Journal of Medicinal Chemistry

Volume 45, Number 21 October 10, 2002

Letters

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

Iyassu K. Sebhat,*,† William J. Martin,‡ Zhixiong Ye,† Khaled Barakat,† Ralph T. Mosley,† David B. R. Johnston,† Raman Bakshi,† Brenda Palucki,† David H. Weinberg,§ Tanya MacNeil,§ Rubana N. Kalyani,§ Rui Tang,§ Ralph A. Stearns,|| Randy R. Miller,|| Constantin Tamvakopoulos,† Alison M. Strack,‡ Erin McGowan,‡ Doreen E. Cashen,‡ Jennifer E. Drisko,‡ Gary J. Hom,‡ Andrew D. Howard,§ D. Euan MacIntyre,‡ Lex H. T. van der Ploeg,§ Arthur A. Patchett,† and Ravi P. Nargund†

Departments of Chemistry, Pharmacology, Obesity Research, and Drug Metabolism, Merck & Co., Inc., P.O. Box 2000, Rahway, New Jersey 07065-0900

Received May 14, 2002

Abstract: Synthetic and natural peptides that act as nonselective melanocortin receptor agonists have been found to be anorexigenic and to stimulate erectile activity. We report the design and development of **1**, a potent, selective (1184-fold vs MC3R, 350-fold vs MC5R), small-molecule agonist of the MC4 receptor. Pharmacological testing confirms the food intake lowering effects of MC4R agonism and suggests another role for the receptor in the stimulation of erectile activity.

Introduction. The melanocortin receptors are a family of seven-transmembrane G-protein-coupled receptors. The five known subtypes interact with their endogenous ligands (the corticotropins and melanocortins) to mediate a wide array of activities, from the control of feeding and sexual behavior to skin pigmentation and neuroendocrine regulation. The melanocortin subtype-4 receptor (MC4R) is expressed in various regions of the brain,[1] and evidence pointing to the role of this receptor in feeding behavior has been summarized.[2−10] This includes obesity observed in animals that overexpress the coat-color regulating protein agouti, a competitive MC4R and MC1R antagonist.[3,4] It was later discovered that injection of MC4R agonists into the brain inhibits food intake in rodents.[5−8] There is a converse increase following intracerebroventricular administration of antagonists.[8−10] This link between MC4R and feeding regulation was strengthened by gene-deletion studies of the receptor resulting in obese mice.[11] Thus, agonism at MC4R produces a lean phenotype while inhibition or inactivation of the receptor results in obesity. These findings have led to intense efforts to identify suitable agonists of the receptor as possible treatments for obesity.

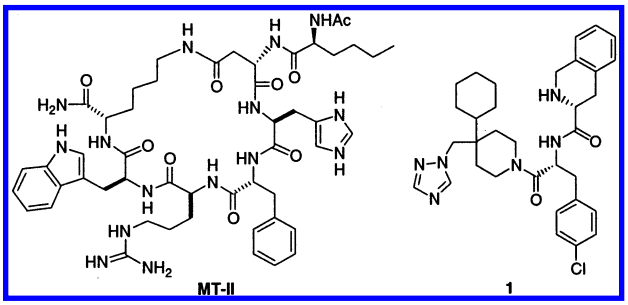


Figure 1. Synthetic ligands MT-II and 1.

Central administration of the endogenous melanocortin receptor agonists ACTH and α-MSH has been shown to elicit erectile activity in rodents.[2,12] More recently, melanotan-II (MT-II, Figure 1), a potent agonist with subnanomolar EC_{50} values at MC1R, MC3R, MC4R, and MC5R, was developed.[13] Wessells and coworkers reported erectile activity on administration of the synthetic peptide to human subjects. Subcutaneous doses resulted in a significant onset of transient erections in 8 out of 10 men with psychogenic erectile

* To whom correspondence should be addressed. Phone: (732) 594 0168. Fax: (732) 594 3007. E-mail: iyassu_sebhat@merck.com.
† Department of Chemistry.
‡ Department of Pharmacology.
§ Department of Obesity Research.
|| Department of Drug Metabolism.

