Phenylguanidines as Selective Nonpeptide Melanocortin-5 Receptor Antagonists

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A series of phenylguanidine analogues represented by **10**, **12**, and **21** were synthesized and found to have high binding affinities for the human melanocortin-5 receptor. Their binding affinities for three other melanocortin receptor subtypes, MC1, MC3, and MC4, were low. Selected compounds were also tested for their functional activity and exhibited inhibition of α-MSH-stimulated cAMP production in cells expressing the human MC5 receptor. Compound **10** had a K_i value of 2.1 nM in the binding assay and an IC_{50} of 72 nM in the functional assay. Some analogues such as **13** from this series possessed weak agonist activity at the human MC4 receptor.

Introduction

Five receptors, MC1−5R, are known to mediate the behavioral and physiological effects of melanocortin peptides. Activation of these G-protein-coupled receptors stimulates the production of cAMP in various tissues.[1] The melanocortin-1 receptor (MC1R) is expressed in epidermal and follicular melanocytes[2] and regulates skin pigmentation and the immune system.[3] The MC2R (ACTH receptor) controls steroid production in the adrenal cortex.[4] The MC3R,[5] MC4R,[6] and MC5R[7] are all found in the brain. While the MC3R may be involved in the central regulation of body weight,[8] the MC4R are known to be important for body weight regulation and influence of sexual behavior.[9] A clear function for the central melanocortin-5 receptor has not been determined. However, the MC5R is found in a wide range of tissues and there is evidence that it may have a role in regulating exocrine gland secretion.[10] Peripherally, the MC5R is expressed abundantly in pheromone-producing exocrine glands, including sebaceous, lachrymal, Harderian, sex accessory, and preputial glands.[11] Mice that are genetically engineered to be MC5R-deficient (MC5R−/−) have defective sebaceous and Harderian glands.[11a] Studies on the behavioral activities in MC5R-deficiet mice have been reported recently.[12,13]

The melanocortin peptides are the natural ligands for the MCRs and consist of the melanotropins α-MSH, *β*-MSH, and *γ*-MSH and the adrenocorticotropin ACTH. Interestingly, agouti-protein and agouti-related protein have been discovered as endogenous antagonists or inverse agonists of the melanocortin receptors.[1] Because of the importance of the MC4R in feeding behavior, metabolism, and energy homeostasis, recent research efforts have been focused on the discovery of selective MC4R agonists or antagonists for potential treatment of human diseases such as obesity and cachexia.[14] Highly potent and selective small molecule MC4R agonists such as **1** have been reported (Figure 1).[15] Moreover, MC1R-selective agonists, such as compound

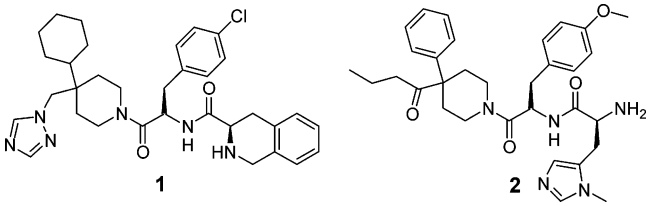

Figure 1. Small molecule agonists for the melanocortin receptors.

2, have been reported recently and demonstrated efficacy in an acute model of inflammation.[16] By contrast, MC5R-selective small molecule ligands, which could be useful to further define the biological role of the melanocortin-5 receptor, have not been described. Here we report a series of *N*-alkyl-*N*′-phenylguanidines as potent and selective antagonists at the human melanocortin-5 receptor.[17]

Chemistry

The synthesis of the targeted phenylguanidines is showed in Scheme 1. The commercially available 4-azidobenzoic acid was coupled with 2,4-dichlorophenethylamine under standard peptide coupling conditions (EDC/DIEA/THF) to give the corresponding amide **3** in 68% isolated yield. This amide **3** was then treated with triphenylphosphine in THF at room temperature to form an azo-Wittig intermediate, which was reacted with cyclohexyl isocyanate at 70 °C to form an iminoimide that was converted to the desired guanidines **4**−**25** by reaction with various primary or secondary amines in a one-pot synthesis.

Results and Discussion

These compounds were then tested in a competitive binding assay using HEK293 cells stably expressing the human melanocortin-5 receptor and [^{125}I]NDP-MSH as the radiolabeled ligand, as previously described.[18] The structure−activity relationships of the phenylguanidines at the human MC5 receptor are depicted in Table 1. Selected compounds were then measured for their

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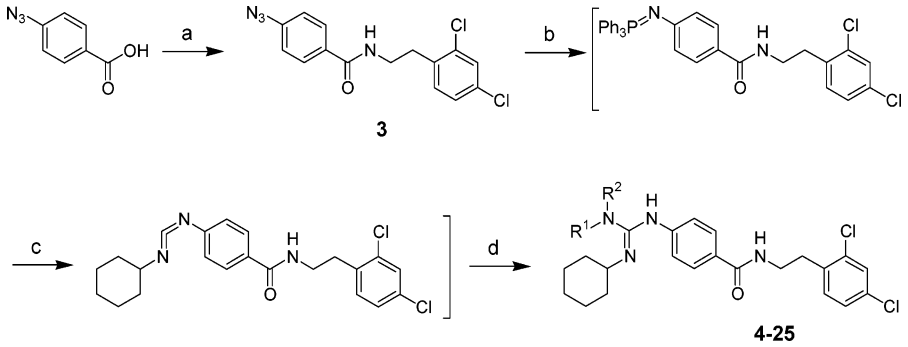
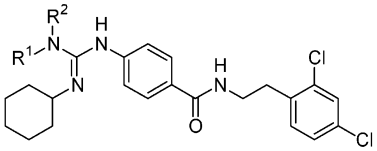
Scheme 1[a]

