3R is postulated to be involved in the regulation of energy and fat homeostasis. However, the underlying mechanism of the MC3R action has not been fully elucidated.[20−22] Therefore, discovery of receptor specific ligands for the MC3R and MC4R are vital for the advancement in the understanding of their overlapping physiological roles and to develop potential treatment for eating disorders.

The endogenous α-MSH is a 13 amino acid long peptide agonist for the melanocortin receptors (MC1R, MC3−5Rs). Structure−activity relationship (SAR) studies of the α-MSH sequence identified an analog containing the Nle and DPhe amino acids substituted at positions 4 and 7, respectively [(Nle[4], DPhe[7])α-MSH, NDP-MSH].[25] This compound is a more potent MCR agonist than α-MSH and has emerged as a valuable biochemical tool in receptor mediated studies. However, endogenous and synthetic peptidic MSH ligands are susceptible to enzymatic degradation in vivo.[26] The poor stability and lack of selectivity limit the use of these peptides to probe and characterize the pharmacological and physiological functions of melanocortin receptors. Rational design based on the conserved peptide core of the melanocortins has formed the basis of designing MC3R/MC4R selective small molecule ligands.

Utilization of nonpeptidic scaffolds has been explored in the past to achieve selectivity and to improve pharmacokinetic properties at the MCRs. Several classes of small molecule scaffolds have been explored for melanocortin receptors including β-turn related motifs, benzodiazepines, benzimidazole pyridazinone, cyclohexylpiperidine, and urea derived compounds.[27−35] In particular, substituted urea derivatives are an important class of carbonyl compounds that are building blocks of several drug candidates such as HIV protease inhibitors,[36] Raf kinase inhibitors,[37] CCK-B receptor antagonists,[38,39] and endothelin antagonists.[40] An orally bioavailable piperazine urea based compound (a selective MC4R partial agonist) has been reported to modulate food intake in animal models without any effect on erectile activity.[41] An earlier study of a "focused" library based on the core tripeptide sequence, "Phe-Trp-Lys" that included a urea linkage resulted in identification of lead compounds **CGJ-7** and **CGJ-9** (Figure 2).[34] Notably, compound **CGJ-9**, containing the 1,2,3,4-tetrahydroisoquinoline (TIQ) functionality displayed full agonist activity at both the mMC3R and mMC4Rs. However, compound **CGJ-7**, containing 4-(aminomethyl)piperidine (AMP) functionality resulted in a 4 μM agonist at the mMC3R and was unable to stimulate the mMC4R at up to 100 μM concentrations. The present study is based on the interesting pharmacology of these two compounds. Three substituted urea-based templates were designed herein to discover small molecules that can be used to probe the molecular mechanisms of the MC3/MC4 receptors. The synthetic strategy was modified from the literature to adopt microwave technology and resulted in efficient overall synthesis.

Template Design. The design strategy included a three component approach including a urea linkage as a core with an amine functionality (R^1) linked to an amino acid residue (R^3) at one end and an aromatic group (R^2) on the other side as delineated in Figure 2. Template 1 was designed based on the compound **CGJ-9** with TIQ moiety at R^2 and variable amino acids (R^3) and amine (R^1) functionalities (Figure 3A). Template 2 contained the fixed AMP group in R^1 position as the amine component (based on **CGJ-7**) and positions R^2 and R^3 were varied (Figure 3B). Template 3 was designed as a compilation of **CGJ-7** and **CGJ-9**, with the AMP group positioned at R^1, the TIQ moiety at R^2, and amino acid substitutions at R^3 (Figure 3C). The amino acid substitutions at R^3 were chosen based on the earlier SAR studies of melanocortin agonists that resulted in potent and/or selective pharmacology at MCRs in different templates. It is hypothesized that diversifying the three components of the urea core

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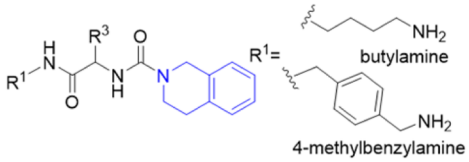
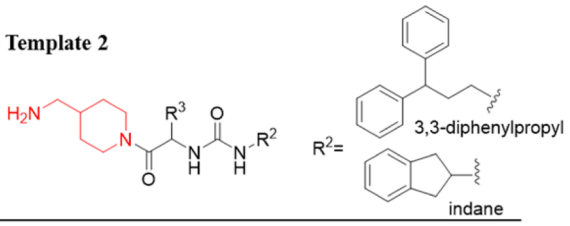
A) Template 1



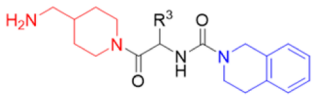
Compound	R^1	R^3
1	butylamine	Phe
2	butylamine	(*p*I)Phe
3	butylamine	(*p*Cl)Phe
4	butylamine	His
5	butylamine	Nal(2')
6	4-methylbenzylamine	Phe
7	4-methylbenzylamine	(*p*I)Phe
8	4-methylbenzylamine	(*p*Cl)Phe
9	4-methylbenzylamine	His
10	4-methylbenzylamine	Nal(2')

B) Template 2



Compound	R^2	R^3
11	3,3-diphenylpropyl	Trp
12	3,3-diphenylpropyl	Phe
13	3,3-diphenylpropyl	(*p*I)Phe
14	3,3-diphenylpropyl	(*p*Cl)Phe
15	3,3-diphenylpropyl	His
16	3,3-diphenylpropyl	Nal(2')
17	indane	Trp
18	indane	Phe
19	indane	(*p*I)Phe
20	indane	(*p*Cl)Phe
21	indane	His

C) Template 3



Compound	R^3
22	Trp
23	Phe
24	(*p*I)Phe
25	(*p*Cl)Phe
26	His
27	Nal(2')

Figure 3. Illustration of the templates and list of compounds in each template in this study. (A) Template 1, (B) Template 2, and (C) Template 3.

may result in more potent and/or selective melanocortin receptor ligands.

RESULTS AND DISCUSSION

Herein, the synthesis and pharmacology of substituted urea derivatives in cells expressing the cloned mouse MC1, MC3, MC4 and MC5 receptors is reported. Twenty seven compounds were designed and synthesized in total, of which 10 compounds (**1**−**10**) were part of Template 1 (Figure 3A), 11 compounds (**11**−**21**) belonged to Template 2 (Figure 3B), and 6 compounds (**22**−**27**) were part of Template 3 (Figure 3C). The solid phase syntheses of the urea compounds were modified from an earlier reported method (Scheme 1).[34,42,43] The synthesis was optimized for a manual microwave synthesizer, which dramatically reduced the reaction time to 3 h compared to the several days in the earlier method at room temperature.[34] The HMPB-MBHA resin was activated with *p*-nitrophenyl chloroformate first and was coupled with the diamine subunit (R^1) through a carbamate linkage under microwave irradiation. The Fmoc-protected amino acid subunit (R^3) was then added to the amine using a standard microwave SPPS protocol with coupling reagents HBTU and DIEA, followed by microwave assisted Fmoc deprotection with 20% piperidine. The aromatic part of the template (R^2, Figure 2) was introduced by coupling the amine subunit (R^2) through a urea linkage using *p*-nitrophenyl chloroformate as a carbonylating agent. The product was cleaved from the resin by treatment with glacial acetic acid using a microwave irradiation. The synthesized compounds were purified by semipreparative RP-HPLC and analyzed for the correct molecular weights by mass spectrometry and the purity by analytical RP-HPLC in two divergent solvent systems (Supporting Information). All compounds were found to be ≥95% pure. The purified compounds were tested for functional activity using a *β*-galactosidase reporter gene bioassay[44] on HEK cells stably expressing the mouse melanocortin receptors (mMC1R, mMC3−5Rs). The MC2R only responds to ACTH and therefore, it was not included in this study.