10.1021/jm025539h CCC: $22.00
Published on Web 09/14/2002

α-MSH
Ac-Ser-Tyr-Ser-Met-Glu-**His-L-Phe-Arg-Trp**-Gly-Lys-pro-ValNH2

MTII (Agonist)
Ac-Nle-c[Asp-**His-D-Phe- Arg-Trp**-Lys]-NH2
SHU-9119 (Antagonist)
Ac-Nle-c[Asp-**His-D-2-Nal-Arg-Trp**-Lys]-NH2

GHS Peptide GHRP-6
His-D-Trp-Ala-Trp-D-Phe-Lys-NH2

Figure 2. Endogenous ligand α-MSH, synthetic cyclic ligands MT-II and SHU-9119, and the growth hormone secretagogue peptide GHRP-6.

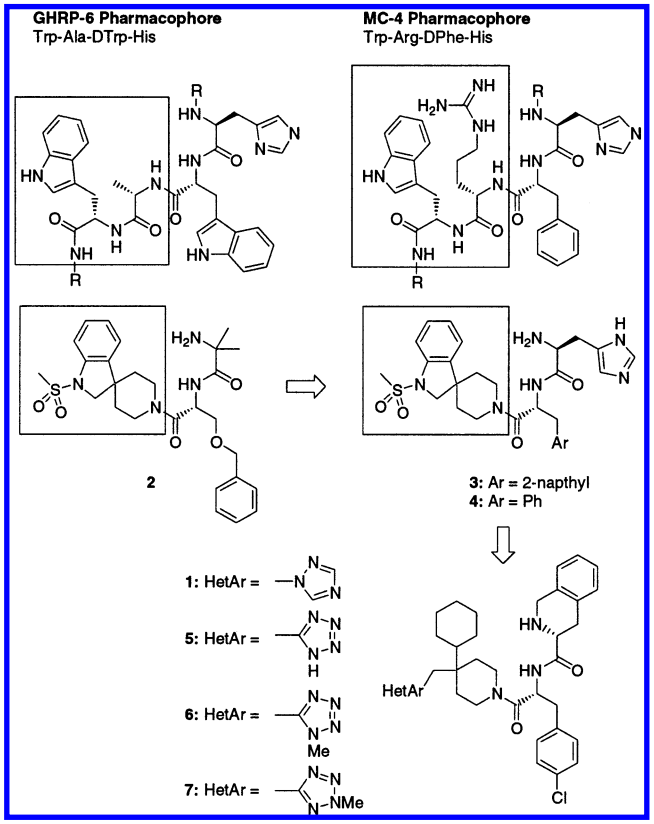


Figure 3. Lead discovery and optimization.

dysfuntion[14] and in 9 out of 10 of those studied with organic dysfunction.[15]

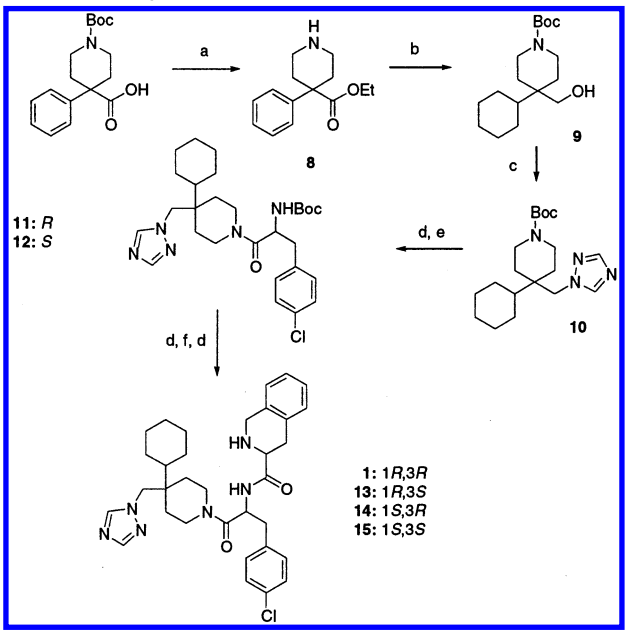
To determine which receptor subtype mediates these observed erectogenic effects, more selective agonists were sought. We report here the design of a highly potent MC4R selective agonist, **1**, and its activity in studies to assess food intake and erectile activity.