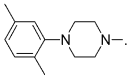
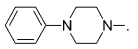
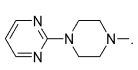
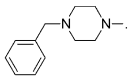
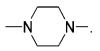
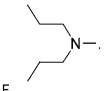
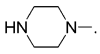
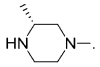
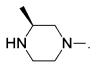
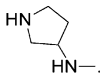
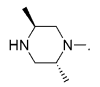
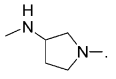
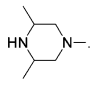
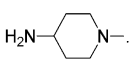
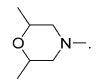
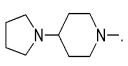


[a] Reagents and conditions: (a) 2,4-dichlorophenethylamine/EDC/THF; (b) Ph_3P/THF; (c) cyclohexyl isocyanate, 70 °C; (d) R^1R^2NH, 70 °C.

Table 1. Binding Affinities of Phenylguanidines at the Human MC5 Receptor[a]



4-25

Compound	R^1NR^2	K_i (nM)[b]	Compound	R^1NR^2	K_i (nM)[b]
4		1760±1040	**15**		144±86
5		290±130	**16**		560±90
6		305±95	**17**		1050±50
7		44±22	**18**		91±12
8		50±33	**19**		360±160
9		18±4	**20**		70±28
10		2.1±0.3	**21**		5.3±0.8
11		21±15	**22**		26±3
12		5.9±1.3	**23**		36±11
13		11±8	**24**		230±47
14		350±300	**25**		77±19

[a] Receptors expressed in HEK293 cells. [b] K_i values ($n > 3$) determined by radioligand binding assay using [^{125}I]NDP-MSH.

binding affinities for the human MC1, MC3, and MC4 receptors to determine selectivity. They were further tested for their abilities to stimulate cAMP production at 10 μM concentration in cells expressing the human MC4 and MC5 receptors for functional agonistic activity. EC_{50} values were obtained from dose-dependent curves for compounds stimulating cAMP release at >25% levels of α-MSH. Compounds with high MC5R affinity from

Table 2. Binding, Selectivity, and Functional Activity of Phenylguanidines[a]

			hMC4		hMC5	
compd	hMC1 K_i (μM)[b]	hMC3 K_i (μM)	K_i (μM)	EC_{50} (μM) (IA)[c]	K_i (μM)	IC_{50} (μM)
9	0.40	2.2	0.65	1.9 (28%)	0.018	0.46
10	(25%)	3.5	0.84	2.7 (37%)	0.0021	0.072
11	(29%)	2.8	0.58	1.8 (65%)	0.021	0.38
12	0.36	2.8	0.84	ND[d]	0.0059	0.38
13	(23%)	3.9	0.59	4.0 (76%)	0.011	0.089
21	(27%)	2.4	1.3	2.9 (45%)	0.0053	0.53[e]
22	1.9	2.5	1.5	ND[d]	0.026	0.53
23	(27%)	2.3	1.4	4.6 (36%)	0.036	0.63

[a] Data are average of at least three independent determinations. [b] Values in parentheses are percentage inhibition at 10 μM concentration. [c] Intrinsic activity: percentage of maximal cAMP production relative to α-MSH. [d] Not determined. [e] Single determination.

the binding experiments were also tested for their abilities to inhibit α-MSH-stimulated cAMP production as functional antagonist activity. These results are summarized in Table 2.

In the competitive binding assay, the nonsubstituted piperazine guanidine **9** bound to the MC5 receptor with a K_i value of 18 nM, and the binding affinity of the corresponding *N*-methylated analogue **8** was slightly lower (K_i = 50 nM). However, the 3-methylpiperazine with an *R*-configuration increased the binding by almost 10-fold (**10**, K_i = 2.1 nM), while the *S*-methyl group had little effect (**11**, K_i = 21 nM). The fact that the (3*R*)-methylpiperazine **10** bound to the MC5 receptor better than the corresponding (3*S*)-isomer **11** demonstrated a strong stereospecific effect at the 3-position of piperazine. The *trans*-2,5-dimethylpiperazine **12** (K_i = 5.9 nM) exhibited slightly higher K_i value than **10**, which may imply that the 2-methyl substituent of the piperazine had minimal effect on the receptor binding.

