The 1,4-Benzodiazepine-2,5-dione Small Molecule Template Results in Melanocortin Receptor Agonists with Nanomolar Potencies

Christine G. Joseph,[†] Krista R. Wilson,[†] Michael S. Wood, Nicholas B. Sorenson, Dong V. Phan, Zhimin Xiang, Rachel M. Witek, and Carrie Haskell-Luevano*

Department of Medicinal Chemistry, College of Pharmacy, University of Florida, Gainesville, Florida 32610

Received October 16, 2007

The melanocortin system consists of five seven-transmembrane spanning G-protein coupled receptors (MC1−5) that are stimulated by endogenous agonists and antagonized by the only two known endogenous antagonists of GPCRs, agouti and agouti-related protein (AGRP). These receptors have been associated with many physiological functions, including the involvement of the MC4R in feeding behavior and energy homeostasis, making this system an attractive target for the treatment of obesity. Small-molecule mimetics of endogenous ligands may result in the development of compounds with properties more suitable for use as therapeutic agents. The research presented herein involves the synthesis and analysis of 12 melanocortin receptor agonists using the 1,4-benzodiazepine-2,5-dione template and is the first report of these derivatives as melanocortin receptor agonists. Structure–activity relationship studies using this privileged structure template has resulted in molecules with molecular weights around 400 that possess nanomolar agonist potency at the melanocortin receptors examined in this study.

Introduction

The melanocortin system consists of five receptors (MC1−5),[1–7] which are members of the superfamily of G-protein coupled receptors (GPCRs).[a] These receptors are seven transmembrane-spanning receptors, which upon agonist binding stimulate the cAMP signal transduction pathway. The melanocortin receptors are stimulated by the endogenous agonists α-MSH, β-MSH, γ1-MSH, γ2-MSH, and ACTH-derived from the proopiomelanocortin (POMC) gene transcript. The melanocortin receptors are antagonized by the only two known endogenous antagonists of GPCRs, agouti[8,9] and agouti-related protein (AGRP).[10,11] The MC1R is expressed in melanocytes and is involved in the production of melanin, the pigment responsible for skin and coat coloration.[2,12–14] The MC2R is expressed in the adrenal cortex where it mediates the production of glucocorticoids and aldosterone.[2] The MC3R is expressed in the brain, heart, gastrointestinal tract, and placenta[4,15] and has been shown to be involved in energy homeostasis, though the mechanism of action remains to be clarified.[16,17] The MC4R is expressed in the brain[6,18] and testis and is involved in energy and weight homeostasis,[19] feeding behavior,[20] and sexual function.[21,22] The MC4R knockout mice exhibit hyperphagia, hyperinsulinemia, hyperglycemia, and increased linear growth when compared with their wild-type littermates.[19] It is hypothesized that potent agonists selective for the MC4R may be used as therapeutic agents to treat obesity. The MC5R is expressed in peripheral tissues and is involved in the secretion of lipids from exocrine glands in mice.[7,23]

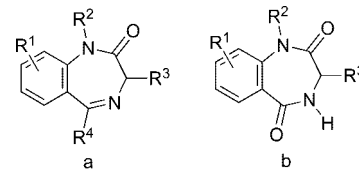


Figure 1. Structures of (a) 1,4-benzodiazepin-2-ones and (b) 1,4-benzodiazepin-2,5-diones.

Information obtained from peptide-related SAR studies may be used for the design of peptidomimetic and small-molecule templates that are potent and melanocortin receptor selective. Peptides are not ideally suited for use as drugs due to their large molecular weight, high rate of first-pass metabolism in the stomach and small intestine, and extreme sensitivity to changes in pH or temperature; therefore it is desirable to obtain a small-molecule drug. In the design of small molecules, the information obtained from the peptide SAR studies is used to create conformationally restrained peptides or small molecules. Further studies are done to optimize the molecule in regards to potency, selectivity, and toxicity. Small-molecule libraries have become an important part of the drug discovery process[24–26] with particular emphasis placed upon the preparation and evaluation of libraries based upon privileged structures. These structures display a number of different functionalities that result in a number of potent and specific drugs or candidates for different therapeutic targets. The 1,4-benzodiazepines are an important class of privileged templates, and numerous derivatives have been identified that have selective activities against a diverse array of biological targets. A subset of the 1,4-benzodiazepines, the 1,4-benzodiazepine-2,5-diones (Figure 1b), are the focus of this work. The 1,4-benzodiazepin-2,5-diones (Figure 1b) are perhaps the next most studied benzodiazepine framework after the 1,4-benzodiazepin-2-ones (Figure 1a). 1,4-Benzodiazepin-2,5-diones have been reported to possess anticonvulsant, anxi-

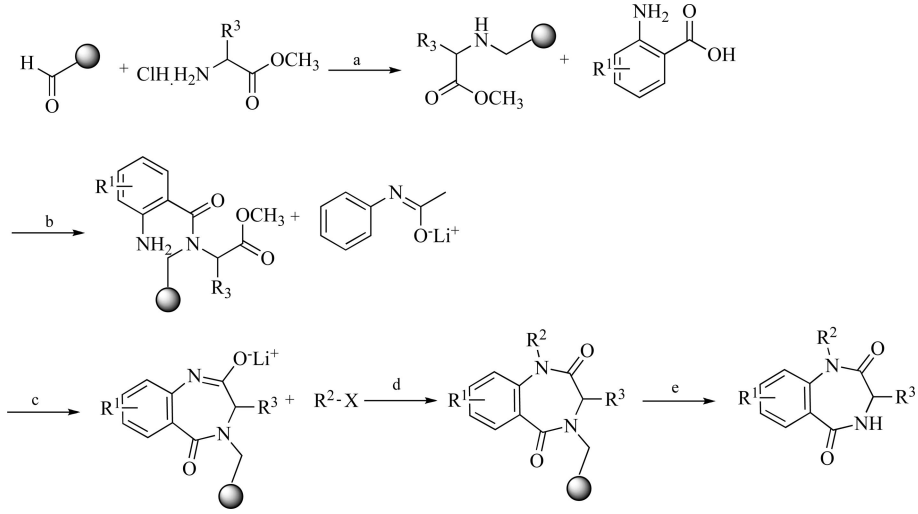
* To whom correspondence should be addressed. Phone: (352)846-2722. Fax: (352) 392-8182. E-mail: Carrie@cop.ufl.edu.

† These are equally contributing first authors.

[a] ACTH, adrenocorticotropic hormone; AGRP, agouti-related protein; BAL, backbone amide linker resin; cAMP, cyclic 5′-adenosine monophosphate; DCM, dichloromethane; DMF, *N*,*N*-dimethylformamide; DMS, dimethylsulfide; DNPH, dinitrophenylhydrazine; EDC, 1-[3-(dimethylamino)propyl]-1′-ethylcarbodiimide hydrochloride; GPCR, G-protein coupled receptor; MC1R, melanocortin-1 receptor; MC2R, melanocortin-2 receptor; MC3R, melanocortin-3 receptor; MC4R, melanocortin-4 receptor; MC5R, melanocortin-5 receptor; MCR, melanocortin receptor; MeOH, methanol; MSH, melanocyte stimulating hormone; $NaBH(OAc)_3$, sodium triacetoxyborohydride; nBuLi, *N*-butyllithium; NMP, *N*-methyl pyrollidinone; POMC, proopiomelanocortin; SAR, structure−activity relationship; SEM, standard error of the mean; THF, tetrahydrofuran; TFA, trifluoroacetic acid; α-MSH, α-melanocyte stimulating hormone; β-MSH, β-melanocyte stimulating hormone; γ-MSH, γ-melanocyte stimulating hormone.

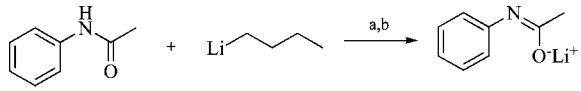
10.1021/jm701303z CCC: $40.75
Published on Web 02/14/2008

Scheme 1[a]



[a] Reaction conditions: (a) $NaBH(OAC)_3$, DMF/1% AcOH, rt, 2 h; (b) EDC in NMP, rt, overnight; (c) rt, 30 h, under argon; (d) rt, until pH = 5; (e) $TFA/Me_2S/H_2O$ (90:5:5), rt, 50 h.

Scheme 2[a]



[a] Reaction conditions: (a)−78 °C, 30 min, under argon; (b) DMF, −78 °C, 15 min.

olytic, and antitumor properties, as well as being cholecystokinin receptor (CCK), opiate receptor, and platelet glycoprotein IIb-IIIa antagonists,[27–29] as well as having herbicidal properties.[30] In addition, the 1,4-benzodiazepine-2,5-dione core appears in a number of natural products.[31–33] We hypothesized that small-molecule 1,4-benzodiazepine-2,5-diones may be created that mimic the side chains of the Phe-Arg-Trp melanocortin agonist core tetrapeptide resulting in potent and full agonist activity at the melanocortin receptors. In order to achieve these goals, a number of the synthetic schemes for the solid-phase organic synthesis of benzodiazepines were evaluated.[29,30,34–42] The optimized synthesis approach that we utilized is shown in Schemes 1 and 2.[24,27,30,35] It begins with the attachment of the α-amino ester to the solid support, followed by acylation with an anthranilic acid, base-catalyzed lactamization, alkylation, and cleavage. The sequence relies on the incorporation of three different components, anthranilic acids (R1), alkylating agents (R2), and α-amino esters (R3), available commercially with appropriate side-chain protection. Synthesis with this scheme also provided high yield and relatively clean crude products. Twelve compounds were synthesized, analytically characterized, and pharmacologically characterized for functional agonist activity at the melanocortin receptors (MC1R, and MC3−5Rs).