Template 1. The agonist EC_{50} values of compounds **1**−**10** (Template 1) at the mouse melanocortin receptors are summarized in Table 1, along with the known agonist NDP-MSH and leads **CGJ-7** and **CGJ-9**. Compound **CGJ-9** was the lead compound for the Template 1 having Trp at the R^3 position, TIQ at the R^2 position and butyl amine at the R^1 position and possessed low micromolar activity at all MCRs

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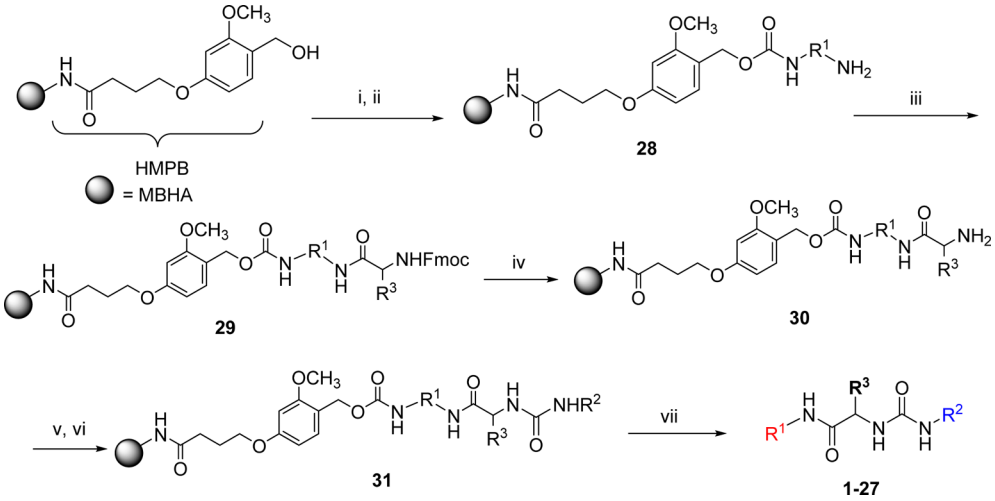
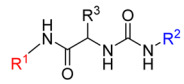
Scheme 1. General Scheme for Microwave-Assisted Urea Based Library Synthesis[a]



[a]Reagents and conditions: (i) *p*-nitrophenyl chloroformate (18.7 equiv), DIEA (18.7 equiv), mW (35 °C, 30 W, 5 min); (ii) diamine, R^1 (12.5 equiv), DIEA (12.5 equiv), DMF, mW (55 °C, 50 W, 10 min); (iii) Fmoc-amino acid, R^3, HBTU, DIEA, mW (75 °C, 30 W, 5 min); (iv) 20% piperidine in DMF, mW (75 °C, 30 W, 4 min); (v) *p*-nitrophenyl chloroformate (1.4 equiv), DIEA (1.4 equiv), mW (35 °C, 30 W, 5 min); (vi) amine, R^2 (1.4 equiv), DIEA (1.4 equiv), DMF, mW (55 °C, 50 W, 10 min); (vii) glacial acetic acid, mW (38 °C, 20 W, 30 min).

Table 1. Pharmacology of Template 1 Urea Compounds at the Mouse Melanocortin Receptors[a]

Compounds				mMC1R	mMC3R	mMC4R	mMC5R
	R^1	R^2	R^3	EC_{50} (μM)			
NDP-MSH				0.05 ± 0.01 nM	0.19 ± 0.04 nM	0.06 ± 0.01 nM	0.20 ± 0.05 nM
CGJ-9*	butylamine	TIQ	Trp	7.9 ± 1.0	1.7 ± 1.0	3.4 ± 1.9	4.9 ± 4.8
CGJ-7*	4-AMP	benzyl	Trp	5.2 ± 2.7	4.4 ± 2.8	> 100	8.2 ± 7.9
1	butylamine	TIQ	Phe	3.8 ± 0.3	> 100	19 ± 2.0	22 ± 4.1
2	butylamine	TIQ	(*p*I)Phe	25 ± 2.8	37 ± 19	17 ± 4.3	43 ± 11
3	butylamine	TIQ	(*p*Cl)Phe	> 100	> 100	> 100	> 100
4	butylamine	TIQ	His	50% @ 100 μM	> 100	60% @ 100 μM	80% @ 100 μM
5	butylamine	TIQ	Nal(2')	> 100	> 100	> 100	> 100
6	4-MBA	TIQ	Phe	60% @ 100 μM	> 100	> 100	70% @ 100 μM
7	4-MBA	TIQ	(*p*I)Phe	> 100	> 100	> 100	> 100
8	4-MBA	TIQ	(*p*Cl)Phe	> 100	> 100	> 100	> 100
9	4-MBA	TIQ	His	> 100	> 100	> 100	40% @ 100 μM
10	4-MBA	TIQ	Nal(2')	> 100	> 100	> 100	> 100

[a]The indicated errors represent the standard error of the mean determined from at least three independent experiments. *The values for compounds have previously been reported and included here for comparison.[34] >100 indicates that the compound was examined but lacked agonist activity at up to 100 μM concentrations. A percentage (%) indicates the percent maximal stimulatory response observed at 100 μM concentrations observed, but not enough stimulation was observed to determine an EC_{50} value.

tested.[34] Compounds **1**−**10** had modifications at the R^1 and R^3 positions compared to **CGJ-9**, while R^2 was fixed as TIQ. TIQ residue has previously been involved in the design of compounds that possessed MC4R agonist pharmacology. The potent MC4R compound first reported by Merck contained a TIQ moiety.[33] More recently, several peptides containing a TIQ residue have been identified to rescue receptor function and signaling in genetically mutated MC4 receptor.[45] Compounds **1**−**5** had the R^1 position substituted with butyl amine and compounds **6**−**10** contained 4-methylbenzylamine (MBA) group at this position. The R^3 position of compounds **1**−**5** contained the amino acid residues Phe, (*p*I)Phe, (*p*Cl)Phe, His, and Nal(2′) residues, respectively. These amino acids have been previously incorporated into the potent melanocortin

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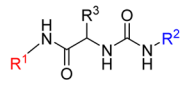
Table 2. Pharmacology of Template 2 Urea Compounds at the Mouse Melanocortin Receptors[a]

Compounds				mMC1R	mMC3R	mMC4R	mMC5R
	R^1	R^2	R^3	EC_{50} (μM)			
NDP-MSH				0.05 ± 0.01 nM	0.19 ± 0.04 nM	0.06 ± 0.01 nM	0.20 ± 0.05 nM
CGJ-9*	butylamine	TIQ	Trp	7.9 ± 1.0	1.7 ± 1.0	3.4 ± 1.9	4.9 ± 4.8
CGJ-7*	4-AMP	benzyl	Trp	5.2 ± 2.7	4.4 ± 2.8	> 100	8.2 ± 7.9
11	4-AMP	3,3-DPP	Trp	14 ± 0.8	40% @ 100 μM	> 100	> 100
12	4-AMP	3,3-DPP	Phe	6.9 ± 2.0	> 100	6.7 ± 3.8	7.7 ± 1.8
13	4-AMP	3,3-DPP	(*p*I)Phe	> 100	> 100	> 100	> 100
14	4-AMP	3,3-DPP	(*p*Cl)Phe	> 100	> 100	> 100	> 100
15	4-AMP	3,3-DPP	His	> 100	> 100	> 100	> 100
16	4-AMP	3,3-DPP	Nal(2')	> 100	> 100	> 100	> 100
17	4-AMP	indane	Trp	14.2 ± 5.1	27.2 ± 10.2	9.1 ± 3.2	16.9 ± 7.3
18	4-AMP	indane	Phe	80% @ 100 μM	> 100	60% @ 100 μM	70% @ 100 μM
19	4-AMP	indane	(*p*I)Phe	30% @ 100 μM	> 100	60% @ 100 μM	70% @ 100 μM
20	4-AMP	indane	(*p*Cl)Phe	30% @ 100 μM	> 100	> 100	> 100
21	4-AMP	indane	His	> 100	> 100	> 100	> 100