Design Considerations. Both ACTH and the MSH peptides contain a common His-Phe-Arg-Trp core unit (Figure 2) essential for their functional activities (EC_{50}). Structure−activity studies about this core have generated various truncated, cyclic analogues including the heptapeptides MT-II and SHU-9119.[13,16−18]

There is close homology between the common pharmacophore and the active core of the growth hormone secretagogue peptide GHRP-6.[19] A clinical candidate, **2** (Figure 3), had been developed in a growth hormone project at Merck. Modeling and molecular calculation suggested that the spiroindanyl piperidine functions as an Ala-Trp mimetic.[20]

We anticipated that such an approach would extend to the design of an MC4R lead. A directed search of the

Scheme 1. Synthesis of **1** and Its Stereoisomers[a]



[a] Reagents: (a) $SOCl_2$/EtOH; (b) (i) LAH/THF, (ii) Rh/Al_2O_3/H_2/HCl/MeOH, (iii) Boc_2O/Et_3N/MeOH; (c) (i) MsCl/Et_3N/CH_2Cl_2, (ii) Na-triazole/DMF; (d) HCl/CH_2Cl_2; (e) Boc-(L or D)-Phe(pCl)-OH/EDC/HOBt/NMM/CH_2Cl_2; (f) Boc-(L or D)-Tic-OH/EDC/HOBt/NMM/CH_2Cl_2.

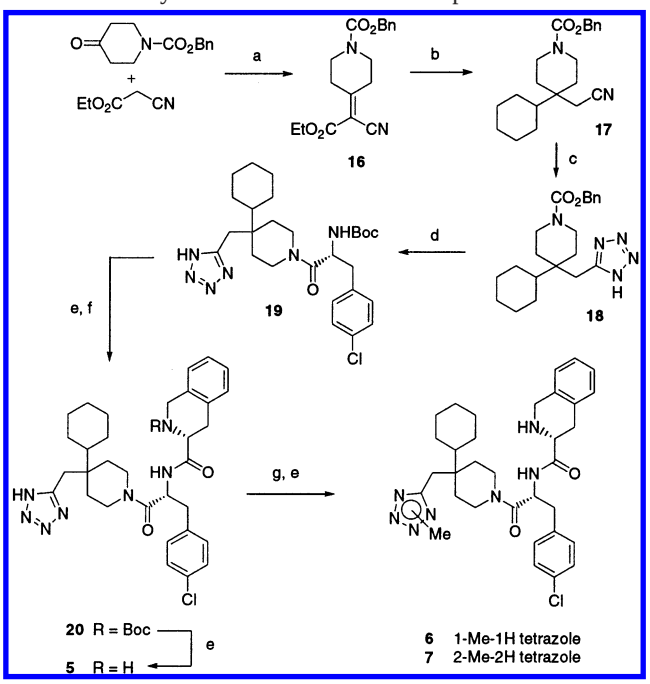
Merck sample collection found **3** containing the piperidine coupled to naphthylalanine and His, closely resembling the antagonist SHU-9119. Compound **3** was found to have a binding IC_{50} of 108 nM at MC4R, inducing little or no activation. Modification of the phenylalanine residue in the cyclic peptides exerts a large influence on agonist activity.[18] Similarly, a change from naphthylalanine to phenylalanine provided our first agonist lead: **4** (EC_{50} = 500 nM; 97% activation). Optimization led to a series of 4-substituted 4-cyclohexylpiperidine-based structures. A very potent class emerged where the piperidine is substituted with a methylene bearing a heterocycle. Replacement of His with 1,2,3,4-tetrahydro-3-isoquinolinecarboxylic acid (Tic) further enhanced potency and selectivity.

Chemistry. The synthesis of the compounds required one of two routes. These are outlined in Schemes 1 and 2.

The synthesis of the triazole-based compounds began with the core skeleton in place. *N*-Boc-4-phenylpiperidine-4-carboxylic acid was initially esterified. The amino ester **8** was then reduced with $LiAlH_4$, the aromatic ring saturated, and the amine protected to generate **9**. Substitution gave triazole **10**, which was deprotected and coupled to L- and D-Phe(*p*Cl) and then to L- and D-Tic to give, after a final deprotection, **1** and its stereoisomers **13**, **14**, and **15**.