Results

Analytical characterization and agonist functional data for the 1,4-benzodiazepine-2,5-dione molecules (Figure 2) synthesized in this study are summarized in Tables 1 and 2, respectively. All functional agonist data are expressed as nanomolar EC_{50} values with the associated standard error of the mean (SEM) derived from at least three independent experiments. Compounds that did not result in agonist activity (>100 000 in Table 2) were not competitive antagonists at any of the melanocortin receptors examined herein. Compound **1** is substituted with a H at position R1, and benzyl groups at positions R2 and R3. This compound is a high micromolar full agonist at the mMC1R but does not exhibit any stimulatory activity at the other three receptors examined at up to 100 μM concentrations. Compounds **2** and **3** are substituted with a H at position R1 and a benzyl group at R2 and differ in their substitution at the R3 position. The R3 propyl guanidine moiety in compound **2** resulted in a molecule that is equipotent (within the 3-fold inherent experimental error) to compound **1** at the mMC1R, but does not contain any agonist activity at the other melanocortin receptors examined. Compound **3**, containing a methyl indole moiety at the R3 position, exhibited only a 70% maximal stimulatory response at the mMC1R as compared with the forskolin control at up to 100 μM concentrations and was unable to stimulate the MC3−5 receptors. Compound **4** contains 9-methyl at R1, benzyl at R2, and 1-benzyl-1*H*-indol-3-yl-methyl at the R3 position. The 1-benzyl-1*H*-indol-3-yl-methyl moiety at the R3 position in **4** was derived from over alkylation with benzyl bromide and alkylation of the 1-nitrogen of the indole ring, and this compound possessed micromolar full agonist activity at the mMC1R and was devoid of stimulatory activity at the MC3−5Rs.

Compound **5**, containing an 8-chloro substitution at R1, a benzyl at R2, and a propyl guanidine group at R3, resulted in full micromolar agonist activity at the mMC1, mMC3, and mMC5 receptors. Substitution of the R3 guanidinyl group with the 4-amino butyl moiety in compound **6** resulted in a full nanomolar agonist activity at all four receptors examined. Compounds **7**−**9** all contain an 8-chloro at the R1 position and a napthylene-2-yl-methyl group in the R2 position, with differential substitution at R3. Comparison of compounds **6** and **8** resulted in decreased potency of **8** by the addition of the naphtyl moiety at the R2 position versus the benzyl moiety of **6**. This difference may be attributed to the bulkier nature of the naphtyl versus benzyl ring that perhaps modifies ligand–receptor interactions or modified putative π−π stacking interactions between the ligand and receptor. Compound **7**, containing a napthyl side chain at R2 and a benzyl group at R3, resulted in a micromolar agonist at the mMC1R, mMC3R, and mMC5R but was only able to stimulate the mMC4R to 70% maximal activity observed for the forskolin control at 100 μM concentrations. Compound **8**, differing from compound **7** by the presence of the 4-aminobutyl moiety at the R3 position, is equipotent

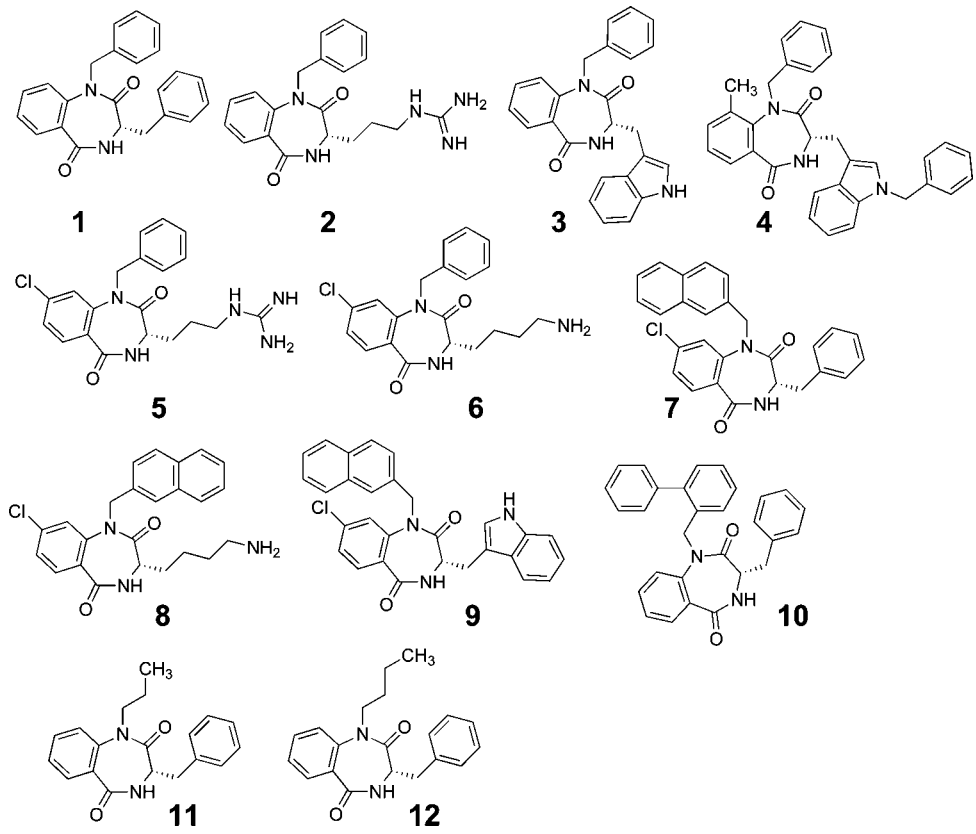


Figure 2. Structures of the ligands designed, synthesized, and pharmacologically tested in this study.

Table 1. Analytical Data of 1,4-Benzodiazepine-2,5-dione Analogues[a]

peptide	R_1	R_2	R_3	HPLC retention time (min) (system 1)	HPLC retention time (min) (system 2)	% purity	*m*/*z* (M, calcd)	*m*/*z* (M + 1, expt)
1	H	benzyl	benzyl	21.5	28.3	>98	356.4	357.2
2	H	benzyl	propyl guanidine	12.7	18.4	>99	365.4	366.4
3	H	benzyl	1*H*-indol-3-yl-methyl	21.2	34.1	>95	395.5	396.0
4	9-methyl	benzyl	1-benzyl-1*H*-indol-3-yl-methyl	27.2	34.3	>96	499.6	500.4
5	8-chloro	benzyl	propyl guanidine	15.3	22.3	>99	399.9	401.1
6	8-chloro	benzyl	4-aminobutyl	14.9	22.3	>99	371.9	372.2
7	8-chloro	naphthalen-2-yl-methyl	benzyl	26.5	41.9[b]	>99	440.9	441.9
8	8-chloro	naphthalen-2-yl-methyl	4-aminobutyl	17.6	26.0	>97	421.9	422.5
9	8-chloro	naphthalene-2-yl-methyl	1*H*-indol-3-yl-methyl	31.1	41.4[b]	>97	480.0	481.5
10	H	biphenyl-2-yl-methyl	benzyl	25.5	40.7[b]	>98	432.5	433.1
11	H	propyl	benzyl	18.5	26.2	>98	308.4	309.5
12	H	butyl	benzyl	20.4	33.2[b]	>99	322.4	323.1

[a] HPLC system 1 (10% acetonitrile in 0.1% trifluoroacetic acid/water and a gradient to 90% acetonitrile over 35 min) or solvent system 2 (10% methanol in 0.1% trifluoroacetic acid/water and a gradient to 90% methanol over 35 min). [b] Indicates solvent system 2 that in which a gradient to 90% methanol over 45 min was used. An analytical Vydac C18 column (Vydac 218TP104) was used with a flow rate of 1.5 mL/min. The peptide purity was determined by HPLC at a wavelength of 214 nm.

with compound **7** at the mMC1R yet was only able to generate weak stimulatory responses at the mMC3R, MC4R, and mMC5R at 100 μM concentrations. Compound **9**, containing the 1*H*-indol-3-yl-methyl moiety at the R3 position instead of the benzyl group in compound **7**, was devoid of any stimulatory activity at any of the receptors at concentrations up to 100 μM.

Compounds **10**−**12** contain a H at the R1 position, a benzyl group at the R3 position, and are differentially modified at the R2 position. Compound **10**, containing a biphenyl-2-yl-methyl moiety at the R2 position, is a nanomolar agonist at the mMC1R, is 18-fold mMC1R versus mMC3R selective, is ca. 9-fold mMC1R versus mMC4R selective, is a micromolar agonist at the mMC3R and the mMC4R, and is only able to stimulate a 60% maximal forskolin response at the mMC5R. When the R2 position contains the propyl moiety in compound **11**, micromolar full agonist activity is observed at the mMC1R, mMC4R, and mMC5R with only an 80% maximal forskolin response at the mMC3R. When the R2 position contains the butyl group in compound **12**, only a 70% maximal forskolin stimulation response is observed at the mMC1R and no stimulatory response is observed at any of the other receptors.

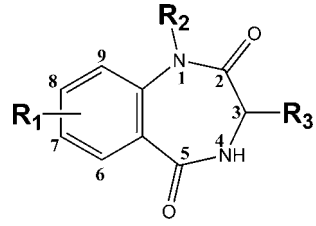
Discussion

The concept of heterocyclic small molecule melanocortin agonists based on a β-turn motif has been reported as early as 1999 and were identified as micromolar mMC1R agonists.[43] Since the association between the MC4R subtype and human obesity and energy homeostasis was recognized, the MC4R has been a target for the design of potent and receptor-selective small-molecule agonists.[44–47] Some of the small-molecule templates possessing nanomolar agonist potency at the mMC4R

Table 2. Functional Activity of 1,4-Benzodiazepine-2,5-dione Analogues at the Mouse Melanocortin Receptors[a]

peptide	structure	agonist EC_{50} (nM) mMC1R	mMC3R	mMC4R	mMC5R
α-MSH	Ac-Ser-Tyr-Ser-Met-Glu-His-Phe-Arg-Trp-Gly-Lys-Pro-Val-NH_2	0.7 ± 0.23	2.14 ± 0.46	2.56 ± 0.29	2.0 ± 0.20

structure



peptide	R_1	R_2	R_3	agonist EC_{50} (nM) mMC1R	mMC3R	mMC4R	mMC5R
1	H	benzyl	benzyl	32850 ± 8200	>100000	>100000	>100000
2	H	benzyl	propyl guanidine	16450±6000	>100000	>100000	>100000
3	H	benzyl	1*H*-indol-3-yl-methyl	70% @ 100 μM	>100000	>100000	>100000
4	9-methyl	benzyl	1-benzyl-1*H*-indol-3-yl-methyl	21600 ± 6900	>100000	>100000	>100000
5	8-chloro	benzyl	propyl guanidine	1800 ± 100	30650 ± 23000	60% @ 100 μM	17000 ± 4500
6	8-chloro	benzyl	4-aminobutyl	48 ± 23	320 ± 80	240 ± 75	87 ± 38
7	8-chloro	naphthalen-2-yl-methyl	benzyl	4700 ± 1270	23300 ± 2500	70% @ 100 μM	4350 ± 1700
8	8-chloro	naphthalen-2-yl-methyl	4-aminobutyl	3400 ± 700	30% @ 100 μM	30% @ 100 μM	65% @ 10 μM
9	8-chloro	naphthalen-2-yl-methyl	1*H*-indol-3-yl-methyl	>100000	>100000	>100000	>100000
10	H	biphenyl-2-yl-methyl	benzyl	230 ± 0.27	4160 ± 70	1970 ± 1230	60% @ 100 μM
11	H	propyl	benzyl	6900 ± 2600	80% @ 100 μM	35000 ± 16000	3267 ± 1010
12	H	butyl	benzyl	70% @ 100 μM	>100000	>100000	>100000