While the size of the 4-substituent of piperazine seemed to have a minimal impact (the *N*-benzyl analogue **7**, K_i = 44 nM, had similar binding affinity to the *N*-methyl analogue **8**, K_i = 50 nM), the basic 4-nitrogen was important for high affinity. Thus, the 4-phenyl (**5**) and 4-pyrimidinyl (**6**) compounds exhibited lower binding affinities (K_i values of **5** and **6** were 290 and 305 nM, respectively) than **7** or **8**. This was also true when the piperazine was replaced by piperidine, morpholine, or piperazinone (compounds **14**−**17**, K_i's > 140 nM). Although the piperazine moiety was important for high affinity binding, it could be replaced by an aminoalkyl group. For example, the 2-aminoisobutyl compound **21** had a K_i value of 5.3 nM, which was comparable to that of the piperazine **10**. Compounds **22** and **23** also possessed good MC5 receptor binding affinities (K_i values of 26 and 36 nM, respectively).

None of these phenylguanidines had appreciable affinity for the human MC1R except compounds **9** and **12**, which had K_i values of 400 and 360 nM, respectively. All compounds tested had weak binding affinities for the human MC3R (K_i values of 2−3 μM, Table 2). Interestingly, these compounds exhibited modest binding affinities for the human MC4 recptor and stimulated cAMP production with various intrinsic activities. For example, compound **9** had a K_i value of 650 nM and stimulated cAMP production but only to the 28% maximal level of α-MSH, with an EC_{50} value of 1.9 μM.

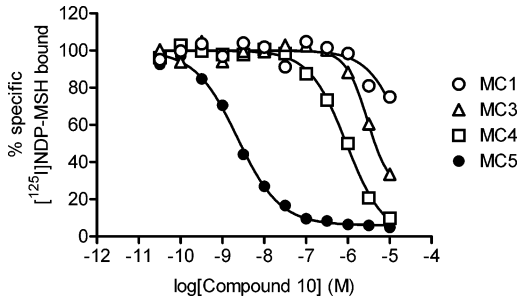


Figure 2. Inhibition curves for compound **10** in radioligand binding assays for human MC1, MC3, MC4, and MC5 receptors expressed in HEK293 cells. Inhibition of [^{125}I]NDP-MSH binding to cell membranes bearing the receptors was measured as described in ref 18.

Compound **13** bound to the MC4R with a K_i of 590 nM; however, it stimulated cAMP production to 76% of the maximal level for α-MSH, with an EC_{50} value of 4.0 μM.

None of these compounds significantly stimulated cAMP production in MC5R-containing cells (<2% of α-MSH, data not shown). We then tested their abilities to inhibit α-MSH-stimulated cAMP production as possible functional antagonistic activity on the MC5R. Several compounds displayed good potency in this assay (Table 2). For example, the IC_{50} values for **10** and **13** were 72 and 89 nM, respectively. These results demonstrated that these compounds were functional antagonists at the human MC5 receptor.

All compounds listed in Table 2 had low binding affinities at the MC1, MC3, and MC4 receptors, although most of them exhibited weak agonistic activities at the MC4 receptor. The K_i ratios of MC5R vs MC1R, MC3R, or MC4R for many compounds were over 100, making this series of compounds, especially **10**, **12**, and **21**, MC5 receptor-selective antagonists (Figure 2). Although the physiological function of the MC5 receptor remains unclear at present, this series of compounds certainly could serve as useful tools for defining and delineating the roles of this receptor.

Conclusion

In summary, we have synthesized a series of phenylguanidines as melanocortin-5 antagonists via an aza-Wittig reaction. These compounds possessed low nanomolar binding affinities and full antagonistic function at the human MC5 receptor. For example, **10** had K_i value of 2.1 nM at the MC5 receptor and >840 nM at the MC1, MC3, and MC4 receptor subtypes. The high MC5 receptor selectivity of these compounds should make them useful as potent MC5 antagonists for further exploring the physiological role of the melanocortin-5 receptor.

Experimental Section

General Procedures. ^{1}H NMR spectra were recorded on Varian spectrometer (Mercury 300 Hz) using TMS as the internal standard and $CDCl_3$ as the solvent, except where indicated. LC−MS analyses were performed on a Perkin-Elmer Sciex API-100 mass spectrometer using the electron spray ionization technique or a SpectraSystem P4000 HPLC system coupled with a Finnigan LCD/Deca mass spectrometer using the electrospray ionization technique. HRMS ESI was carried out on a Bruker 4.7T BIOAPEX FTMS mass spectrometer. All commercially available reagents were used without further purification.

***N*-[2-(2,4-Dichlorophenyl)ethyl]-4-azadophenylcarboxamide 3**. To a mixture of 2-(2,4-dichlorophenyl)ethylamine (19.9 mmol), 4-azidobenzoic acid (21.9 mmol), and EDC (21.9 mmol) in THF was added DIEA (43.8 mmol) at room temperature. The mixture was stirred overnight and the solvent was removed. The residue was diluted with ethyl acetate; washed with 1 N HCl, brine, and saturated $NaHCO_3$; dried over $MgSO_4$; and concentrated to give a solid, which was purified on silica gel with ethyl acetate/hexane (1:4) to give the title compound (68%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.06 (t, J = 6.9 Hz, 2H), 3.71 (dt, J = 7.2, 6.0 Hz, 2H), 6.17 (t, J = 6.0 Hz, 1H), 7.07 (d, J = 8.7 Hz, 2H), 7.20 (d, J = 1.5 Hz, 2H), 7.41 (s, 1H), 7.72 (d, J = 8.7 Hz, 2H) ppm; APCI MS m/z 335.2 $[MH]^+$, 307.2 $[MH^+ - N_2]$.