[a]The indicated errors represent the standard error of the mean determined from at least three independent experiments. *The values for compounds have previously been reported and included here for comparison.[34] >100 indicates that the compound was examined but lacked agonist activity at up to 100 μM concentrations. A percentage (%) indicates the percent maximal stimulatory response observed at 100 μM concentrations observed, but not enough stimulation was observed to determine an EC_{50} value.

tetrapeptide agonist sequence Ac-His-DPhe-Arg-Trp-NH_2 and were shown to differentiate functional activity between the mMC3R and mMC4R.[46−48] Compound **1** with Phe substituent at the R^3, was equipotent at the mMC1R (within 3-fold experimental error), 6- and 4-fold less potent at the mMC4R and mMC5R, respectively compared to the **CGJ-9**. Interestingly, replacement of the Trp residue with Phe abolished ability of compound **1** to stimulate the mMC3R up to 100 μM concentrations. Similar observations were reported in other templates, where perturbation of Trp in the postulated pharmacophore region resulted in reduced, or no agonist activity at the MC3R.[49,50] Substitution of the (*p*I)Phe residue at the R^3 position (compound **2**) resulted in a full agonist at all four MCRs with 3-, 22-, 5-, and 9- fold decreased potency at the mMC1R, mMC3−5R, respectively as compared to **CGJ-9**. The (*p*Cl)Phe residue substitution at the R^3 group (compound **3**) abolished agonist activity at all MCRs. The placement of polar and hydrophilic residue His at this position (compound **4**) resulted in only 50%, 60%, and 80% of the maximum cAMP response (relative to the control NDP-MSH) at 100 μM concentrations at the mMC1R, mMC4R, and mMC5R, respectively, but showed no activity at the mMC3R. The bulky aromatic group Nal(2′) substitution of Trp (compound **5**) in compound **CGJ-9** resulted in no agonist activity at MCRs.

Compounds **6−10** possess the 4-methylbenzylamine group at the R^1 position and retained the TIQ moiety at the R^2 position. SAR of ligands **6−10** was selected to examine the effect of an aromatic ring in the place of aliphatic amine chain. The aromatic cyclic ring substitution at the R^1 position was not tolerated well in the template (Table 1). Compound **6** with Phe residue at the R^3 position resulted in some partial stimulatory activity at the mMC1R (60%) and mMC5R (70%), but showed no activity at the mMC3R and mMC4R. When the halogen groups were added to the phenyl ring in compounds **7** and **8**, a complete loss of activity was observed at all four receptors tested at 100 μM concentrations. The His residue containing compound **9** resulted in 40% agonist activity at the mMC5R, but no activity at the mMC1R, mMC3R, and mMC4R resulted. The substitution of the bulky aromatic group Nal(2′) (compound **10**) showed no activity at all four receptors at up to 100 μM concentrations.

Template 2. Compounds **11−21** had modifications made at the R^2 position, while R^1 was held constant as 4-(aminomethyl)piperidinyl (AMP) in the lead compound **CGJ-7**. Compounds **11−16** contained the bulkier 3,3-diphenylpropyl (DPP) group at the R^2 position as compared to the benzyl group in **CGJ-7**, and the R^3 position was substituted with Trp, Phe, (*p*I)Phe, (*p*Cl)Phe, His, and Nal(2′), respectively. The agonist EC_{50} values for Template 2 urea derivatives are provided in Table 2. Compound **11**, chemically similar to the lead compound **CGJ-7** except at the R^2 position, showed equipotent activity (within experimental error) at the mMC1R, some agonist activity at the mMC3R compared to **CGJ-7**, but no activity at the mMC4R and mMC5R. Substitution with the Phe residue (**12**) resulted in equipotent agonist activity at the mMC1R and mMC5R, as compared to the lead compound. Interestingly, compound **12** possessed full agonist activity at the mMC4R (6.7 ± 3.8 μM) and no activity at the mMC3R, which was the opposite pharmacology with respect to **CGJ-7**. Replacement of the indole ring (compound **11**) to the phenyl ring (compound **12**) resulted in same potency at the mMC1R, restored activity at the mMC4R and mMC5R, but abolished activity at the mMC3R. All other amino acid substitutions (compounds **13−16**) did not show agonist

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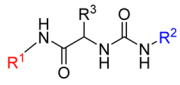
Table 3. Pharmacology of Template 3 Urea Compounds at the Mouse Melanocortin Receptors[a]

Compound				mMC1R	mMC3R	mMC4R	mMC5R
	R^1	R^2	R^3	EC_{50} (μM)			
NDP-MSH				0.05 ± 0.01 nM	0.19 ± 0.04 nM	0.06 ± 0.01 nM	0.20 ± 0.05 nM
CGJ-9*	butylamine	TIQ	Trp	7.9 ± 1.0	1.7 ± 1.0	3.4 ± 1.9	4.9 ± 4.8
CGJ-7*	4-AMP	benzyl	Trp	5.2 ± 2.7	4.4 ± 2.8	> 100	8.2 ± 7.9
22	4-AMP	TIQ	Trp	40% @ 100 μM	> 100	30% @ 100 μM	> 100
23	4-AMP	TIQ	Phe	> 100	> 100	> 100	> 100
24	4-AMP	TIQ	(*p*I)Phe	> 100	> 100	> 100	> 100
25	4-AMP	TIQ	(*p*Cl)Phe	> 100	> 100	> 100	> 100
26	4-AMP	TIQ	His	6.4 ± 1.1	14 ± 0.4	7.7 ± 1.7	12 ± 1.3
27	4-AMP	TIQ	Nal(2')	> 100	> 100	> 100	> 100

[a]The indicated errors represent the standard error of the mean determined from at least three independent experiments. *The values for compounds have previously been reported and included here for comparison.[34] >100 indicates that the compound was examined but lacked agonist activity at up to 100 μM concentrations. A percentage (%) indicates the percent maximal stimulatory response observed at 100 μM concentrations observed, but not enough stimulation was observed to determine an EC_{50} value.

activity at any of the four MCRs when tested at up to 100 μM concentrations.

Compounds **17**−**21** contained the Indane group at the R^2 position were designed to determine whether constraining the aromatic group at this position would change the activity of the template. Compound **17**, with a Trp residue at the R^3 position, resulted in full micromolar agonist activity at all four receptors. Diminished activity was observed when Phe was substituted at the R^3 position (**18**), with 80% (mMC1R), 60% (mMC4R), and 70% (mMC5R) of the maximum cAMP response at 100 μM concentrations resulted. No stimulatory activity was observed at the mMC3R at 100 μM concentrations. When the Phe ring was substituted by a *p*-iodo substitution (compound **19**), some agonist activity was retained at the mouse MC1, MC4, and MC5 receptors while no activity was observed at the mMC3R. The *p*-chloro substitution at the Phe ring, compound **20**, lost activity at the mMC3−5Rs and possessed slight agonist activity at the mMC1R. Compound **21** having the His residue at the R^3 position, lacked agonist activity at any of the studied receptors.