[a] Indicated errors represent the standard error of the mean determined from at least four independent experiments. >100000 indicates that no agonist activity was observed at up to 100 μM. Agonists that possessed some stimulatory response at 100 μM concentrations are indicated as percent response relative to the maximal response observed for the forskolin control.

contain similar features such as a para(chloro)-substituted phenyl ring and a heterocyclic scaffold.[46]

The benzodiazepine template has been widely studied as a scaffold for nonpeptide drugs. Derivatives have been identified that have properties as anxiolytics, cholecystokinin receptor antagonists,[48] κ-opioid receptor agonists,[49] oxytocin receptor antagonists,[50,51] endothelin antagonists,[52] HIV Tat antagonists,[53] and reverse transcriptase inhibitors,[54] among others. Because many targets are members of the G-protein coupled receptor superfamily (i.e., cholecystokinin, κ-opioid, oxytocin, and endothelin receptors), we hypothesized that benzodiazepine derivatives could be designed as melanocortin ligands. The 1,4-benzodiazepine-2,5-dione molecules synthesized in this study was designed to interact with proposed binding sites in the melanocortin receptors. Consistent with melanocortin *in vitro* receptor mutagenesis studies,[14,55–57] homology molecular modeling of the mMC1R[58] and the MC4R[59–62] suggest the presence of hydrophobic and electrostatic domains within the putative binding pocket that are important for melanocortin receptor agonist molecular recognition and stimulation. Thus, using this information and the design strategy of mimicking the melanocortin agonist Phe-Arg-Trp side chains, we designed the ligands reported herein. Based on the results of this study, we speculate that the p(Cl)-phenyl ring of the benzodiazepine and an aryl side chain putatively interact with the hydrophobic receptor domains and that a cationic component interacts with the anionic receptor binding domain.

The compounds synthesized in this study have provided information about the mMC1R and mMC4R small-molecule pharmacophore. There were several compounds synthesized that only showed agonist activity at the mMC1R. These compounds all had either a hydrogen or a methyl group in the R1 position, a benzyl group in the R2 position, and either an aromatic or a basic group in the R3 position. Interestingly, the addition of chlorine at the R1 position converts the molecule into an agonist at all four receptors. Studies of $\pi-\pi$ stacking in peptides have suggested that the addition of an electron-withdrawing group in the para position on a phenyl ring increases activity.[63–65] Addition of chlorine creates an electron-deficient ring, which interacts favorably with the relatively electron-rich ring of tyrosine.[66] Additional studies have shown that in proteins, a T-shaped conformation is favored in π-stacking, resulting in a greater decrease in free energy than the sandwich conformation.[67,68] Based on this information, it may be proposed that the halogenated ring of the benzodiazepine template may interact with the tyrosine of the receptor in a T-shaped conformation to create a favorable π-stacking charge-transfer relationship, resulting in increased agonist activity.

The most interesting compound discovered in this study is compound **6**, which is a low nanomolar agonist at all four melanocortin receptors (Table 2). This compound has a chlorine in position R1, a benzyl group in position R2, and a 4-aminobutyl group in position R3. It is interesting to note that when a propyl guanidine was substituted at position R3 (compound **5**), the resulting compound exhibited a significant decrease in agonist activity at all melanocortin receptors. It appears, therefore, that the decreased cationic character of the 4-aminobutyl group compared with the guanidine provides optimal intermolecular interactions with the electrostatic binding pocket of the melanocortin receptors.

When a naphthalene group was inserted at position R2 (compounds **7–9**), the compounds showed decreased agonist activity at all receptors. This is consistent with previous SAR studies on peptide agonists, which showed that when the DPhe of the tetrapeptide His-DPhe-Arg-Trp was replaced with a bulky aromatic group such as DNal(2′), the peptide was converted into an antagonist at the mMC3R and the mMC4R.[69] The most significant example of this is the mMC3−4R antagonist SHU9119, which exhibits partial agonist activity and potent antagonist activity at the mMC3R and mMC4R.[69] When a

biphenyl was inserted at position R2 (compound **10**), the compound was a low nanomolar agonist at the mMC1R and a low micromolar agonist at the mMC3−4R. This suggests that there may be an additional interaction with the greater negative potential on the face of the conjugated π system of napthylene that is not present with the nonconjugated rings of the biphenyl group that results in loss of agonist activity.

It should also be observed that an aromatic group in the R2 position provides for increased agonist activity. When this position contained a propyl (compound **11**) or butyl (compound **12**) group, the agonist activity at all four receptors was decreased. It is also interesting to note that the shorter propyl chain in compound **11** was significantly more potent that the butyl chain in compound **12** at all receptors.

Conclusions

This research has resulted in the identification and first report of several 1,4-benzodiazepine-2,5-dione derivatives that exhibit nanomolar agonist activity at the mouse melanocortin receptors. These compounds are easy to synthesize due to the use of solid-phase organic synthesis and may be readily purified allowing for the design of a large number of compounds. When substituted with building blocks that mimic the active sequence of peptide melanocortin ligands, this template has the potential to create compounds with increasing potency that may be used in the future as drugs to treat obesity.

Experimental Design and Methods

Synthesis was performed in a manual reaction vessel (Peptides International, Louisville, KY); mixing was accomplished with nitrogen. All reagents used were ACS grade or better and used without further purification. BAL resin (Backbone amide linker resin) was purchased from Advanced ChemTech (Louisville, KY). Dichloromethane, acetic acid, *N*-methyl pyrollidinone, acetonitrile, acetone, and methanol were purchased from Fisher (Fair Lawn, NJ). *N*,*N*-Dimethylformamide was purchased from Burdick and Jackson (McGaw Park, IL). Sodium triacetoxyborohydride, 1-[3-(dimethylamino)propyl]-1′-ethylcarbodiimide hydrochloride, acetanilide, *N*-butyllithium (10 M in hexanes), benzyl bromide, 2-(bromomethyl)-napthalene, 2-phenylbenzylbromide, dimethylsulfide, 1-iodopropane, 1-iodobutane, tetrahydrofuran, and trifluoroacetic acid were purchased from Sigma-Aldrich (St. Louis, MO). 2-Amino-4-chlorobenzoic acid and 2-amino-3-methylbenzoic acid were purchased from Acros Organics (Morris Plains, NJ). Arginine-(Pbf) methyl ester, and lysine-(Boc) methyl ester were purchased from Bachem (Torrence, CA). Phenylalanine methyl ester was purchased from Advanced Chemtech (Louisville, KY). Tryptophan methyl ester was purchased from Fluka (St. Louis, MO). 2-Aminobenzoic acid was purchased from Alfa Aesar (Ward Hill, MA).

Ligand Synthesis. Synthesis was performed on solid phase using the method described in Scheme 1. Bal resin was swollen in DCM for 1 h and tested for the presence of an aldehyde using the DNPH method. To a reaction vessel containing 0.3 mmol of BAL resin was added $NaBH(OAc)_3$ (10 equiv, 3.0 mmol) dissolved in 1% AcOH/DMF, generating a white suspension. The suspension was mixed until the mixture became clear. Methyl ester (10 equiv, 3.0 mmol) was then added, and the solution was stirred for 3–4 h. The resin was filtered and washed with DMF (3 × 20 mL), DCM (3 × 20 mL), MeOH (2 × 20 mL), and DCM (3 × 20 mL). A sample of the resin was removed and tested for the presence of a secondary amine (chloranil test) and the absence of an aldehyde (DNPH test for aldehydes). The resin was dissolved in *N*-methyl pyrollidinone (NMP) followed by the addition of 1-[3-(dimethylamino)propyl]-1′-ethylcarbodiimide hydrochloride (EDC). After the EDC was completely dissolved, the 2-aminobenzoic acid was added slowly to minimize side reaction that may occur with an unprotected amine, and the reaction was mixed with nitrogen overnight. Resin was filtered and washed with NMP (2 × 20 mL), DMF (3 × 20 mL), DCM (3 × 20 mL), MeOH (2 × 20 mL), and DCM (3 × 20 mL). A sample of the resin was removed and tested for the absence of a secondary amine (chloranil test) and the presence of a tertiary amine (bromophenol blue test). To an oven-dried round-bottomed flask was added a magnetic stir bar and acetanilide (24 equiv, 7.2 mmol) followed by 10 mL of THF. The flask was purged with argon gas and cooled to −78 °C in a dry ice/acetone bath. A hexane solution of n-BuLi (20 equiv, 6.0 mmol) was added dropwise over 10 min followed by rapid stirring for 30 min at −78 °C (Scheme 2). After 30 min, 10 mL of DMF was added to homogenize the solution, and the solution was stirred gently for 15 min. The flask was then allowed to slowly come to rt. The acylated resin was transferred to a round-bottomed flask and purged with argon gas. The lithium salt was transferred to the flask containing the resin and mixed gently for 30 h at rt. Following cyclization, the pH of the solution was tested (pH ≈ 13–14) and then the alkylating agent (40 equiv, 12.0 mmol) was added, and the solution was stirred at rt until the pH of the solution was around 5. The resin was then transferred back to a reaction vessel and washed with DMF (7 × 20 mL), DCM (7 × 20 mL), MeOH (3 × 20 mL), and DCM (3 × 20 mL). The resin was air-dried and then cleaved with a cleavage cocktail of TFA/Me_2S/H_2O (90:5:5) for 50 h. Resin was removed by filtration, and the filtrate was evaporated and lyophilized.