The synthesis of the tetrazoles required construction of the core skeleton and proceeded initially with the Knoevenalgel condensation of Cbz-protected 4-piperidone with ethyl cyanoacetate. The resultant unsaturated cyanoacetate **16** was treated with a cuprate generated from cyclohexylmagnesium chloride. The 4-cyclohexylpiperidine was then thermally decarboxylated to generate nitrile **17**. Cycloaddition of azide afforded the free tetrazole, which was deprotected and coupled to the

Scheme 2. Synthesis of Tetrazole Compounds **5**−**7**[a]



[a] Reagents: (a) $NH_4Cl/AcOH/C_6H_6$; (b) (i) cyclohexylmagnesium chloride/CuCN/THF, (ii) $LiCl/H_2O/DMSO$; (c) $TMSN_3/Bu_2SnO$/toluene; (d) (i) $Pd(OH)_2/H_2/HCl/MeOH$, (ii) Boc-D-Phe(pCl)-OH/EDC/HOBt/NMM/CH_2Cl_2; (e) HCl/CH_2Cl_2; (f) Boc-D-Tic-OH/EDC/HOBt/NMM/CH_2Cl_2; (g) K_2CO_3/MeI/DMF.

Table 1. Binding Affinity and Selectivity of Compounds for the Human Melanocortin 4 Receptor[a]

	IC_{50} [b] (nM)		
compd	MC4R	MC3R/4R	MC5R/4R
1	1.2 ± 0.11	634	272
13	0.5 ± 0.14	336	130
14	3050 ± 150	0.3	1.3
15	127 ± 32	57	9.4
5	238 ± 26	53	19
6	0.3 ± 0.09	277	100
7	1.2 ± 0.15	601	258

[a] Values (n = 2) with standard errors. [b] Displacement of [^{125}I]-NDP-α-MSH from human receptors expressed in CHO cells.

dipeptide cap. Methylation prior to final deprotection gave the 1- and 2-methyltetrazoles **6** and **7**.

Results and Discussion

The compounds were evaluated in two primary assays: affinity to the melanocortin subtypes was assessed in a competitive binding assay (Table 1); agonist potency was determined in cAMP release assays using CHO cells expressing the relevant receptors (Table 2).[21] The compounds bearing *S* (or L) stereochemistry at the Phe(*p*Cl) residue (**14** and **15**) bind poorly at MC4R. cAMP levels generated in functional assays suggest that the compounds are at best only partial agonists (9−34%). Conversely the 1*R* (or D) isomers **1** and **13** show high affinity for the receptor and activation at a concentration of 10 μM approaching that of α-MSH. The 3*R* (D-Tic) isomer **1** exhibits marginally higher IC_{50} but improved functional activity.

The tetrazoles were synthesized bearing the optimized 1*R*,3*R* stereochemistry. The parent compound **5** shows poor potency, suggesting that binding is disrupted by acidity in the region. The two methylated compounds **6** and **7** show very high potency with strong activation of the receptor. While the 1-methyltetrazole **6** is the most potent compound in the series, its reduced selectivity makes it less attractive than triazole **1**. Data for this compound are outlined in more detail in Table 3. **1** is over 100-fold selective vs the other human receptor subtypes. It is a full agonist at MC1R (binding only weakly to the receptor) and at MC4R. Selectivity is better still at the rat receptors.

Table 2. Functional Activity of Compounds at Human Melanocortin Receptors[a]

	EC_{50} [b] (nM) [% max][c]		
compd	MC4R	MC3R	MC5R
1	2.1 ± 0.24 [97]	2487 ± 43 [32]	736 ± 65 [61]
13	8.7 ± 4 [67]	[7]	271 ± 7 [19]
14	[9]	[1]	[2]
15	[34]	[4]	[5]
5	771 ± 32 [79]	[10]	[15]
6	0.6 ± 0.2[103]	59 ± 1 [50]	127 ± 6 [51]
7	9.6 ± 1.5 [87]	[24]	1004 ± 46 [38]

[a] Values (n = 2) with standard errors. [b] Concentration of compound at 50% maximum cAMP accumulation. [c] Percentage of cAMP accumulation at 10 μM compound relative to α-MSH.