Template 3. Compounds in Template 3 had fixed R^1 (4-AMP group) and R^2 (TIQ) positions that were present in lead compounds **CGJ-7** and **CGJ-9**. The R^3 position was substituted by the Trp, Phe, (*p*I)Phe, (*p*Cl)Phe, His and Nal(2′) residues in compounds **22**−**27**, respectively. Table 3 summarizes the agonist activity for this template at the melanocortin receptors. Compound **26**, with a His substitution, resulted in a full μM agonist at all four studied melanocortin receptors and showed equipotency (within 3-fold experimental error) at the mMC1R and mMC5R compared to both **CGJ-7** and **CGJ-9**. Compound **22**, with Trp at the R^3 position, showed 40% mMC1R and 30% mMC4R agonist activity as compared to the maximum cAMP response at 100 μM concentrations. All other substitutions in compounds **23**−**25** and **27** resulted in a lack of agonist activity at the MCRs tested.

Central melanocortin signaling has been implicated to regulate feeding behavior and energy homeostasis. There has been intensive research on identifying small molecules based on peptidic and nonpeptidic template to selectively target centrally located melanocortin receptors. Nonpeptidic small molecules may be favorable as potential drugs because of increased metabolic stability, oral bioavailability and ability to cross blood-brain barrier (BBB). The first small molecule template for melanocortins was identifed in a β-turn-based small molecules library screening by Haskell-Luevano and colleagues.[27] Following this, a potent and selective small molecule MC4R agonist molecule was reported by Merck & Co.[33] This compound was shown to reduce food intake in rats as well as stimulate erectile activity. Moreover, it was shown that these effects of the compound were mediated by MC4R since MC3R/4R double knockout mice did not show any of the above effects.[33] Several other small molecules have been reported from the Merck Research Laboratories including a report on a piperazine urea based MC4R partial agonist with nM potency at the human MC4R, but without an effect on erectile activity in the rodents.[41] A focused urea-based nonpeptide agonist library has previously been reported by our lab.[34] Compound **CGJ-7** was identified in that study as selective for the mMC3R (4.4 μM) as compared to the mMC4R, which showed no agonist activity up to 100 μM. Another compound **CGJ-9** with TIQ moiety at the R^1 position and aliphatic amine at the R^2 position resulted in a μM agonist at all four melanocortin receptors. Both of the compounds had Trp at the R^3 position and a urea core in the backbone. The urea core presents an advantage due to its similarity to an amide and it can also form hydrogen bonds to form secondary structures. The formation of intramolecular hydrogen bonds between urea and amide moieties in peptides has also been reported.[51] The introduction of an additional heteroatom in the peptidyl backbone by a urea derivative may also influence ligand−receptor interactions.

The present study was undertaken to take advantage of previously published SAR and utilize a three component diversity approach to identify selective MC3R/MC4R receptor

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small molecules. In the current study, five compounds (**1**, **2**, **12**, **17**, **26**) resulted in full agonist activity and another five compounds (**4**, **11**, **18**, **19**, **22**) showed a percent of maximal agonist activity either at the mMC3R, mMC4R or both. In general, aliphatic and cyclic amine groups at the R^1 position resulted in active compounds, while aromatic rings were not tolerated well and resulted mostly in inactive compounds. The R^2 position was modified with constrained (TIQ, Indane) and flexible (3,3-diphenyl propyl) aromatic rings and gave varied results on the melanocortin receptor subtypes. The R^3 position was substituted either with nonpolar hydrophobic amino acids Trp, Phe, (*p*I)Phe, (*p*Cl)Phe, Nal(2′), or His residue, which contained a polar imidazole side chain. When the Trp residue at the R^3 position in lead compound **CGJ-9** was replaced with Phe residue along with the bulkier aromatic group (compound **1**), loss of activity at the mMC3R resulted. This observation support the hypothesis that an indole ring is important for maintaining agonist activity at the mMC3R in this urea-based template and may be harnessed to generate selectivity between centrally expressed MC3 and MC4 receptors. Similar reports of Trp substitution by Ala and Pro in the cyclic peptide MTII resulted in only a 2% cAMP stimulation at the hMC3R.[52,53] In a shorter tetrapeptide sequence Ac-His-DPhe-Arg-Ala-NH_2, Trp to Ala substitution resulted in total loss of activity.[47] However, compound **1** retained full agonist activity at the mMC1R, mMC4R, and mMC5R and showed only 5-fold selectivity for the mMC1R and was equipotent at the mMC5R versus the mMC4R. The replacement of Trp with *p*-iodo substituted Phe residue (compound **2**) resulted in full agonist activity at all four MCRs, albeit with decreased potency compared to the lead compound **CGJ-9**. However, no activity was observed for *p*-chloro substitution (compound **3**) at this position. The difference between (*p*I)Phe and (*p*Cl)Phe pharmacology could be due to the difference in lipophilicity of these two compounds. The lipophilicity of halogens increases as *F* < Cl < Br < I. This may affect the interactions of these two compounds in the putative hydrophobic binding pocket of melanocortin receptors. Compound **4** with an imdiazole ring showed some stimulatory activity at the mMC1R, mMC4R, and MC5R but not enough to determine an EC_{50} value. The lack of activity at the mMC3R was similar to the tetrapeptide Trp to His substitution SAR.[47] In the previous urea-based study, compound **CGJ-6** (Figure 2) with 4-methylbenzylamine group at the R^1 position resulted in the most potent compound of that series with high nM activity at the mMC4R.[34] Unfortunately, when an aromatic 4-methylbenzyl group was introduced at the R^1 position in Template 1, only compound **6** showed a very slight agonist activity at the mMC1R and mMC5R and compound **9** was only able to stimulate the mMC5R to 40% maximum stimulation. No other compound from the Template 1 series was found active at the MCRs. The steric hindrance caused by the bulky aromatic ring at this position may be the reason for the nonactive compounds in this series. This may also suggests that the mMC1R and mMC5R putative binding pockets may be more flexible to accommodate bulkier side chain than the mMC3R and mMC4R.

Compounds **11**−**21** had a cyclic piperidine ring at the R^1 position, and the R^2 position was varied to aromatic rings that were attached either to a flexible propyl linker in 3,3-diphenylpropyl or a constrained cyclic frame in Indane. Compound **11** with the R^2 modified to 3,3-diphenylpropyl and Trp residue at the R^3 position showed selectivity for the mMC1R. When Trp residue was replaced with Phe, resulting in compound **12**, equimolar potency at the mMC1R and mMC5R resulted, as compared to the lead compound **CGJ-7**, but no activity at the mMC3R was observed. Compound **12** showed 6.7 μM full agonist activity at the mMC4R and was selective for the centrally located mMC4R versus the mMC3R. However, this analog was not selective when compared to the predominantly peripheral receptors mMC1R and mMC5R. When the aromatic ring was constrained by a cyclic frame as opposed to a diphenyl ring that was attached to a flexible propyl linker, compound **17** containing Trp residue showed full agonist activity at all four receptors. Compounds **18** and **19**, containing Phe and (*p*I)Phe, respectively, did not show full agonist activities and contained varied stimulatory activity at the mMC1R, mMC4R, and mMC5R. Both of these compounds were inactive at the mMC3R. Compound **18** showed more activity than the compound **19** at the mMC1R. The differences in SAR for theses two groups can be attributed to the relatively smaller size of Indane and the propyl linker of the 3,3-DPP group, which allows increased rotational freedom of the aromatic rings and renders it less rigid as compared to Indane substitution.