1,3-Dibenzyl-3,4-dihydro-1*H*-benzo[*e*][1,4]diazepine-2,5-dione (1). BAL resin (333 mg, 0.9 mmol/g) was mixed with sodium triacetoxyborohydride [$NaBH(OAc)_3$] (636 mg, 3.0 mmol) and L-Phe-OMe·HCl (647 mg, 3.0 mmol) in 1% acetic acid/DMF for 2 h and washed with DMF, DCM, and MeOH. The resin containing the amino ester was then treated with EDC (690 mg, 3.6 mmol) in NMP and 2-aminobenzoic acid (411 mg, 3.0 mmol) overnight and washed as before. A solution of acetanilide (973 mg, 7.2 mmol) and *N*-butyllithium (252 μL, 3.0 mmol) in THF was stirred for 30 min, and then 15 mL of DMF was added; this solution was then added to the acylated resin and stirred under argon for 30 h. Benzyl bromide (1.4 mL, 12 mmol) was added following lactamization, and the solution was stirred until pH = 5. The resin was then washed and dried in a desiccator. The compound was cleaved from the resin using 90% TFA, 5% dimethylsulfide, and 5% H_2O for 50 h. Semipreparative HPLC on an RP-HPLC C18 bonded silica column (flow rate = 5.0 mL/min, Vydac 218TP1010, 1.0 cm × 25 cm) using a gradient of 46–56% acetonitrile in 0.1% TFA/H_2O over 10 min and collection from 8.0 to 8.7 min gave a compound that was >95% pure. M + 1 = 357.2 by MALDI-TOF mass spectrometry. ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.77 (d, J = 1.83 Hz, 1H), 7.56 (d, J = 2.0 Hz, 1H), 7.53–7.51 (m, 1H), 7.47 (d, J = 2.3 Hz, 1H), 7.32 (d, J = 2.0 Hz, 2H), 7.26–7.22 (m, 5H), 7.19–7.17 (m, 2H), 7.08 (d, J = 2.0 Hz, 2H), 5.34 (d, J = 4.3 Hz, 1H), 4.98 (d, J = 4.3 Hz, 1H), 4.03 (dt, J = 2.3, 1.7 Hz, 1H), 4.19 (dd, J = 1.5, 4.0 Hz, 1H), 2.96 (dd, J = 4.0, 2.5 Hz, 1H).

***N*-[3-(1-Benzyl-2,5-dioxo-2,3,4,5-tetrahydro-1*H*-benzo[*e*][1,4]-diazepin-3-yl)-propyl]-guanidine (2).** BAL resin (333 mg, 0.9 mmol/g) was mixed with sodium triacetoxyborohydride [$NaBH(OAc)_3$] (636 mg, 3.0 mmol) and L-Arg(Pbf)-OMe·HCl (1321 mg, 3.0 mmol) in 1% acetic acid/DMF for 2 h and washed with DMF, DCM, and MeOH. The resin containing the amino ester was then treated with EDC (690 mg, 3.6 mmol) in NMP and 2-aminobenzoic acid (411 mg, 3.0 mmol) overnight and washed as before. A solution of acetanilide (973 mg, 7.2 mmol) and *N*-butyllithium (252 μL, 3.0 mmol) in THF was stirred for 30 min, and then 15 mL of DMF was added; this solution was then added to the acylated resin and stirred under argon for 30 h. Benzyl bromide (1.4 mL, 12 mmol) was added following lactamization, and the solution was stirred until pH = 5. The resin was then washed and dried in a desiccator. The compound was cleaved from the resin using 90% TFA, 5% dimethylsulfide, and 5% H_2O for 50 h. Semipreparative HPLC on an RP-HPLC C18 bonded silica column (flow rate = 5.0 mL/min, Vydac 218TP1010, 1.0 cm × 25 cm) using a gradient of 23–33% acetonitrile in 0.1% TFA/H_2O over 10 min and collection from 7.8 to 9.0 min gave a compound that was >95% pure. M + 1 = 366.4 by MALDI-TOF mass spectrometry. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.68 (d, J = 3.5 Hz, 1H), 7.31 (dd, J =

5.0, 1.0 Hz, 1H), 7.54–7.50 (m, 1H), 7.44 (d, J = 4.75 Hz), 7.29–7.17 (m, 4H), 7.07 (d, J = 4.25 Hz, 2H), 5.29 (d, J = 10 Hz, 1H), 4.98 (d, J = 10.0 Hz, 1H), 3.80–3.78 (m, 1H), 3.32–3.30 (m, 2H), 3.29 (s, 2H), 3.07–3.05 (m, 2H), 1.86–1.78 (m, 1H), 1.70–1.62 (m, 1H), 1.68–1.62 (m, 1H), 1.58–1.45 (m, 2H).

1-Benzyl-3-(1*H*-indol-3-ylmethyl)-3,4-dihydro-1*H*-benzo[*e*][1,4]-diazepine-2,5-dione (3). BAL resin (333 mg, 0.9 mmol/g) was mixed with sodium triacetoxyborohydride [$NaBH(OAc)_3$] (636 mg, 3.0 mmol) and L-Trp-OMe·HCl (764 mg, 3.0 mmol) in 1% acetic acid/DMF for 2 h and washed with DMF, DCM, and MeOH. The resin containing the amino ester was then treated with EDC (690 mg, 3.6 mmol) in NMP and 2-aminobenzoic acid (411 mg, 3.0 mmol) overnight and washed as before. A solution of acetanilide (973 mg, 7.2 mmol) and *N*-butyllithium (252 μL, 3.0 mmol) in THF was stirred for 30 min, and then 15 mL of DMF was added; this solution was then added to the acylated resin and stirred under argon for 30 h. Benzyl bromide (1.4 mL, 12 mmol) was added following lactamization, and the solution was stirred until pH = 5. The resin was then washed and dried in a desiccator. The compound was cleaved from the resin using 90% TFA, 5% dimethylsulfide, and 5% H_2O for 50 h. Semipreparative HPLC on an RP-HPLC C18 bonded silica column (flow rate = 5.0 mL/min, Vydac 218TP1010, 1.0 cm × 25 cm) using a gradient of 43–53% acetonitrile in 0.1% TFA/H_2O over 10 min and collection from 6.7 to 7.0 min gave a compound that was >95% pure. M + 1 = 396.0 by MALDI-TOF mass spectrometry. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.83 (d, J = 1.0 Hz, 1H), 8.70 (d,J = 4.0 Hz, 1H), 7.52 (d,J = 1.0 Hz 1H), 7.50–7.44 (m, 3H), 7.29 (d, J = 5.3 Hz, 2H), 7.23–7.16 (m, 4H), 7.08–7.06 (m, 2H), 7.00 (t, J = 4.5 Hz, 1H), 6.87 (t, J = 4.5 Hz, 1H), 5.34 (d, J = 10 Hz, 1H), 4.95 (d, J = 10 Hz, 1H), 4.00–3.95 (m, 1H), 3.07 (dd, J = 5.5, 9.5 Hz, 2H).

1-Benzyl-3-(1-benzyl-1*H*-indol-3-ylmethyl)-9-methyl-3,4-dihydro-1*H*-benzo[*e*][1,4]diazepine-2,5-dione (4). BAL resin (333 mg, 0.9 mmol/g) was mixed with sodium triacetoxyborohydride [$NaBH(OAc)_3$] (636 mg, 3.0 mmol) and L-Trp-OMe·HCl (764 mg, 3.0 mmol) in 1% acetic acid/DMF for 2 h and washed with DMF, DCM, and MeOH. The resin containing the amino ester was then treated with EDC (690 mg, 3.6 mmol) in NMP and 2-amino-3-methylbenzoic acid (453 mg, 3.0 mmol) overnight and washed as before. A solution of acetanilide (973 mg, 7.2 mmol) and *N*-butyllithium (252 μL, 3.0 mmol) in THF was stirred for 30 min, and then 15 mL of DMF was added; this solution was then added to the acylated resin and stirred under argon for 30 h. Benzyl bromide (1.4 mL, 12 mmol) was added following lactamization, and the solution was stirred until pH = 5. The resin was then washed and dried in a desiccator. The compound was cleaved from the resin using 90% TFA, 5% dimethylsulfide, and 5% H_2O for 50 h. Semipreparative HPLC on an RP-HPLC C18 bonded silica column (flow rate = 5.0 mL/min, Vydac 218TP1010, 1.0 cm × 25 cm) using a gradient of 52–67% acetonitrile in 0.1% TFA/H_2O over 10 min and collection from 9.7 to 10.4 min gave a compound that was >95% pure. M + 1 = 500.4 by MALDI-TOF mass spectrometry. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.62 (d, J = 3.8 Hz, 1H), 7.51 (d, J = 5.5 Hz, 1H), 7.46 (d, J = 5.3 Hz, 1H), 7.35 (d, J = 4.8 Hz, 2H), 7.30–7.17 (m, 8H), 7.12–7.10 (m, 2H), 7.05–7.01 (m, 3H), 6.92 (t, J = 4.5 Hz, 1H), 5.40 (d, J = 9.4 Hz, 1H), 5.33 (s, 2H), 4.28 (d, J = 9.4 Hz, 1H), 3.86–3.85 (m, 1H), 3.00 (dd, J = 5.3, 9.5 Hz, 2H), 2.40 (s, 3H).