General Procedure for Synthesis of 4−25. To a solution of *N*-[2-(2,4-dichlorophenyl)ethyl]-4-azadophenylcarboxamide (1 equiv) in THF was added triphenylphosphine (1.2 equiv) at room temperature. After 10 min, cyclohexyl isocyanate (1.2 equiv) was added. The solution was heated at 70 °C overnight. An appropriated amine (2 equiv) was then added and the mixture was further heated at 70 °C for 2 h. THF was removed in vacuo, and the residue was dissolved in 1 N HCl and water and extracted with ether. The aqueous layer was treated with saturated $NaHCO_3$ and extracted with ethyl acetate. The combined ethyl acetate layers were dried over $MgSO_4$ and concentrated, and the residue was purified on silica gel (aqueous NH_4OH:MeOH:DCM, 1:5:94) to give the desired produce in 17−100% yield.

***N*-[2-(2,4-Dichlorophenyl)ethyl]-4-{[cyclohexylimino-(4-(2,5-dimethylphenyl)-1-piperazinyl)methyl]amino}benzamide (4):** white solid, 87 mg (90%); ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.90−1.34 (m, 4H), 1.52−1.75 (m, 4H), 1.91 (m, 2H), 2.26 (s, 3H), 2.31 (s. 3H), 2.92 (t, J = 4.2 Hz, 4H), 3.05 (t, J = 6.6 Hz, 2H), 3.18 (brs, 1H), 3.38 (t, J = 4.5 Hz, 4H), 3.79 (dt, J = 6.6, 6.3 Hz, 2H), 6.16 (t, J = 5.7 Hz, 1H), 6.82 (d, J = 7.2 Hz, 1H), 6.84 (s, 1H), 6.88 (d, J = 8.4 Hz, 2H), 7.07 (d, J = 7.2 Hz, 1H), 7.19 (dd, J = 8.0, 1.8 Hz, 1H), 7.21 (d, J = 8.0 Hz, 1H), 7.39 (d, J = 1.2 Hz, 1H), 7.64 (d, J = 8.4 Hz, 2H); LC−MS m/z 606.4 $[MH]^+$, t_R 8.31 min. Anal. ($C_{34}H_{41}Cl_2N_5O$) C, H, N.

***N*-[2-(2,4-Dichlorophenyl)ethyl]-4-{[cyclohexylimino-(4-phenyl-1-piperazinyl)methyl]amino}benzamide (5):** white solid, 79 mg (85%); ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.90−1.30 (m, 4H), 1.52−1.76 (m, 4H), 1.90 (m, 2H), 3.05 (t, J = 6.6 Hz, 2H), 3.15 (brs, 1H), 3.21 (t, J = 5.4 Hz, 4H), 3.41 (t, J = 5.4 Hz, 4H), 3.69 (dt, J = 7.2, 6.0 Hz, 2H), 6.15 (t, J = 5.4 Hz, 1H), 6.86 (d, J = 8.4 Hz, 2H), 6.89 (t, J = 7.5 Hz, 1H), 6.95 (d, J = 8.4 Hz, 2H), 7.19 (dd, J = 8.0, 1.8 Hz, 1H), 7.21 (d, J = 8.0 Hz, 1H), 7.28 (dd, J = 8.4, 7.5 Hz, 2H), 7.39 (d, J = 1.5 Hz, 1H), 7.64 (d, J = 8.4 Hz, 2H); LC−MS m/z 578.5 $[MH]^+$, t_R 7.51 min. Anal. ($C_{32}H_{37}Cl_2N_5O$) C, H, N.

***N*-[2-(2,4-Dichlorophenyl)ethyl]-4-{[cyclohexylimino-(4-(1-pyrimidinyl)-1-piperazinyl)methyl]amino}benzamide 4-(1-pyrimidine)piperazine (6):** white solid, 90 mg (97%); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.00−1.30 (m, 4H), 1.52−1.98 (m, 6H), 3.05 (t, J = 6.9 Hz, 2H), 3.27 (brs, 1H), 3.35 (t, J = 4.5 Hz, 4H), 3.69 (dt, J = 7.2, 6.0 Hz, 2H), 3.86 (t, J = 4.8 Hz, 4H), 6.34 (brs, 1H), 6.53 (t, J = 5.1 Hz, 1H), 6.92 (d, J = 7.5 Hz, 2H), 7.18 (dd, J = 8.0, 1.8 Hz, 1H), 7.21 (d, J = 8.0 Hz, 1H), 7.39 (d, J = 1.5 Hz, 1H), 7.67 (d, J = 8.4 Hz, 2H), 8.31 (d, J = 4.8 Hz, 2H); LC−MS m/z 580.5 $[MH]^+$, t_R 6.66 min. Anal. ($C_{30}H_{35}Cl_2N_7O\cdot{}^1/_3H_2O$) C, H, N.