Finally, template 3 (compounds **22**−**27**) was designed to see effects of removing the NH proton by placing a ring system directly attached to nitrogen atoms at both ends. The TIQ residue was positioned at the R^1 and 4-AMP at the R^2 position and were both directly attached to nitrogen atoms. Interestingly, compound **26** with the His substitution at the R^3 position resulted in full micromolar agonist at all four melanocortin receptors. The Trp substitution (compound **22**) resulted in only slight agonist activity at the mMC1R and mMC4R with the Trp residue. All other compounds **23**−**25** and **27** having nonpolar hydrophobic substitutions did not show any activity at MCRs. This observation suggests that the hydrogen bond may play an important role in ligand−receptor interactions or stabilizing the ligand in bioactive conformation in this small molecule template.

In summary, we have designed, synthesized, and characterized three novel series of melanocortin agonists based on an unsymmetrical substituted urea core. The synthesis was performed on solid phase using microwave irradiation, resulting in significantly faster syntheses than previously reported. The structure−activity relationship studies based on urea scaffold presented herein provides insight into important ligand−receptor interactions for MC3R vs MC4R activity. In particular, compounds having the aliphatic or saturated cyclic group at the R^1 position, bulky aromatic group at the R^2 position and indole or phenyl group at the R^3 position showed activity at MCRs in the small urea based molecule template. Compounds **1** and **12** showed selectivity for the centrally expressed mMC4R, as compared to the mMC3R, but no significant selectivity profile was observed at the mMC1R and mMC5R versus the mMC4R. These results may be useful in the further design of selective nonpeptide small molecule ligands for the melanocortin receptors.

METHODS

Chemistry. The amino acids Nα 9-fluorenylmethyloxycarbonyl (Fmoc)-Trp-OH, Fmoc-Phe-OH, Fmoc-(*p*Cl)Phe-OH, and Fmoc-Nal(2′)-OH, the resin 4-(4-hydroxymethyl-3-methoxyphenoxy)-butyryl-MBHA (HMPB-MBHA Resin, 0.47 mequiv/g substitution), and the coupling reagent *O*-(benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate (HBTU) were purchased from

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Peptides International (Louisville, KY). Fmoc-(*p*I)Phe-OH was purchased from Synthetech (Albany, OR). Fmoc-His-OH was purchased from Bachem (Torrance, CA). 4-Nitrophenyl chloroformate, 1,4-diaminobutane, 1,2,3,4-tetrahydroisoquinoline, 4-(aminomethyl)piperidine, 3,3-diphenylpropylamine, and 2-aminoindane, diisopropylethylamine (DIEA) were purchased from Sigma-Aldrich (St. Louis, MO). Dichloromethane (DCM), tetrahydrofuran (THF), glacial acetic acid, methanol, acetonitrile, isopropanol (*i*-PrOH), and anhydrous diethyl ether were purchased from Fisher (Fair Lawn, NJ). *N*,*N*-Dimethylformamide (DMF) was purchased from Burdick and Jackson (McGaw Park, IL). All chemicals were obtained from commercial suppliers and were used without further purification.

General Procedure for Compounds 1−27. The solid-phase synthesis (SPS) of the library compounds used in this study is modified from the previously published method.[34] Microwave synthesizer (CEM Discover SPS) was used instead of conventional SPS synthesis. Discover SPS is equipped with a fiber-optic temperature probe for controlling the microwave power delivery. Agitation is accomplished by nitrogen gas bubbling through external setup. A separate vacuum manifold is provided with the assembly for solvent drainage.

Coupling of Diamine Unit (R^1) on Solid Phase. HMPB-MBHA resin (0.1 mmol) was swelled in 25 mL polypropylene reaction vessel (CEM) with THF/DCM (1:1) for 1 h. The solvent was drained, and then *p*-nitrophenyl chloroformate (18.7 equiv in THF/DCM (1:1), 2 mmol) and DIEA (18.7 equiv in THF/DCM (1:1), 2 mmol) were added. The reaction mixture was bubbled with nitrogen for mixing and subjected to the microwave irradiation (35 °C, 30 W) for 5 min. After which, the resins were washed with 2 × THF/DCM (1:1), 2 × DCM, 1 × DMF. The appropriate diamine, R^1 subunit (12.5 eq, 1.2 mmol) was then added, along with DIEA (12.5 equiv) in DMF under microwave irradiation (55 °C, 50W) for 10 min to generate **28**. The presence of the primary amine group was tested using the Kaiser test.[54] The resins were washed with 3 × DMF.

Coupling of Amino Acid Unit (R^3) on Solid Phase. The diamine addition was followed by coupling of amino acid (3.0 eq, 0.3 mmol) under the microwave irradiation (75 °C, 30 W) for 5 min using HBTU (3.0 equiv) and DIEA (5.1 equiv) to the terminal *N*-Fmoc protected intermediate **29**. The coupling of the His amino acid was performed at lower temperature in the microwave synthesizer (50 °C, 30 W) for 5 min. The coupling of appropriate amino acid was confirmed by a negative Kaiser test. The resins were washed with 5 × DMF and subjected to the Fmoc deprotection by 20% piperidine in DMF 1 × 2 min at room temperature and then by irradiating the sample at 75 °C, 30 W for 4 min in the CEM Discover SPS instrument to generate free amine compound **30** on the resin. The removal of Fmoc was confirmed by a positive Kaiser test.

Coupling of R^2 Subunit on Solid Phase. The *N*-Fmoc deprotection was followed by activation with *p*-nitrophenyl chloroformate (1.4 equiv) and DIEA (1.4 equiv) in 1:1 THF/DCM for 5 min under same conditions as described in the first step. The resins were washed with 2 × THF/DCM (1:1), 3 × DCM, and 2 × DMF, and respective amine group (R^2 subunit) (1.4 equiv) was then added, along with DIEA (1.4 equiv) in DMF and irradiate under microwave irradiation (55 °C, 50 W) for 10 min to get **31**. The resins were washed with 3 × DMF and 2 × THF/DCM (1:1), 2 × THF and DCM, 2 × acetic acid and *i*-PrOH, 1 × methanol, and 3 × DCM and dried under vacuum before cleavage.

Cleavage from the Solid Phase. The cleavage of compounds from resin was performed by adding glacial acetic acid (5 mL) to the resin and subjected it to microwave irradiation for 30 min at 38 °C, 20W. Following 30 min, the cleaved product was collected, the resins were washed with an additional 1 mL of glacial acetic acid. The solutions were then frozen at −78 °C and lyophilized to achieve crude compounds **1**−**27**.

The crude compounds were purified by reversed-phase HPLC (flow rate of 5 mL/min, acetonitrile/water 0.1% TFA) using a Shimadzu chromatography system with a photodiode array detector and a semipreparative RP-HPLC C18 bonded silica column (Vydac 218TP1010, 1.0 × 25 cm). The purified compounds were >95% pure as determined by RP-HPLC in two diverse solvent systems and had the correct molecular mass (Supporting Information). Electrospray ionization mass spectrometry (ESI-MS) was used to record spectra on an ABI 3200Q TRAP instrument at the University of Florida. The lead compounds **1** and **12** were also characterized by NMR and HRMS. NMR spectra was acquired in MeOH-d_4 with Varian 400 MHz spectrometer with autosampler and HRMS (ESI positive) were obtained with a Bruker BioTof II mass Spectrometer (University of Minnesota).