***N*-[3-(1-Benzyl-8-chloro-2,5-dioxo-2,3,4,5-tetrahydro-1*H*-benzo[*e*][1,4]diazepin-3-yl)-propyl]-guanidine (5).** BAL resin (333 mg, 0.9 mmol/g) was mixed with sodium triacetoxyborohydride [$NaBH(OAc)_3$] (636 mg, 3.0 mmol) and L-Arg(Pbf)-OMe·HCl (1321 mg, 3.0 mmol) in 1% acetic acid/DMF for 2 h and washed with DMF, DCM, and MeOH. The resin containing the amino ester was then treated with EDC (690 mg, 3.6 mmol) in NMP and 2-amino-4-chlorobenzoic acid (515 mg, 3.0 mmol) overnight and washed as before. A solution of acetanilide (973 mg, 7.2 mmol) and *N*-butyllithium (252 μL, 3.0 mmol) in THF was stirred for 30 min and then 15 mL of DMF was added; this solution was then added to the acylated resin and stirred under argon for 30 h. Benzyl bromide (1.4 mL, 12 mmol) was added following lactamization, and the solution was stirred until pH = 5. The resin was then washed and dried in a desiccator. The compound was cleaved from the resin using 90% TFA, 5% dimethylsulfide, and 5% H_2O for 50 h. Semipreparative HPLC on an RP-HPLC C18 bonded silica column (flow rate = 5.0 mL/min, Vydac 218TP1010, 1.0 cm × 25 cm) using a gradient of 28–38% acetonitrile in 0.1% TFA/H_2O over 10 min and collected from 6.9 to 7.5 min gave a compound that was >95% pure. M + 1 = 401.1 by MALDI-TOF mass spectrometry. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.77 (d, J = 3.25 Hz, 1H), 7.63 (d, J = 5.25 Hz, 1H), 7.58 (d, J = 1.25 Hz, 1H), 7.34 (dd, J = 5.5, 1.25 Hz, 1H), 7.26–7.22 (m, 2H), 7.20–7.16 (m, 1H), 7.06 (d, J = 4.25 Hz, 2H), 5.34 (d, J = 10.25 Hz, 1H), 4.99 (d, J = 10.0 Hz, 1H), 3.10–3.03 (m, 2H), 1.88 (s, 5H), 1.84–1.78 (m, 1H), 1.71–1.59 (m, 1H), 1.58–1.42 (m, 2H).

3-(4-Aminobutyl)-1-benzyl-8-chloro-3,4-dihydro-1*H*-benzo[*e*][1,4]diazepine-2,5-dione (6). BAL resin (333 mg, 0.9 mmol/g) was mixed with sodium triacetoxyborohydride [$NaBH(OAc)_3$] (636 mg, 3.0 mmol) and L-Lys(Boc)-OMe·HCl (1187 mg, 3.0 mmol) in 1% acetic acid/DMF for 2 h and washed with DMF, DCM, and MeOH. The resin containing the amino ester was then treated with EDC (690 mg, 3.6 mmol) in NMP and 2-amino-4-chlorobenzoic acid (515 mg, 3.0 mmol) overnight and washed as before. A solution of acetanilide (973 mg, 7.2 mmol) and *N*-butyllithium (252 μL, 3.0 mmol) in THF was stirred for 30 min and then 15 mL of DMF was added; this solution was then added to the acylated resin and stirred under argon for 30 h. Benzyl bromide (1.4 mL, 12 mmol) was added following lactamization, and the solution was stirred until pH = 5. The resin was then washed and dried in a desiccator. The compound was cleaved from the resin using 90% TFA, 5% dimethylsulfide, and 5% H_2O for 50 h. Semipreparative HPLC on an RP-HPLC C18 bonded silica column (flow rate = 5.0 mL/min, Vydac 218TP1010, 1.0 cm × 25 cm) using a gradient of 26–36% acetonitrile in 0.1% TFA/H_2O over 10 min and collected from 7.8 to 8.5 min gave a compound that was >95% pure. M + 1 = 372.2 by MALDI-TOF mass spectrometry. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.75 (d, J = 3.75 Hz, 1H), 7.65–7.61 (m, 2H), 7.36 (dd, J = 5.25, 1.0 Hz, 1H), 7.28–7.18 (m, 3H), 7.09 (d, J = 4.5 Hz, 2H), 5.39 (d, J = 10.0 Hz, 1H), 5.00 (d, J = 10.0 Hz, 1H), 3.82–3.77 (m, 1H), 2.76 (t, J = 3.65 Hz, 2H), 1.84–1.66 (m, 2H), 1.57–1.48 (m, 2H), 1.38–1.28 (m, 4H).

3-Benzyl-8-chloro-1-naphthalen-2-ylmethyl-3,4-dihydro-1*H*-benzo[*e*][1,4]diazepine-2,5-dione (7). BAL resin (333 mg, 0.9 mmol/g) was mixed with sodium triacetoxyborohydride [$NaBH(OAc)_3$] (636 mg, 3.0 mmol) and L-Phe-OMe·HCl (647 mg, 3.0 mmol) in 1% acetic acid/DMF for 2 h and washed with DMF, DCM, and MeOH. The resin containing the amino ester was then treated with EDC (690 mg, 3.6 mmol) in NMP and 2-amino-4-chlorobenzoic acid (515 mg, 3.0 mmol) overnight and washed as before. A solution of acetanilide (973 mg, 7.2 mmol) and *N*-butyllithium (252 μL, 3.0 mmol) in THF was stirred for 30 min, and then 15 mL of DMF was added; this solution was then added to the acylated resin and stirred under argon for 30 h. 2-(Bromomethyl)-napthalene (2.7 g, 12 mmol) was added following lactamization, and the solution was stirred until pH = 5. The resin was then washed and dried in a desiccator. The compound was cleaved from the resin using 90% TFA, 5% dimethylsulfide, and 5% H_2O for 50 h. Semipreparative HPLC on an RP-HPLC C18 bonded silica column (flow rate = 5.0 mL/min, Vydac 218TP1010, 1.0 cm × 25 cm) using a gradient of 55–65% acetonitrile in 0.1% TFA/H_2O over 10 min and collection from 7.5 to 8.2 min gave a compound that was >95% pure. M + 1 = 441.9 by MALDI-TOF mass spectrometry. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.92 (d, J = 4.0 Hz, 1H), 7.86–7.83 (m, 1H), 7.81 (d, J = 5.5 Hz, 1H), 7.75–7.72 (m, 1H), 7.68 (d, J = 1.3 Hz, 1H), 7.58 (s, 1H), 7.55 (d, J = 5.3 Hz, 1H), 7.51–7.45 (m, 2H), 7.36–7.34 (m, 2H), 7.31–7.24 (m, 3H), 7.23–7.18 (m, 2H), 5.56 (d, J = 10.0 Hz, 1H), 5.15 (d, J = 10.0 Hz, 1H), 4.19–4.14 (m, 1H), 3.19 (dd, J = 3.5, 8.8 Hz, 1H), 2.98 (dd, J = 8.8, 5.5 Hz, 1H).

3-(4-Aminobutyl)-8-chloro-1-naphthalen-2-ylmethyl-3,4-dihydro-1*H*-benzo[*e*][1,4]diazepine-2,5-dione (8). BAL resin (333 mg, 0.9 mmol/g) was mixed with sodium triacetoxyborohydride [NaBH

$(OAc)_3$] (636 mg, 3.0 mmol) and L-Lys(Boc)-OMe·HCl (890 mg, 3.0 mmol) in 1% acetic acid/DMF for 2 h and washed with DMF, DCM, and MeOH. The resin containing the amino ester was then treated with EDC (690 mg, 3.6 mmol) in NMP and 2-amino-4-chlorobenzoic acid (515 mg, 3.0 mmol) overnight and washed as before. A solution of acetanilide (973 mg, 7.2 mmol) and *N*-butyllithium (252 μL, 3.0 mmol) in THF was stirred for 30 min, and then 15 mL of DMF was added; this solution was then added to the acylated resin and stirred under argon for 30 h. 2-(Bromomethyl)-napthalene (2.7 g, 12 mmol) was added following lactamization, and the solution was stirred until pH = 5. The resin was then washed and dried in a desiccator. The compound was cleaved from the resin using 90% TFA, 5% dimethylsulfide, and 5% H_2O for 50 h. Semipreparative HPLC on an RP-HPLC C18 bonded silica column (flow rate = 5.0 mL/min, Vydac 218TP1010, 1.0 cm × 25 cm) using a gradient of 35–45% acetonitrile in 0.1% TFA/H_2O over 10 min and collection from 6.0 to 6.8 min gave a compound that was >95% pure. M + 1 = 422.5 by MALDI-TOF mass spectrometry. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.83–8.81 (m, 1H), 7.87–7.85 (m, 1H), 7.82 (d, J = 5.25 Hz, 1H), 7.78–7.75 (m, 1H), 7.70 (d, J = 0.75 Hz, 1H), 7.64–7.61 (m, 1H), 7.52–7.46 (m, 1H), 7.34 (dd, J = 5.25, 1.25 Hz, 1H), 7.21 (dd, J = 5.0, 1.0 Hz, 1H), 5.58 (d, J = 10.25 Hz, 1H), 5.15 (d, J = 10.0 Hz, 1H), 2.70–2.67 (m, 2H), 1.90 (s, 2H), 1.86–1.80 (m, 1H), 1.77–1.71 (m, 1H), 1.47–1.34 (m, 4H).

8-Chloro-3-(1*H*-indol-3-ylmethyl)-1-naphthalen-2-ylmethyl-3,4-dihydro-1*H*-benzo[*e*][1,4]diazepine-2,5-dione (9). BAL resin (333 mg, 0.9 mmol/g) was mixed with sodium triacetoxyborohydride [$NaBH(OAc)_3$] (636 mg, 3.0 mmol) and L-Trp-OMe·HCl (764 mg, 3.0 mmol) in 1% acetic acid/DMF for 2 h and washed with DMF, DCM, and MeOH. The resin containing the amino ester was then treated with EDC (690 mg, 3.6 mmol) in NMP and 2-amino-4-chlorobenzoic acid (515 mg, 3.0 mmol) overnight and washed as before. A solution of acetanilide (973 mg, 7.2 mmol) and *N*-butyllithium (252 μL, 3.0 mmol) in THF was stirred for 30 min, and then 15 mL of DMF was added; this solution was then added to the acylated resin and stirred under argon for 30 h. 2-(Bromomethyl)-napthalene (2.7 g, 12 mmol) was added following lactamization, and the solution was stirred until pH = 5. The resin was then washed and dried in a desiccator. The compound was cleaved from the resin using 90% TFA, 5% dimethylsulfide, and 5% H_2O for 50 h. Semipreparative HPLC on an RP-HPLC C18 bonded silica column (flow rate = 5.0 mL/min, Vydac 218TP1010, 1.0 cm × 25 cm) using a gradient of 52–62% acetonitrile in 0.1% TFA/H_2O over 10 min and collected from 8.0 to 8.6 min gave a compound that was >95% pure. M + 1 = 481.5 by MALDI-TOF mass spectrometry. ^{1}H NMR (400 MHz, DMSO- d_6) δ 10.89 (s, 1H), 8.85 (d, J = 4 Hz, 1H), 7.86–7.84 (m, 1H), 7.81 (d, J = 5.25 Hz, 1H), 7.74–7.72 (m, 1H), 7.66 (d, J = 1.25 Hz, 1H), 7.59–7.45 (m, 5H), 7.32 (d, J = 4.75 Hz, 1H), 7.28–7.21 (m, 3H), 7.04 (t, J = 4.5 Hz, 1H), 6.92 (t, J = 5.0, 4.25 Hz, 1H), 5.59 (d, J = 10 Hz, 1H), 5.15 (d, J = 9.75 Hz, 1H), 4.14–4.09 (m, 1H), 3.12 (dd, J = 5.50, 9.5 Hz, 2H).