***N*-[2-(2,4-Dichlorophenyl)ethyl]-4-{[cyclohexylimino-(4-benzyl-1-piperazinyl)methyl]amino}benzamide (7):** white solid, 55 mg (58%); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.04−1.34 (m, 4H), 1.50−1.94 (m, 6H), 2.45(brs, 4H), 3.06 (t, J = 6.9 Hz, 2H), 3.29 (brs, 5H), 3.51 (s, 2H), 3.69 (dt, J = 6.9, 6.6 Hz, 2H), 6.46 (brs, 1H), 6.95 (d, J = 6.3 Hz, 2H), 7.17 (dd, J = 8.0, 1.8 Hz, 1H), 7.21 (d, J = 8.0 Hz, 1H), 7.28 (m, 5H), 7.38 (d, J = 1.5 Hz, 1H), 7.68 (d, J = 8.4 Hz, 2H); LC−MS m/z 592.3 $[MH]^+$, t_R 5.75 min. Anal. ($C_{33}H_{39}Cl_2N_5O\cdot H_2O$) C, H, N.

***N*-[2-(2,4-Dichlorophenyl)ethyl]-4-{[cyclohexylimino-(4-methyl-1-piperazinyl)methyl]amino}benzamide 4-methylpiperazine (8):** white solid, 27 mg (35%); ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.90−1.30 (m, 4H), 1.50−1.95 (m, 6H), 2.30 (s, 3H), 2.42 (m, 4H), 3.10 (t, J = 7.0 Hz, 2H), 3.20 (brs, 1H), 3.29 (m, 4H), 3.69 (dt, J = 7.2, 6.0 Hz, 2H), 6.40 (brs, 1H), 6.91 (d, J = 8.7 Hz, 2H), 7.19 (dd, J = 8.0, 1.8 Hz, 1H), 7.21 (d, J = 8.0 Hz, 1H), 7.39 (d, J = 1.5 Hz, 1H), 7.67 (d, J = 8.7 Hz, 2H); LC−MS m/z 516.1 $[MH]^+$, t_R 5.18 min; HRMS (MALDI−FTMS) calcd for $C_{27}H_{35}Cl_2N_5O$ 516.2291, found 516.2287.

***N*-[2-(2,4-Dichlorophenyl)ethyl]-4-{[cyclohexylimino-(1-piperazinyl)methyl]amino}benzamide (9):** white solid, 38 mg (47%); ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.90−1.30 (m, 4H), 1.52−1.94 (m, 6H), 2.89 (t, J = 5.4 Hz, 4H), 3.04 (t, J = 7.2 Hz, 2H), 3.11 (brs, 1H), 3.20 (t, J = 5.4 Hz, 4H), 3.68 (dt, J = 6.9, 6.3 Hz, 2H), 6.16 (t, J = 6.0 Hz, 1H), 6.84 (d, J = 9.0 Hz, 2H), 7.18 (dd, J = 8.0, 1.8 Hz, 1H), 7.20 (d, J = 8.0 Hz, 1H), 7.39 (d, J = 1.5 Hz, 1H), 7.63 (d, J = 8.4 Hz, 2H); LC−MS m/z 502.3 $[MH]^+$, t_R 5.26 min. Anal. ($C_{26}H_{33}Cl_2N_5O$) C, H, N.

***N*-[2-(2,4-Dichlorophenyl)ethyl]-4-{[cyclohexylimino-(3*R*-methyl-1-piperazinyl)methyl]amino}benzamide (10):** white solid, 18 mg (22%); ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.94−1.30 (m, 4H), 1.05 (d, J = 6.6 Hz, 3H), 1.50−1.94 (m, 6H), 2.47 (dd, J = 10.2, 12.9 Hz, 1H), 2.72−3.01 (m, 5H), 3.05 (t, J = 7.5 Hz, 2H), 3.10 (brs, 1H), 3.53 (d, J = 10.8 Hz, 2H), 3.68 (dt, J = 6.9, 6.3 Hz, 2H), 6.16 (t, J = 5.7 Hz, 1H), 6.85 (d, J = 8.4 Hz, 2H), 7.18 (dd, J = 8.0, 1.8 Hz, 1H), 7.22 (d, J = 8.0 Hz, 1H), 7.39 (d, J = 1.2 Hz, 1H), 7.63 (d, J = 8.4 Hz, 2H); LC−MS m/z 516.4 $[MH]^+$, t_R 5.32 min; HRMS (MALDI−FTMS) calcd for $C_{27}H_{35}Cl_2N_5O$: 516.2291, found 516.2287.

***N*-[2-(2,4-Dichlorophenyl)ethyl]-4-{[cyclohexylimino-(3*S*-methyl-1-piperazinyl)methyl]amino}benzamide (11):** white solid, 59 mg (72%); ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.88−1.30 (m, 4H), 1.05 (d, J = 6.3 Hz, 3H), 1.50−1.92 (m, 6H), 2.47 (dd, J = 10.2, 12.9 Hz, 1H), 2.70−3.00 (m, 5H), 3.05 (t, J = 6.9 Hz, 2H), 3.12 (brs, 1H), 3.53 (d, J = 11.4 Hz, 2H), 3.68 (dt, J = 6.9, 6.3 Hz, 2H), 6.14 (t, J = 6.3 Hz, 1H), 6.85 (d, J = 8.4 Hz, 2H), 7.18 (dd, J = 8.0, 1.8 Hz, 1H), 7.21 (d, J = 8.0 Hz, 1H), 7.39 (d, J = 1.8 Hz, 1H), 7.63 (d, J = 8.4 Hz, 2H); LC−MS m/z 516.3 $[MH]^+$, t_R 5.32 min. Anal. ($C_{27}H_{35}Cl_2N_5O\cdot 0.5H_2O$) C, H, N.