Functional Bioassay. HEK-293 cells stably expressing the mouse melanocortin receptors were maintained in Dulbecco's modified Eagle's medium (DMEM) with 10% fetal calf serum and transfected with 4 μg of the CRE/β-galactosidase reporter gene as previously described.[44] Briefly, 5000−15 000 post-transfection cells were plated into collagen treated 96-well plates (Nunc) and incubated overnight. At 48 h post-transfection, the cells were stimulated with 100 μL of peptide (10^{-4}−10^{-12} M) or forskolin (10^{-4} M) control in assay medium (DMEM containing 0.1 mg/mL BSA and 0.1 mM isobutylmethylxanthine) for 6 h. The assay media was aspirated and 50 μL of lysis buffer (250 mM Tris-HCl pH = 8.0 and 0.1% Triton X-100) was added. The plates were stored at −80 °C overnight. The plates containing the cell lysates were thawed the following day. Aliquots of 10 μL were taken from each well and transferred to another 96-well plate for relative protein determination. To the cell lysate plates, 40 μL of phosphate-buffered saline with 0.5% BSA was added to each well. Subsequently, 150 μL of substrate buffer (60 mM sodium phosphate, 1 mM $MgCl_2$, 10 mM KCl, 5 mM β-mercaptoethanol, 2 mg/mL ortho-nitrophenyl-β-galactoside [ONPG]) was added to each well and the plates were incubated at 37 °C. The sample absorbance, OD_{405}, was measured using a 96-well plate reader (Molecular Devices). The relative protein was determined by adding 200 μL of 1:5 dilution Bio Rad G250 protein dye/water to the 10 μL cell lysate sample taken previously, and the OD_{595} was measured on a 96-well plate reader (Molecular Devices). Data points were normalized both to the relative protein content and nonreceptor dependent forskolin stimulation. Maximal efficacy was compared to that observed for the NDP-MSH control peptide tested simultaneously on each 96-well plate.

Data Analysis. The agonist EC_{50} values represent the mean of duplicate wells performed in three or more independent experiments. The EC_{50} value estimates, and their associated standard errors, were determined by fitting the data to a nonlinear least-squares analysis using the PRISM software program (v4.0, GraphPad Inc.). The compounds were assayed as TFA salts.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acschemneuro.5b00273.

Analytical data table of all synthesized compounds, characterization data of compounds **1** and **12**, and NMR spectra (PDF)

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Notes

The authors declare no competing financial interest.

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A.S. and C.H.-L. designed the research. A.S., J.K., M.L.S.D. and H.H performed the experiments. A.S. and C.H.-L. analyzed the data. A.S. wrote the manuscript with the help of C.H.-L.

■ ABBREVIATIONS

MCR, melanocortin receptor; MC3R, melanocortin-3 receptor; MC4R, melanocortin-4 receptor; POMC, proopiomelanocortin; SAR, structure−activity relationship; TIQ, 1,2,3,4,-tetrahydroisoquinoline; AMP, 4-(aminomethyl)piperidine; MBA, (4-methyl)benzylamine; DPP, diphenylpropyl; RP-HPLC, reverse-phase high-pressure liquid chromatography; MBHA, 4-methylbenzhydrylamine

■ REFERENCES

(1) Chhajlani, V., and Wikberg, J. E. (1992) Molecular Cloning and Expression of the Human Melanocyte Stimulating Hormone Receptor cDNA. *FEBS Lett. 309*, 417−420.

(2) Mountjoy, K. G., Robbins, L. S., Mortrud, M. T., and Cone, R. D. (1992) The Cloning of a Family of Genes that Encode the Melanocortin Receptors. *Science 257*, 1248−1251.

(3) Roselli-Rehfuss, L., Mountjoy, K. G., Robbins, L. S., Mortrud, M. T., Low, M. J., Tatro, J. B., Entwistle, M. L., Simerly, R. B., and Cone, R. D. (1993) Identification of a Receptor for Gamma Melanotropin and Other Proopiomelanocortin Peptides in the Hypothalamus and Limbic System. *Proc. Natl. Acad. Sci. U. S. A. 90*, 8856−8860.

(4) Gantz, I., Konda, Y., Tashiro, T., Shimoto, Y., Miwa, H., Munzert, G., Watson, S. J., DelValle, J., and Yamada, T. (1993) Molecular Cloning of a Novel Melanocortin Receptor. *J. Biol. Chem. 268*, 8246−8250.

(5) Gantz, I., Miwa, H., Konda, Y., and Shimoto, Y. (1993) Molecular Cloning, Expression, and Gene Localization of a Fourth Melanocortin Receptor. *J. Biol. Chem. 268*, 15174−15179.

(6) Chhajlani, V., Muceniece, R., and Wikberg, J. E. (1993) Molecular Cloning of a Novel Human Melanocortin Receptor. *Biochem. Biophys. Res. Commun. 195*, 866−873.

(7) Gantz, I., Shimoto, Y., Konda, Y., Miwa, H., Dickinson, C., and Yamada, T. (1994) Molecular Cloning, Expression, and Characterization of a Fifth Melanocortin Receptor. *Biochem. Biophys. Res. Commun. 200*, 1214−1220.

(8) Griffon, N., Mignon, V., Facchinetti, P., Diaz, J., Schwartz, J. C., and Sokoloff, P. (1994) Molecular Cloning and Characterization of the Rat Fifth Melanocortin Receptor. *Biochem. Biophys. Res. Commun. 200*, 1007−1014.

(9) Huszar, D., Lynch, C. A., Fairchild-Huntress, V., Dunmore, J. H., Fang, Q., Berkemeier, L. R., Gu, W., Kesterson, R. A., Boston, B. A., Cone, R. D., Smith, F. J., Campfield, L. A., Burn, P., and Lee, F. (1997) Targeted Disruption of the Melanocortin-4 receptor Results in Obesity in Mice. *Cell 88*, 131−141.

(10) Fan, W., Boston, B. A., Kesterson, R. A., Hruby, V. J., and Cone, R. D. (1997) Role of Melanocortinergic Neurons in Feeding and the Agouti Obesity Syndrome. *Nature 385*, 165−168.

(11) Smith, P. E. (1916) Experimental Ablation of the Hypophysis in the Frog Embryo. *Science 44*, 280−282.

(12) Haynes, R. C., Jr., and Berthet, L. (1957) Studies on the Mechanism of Action of the Adrenocorticotropic Hormone. *J. Biol. Chem. 225*, 115−124.

(13) Greenfield, J. R., Miller, J. W., Keogh, J. M., Henning, E., Satterwhite, J. H., Cameron, G. S., Astruc, B., Mayer, J. P., Brage, S., See, T. C., Lomas, D. J., O'Rahilly, S., and Farooqi, I. S. (2009) Modulation of Blood Pressure by Central Melanocortinergic Pathways. *N. Engl. J. Med. 360*, 44−52.

(14) Wessells, H., Hruby, V. J., Hackett, J., Han, G., Balse-Srinivasan, P., and Vanderah, T. W. (2003) Ac-Nle-c[Asp-His-DPhe-Arg-Trp-Lys]-NH2 Induces Penile Erection Via Brain and Spinal Melanocortin Receptors. *Neuroscience 118*, 755−762.

(15) Wessells, H., Levine, N., Hadley, M., Dorr, R., and Hruby, V. (2000) Melanocortin Receptor Agonists, Penile Erection, and Sexual Motivation: Human Studies With Melanotan II. *Int. J. Impotence Res. 12*, S74−S79.

(16) Wessells, H., Fuciarelli, K., Hansen, J., Hadley, M. E., Hruby, V. J., Dorr, R., and Levine, N. (1998) Synthetic Melanotropic Peptide Initiates Erections in Men with Psychogenic Erectile Dysfunction: Double-Blind, Placebo Controlled Crossover Study. *J. Urol. 160*, 389−393.

(17) Eipper, B. A., and Mains, R. E. (1980) Structure and Biosynthesis of Pro-Adrenocorticotropin/Endorphin and Related Peptides. *Endocr. Rev. 1*, 1−26.