3-Benzyl-1-biphenyl-2-ylmethyl-3,4-dihydro-1*H*-benzo[*e*][1,4]-diazepine-2,5-dione (10). BAL resin (333 mg, 0.9 mmol/g) was mixed with sodium triacetoxyborohydride [$NaBH(OAc)_3$] (636 mg, 3.0 mmol) and L-Phe-OMe·HCl (647 mg, 3.0 mmol) in 1% acetic acid/DMF for 2 h and washed with DMF, DCM, and MeOH. The resin containing the amino ester was then treated with EDC (690 mg, 3.6 mmol) in NMP and 2-aminobenzoic acid (411 mg, 3.0 mmol) overnight and washed as before. A solution of acetanilide (973 mg, 7.2 mmol) and *N*-butyllithium (252 μL, 3.0 mmol) in THF was stirred for 30 min, and then 15 mL of DMF was added; this solution was then added to the acylated resin and stirred under argon for 30 h. 2-Phenylbenzylbromide (2.2 mL, 12 mmol) was added following lactamization, and the solution was stirred until pH = 5. The resin was then washed and dried in a desiccator. The compound was cleaved from the resin using 90% TFA, 5% dimethylsulfide, and 5% H_2O for 50 h. Semipreparative HPLC on an RP-HPLC C18 bonded silica column (flow rate = 5.0 mL/min, Vydac 218TP1010, 1.0 cm × 25 cm) using a gradient of 55–65% acetonitrile in 0.1% TFA/H_2O over 10 min and collection from 7.1 to 7.9 min gave a compound that was >95% pure. M + 1 = 433.1 by MALDI-TOF mass spectrometry. ^{1}H NMR (400 MHz, DMSO- d_6) δ 8.79 (d, J = 4.0 Hz, 1H), 7.56 (dd, J = 1.0, 2.25 Hz, 1H), 7.48- 7.37 (m, 4H), 7.31–7.22 (m, 9H), 7.20–7.15 (m, 2H), 7.09–7.04 (m, 2H), 5.16 (d, J = 10.0 Hz, 1H), 4.89 (d, J = 10.0 Hz, 1H), 4.02–3.97 (m, 1H), 3.14 (dd, J = 3.5, 8.8 Hz, 1H), 2.92 (d, J = 8.8, 5.25 Hz, 1H).

3-Benzyl-1-propyl-3,4-dihydro-1*H*-benzo[*e*][1,4]diazepine-2,5-dione (11). BAL resin (333 mg, 0.9 mmol/g) was mixed with sodium triacetoxyborohydride [$NaBH(OAc)_3$] (636 mg, 3.0 mmol) and L-Phe-OMe·HCl (647 mg, 3.0 mmol) in 1% acetic acid/DMF for 2 h and washed with DMF, DCM, and MeOH. The resin containing the amino ester was then treated with EDC (690 mg, 3.6 mmol) in NMP and 2-aminobenzoic acid (411 mg, 3.0 mmol) overnight and washed as before. A solution of acetanilide (973 mg, 7.2 mmol) and *N*-butyllithium (252 μL, 3.0 mmol) in THF was stirred for 30 min, and then 15 mL of DMF was added; this solution was then added to the acylated resin and stirred under argon for 30 h. 1-Iodopropane (1.2 mL, 12 mmol) was added following lactamization, and the solution was stirred until pH = 5. The resin was then washed and dried in a desiccator. The compound was cleaved from the resin using 90% TFA, 5% dimethylsulfide, and 5% H_2O for 50 h. Semipreparative HPLC on an RP-HPLC C18 bonded silica column (flow rate = 5.0 mL/min, Vydac 218TP1010, 1.0 cm × 25 cm) using a gradient of 40–50% acetonitrile in 0.1% TFA/H_2O over 10 min and collection from 6.4 to 7.1 min gave a compound that was >95% pure. M + 1 = 309.5 by MALDI-TOF mass spectrometry. ^{1}H NMR (400 MHz, DMSO- d_6) δ 8.73 (d, J = 4.0 Hz, 1H), 7.61–7.57 (m, 2H), 7.49 (d, J = 5.25 Hz, 1H), 7.33–7.28 (m, 3H), 7.23 (t, J = 4.5 Hz, 2H), 7.18–7.14 (m, 1H), 4.23 (dt, J = 5.0, 8.5 Hz, 1H), 3.89–3.84 (m, 1H), 3.61 (dt, J = 5.0, 7.0 Hz, 2H), 3.13 (dd, J = 3.25, 8.75 Hz, 1H), 2.91 (dd, J = 8.75, 5.5 Hz, 1H), 1.47–1.38 (m, 1H), 1.33–1.24 (m, 1H), 0.70 (t, J = 4.5 Hz, 3H).

3-Benzyl-1-butyl-3,4-dihydro-1*H*-benzo[*e*][1,4]diazepine-2,5-dione (12). BAL resin (333 mg, 0.9 mmol/g) was mixed with sodium triacetoxyborohydride [$NaBH(OAc)_3$] (636 mg, 3.0 mmol) and L-Phe-OMe·HCl (647 mg, 3.0 mmol) in 1% acetic acid/DMF for 2 h and washed with DMF, DCM, and MeOH. The resin containing the amino ester was then treated with EDC (690 mg, 3.6 mmol) in NMP and 2-aminobenzoic acid (411 mg, 3.0 mmol) overnight and washed as before. A solution of acetanilide (973 mg, 7.2 mmol) and *N*-butyllithium (252 μL, 3.0 mmol) in THF was stirred for 30 min, and then 15 mL of DMF was added; this solution was then added to the acylated resin and stirred under argon for 30 h. 1-Iodobutane (1.4 mL, 12 mmol) was added following lactamization, and the solution was stirred until pH = 5. The resin was then washed and dried in a desiccator. The compound was cleaved from the resin using 90% TFA, 5% dimethylsulfide, and 5% H_2O for 50 h. Semipreparative HPLC on an RP-HPLC C18 bonded silica column (flow rate = 5.0 mL/min, Vydac 218TP1010, 1.0 cm × 25 cm) using a gradient of 55–65% acetonitrile in 0.1% TFA/H_2O over 10 min and collection from 4.0 to 4.5 min gave a compound that was >95% pure. M + 1 = 323.1 by MALDI-TOF mass spectrometry. ^{1}H NMR (400 MHz, DMSO- d_6) δ 8.72 (d, J = 4.25 Hz, 1H), 7.62–7.57 (m, 1H), 7.51 (d, J = 5.0 Hz, 1H), 7.33–7.29 (m, 2H), 7.25–7.21 (m, 1H), 7.19–7.15 (m, 1H), 4.30 (dt, J = 8.75, 4.5 Hz, 1H), 3.87–3.84 (m, 1H), 3.66–3.59 (m, 1H), 3.13 (dd, J = 8.75, 3.50 Hz, 1H), 2.91 (dd, J = 4.5, 5.75 Hz, 1H), 1.39–1.24 (m, 2H), 1.16–1.11 (m, 2H), 0.78 (t, J = 4.5 Hz, 3H).

Ligand Purification and Analysis. Ligands were purified by reverse phase high-performance liquid chromatography (RP-HPLC) using a Shimadzu chromatography system with a photodiode array detector and a semipreparative RP-HPLC C18 bonded silica column (Vydac 218TP1010, 1.0 cm × 25 cm) and lyophilized. The purified peptides were analyzed using RP-HPLC with an analytical Vydac C18 column (Vydac 218TP104). Molecular mass was determined by MALDI-TOF mass spectrometry (University of Florida protein core facility). ^{1}H NMR was performed on a 400 MHz Varian instrument using DMSO-d_6 as solvent and TMS as reference. ^{1}H

NMR of compound **2** was performed on a 600 MHz Bruker Avance Console using DMSO-d_6 as solvent and TMS as reference.

Melanocortin Receptor Agonist Pharmacology. Pharmacological analysis was performed by β-galactosidase reporter gene assay.[70] HEK-293 cells stably expressing the melanocortin receptors were transiently transfected with CRE/β-galactosidase reporter gene. Forty-eight hours post-transfection, the cells were stimulated with 100 μL of peptide (10^{-4} to 10^{-12} M) or forskolin (10^{-4} M) control in assay medium (DMEM containing 0.1 mg/mL BSA and 0.1 mM IBMX) for 6 h. To the cell lysate plates, 150 μL of substrate buffer (60 mM sodium phosphate, 1 mM $MgCl_2$, 10 mM KCl, 5 mM β-mercaptoethanol, 2 mg/mL 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). Data points were normalized both to the relative protein content and to non-receptor-dependent forskolin stimulation.

Acknowledgment. This work was supported by NIH Grant DK57080 and an American Diabetes Association Research Award (C.H.L.). We also thank Mic E. Mouse for graciously providing the inspiration for compound **1**.