Table 3. Binding Affinity and Functional Activity of **1** at Human and Rat Melanocortin Receptors

receptor	binding[a] IC_{50} (nM)	cAMP[b] EC_{50} (nM)	activation[c] at 10 μM (%)
hMC1R	2067 ± 73	2850 ± 450	95
hMC3R	761 ± 31	2487 ± 43	32
hMC4R	1.2 ± **0.11**	2.1 ± **0.2**	97
hMC5R	326 ± 9	737 ± 65	61
rMC3R	1883 ± 478	1325 ± 607	65
rMC4R	0.6 ± **0.16**	2.9 ± **0.8**	99
rMC5R	1575 ± 372	>3000	58

[a] Displacement of [^{125}I]-NDP-α-MSH from receptors expressed in CHO cells (n > 15). [b] Concentration of compound at 50% maximum cAMP accumulation (n > 5). [c] Percentage of cAMP accumulation at 10 μM relative to α-MSH.

Table 4. Pharmacokinetic Data for **1**

PK parameter	rat[a]	dog[b]
F (%)	14	16
Cl (mL min^{-1} kg^{-1})	84 ± 2	27.55 ± 0.2
V_{dss} (L kg^{-1})	3.6 ± 0.2	2.79 ± 0.02
$t_{1/2}$ (h)	0.6 ± 0.05	1.2 ± 0.1
T_{max} (h)	1 ± 0.05	1.5 ± 0.5

[a] Compound dosed in Sprague−Dawley rats as a solution in EtOH/PEG/saline (1:4:5) at 1 mg/kg, iv (n = 2) and 10 mg/kg, po (n = 2). [b] Compound dosed in beagles as a solution in EtOH/PEG/saline (1:4:5) at 0.2 mg/kg, iv (n = 2) and 0.5 mg/kg, po (n = 2).

The compound was characterized in pharmacokinetic (PK) studies (Table 4). Bioavailability was moderate for the rat and dog. The compound is rapidly absorbed (short T_{max}), but relatively fast plasma clearance results in short duration in both species ($t_{1/2}$(rat) = 30 min).

Figure 4 shows the superposition of a low-energy conformer of **1** onto a low-energy conformation of MT-II. The residues common to MT-II and α-MSH (in purple) were used as a probe over multiple energy-minimized conformations of **1** using SQ[22] to identify the energetically most stable conformer.

Compound **1** was evaluated for its effects on food intake. In line with the observed effects of other MC4R agonists, food intake was significantly reduced on administration of the compound.[23]

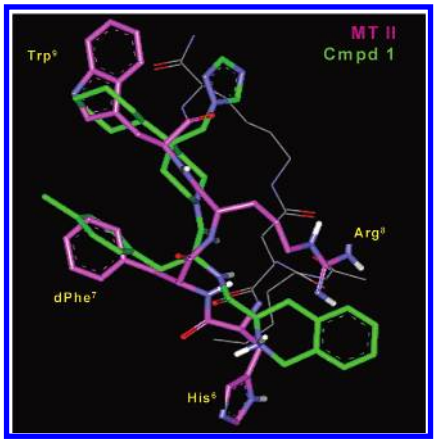


Figure 4. Superposition of **1** (green) with MT-II (purple).

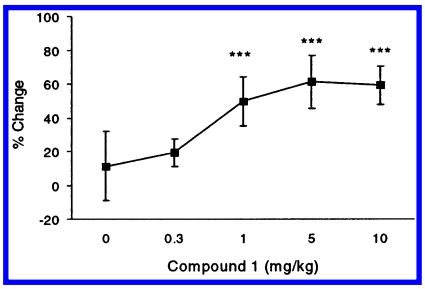


Figure 5. Penile erections in ex copula model after iv dosing. Vehicle ($n = 18$) and 0.3 mg/kg ($n = 6$) did not significantly increase the number of erections; 1 ($n = 16$), 5 ($n = 18$), and 10 ($n = 6$) mg/kg produced comparable increases. (***) $p < 0.001$, compared to vehicle treatment (paired t test).

Erectogenic activity was evaluated using an established rodent model[24−26] such that each rat served as its own control. The mean number of erections elicited over a 15 min period (direct visual count) in vehicle-treated rats was 27.07 ± 1.2, $n = 64$. Apomorphine, a known erectile stimulant,[24] was used as a positive control. At 0.01 mg/kg, sc, apomorphine increased penile erections by 37% ($p < 0.001$; $n = 20$). Higher doses (0.05−0.1 mg/kg, sc) did not further increase erectile responses. Following iv administration, **1** dose-dependently (0.3−10 mg/kg) increased erections ($ED_{50} = 0.87$ mg/kg; Figure 5). The maximal increase in the number of erections (60%) was detected at 5 mg/kg but was not significantly different from that produced by 1 mg/kg. Following oral administration, **1** (20 mg/kg, po) also produced statistically significant increases in erectile responses with a mean increase of 31 ± 4% ($p < 0.001$; $n = 6$).[25]