***N*-[2-(2,4-Dichlorophenyl)ethyl]-4-{[cyclohexylimino-(2,5-*trans*-dimethyl-1-piperazinyl)methyl]amino}benzamide (12):** white solid, 39 mg (46%); ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.90−1.34 (m, 4H), 1.08 (d, J = 6.6 Hz, 3H), 1.18 (d, J = 6.0 Hz, 3H), 1.52−1.96 (m, 6H), 2.55 (m, 2H), 2.84−3.38 (m, 8H), 3.63−3.73 (dt, J = 6.6, 6.6 Hz, 2H), 6.16 (t, J = 5.7 Hz, 1H), 6.86 (d, J = 8.4 Hz, 2H), 7.18 (dd, J = 8.0, 1.8 Hz, 1H), 7.21 (d, J = 8.0 Hz, 1H), 7.39 (d, J = 1.8 Hz, 1H), 7.64 (d, J = 8.4 Hz, 2H); LC−MS m/z 530.3 $[MH]^+$, t_R 5.43 min. Anal. ($C_{28}H_{37}Cl_2N_5O\cdot 0.5H_2O$) C, H, N.

***N*-[2-(2,4-Dichlorophenyl)ethyl]-4-{[cyclohexylimino-(3,5-dimethyl-1-piperazinyl)methyl]amino}benzamide (13):** white solid, 82 mg (97%); ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.89−1.29 (m, 4H), 1.06 (d, J = 6.0 Hz, 6H), 1.50−1.92 (m, 6H), 2.38 (dd, J = 12.3, 10.5 Hz, 2H), 2.90 (m, 2H), 3.04 (t, J = 7.2 Hz, 2H), 3.12 (brs, 1H), 3.42 (brs, 1H), 3.54 (d, J = 12.9 Hz, 2H), 3.68 (dt, J = 6.9, 6.3 Hz, 2H), 6.16 (t, J = 6.0 Hz, 1H), 6.82 (d, J = 8.4 Hz, 2H), 7.18 (dd, J = 8.0, 1.8 Hz, 1H), 7.21 (d, J = 8.0 Hz, 1H), 7.38 (d, J = 1.2 Hz, 1H), 7.62 (d, J = 8.4 Hz, 2H); LC−MS m/z 530.5 $[MH]^+$, t_R 5.36 min. Anal. ($C_{28}H_{37}Cl_2N_5O$) C, H, N.

***N*-[2-(2,4-Dichlorophenyl)ethyl]-4-{[cyclohexylimino-(3,5-dimethyl-1-morpholinyl)methyl]amino}benzamide (14):** white solid, 71 mg (89%); ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.88−1.30 (m, 10H), 1.50−1.96 (m, 6H), 2.40−4.10 (m, 11H), 6.16 (brs. 1H), 6.82 (d, J = 8.7 Hz, 2H), 7.18 (dd, J = 8.0, 1.8 Hz, 1H), 7.21 (d, J = 8.0 Hz, 1H),7.39 (d, J = 1.8 Hz, 1H), 7.63 (d, J = 7.5 Hz, 2H); LC−MS m/z 531.1 $[MH]^+$, t_R 6.77 min; HRMS (MALDI−FTMS) calcd for $C_{28}H_{36}Cl_2N_4O_2$ 531.2288, found 531.2263.

***N*-[2-(2,4-Dichlorophenyl)ethyl]-4-{[cyclohexylimino-(3,5-dimethyl-1-piperazinyl)methyl]amino}benzamide (15):** white solid, 27 mg (34%); ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.61−1.60 (m, 12H), 1.50−1.96 (m, 6H), 2.20−3.80 (m, 11H), 6.13 (t, J = 6.0 Hz, 1H), 6.84 (m, 2H), 7.18 (dd, J = 8.0, 1.8 Hz, 1H), 7.21 (d, J = 8.0 Hz, 1H), 7.39 (d, J = 1.8 Hz, 1H),

7.62 (d, J = 8.4 Hz, 2H); LC−MS m/z 529.1 $[MH]^+$, t_R 7.56 min; HRMS (MALDI−FTMS) calcd for $C_{29}H_{38}Cl_2N_4O$ 529.2495, found 529.2479.