References

(1) Chhajlani, V.; Wikberg, J. E. S. Molecular Cloning and Expression of the Human Melanocyte Stimulating Hormone Receptor cDNA. *FEBS Lett.* **1992**, *309*, 417–420.
(2) Mountjoy, K. G.; Robbins, L. S.; Mortrud, M. T.; Cone, R. D. The Cloning of a Family of Genes that Encode the Melanocortin Receptors. *Science* **1992**, *257*, 1248–1251.
(3) Mountjoy, K. G. The Human Melanocyte Stimulating Hormone Receptor has Evolved to Become "Super-Sensitive" to Melanocortin Peptides. *Mol. Cell. Endocrinol.* **1994**, *102*, R7–R11.
(4) Roselli-Rehfuss, L.; Mountjoy, K. G.; Robbins, L. S.; Mortrud, M. T.; Low, M. J.; et al. Identification of a Receptor for γ Melanotropin and Other Proopiomelanocortin Peptides in the Hypothalamus and Limbic System. *Proc. Natl. Acad. Sci. U.S.A.* **1993**, *90*, 8856–8860.
(5) Gantz, I.; Konda, Y.; Tashiro, T.; Shimoto, Y.; Miwa, H.; et al. Molecular Cloning of a Novel Melanocortin Receptor. *J. Biol. Chem.* **1993**, *268*, 8246–8250.
(6) Gantz, I.; Miwa, H.; Konda, Y.; Shimoto, Y.; Tashiro, T.; et al. Molecular Cloning, Expression, and Gene Localization of a Fourth Melanocortin Receptor. *J. Biol. Chem.* **1993**, *268*, 15174–15179.
(7) Gantz, I.; Shimoto, Y.; Konda, Y.; Miwa, H.; Dickinson, C. J.; et al. Molecular Cloning, Expression, and Characterization of a Fifth Melanocortin Receptor. *Biochem. Biophys. Res. Commun.* **1994**, *200*, 1214–1220.
(8) Bultman, S. J.; Michaud, E. J.; Woychick, R. P. Molecular Characterization of the Mouse Agouti Locus. *Cell* **1992**, *71*, 1195–1204.
(9) Miller, M. W.; Duhl, D. M.; Vrieling, H.; Cordes, S. P.; Ollmann, M. M.; et al. Cloning of the Mouse Agouti Gene Predicts a Secreted Protein Ubiquitously Expressed in Mice Carrying the Lethal Yellow Mutation. *Genes Dev.* **1993**, *7*, 454–467.
(10) Ollmann, M. M.; Wilson, B. D.; Yang, Y.-K.; Kerns, J. A.; Chen, Y.; et al. Antagonism of Central Melanocortin Receptors in Vitro and in Vivo by Agouti-Related Protein. *Science* **1997**, *278*, 135–138.
(11) Shutter, J. R.; Graham, M.; Kinsey, A. C.; Scully, S.; Lüthy, R.; et al. Hypothalamic Expression of ART, a Novel Gene Related to Agouti, is Up-Regulated in Obese and Diabetic Mutant Mice. *Genes Dev.* **1997**, *11*, 593–602.
(12) Lerner, A. B.; McGuire, J. S. Effect of Alpha- and Beta-Melanocyte Stimulating Hormones on the Skin Colour of Man. *Nature* **1961**, *189*, 176–179.
(13) Robbins, L. S.; Nadeau, J. H.; Johnson, K. R.; Kelly, M. A.; Roselli-Rehfuss, L.; et al. Pigmentation Phenotypes of Variant Extension Locus Alleles Result From Point Mutations That Alter MSH Receptor Function. *Cell* **1993**, *72*, 827–834.
(14) Lu, D.; Väge, D. I.; Cone, R. D. A Ligand-Mimetic Model for Constitutive Activation of the Melanocortin-1 Receptor. *Mol. Endocrinol.* **1998**, *12*, 592–604.
(15) Gantz, I.; Tashiro, T.; Barcroft, C.; Konda, Y.; Shimoto, Y.; et al. Localization of the Genes Encoding the Melanocortin-2 (Adrenocorticotropic Hormone) and Melanocortin-3 Receptors to Chromosomes 18p11.2 and 20q13.2-q13.3 by Fluorescence In Situ Hybridization. *Genomics* **1993**, *18*, 166–167.
(16) Butler, A. A.; Kesterson, R. A.; Khong, K.; Cullen, M. J.; Pelleymounter, M. A.; et al. A Unique Metabolic Syndrome Causes Obesity in the Melanocortin-3 Receptor-deficient Mouse. *Endocrinology* **2000**, *141*, 3518–3521.
(17) Chen, A. S.; Marsh, D. J.; Trumbauer, M. E.; Frazier, E. G.; Guan, X. M.; et al. Inactivation of the Mouse Melanocortin-3 Receptor Results in Increased Fat Mass and Reduced Lean Body Mass. *Nat. Genet.* **2000**, *26*, 97–102.
(18) Mountjoy, K. G.; Mortrud, M. T.; Low, M. J.; Simerly, R. B.; Cone, R. D. Localization of the Melanocortin-4 Receptor (MC4-R) in Neuroendocrine and Autonomic Control Circuits in the Brain. *Mol. Endocrinol.* **1994**, *8*, 1298–1308.
(19) Huszar, D.; Lynch, C. A.; Fairchild-Huntress, V.; Dunmore, J. H.; Smith, F. J.; et al. Targeted Disruption of the Melanocortin-4 Receptor Results in Obesity in Mice. *Cell* **1997**, *88*, 131–141.
(20) Fan, W.; Boston, B. A.; Kesterson, R. A.; Hruby, V. J.; Cone, R. D. Role of Melanocortinergic Neurons in Feeding and the *agouti* Obesity Syndrome. *Nature* **1997**, *385*, 165–168.
(21) Van Der Ploeg, L. H.; Martin, W. J.; Howard, A. D.; Nargund, R. P.; Austin, C. P.; et al. A Role for the Melanocortin 4 Receptor in Sexual Function. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 11381–11386.
(22) Hadley, M. E. Discovery That a Melanocortin Regulates Sexual Functions in Male and Female Humans. *Peptides* **2005**, *26*, 1687–1689.
(23) Chen, W.; Kelly, M. A.; Opitz-Araya, X.; Thomas, R. E.; Low, M. J.; et al. Exocrine Gland Dysfunction in MC5-R Deficient Mice: Evidence for Coordinated Regulation of Exocrine Gland Functions by Melanocortin Peptides. *Cell* **1997**, *91*, 789–798.
(24) Ellman, J. A. Design, Synthesis, and Evaluations of Small-Molecule Libraries. *Acc. Chem. Res.* **1996**, *29*, 132–143.
(25) Gordon, E. M.; Barrett, R. W.; Dower, W. J.; Fodor, S. P. A.; Gallop, M. A. Applications of Combinatorial Technologies to Drug Discovery. 2. Combinatorial Organic Synthesis, Library Screening Strategies, and Future Directions. *J. Med. Chem.* **1994**, *37*, 1385–1401.
(26) Terrett, N. K.; Gardner, M.; Gordon, D. W.; Kobylecki, R. J.; Steele, J. Combinatorial Synthesis-the Design of Compound Libraries and Their Application to Drug Discovery. *Tetrahedron* **1995**, *51*, 1385–1401.
(27) Horton, D. A.; Bourne, G. T.; Smythe, M. L. The Combinatorial Synthesis of Bicyclic Privileged Structrues or Privileged Substructrures. *Chem. Rev.* **2003**, *103*, 893–930.
(28) Hulme, C.; Peng, J.; Tang, S. Y.; Burns, C. J.; Morize, I.; et al. Improved Procedure for the Solution Phase Preparation of 1,4-Benzodiazepine-2,5-Dione Libraries via Armstrong's Convertible Isonitrile and the Ugi Reaction. *J. Org. Chem.* **1998**, *63*, 8021–8023.
(29) Keating, T. A.; Armstrong, R. W. A Remarkable Two-Step Synthesis of Diverse 1,4-Benzodiazepine-2,5-Diones Using the Ugi Four-Component Condensation. *J. Org. Chem.* **1996**, *61*, 8935–8939.
(30) Boojamra, C. G.; Burrow, K. M.; Thompson, L. A.; Ellman, J. A. Solid-Phase Synthesis of 1,4-Benzodiasepine-2,5-diones. Library Preparation and Emonstration of Synthesis Generality. *J. Org. Chem.* **1997**, *62*, 1240–1256.
(31) Martin, P. K.; Rapoport, H.; Smith, H. W.; et al. Synthesis of Cyclopenin and Isocyclopenin. *J. Org. Chem.* **1969**, *34*, 1359–1363.
(32) Smith, H.; Wegfahrt, P.; Rapoport, H. The synthesis of cyclopenin. *J. Am. Chem. Soc.* **1968**, *90*, 1668–1669.
(33) White, J. D.; Haefliger, W. E.; Dimsdale, M. J. Stereospecific Synthesis of DL-Cyclopenin and DL-Cyclopenol. *Tetrahedron* **1970**, *26*, 233–242.
(34) Bhalay, G.; Blaney, P.; Palmer, V. H.; Baxter, A. D. Solid-Phase Synthesis of Diverse Tetrahydro-1,4-benzodiazepine-2-ones. *Tetrahedron Lett.* **1997**, *38*, 8375–8378.
(35) Boojamra, C. G.; Burow, K. M.; Ellman, J. A. An Expedient and High-Yielding Method for the Solid-Phase Synthesis of Diverse 1,4-Benzodiazepine-2,5-Diones. *J. Org. Chem.* **1995**, *60*, 5742–5743.
(36) Bunin, B. A.; Plunkett, M. J.; Ellman, J. A. The Combinatorial Synthesis and Chemical and Biological Evaluation of a 1,4-Benzodiazepine Library. *Proc. Natl. Acad. Sci. U.S.A.* **1994**, *91*, 4708–4712.
(37) Bunin, B. A.; Plunkett, M. J.; Ellman, J. A. *Comb. Chem.* **1996**, 448–465.
(38) Bunin, B. A.; Plunkett, M. J.; Ellman, J. A.; Bray, A. M. The Synthesis of a 1680 Member 1,4-Benzodiazepine Library. *New J. Chem.* **1997**, *21*, 125–130.
(39) Hulme, C.; Ma, L.; Kumar, N. V.; Krolikowski, P. H.; Allen, A. C.; et al. Novel Applications of Resin Bound α-Amino Acids for the Synthesis of Benzodiazepines (via Wang Resin) and Ketopiperazines (via Hydroxymethyl Resin). *Tetrahedron Lett.* **2000**, *41*, 1509–1514.
(40) Mayer, J. P.; Zhang, J.; Bjergarde, K.; Lenz, D. M.; Gaudino, J. J. Solid Phase Synthesis of 1,4-Benzodiazepine-2,5-diones. *Tetrahedron Lett.* **1996**, *37*, 8081–8084.
(41) Lee, J.; Gauthier, D.; Rivero, R. A. Solid-Phase Synthesis of 3,4,5-Substituted 1,5-Benzodiazepin-2-ones. *J. Org. Chem.* **1999**, *64*, 3060–3065.
(42) Zhang, J.; Goodloe, W. P.; Lou, B.; Saneii, H. Solid-Phase Synthesis of Tetrahydro-1,4-benzodiazepin-2-one Derivatives. *Mol. Diversity* **2000**, *5*, 127–130.