(18) Hruby, V. J., Wilkes, B. C., Hadley, M. E., Al-Obeidi, F., Sawyer, T. K., Staples, D. J., de Vaux, A. E., Dym, O., and Castrucci, A. M. (1987) alpha-Melanotropin: the Minimal Active Sequence in the Frog Skin Bioassay. *J. Med. Chem. 30*, 2126−2130.

(19) Schioth, H. B., Chhajlani, V., Muceniece, R., Klusa, V., and Wikberg, J. E. (1996) Major Pharmacological Distinction of the ACTH Receptor from Other Melanocortin Receptors. *Life Sci. 59*, 797−801.

(20) Irani, B. G., Xiang, Z., Yarandi, H. N., Holder, J. R., Moore, M. C., Bauzo, R. M., Proneth, B., Shaw, A. M., Millard, W. J., Chambers, J. B., Benoit, S. C., Clegg, D. J., and Haskell-Luevano, C. (2011) Implication of the Melanocortin-3 Receptor in the Regulation of Food Intake. *Eur. J. Pharmacol. 660*, 80−87.

(21) Butler, A. A., Kesteson, R. A., Khong, K., Cullen, M. J., Pelleymounter, M. A., Dekoning, J., Baetscher, M., and Cone, R. D. (2000) A Unique Metabolic Syndrome Causes Obesity in the Melanocortin-3 Receptor-Deficient Mouse. *Endocrinology 141*, 3518−3521.

(22) Chen, A. S., Marsh, D. J., Trumbauer, M. E., Frazier, E. G., Guan, X. M., Yu, H., Rosenblum, C. I., Vongs, A., Feng, Y., Cao, L., Metzger, J. M., Strack, A. M., Camacho, R. E., Mellin, T. N., Nunes, C. N., Min, W., Fisher, J., Gopal-Truter, S., MacIntyre, D. E., Chen, H. Y., and Van der Ploeg, L. H. (2000) Inactivation of the Mouse Melanocortin-3 Receptor Results in Increased Fat Mass and Reduced Lean Body Mass. *Nat. Genet. 26*, 97−102.

(23) Benoit, S. C., Schwartz, M. W., Lachey, J. L., Hagan, M. M., Rushing, P. A., Blake, K. A., Yagaloff, K. A., Kurylko, G., Franco, L., Danhoo, W., and Seeley, R. J. (2000) A Novel Selective Melanocortin-4 Receptor Agonist Reduces Fod Intake in Rats and Mice Without Producing Aversive Consequences. *J. Neurosci. 20*, 3442−3448.

(24) Farooqi, I. S., Keogh, J. M., Yeo, G. S., Lank, E. J., Cheetham, T., and O'Rahilly, S. (2003) Clinical Spectrum of Obesity and Mutations in the Melanocortin 4 Receptor Gene. *N. Engl. J. Med. 348*, 1085−1095.

(25) Sawyer, T. K., Sanfilippo, P. J., Hruby, V. J., Engel, M. H., Heward, C. B., Burnett, J. B., and Hadley, M. E. (1980) 4-Norleucine, 7-D-Phenylalanine-alpha-Melanocyte-Stimulating Hormone: a Highly Potent alpha-Melanotropin with Ultralong Biological Activity. *Proc. Natl. Acad. Sci. U. S. A. 77*, 5754−5758.

(26) Castrucci, A. M., Hadley, M. E., Sawyer, T. K., and Hruby, V. J. (1984) Enzymological Studies of Melanotropins. *Comp. Biochem. Physiol. B 78*, 519−524.

(27) Haskell-Luevano, C., Rosenquist, A., Souers, A., Khong, K. C., Ellman, J. A., and Cone, R. D. (1999) Compounds that Activate the Mouse Melanocortin-1 Receptor Identified by Screening a Small Molecule Library Based Upon the beta-Turn. *J. Med. Chem. 42*, 4380−4387.

(28) Bondebjerg, J., Xiang, Z., Bauzo, R. M., Haskell-Luevano, C., and Meldal, M. (2002) A Solid-Phase Approach to Mouse Melanocortin Receptor Agonists Derived from a Novel Thioether Cyclized Peptidomimetic Scaffold. *J. Am. Chem. Soc. 124*, 11046−11055.

(29) Ujjainwalla, F., Warner, D., Walsh, T. F., Wyvratt, M. J., Zhou, C., Yang, L., Kalyani, R. N., MacNeil, T., Van der Ploeg, L. H., Rosenblum, C. I., Tang, R., Vongs, A., Weinberg, D. H., and Goulet, M. T. (2003) Design and Syntheses of Melanocortin Subtype-4 Receptor Agonists: Evolution of the Pyridazinone Archetype. *Bioorg. Med. Chem. Lett. 13*, 4431−4435.