***N*-[2-(2,4-Dichlorophenyl)ethyl]-4-{[cyclohexylimino-(3-oxo-1-piperazinyl)methyl]amino}benzamide (16):** white solid, 73 mg (88%); ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.90−1.30 (m, 4H), 1.50−1.90 (m, 6H), 3.00 (brs, 1H), 3.05 (t, J = 6.9 Hz, 2H), 3.45−3.57 (m, 4H), 3.70 (dt, J = 6.3, 4.8 Hz, 2H), 3.96 (s, 2H), 6.20 (m, 2H), 6.83 (d, J = 8.4 Hz, 2H), 7.18 (dd, J = 8.0, 1.8 Hz, 1H), 7.21 (d, J = 8.0 Hz, 1H), 7.39 (d, J = 0.9 Hz, 1H), 7.65 (d, J = 8.4 Hz, 2H); LC−MS m/z 516.4 $[MH]^+$, t_R 5.91 min. Anal. ($C_{26}H_{31}Cl_2N_5O_2 \cdot {}^1/_3H_2O$) C, H, N.

***N*-[2-(2,4-Dichlorophenyl)ethyl]-4-{[cyclohexylimino-(2-methyl-3-oxo-1-piperazinyl)methyl]amino}benzamide (17):** white solid, 92 mg (100%); ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.84−1.26 (m, 4H), 1.52 (d, J = 7.5 Hz, 3H), 1.50−1.92 (m, 6H), 3.00 (m, 1H), 3.05 (t, J = 6.9 Hz, 2H), 3.23−3.36 (m, 3H), 3.68 (m, 2H), 3.78 (m, 1H), 4.34 (q, J = 7.5 Hz, 1H), 6.20 (m, 2H), 6.81 (d, J = 8.4 Hz, 2H), 7.18 (dd, J = 8.0, 1.8 Hz, 1H), 7.21 (d, J = 8.0 Hz, 1H), 7.39 (d, J = 1.2 Hz, 1H), 7.64 (d, J = 8.4 Hz, 2H); LC−MS m/z 530.5 $[MH]^+$, t_R 5.97 min. Anal. ($C_{27}H_{33}Cl_2N_5O$) C, H, N.

***N*-[2-(2,4-Dichlorophenyl)ethyl]-4-{[cyclohexylimino-(*N*-cyclohexyl-*N*-methylamino)methyl]amino}benzamide (18):** white solid, 78 mg (92%); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.0−1.36 (m, 8H), 1.40−1.96 (m, 12H), 2.71 (s, 3H), 3.06 (t, J = 6.9 Hz, 2H), 3.29 (brs, 1H), 3.57 (brs, 1H), 3.69 (dt, J = 7.2, 6.0 Hz, 2H), 6.48 (brs, 1H), 6.92 (brs, 2H), 7.16 (dd, J = 8.0, 1.8 Hz, 1H), 7.22 (d, J = 8.0 Hz, 1H), 7.37 (d, J = 1.8 Hz, 1H), 7.68 (d, J = 6.9 Hz, 2H); LC−MS m/z 529.3 $[MH]^+$, t_R 7.52 min. Anal. ($C_{29}H_{38}Cl_2N_4O \cdot H_2O$) C, H, N.

***N*-[2-(2,4-Dichlorophenyl)ethyl]-4-{[cyclohexylimino-(dipropylamino)methyl]amino}benzamide (19):** white solid, 62 mg (75%); ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.90 (t, J = 6.3 Hz, 6H), 0.93−1.26 (m, 4H), 1.50−1.68 (m, 8H) 1.84 (m, 2H), 3.05 (t, J = 6.6 Hz, 2H), 3.13 (t, J = 7.5 Hz, 4H), 3.52 (brs, 1H), 3.69 (dt, J = 7.0, 6.3 Hz, 2H), 6.11 (t, J = 5.7 Hz, 1H), 6.80 (d, J = 8.4 Hz, 2H), 7.18 (dd, J = 8.0, 1.8 Hz, 1H), 7.21 (d, J = 8.0 Hz, 1H), 7.39 (d, J = 1.5 Hz, 1H), 7.69 (d, J = 8.4 Hz, 2H); LC−MS m/z 517.3 $[MH]^+$, t_R 7.38 min. Anal. ($C_{28}H_{38}Cl_2N_4O$) C, H, N.

***N*-[2-(2,4-Dichlorophenyl)ethyl]-4-{[cyclohexylimino-(3,5-difluorobenzylamino)methyl]amino}benzamide (20):** white solid, 18 mg (20%); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.0−1.40 (m, 4H), 1.52−2.0 (m, 6H), 3.04 (t, J = 7.2 Hz, 2H), 3.28 (brs, 1H), 3.68 (dt, J = 6.9, 6.3 Hz, 2H), 3.74 (brs, 1H), 4.45 (s, 2H), 6.13 (t, J = 6.0 Hz, 1H), 6.72 (m, 1H), 6.89 (m, 4H), 7.18 (dd, J = 8.0, 1.8 Hz, 1H), 7.21 (d, J = 8.0 Hz, 1H), 7.39 (d, J = 1.2 Hz, 1H), 7.63 (d, J = 8.5 Hz, 2H); LC−MS m/z 559.3 $[MH]^+$, t_R 7.55 min; HRMS (MALDI−FTMS) calcd for $C_{29}H_{30}Cl_2F_2N_4O$ 559.1837, found 559.1822.