(43) Haskell-Luevano, C.; Rosenquist, Å.; Souers, A.; Kong, K.; Ellman, J.; et al. Compounds That Activate the Mouse Melanocortin-1 Receptor Identified by Screening a Small Molecule Library Based upon the β-Turn. *J. Med. Chem.* **1999**, *42*, 4380–4387.

(44) Dyck, B.; Parker, J.; Phillips, T.; Carter, L.; Murphy, B.; et al. Aryl Piperazine Melanocortin MC4 Receptor Agonists. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 3793–3796.

(45) Richardson, T. I.; Ornstein, P. L.; Briner, K.; Fisher, M. J.; Backer, R. T.; et al. Synthesis and Structure-Activity Relationships of Novel Arylpiperazines as Potent and Selective Agonists of the Melanocortin Subtype-4 Receptor. *J. Med. Chem.* **2004**, *47*, 744–755.

(46) Sebhat, I. K.; Martin, W. J.; Ye, Z.; Barakat, K.; Mosley, R. T.; et al. Design and Pharmacology of *N*-[(3*R*)-1,2,3,4-Tetrahydroisoquinolinium- 3-ylcarbonyl]-(1*R*)-1-(4-chlorobenzyl)- 2-[4-cyclohexyl-4-(1*H*-1,2,4-triazol- 1-ylmethyl)piperidin-1-yl]-2-oxoethylamine (**1**), a Potent, Selective, Melanocortin Subtype-4 Receptor Agonist. *J. Med. Chem.* **2002**, *45*, 4589–4593.

(47) Xi, N.; Hale, C.; Kelly, M. G.; Norman, M. H.; Stec, M.; et al. Synthesis of Novel Melanocortin 4 Receptor Agonists and Antagonists Containing a Succinamide Core. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 377–381.

(48) Huang, S. C.; Zhang, L.; Chiang, H. C.; Wank, S. A.; Maton, P. N.; et al. Benzodiazepine Analogues L365,260 and L364,718 as Gastrin and Pancreatic CCK Receptor Antagonists. *Am. J. Physiol.* **1989**, *257*, G169–174.

(49) Anzini, M.; Canullo, L.; Braile, C.; Cappelli, A.; Gallelli, A.; et al. Synthesis, Biological Evaluation, and Receptor Docking Simulations of 2-[(Acylamino)Ethyl]-1,4-Benzodiazepines as κ-Opioid Receptor Agonists Endowed with Antinociceptive and Antiamnesic Activity. *J. Med. Chem.* **2003**, *46*, 3853–3864.

(50) Wyatt, P. G.; Allen, M. J.; Chilcott, J.; Hickin, G.; Miller, N. D.; et al. Structure-Activity Relationship Investigations of a Potent and Selective Benzodiazepine Oxytocin Antagonist. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 1301–1305, et al.

(51) Evans, B.; Pipe, A.; Clark, L.; Banks, M. Identification of a Potent and Selective Oxytocin Antagonist, from Screening a Fully Encoded Differential Release Combinatorial Chemical Library. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 1297–1300.

(52) Cheng, M. F.; Fang, J. M. Liquid-Phase Combinatorial Synthesis of 1,4-Benzodiazepine-2,5-diones as the Candidates of Endothelin Receptor Antagonism. *J. Comb. Chem.* **2004**, *6*, 99–104.

(53) Hsu, M. C.; Schutt, A. D.; Holly, M.; Slice, L. W.; Sherman, M. I.; et al. Inhibition of HIV Replication in Acute and Chronic Infections in Vitro by a Tat Antagonist. *Science* **1991**, *254*, 1799–1802.

(54) Pauwels, R.; Andries, K.; Desmyter, J.; Schols, D.; Kukla, M. J.; et al. Potent and Selective Inhibition of HIV-1 Replication in Vitro by a Novel Series of TIBO Derivatives. *Nature* **1990**, *343*, 470–474.

(55) Yang, Y.-K.; Dickinson, C.; Haskell-Luevano, C.; Gantz, I. Molecular Basis for the Interaction of [Nle^4, D$\underline{\text{P}}$he^7] Melanocyte Stimulating Hormone with the Human Melanocortin-1 Receptor (Melanocyte α-MSH Receptor). *J. Biol. Chem.* **1997**, *272*, 23000–23010.

(56) Yang, Y.; Fong, T. M.; Dickinson, C. J.; Mao, C.; Li, J. Y.; et al. Molecular Determinants of Ligand Binding to the Human Melanocortin-4 Receptor. *Biochemistry* **2000**, *39*, 14900–14911.

(57) Haskell-Luevano, C.; Cone, R. D.; Monck, E. K.; Wan, Y.-P. Structure Activity Studies of the Melanocortin-4 Receptor by *in Vitro* Mutagenesis: Identification of Agouti-Related Protein (AGRP), Melanocortin Agonist and Synthetic Peptide Antagonist Interaction Determinants. *Biochemistry* **2001**, *40*, 6164–6179.

(58) Haskell-Luevano, C.; Sawyer, T. K.; Trumpp-Kallmeyer, S.; Bikker, J.; Humblet, C.; et al. Three-Dimensional Molecular Models of the hMC1R Melanocortin Receptor: Complexes with Melanotropin Peptide Agonists. *Drug Des. Discovery* **1996**, *14*, 197–211.

(59) Wilczynski, A.; Wang, X. S.; Joseph, C. G.; Xiang, Z.; Bauzo, R. M.; et al. Identification of Putative Agouti-Related Protein(87–132)-Melanocortin-4 Receptor Interactions by Homology Molecular Modeling and Validation Using Chimeric Peptide Ligands. *J. Med. Chem.* **2004**, *47*, 2194–2207.

(60) Chai, B. X.; Pogozheva, I. D.; Lai, Y. M.; Li, J. Y.; Neubig, R. R.; et al. Receptor-Antagonist Interactions in the Complexes of Agouti and Agouti-Related Protein with Human Melanocortin 1 and 4 Receptors. *Biochemistry* **2005**, *44*, 3418–3431.

(61) Pogozheva, I. D.; Chai, B. X.; Lomize, A. L.; Fong, T. M.; Weinberg, D. H.; et al. Interactions of Human Melanocortin 4 Receptor with Nonpeptide and Peptide agonists. *Biochemistry* **2005**, *44*, 11329–11341.

(62) Hogan, K.; Peluso, S.; Gould, S.; Parsons, I.; Ryan, D.; et al. Mapping the Binding Site of Melanocortin 4 Receptor Agonists: A Hydrophobic Pocket Formed by I3.28(125), I3.32(129), and I7.42(291) Is Critical for Receptor Activation. *J. Med. Chem.* **2006**, *49*, 911–922.

(63) Barlow, J. J.; Blackburn, T. P.; Costello, G. F.; James, R.; Le Count, D. J.; et al. Structure/Activity Studies Related to 2-(3,4-Dichlorophenyl)-*N*-methyl-*N*-[2-(1-pyrrolidinyl)-1-substituted- ethyl]acetamides: A Novel Series of Potent and Selective κ-Opioid Agonists. *J. Med. Chem.* **1991**, *34*, 3149–3158.

(64) Clark, C. R.; Halfpenny, P. R.; Hill, R. G.; Horwell, D. C.; Hughes, J.; et al. Highly Selective κ-Opioid Analgesics. Synthesis and Structure-Activity Relationships of Novel *N*-[(2-Aminocyclohexyl)aryl]acetamide and *N*-[(2-Aminocyclohexyl)aryloxy]acetamide Derivatives. *J. Med. Chem.* **1988**, *31*, 831–836.

(65) Vecchietti, V.; Clarke, G. D.; Colle, R.; Giardina, G.; Petrone, G.; et al. (1*S*)-1-(Aminomethyl)-2-(arylacetyl)-1,2,3,4-tetrahydroisoquinoline and Heterocycle-Condensed Tetrahydropyridine Derivatives: Members of a Novel Class of Very Potent κ-Opioid Analgesics. *J. Med. Chem.* **1991**, *34*, 2624–2633.

(66) Lavecchia, A.; Greco, G.; Novellino, E.; Vittorio, F.; Ronsisvalle, G. Modeling of κ-Opioid Receptor/Agonists Interactions Using Pharmacophore-Based and Docking Simulations. *J. Med. Chem.* **2000**, *43*, 2124–2134.

(67) Chipot, C.; Jaffe, R.; Maigret, B.; Pearlman, D. A.; Kollman, P. A. Benzene Dimer: A Good Model for $\pi-\pi$ Interactions in Proteins? A Comparison between the Benzene and the Toluene Dimers in the Gas Phase and in an Aqueous Solution. *J. Am. Chem. Soc.* **1996**, *118*, 11217–11224.

(68) Hunter, C. A.; Singh, J.; Thornton, J. M. Pi-Pi Interactions: The Geometry and Energetics of Phenylalanine-Phenylalanine Interactions in Proteins. *J. Mol. Biol.* **1991**, *218*, 837–846.

(69) Hruby, V. J.; Lu, D.; Sharma, S. D.; Castrucci, A. M. L.; Kesterson, R. A.; et al. Cyclic Lactam α-Melanotropin Analogues of Ac-Nle^4-c[Asp^5, D-Phe^7, Lys^{10}]-α-MSH(4-10)-NH$_2$ with Bulky Aromatic Amino Acids at Position 7 Show High Antagonist Potency and Selectivity at Specific Melanocortin Receptors. *J. Med. Chem.* **1995**, *38*, 3454–3461.

(70) Chen, W.; Shields, T. S.; Stork, P. J. S.; Cone, R. D. A Colorimetric Assay for Measuring Activation of Gs- and Gq-Coupled Signaling Pathways. *Anal. Biochem.* **1995**, *226*, 349–354.

JM701303Z