Conclusion. Compound **1** is the first selective small-molecule agonist of the MC4 receptor with submicromolar potency to be disclosed in the literature. In vivo testing of **1** suggests that MC4R agonism plays a role in eliciting erectile activity in rodents. While we cannot exclude the possibility of MC3R participation, **1** maintains low potency at the rodent MC3 receptor. Additionally, augmentation of erectile activity observed in wild-type mice is absent in MC4R null mice.[26]

Acknowledgment. The authors are grateful to Falguni Patel and Mark Levorse for assistance.

References

(1) Mountjoy, K. G.; Mortrud, M. T.; Low, M. J.; Simerley, R. B.; Cone, R. D. Localization of the MC-4 Receptor in Neuroendocrine and Autonomic Control Circuits in the Brain. *Mol. Endocrinol.* **1994**, *8*, 1298−1308.
(2) Vergoni, A. V.; Bertolini, A. Role of Melanocortins in the Central Control of Feeding. *Eur. J. Pharmacol.* **2000**, *405*, 25−32.
(3) Bultman, S. J.; Michaud, E. J.; Woychik, R. P. Molecular Characterization of the Mouse Agouti Locus. *Cell* **1992**, *71*, 1195−1204.
(4) Lu, D.; Willard, D.; Patel, I. R.; Kandell, S.; Overton, L.; Kost, T.; Luther, M.; Chen, W.; Woychik, R. P.; Wilkison, W. O.; Cone, R. Agouti Protein is an Antagonist of the Melanocyte Stimulating Hormone Receptor. *Nature* **1994**, *371*, 799−802.
(5) Kask, A.; Rago, L.; Wikberg, J. E. S.; Schiotch, H. B. Evidence of Involvement of the Melanocortin MC4 Receptor in the Effects of Leptin on Food Intake and Body Weight. *Eur. J. Pharmacol.* **1998**, *360*, 15−19.
(6) 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.
(7) Murphy, B.; Nunes, C. N.; Ronan, J. J.; Harper, C. M.; Beall, M. J.; Hanaway, M.; Fairhurst, A. M.; Van der Ploeg, L. H. T.; MacIntyre, D. E.; Mellin, T. N. Melanocortin Mediated Inhibition of Feeding Behavior in Rats. *Neuropeptides* **1998**, *32*, 491−497.
(8) Giraudo, S. Q.; Billington, C. J.; Levine, A. S. Feeding Effects of Hypothalmic Injection of Melanocortin 4 Receptor Ligands. *Brain Res.* **1998**, *809*, 302−306.
(9) Ollmann, M. M.; Wilson, B. D.; Yang, Y.; Kerns, J. A.; Chen, Y.; Gantz, I.; Barsh, G. S. Antagonism of Central Melanocortin Receptors in Vitro and in Vivo by Agouti-Related Protein. *Science* **1997**, *278*, 135−138.
(10) Kask, A.; Rago, L.; Mutulis, F.; Pahkla, R.; Wikberg, J. E. S.; Schiotch, H. B. Selective Antagonists for the Melanocortin 4 Receptor (HS014) Increases Food Intake in Free-Feeding Rats. *Biochem. Biophys. Res. Commun.* **1998**, *245*, 90−93.
(11) Huszar, D.; Lynch, C. A.; Fairchild-Huntress; 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.; Lee, F. Targeted Disruption of the Melanocortin-4 Receptor Results in Obesity in Mice. *Cell* **1997**, *88*, 131−141.
(12) Bertolini, A.; Gessa, G. L.; Ferrari, W. Penile Erection and Ejaculation: A Central Effect of ACTH-like peptides in Mammals. In *Pharmacology and Biochemistry*; Sandler, M., Gessa, G. L., Eds.; Raven Press: New York 1975.
(13) Al-Obeidi, F.; Hadley, M. E.; Pettitt, B. M.; Rhuby, V. J. Design of a New Class of Superpotent Cyclic α-Melanotropins Based on Quenched Dynamic Simulations. *J. Am. Chem. Soc.* **1989**, *111*, 3413−3416.