DOI: 10.1021/acschemneuro.5b00273
ACS Chem. Neurosci. 2016, 7, 196−205

(30) Joseph, C. G., Wilson, K. R., Wood, M. S., Sorenson, N. B., Phan, D. V., Xiang, Z., Witek, R. M., and Haskell-Luevano, C. (2008) The 1,4-Benzodiazepine-2,5-Dione Small Molecule Template Results in Melanocortin Receptor Agonists With Nanomolar Potencies. *J. Med. Chem. 51*, 1423−1431.
(31) Nargund, R. P., Strack, A. M., and Fong, T. M. (2006) Melanocortin-4 receptor (MC4R) Agonists for the Ttreatment of Obesity. *J. Med. Chem. 49*, 4035−4043.
(32) Ujjainwalla, F., and Sebhat, I. K. (2007) Small Molecule Ligands of the Human Melanocortin-4 Receptor. *Curr. Top. Med. Chem. 7*, 1068−1084.
(33) Sebhat, I. K., Martin, W. J., Ye, Z., Barakat, K., Mosley, R. T., Johnston, D. B., Bakshi, R., Palucki, B., Weinberg, D. H., MacNeil, T., Kalyani, R. N., Tang, R., Stearns, R. A., Miller, R. R., Tamvakopoulos, C., Strack, A. M., McGowan, E., Cashen, D. E., Drisko, J. E., Hom, G. J., Howard, A. D., MacIntyre, D. E., van der Ploeg, L. H., Patchett, A. A., and Nargund, R. P. (2002) Design and Pharmacology of N-[(3R)-1,2,3,4-tetrahydroisoquinolinium- 3-ylcarbonyl]-(1R)-1-(4-chlorobenzyl)- 2-[4-cyclohexyl-4-(1H-1,2,4-triazol- 1-ylmethyl)piperidin-1-yl]-2-oxoethylamine (1), A Potent, Selective, Melanocortin Subtype-4 Receptor Agonist. *J. Med. Chem. 45*, 4589−4593.
(34) Joseph, C. G., Bauzo, R. M., Xiang, Z., and Haskell-Luevano, C. (2003) Urea Small Molecule Agonists on Mouse Melanocortin Receptors. *Bioorg. Med. Chem. Lett. 13*, 2079−2082.
(35) Todorovic, A., Joseph, C. G., Sorensen, N. B., Wood, M. S., and Haskell-Luevano, C. (2007) Structure-Activity Relationships of Melanocortin Agonists Containing the Benzimidazole Scaffold. *Chem. Biol. Drug Des. 69*, 338−349.
(36) Lam, P. Y., Jadhav, P. K., Eyermann, C. J., Hodge, C. N., Ru, Y., Bacheler, L. T., Meek, J. L., Otto, M. J., Rayner, M. M., Wong, Y. N., et al. (1994) Rational Design of Potent, Bioavailable, Nonpeptide Cyclic Ureas as HIV Protease Inhibitors. *Science 263*, 380−384.
(37) Zhan, W., Li, Y., Huang, W., Zhao, Y., Yao, Z., Yu, S., Yuan, S., Jiang, F., Yao, S., and Li, S. (2012) Design, Synthesis and Antitumor Activities of Novel bis-Aryl Ureas Derivatives as Raf kinase Inhibitors. *Bioorg. Med. Chem. 20*, 4323−4329.
(38) Castro, J. L., Ball, R. G., Broughton, H. B., Russell, M. G., Rathbone, D., Watt, A. P., Baker, R., Chapman, K. L., Fletcher, A. E., Patel, S., Smith, A. J., Marshall, G. R., Ryecroft, W., and Matassa, V. G. (1996) Controlled Modification of Acidity in Cholecystokinin B Receptor Antagonists: N-(1,4-benzodiazepin-3-yl)-N′-[3-(tetrazol-5-ylamino) Phenyl]ureas. *J. Med. Chem. 39*, 842−849.
(39) Semple, G., Ryder, H., Rooker, D. P., Batt, A. R., Kendrick, D. A., Szelke, M., Ohta, M., Satoh, M., Nishida, A., Akuzawa, S., and Miyata, K. (1997) (3R)-N-(1-(tert-butylcarbonylmethyl)-2,3-dihydro-2-oxo-5-(2-pyridyl)-1H-1,4-benzo diazepin-3-yl)-N′-(3-(methylamino)phenyl)urea (YF476): A Potent and Orally Active Gastrin/CCK-B Antagonist. *J. Med. Chem. 40*, 331−341.
(40) von Geldern, T. W., Kester, J. A., Bal, R., Wu-Wong, J. R., Chiou, W., Dixon, D. B., and Opgenorth, T. J. (1996) Azole Endothelin Antagonists. 2. Structure-Activity Studies. *J. Med. Chem. 39*, 968−981.
(41) Hong, Q., Bakshi, R. K., Palucki, B. L., Park, M. K., Ye, Z., He, S., Pollard, P. G., Sebhat, I. K., Liu, J., Guo, L., Cashen, D. E., Martin, W. J., Weinberg, D. H., MacNeil, T., Tang, R., Tamvakopoulos, C., Peng, Q., Miller, R. R., Stearns, R. A., Chen, H. Y., Chen, A. S., Strack, A. M., Fong, T. M., MacIntyre, D. E., Wyvratt, M. J., and Nargund, R. P. (2011) Discovery of a Piperazine Urea Based Compound as a Potent, Selective, Orally Bioavailable Melanocortin Subtype-4 Receptor Partial Agonist. *Bioorg. Med. Chem. Lett. 21*, 2330−2334.
(42) Berk, S. C., Rohrer, S. P., Degrado, S. J., Birzin, E. T., Mosley, R. T., Hutchins, S. M., Pasternak, A., Schaeffer, J. M., Underwood, D. J., and Chapman, K. T. (1999) A Combinatorial Approach Toward the Discovery of Non-Peptide, Subtype-Selective Somatostatin Receptor Ligands. *J. Comb. Chem. 1*, 388−396.
(43) Rohrer, S. P., Birzin, E. T., Mosley, R. T., Berk, S. C., Hutchins, S. M., Shen, D. M., Xiong, Y., Hayes, E. C., Parmar, R. M., Foor, F., Mitra, S. W., Degrado, S. J., Shu, M., Klopp, J. M., Cai, S. J., Blake, A., Chan, W. W., Pasternak, A., Yang, L., Patchett, A. A., Smith, R. G., Chapman, K. T., and Schaeffer, J. M. (1998) Rapid Identification of Subtype-Selective Agonists of the Somatostatin Receptor Through Combinatorial Chemistry. *Science 282*, 737−740.
(44) Chen, W., Shields, T. S., Stork, P. J., and Cone, R. D. (1995) A Colorimetric Assay for Measuring Activation of Gs- and Gq-Coupled Signaling Pathways. *Anal. Biochem. 226*, 349−354.
(45) Haslach, E. M., Huang, H., Dirain, M., Debevec, G., Geer, P., Santos, R. G., Giulianotti, M. A., Pinilla, C., Appel, J. R., Doering, S. R., Walters, M. A., Houghten, R. A., and Haskell-Luevano, C. (2014) Identification of Tetrapeptides from a Mixture Based Positional Scanning Library that can Restore nM Full Agonist Function of the L106P, I69T, I102S, A219V, C271Y, and C271R Human Melanocortin-4 Polymorphic Receptors (hMC4Rs). *J. Med. Chem. 57*, 4615−4628.
(46) Holder, J. R., Bauzo, R. M., Xiang, Z., and Haskell-Luevano, C. (2002) Structure-Activity Relationships of the Melanocortin Tetrapeptide Ac-His-DPhe-Arg-Trp-NH(2) at the Mouse Melanocortin Receptors: part 2 Modifications at the Phe Position. *J. Med. Chem. 45*, 3073−3081.
(47) Holder, J. R., Xiang, Z., Bauzo, R. M., and Haskell-Luevano, C. (2002) Structure-Activity Relationships of the Melanocortin Tetrapeptide Ac-His-D-Phe-Arg-Trp-NH2 at the Mouse Melanocortin Receptors. 4. Modifications at the Trp Position. *J. Med. Chem. 45*, 5736−5744.
(48) Proneth, B., Pogozheva, I. D., Portillo, F. P., Mosberg, H. I., and Haskell-Luevano, C. (2008) Melanocortin Tetrapeptide Ac-His-DPhe-Arg-Trp-NH2Modified at the Para Position of the Benzyl Side Chain (DPhe): Importance for Mouse Melanocortin-3 Receptor Agonist Versus Antagonist Activity. *J. Med. Chem. 51*, 5585−5593.
(49) Singh, A., Tala, S. R., Flores, V., Freeman, K., and Haskell-Luevano, C. (2015) Synthesis and Pharmacology of alpha/beta(3)-Peptides Based on the Melanocortin Agonist Ac-His-dPhe-Arg-Trp-NH2 Sequence. *ACS Med. Chem. Lett. 6*, 568−572.
(50) Singh, A., Dirain, M. L., Wilczynski, A., Chen, C., Gosnell, B. A., Levine, A. S., Edison, A. S., and Haskell-Luevano, C. (2014) Synthesis, Biophysical, and Pharmacological Evaluation of the Melanocortin Agonist AST3−88: Modifications of Peptide Backbone at Trp 7 Position Lead to a Potent, Selective, and Stable Ligand of the Melanocortin 4 Receptor (MC4R). *ACS Chem. Neurosci. 5*, 1020−1031.
(51) Nowick, J. S. (1999) Chemical Models of Protein beta-Sheets. *Acc. Chem. Res. 32*, 287−296.
(52) Bednarek, M. A., Macneil, T., Kalyani, R. N., Tang, R., Van der Ploeg, L. H., and Weinberg, D. H. (1999) Analogs of MTII, lactam Derivatives of alpha-Melanotropin, Modified at the N-Terminus, and Their Selectivity at Human Melanocortin Receptors 3, 4, and 5. *Biochem. Biophys. Res. Commun. 261*, 209−213.
(53) Bednarek, M. A., Silva, M. V., Arison, B., MacNeil, T., Kalyani, R. N., Huang, R. R., and Weinberg, D. H. (1999) Structure-Function Studies on the Cyclic Peptide MT-II, Lactam Derivative of alpha-Melanotropin. *Peptides 20*, 401−409.
(54) Kaiser, E., Colescott, R. L., Bossinger, C. D., and Cook, P. I. (1970) Color Test for Detection of Free Terminal Amino Groups in the Solid-Phase Synthesis of Peptides. *Anal. Biochem. 34*, 595−598.

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