(14) Wessells, H.; Fuciarelli, K.; Hansen, J.; Hadley, M. E.; Hruby, V. J.; Dorr, R.; Levine, N. Synthetic Melanotropic Peptide Initiates Erections in Men with Psychogenic Erectile Dysfunction. *J. Urol.* **1998**, *160*, 389−393.
(15) Wessells, H.; Gralnek, D.; Dorr, R.; Hruby, V. J.; Hadley, M. E.; Levine, N. Effect of an Alpha-Melanocyte Stimulating Hormone Analog on Penile Erection and Sexual Desire in Men with Organic Erectile Dysfunction. *Urologia* **2000**, *56*, 641−646.
(16) Bednarek, M. A.; MacNeil, T.; Kalyani, R. N.; Tang, R.; van der Ploeg, L. H. T.; Weinberg, D. H. Selective, High Affinity Peptide Antagonists of α-Melanotropin Action at Human Melanocortin Receptor 4: Their Synthesis and Biological Evaluation in Vitro. *J. Med. Chem.* **2001**, *44*, 3665−3672.
(17) Al-Obeidi, F.; de L. Castrucci, A. M.; Hadley, M. E.; Rhuby, V. J. Potent and Prolonged Acting Cyclic Lactam Analogues of α-Melanotropin: Design Based on Molecular Dynamics. *J. Med. Chem.* **1989**, *32*, 2555−2561.
(18) Hruby, V. J.; Lu, D.; Sharma, S. D.; de L. Castrucci, A.; Kesterson, R. A.; Al-Obeidi, F. A.; Hadley, M. E.; Cone, R. D. Cyclic Lactam α-Melanotropin Analogues of Ac-4-Cyclo[Asp5,D-Phe7,Lys10] α-Melanocyte-Stimulating-(4−10)-NH2 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.
(19) Castrucci, A. M.; Sherbrooke, W. C.; Sawyer, T. K.; Staples, D. J.; Tuma, M. C.; Hadley, M. E. Discovery of an alpha-melanotropin antagonist effective in vivo. *Peptides* **1994**, *15*, 627−632.
(20) Nargund, R. P.; Patchett, A. A.; Bach, M. A.; Murphy, M. G.; Smith, R. Peptidomimetic Growth Hormone Secretagogues. Design Considerations and Therapeutic Potential. *J. Med. Chem.* **1998**, *41*, 3103−3127.
(21) Bednarek, M. A.; Silve, M. V.; Arison, B.; MacNeil, T.; Kalyani, R. N.; Huang, R. C.; Weinberg, D. H. Structure−Function Studies on the Cyclic Peptide MT-II, Derivative of α-Melanotropin. *Peptides* **1999**, *20*, 401−409.
(22) Miller, M. D.; Sheridan, R. P.; Kearsley, S. K. SQ—a program for rapidly producing pharmacophorically relevant molecular superpositions. *J. Med. Chem.* **1999**, *42*, 1505−1514.
(23) Strack, A. M.; et al. Unpublished results.

(24) Sachs, B. D. Contextual approaches to the physiology and classification of erectile function, erectile dysfunction, and sexual arousal. *Neurosci. Biobehav. Rev.* **2000**, *24*, 541−560.
(25) Martin, W. J.; et al. Activation of MC4R Increases Erectile Activity in Rats Ex Copula. *Eur. J. Pharmacol.*, in press.
(26) van der Ploeg, L. H. T.; Martin, W. J.; Howard, A. D.; Nargund, R. P.; Austin, C. P.; Guan, X.; Drisko, J.; Cashen, D.; Sebhat, I.; Patchett, A. A.; Figueroa, D. J.; DiLella, A. G.; Connolly, B. M.; Weinberg, D. H.; Tan, C. T.; Palyha, O. C.; Pong, S.; MacNeil, T.; Rosenblum, C.; Vongs, A.; Tang, R.; Yu, H.; Sailer, A. W.; Fong, T. M.; Huang, C.; Tota, M.; Chang, R. S.; Stearns, R.; Tamvakopoulos, C.; Christ, G.; Drazen, D. L.; Spar, B. D.; Nelson, R. J.; MacIntyre, D. E. A role for the melanocortin 4 receptor in sexual function. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 11381.

JM025539H