***N*-[2-(2,4-Dichlorophenyl)ethyl]-4-{[cyclohexylimino-(2-amino-2-methylpropylamino)methyl]amino}benzamide (21):** white solid, 37 mg (46%); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.06−1.42 (m, 10H), 1.54−1.74 (m, 4H), 1.92−2.03 (m, 2H), 3.0−3.09 (m, 4H), 3.43 (brs, 1H), 3.68 (dt, J = 6.9, 6.3 Hz, 2H), 6.12 (t, J = 5.7 Hz, 1H), 6.90 (d, J = 8.4 Hz, 2H), 7.18 (dd, J = 8.0, 1.8 Hz, 1H), 7.21 (d, J = 8.0 Hz, 1H),7.39 (d, J = 1.8 Hz, 1H), 7.62 (d, J = 8.4 Hz, 2H); LC−MS m/z 504.4 $[MH]^+$, t_R 5.35 min. Anal. ($C_{26}H_{35}Cl_2N_5O \cdot {}^1/_3H_2O$) C, H, N.

***N*-[2-(2,4-Dichlorophenyl)ethyl]-4-{[cyclohexylimino-(pyrrolidin-3-ylamine)methyl]amino}benzamide (22):** white solid, 78 mg (97%); ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.98−1.30 (m, 4H), 1.50−1.72 (m, 4H), 1.90 (m, 2H), 2.06 (m, 1H), 3.00 (m, 1H), 3.04 (t, J = 6.9 Hz, 2H), 3.29 (brs, 1H), 3.35 (m, 1H), 3.44−3.63 (m, 3H), 3.69 (dt, J = 6.6, 6.3 Hz, 2H), 3.87 (brs, 1H), 6.14 (t, J = 6.0 Hz, 1H), 6.82 (d, J = 9.0 Hz, 2H), 7.18 (dd, J = 8.0, 1.8 Hz, 1H), 7.21 (d, J = 8.0 Hz, 1H), 7.39 (d, J = 1.8 Hz, 1H), 7.58 (d, J = 9.0 Hz, 2H); LC−MS m/z 502.3 $[MH]^+$, t_R 5.32 min. Anal. ($C_{26}H_{33}Cl_2N_5O \cdot {}^1/_3H_2O$) C, H, N.

***N*-[2-(2,4-Dichlorophenyl)ethyl]-4-{[cyclohexylimino-(3-methylamino-1-pyrrolidinyl)methyl]amino}benzamide (23):** white solid, 14 mg (17%); ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.96−1.30 (m, 4H), 1.50−1.80 (m, 4H), 1.90 (m, 2H), 2.05 (m, 1H), 2.41 (s, 3H), 3.04 (t, J = 6.9 Hz, 2H), 3.08 (m, 1H), 3.18−3.54 (m, 5H), 3.67 (dt, J = 6.9, 6.3 Hz, 2H), 3.91 (brs, 1H), 6.16 (t, J = 5.7 Hz, 1H), 6.83 (d, J = 8.7 Hz, 2H), 7.18 (dd, J = 8.0, 1.8 Hz, 1H), 7.21 (d, J = 8.0 Hz, 1H), 7.38 (d, J = 1.8 Hz, 1H), 7.59 (d, J = 8.4 Hz, 2H); LC−MS m/z 516.3 $[MH]^+$, t_R 5.33 min; HRMS (MALDI−FTMS) calcd for $C_{27}H_{35}Cl_2N_5O$ 516.2291, found 516.2305.

***N*-[2-(2,4-Dichlorophenyl)ethyl]-4-{[cyclohexylimino-(4-amino-1-piperidinyl)methyl]amino}benzamide (24):** white solid, 21 mg (25%); ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.92−1.94 (m, 14H), 2.74−2.90 (m, 3H), 3.04 (t, J = 7.2 Hz, 2H), 3.11 (brs, 1H), 3.59−3.73 (m, 4H), 6.18 (t, J = 5.7 Hz, 1H), 6.84 (d, J = 8.1 Hz, 2H), 7.18 (dd, J = 8.0, 1.8 Hz, 1H), 7.21 (d, J = 8.0 Hz, 1H), 7.39 (d, J = 1.8 Hz, 1H), 7.63 (d, J = 8.4 Hz, 2H); LC−MS m/z 516.3 $[MH]^+$, t_R 5.36 min. Anal. ($C_{27}H_{35}Cl_2N_5O \cdot {}^1/_3H_2O$) C, H, N.

***N*-[2-(2,4-Dichlorophenyl)ethyl]-4-{[cyclohexylimino-(4-(1-pyrrolidinyl)-1-piperidinyl)methyl]amino}benzamide (25):** white solid, 85 mg (93%); ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.90−1.30 (m, 4H), 1.42−2.20 (m, 14H), 2.59 (brs, 4 H), 2.77 (t, J = 12.3 Hz, 2H), 3.04 (t, J = 7.2 Hz, 2H), 3.10 (brs, 1H), 3.46 (brs, 1H), 3.60−3.74 (m, 4H), 6.12 (t, J = 5.7 Hz, 1H), 6.83 (d, J = 8.1 Hz, 2H), 7.18 (dd, J = 8.0, 1.8 Hz, 1H), 7.21 (d, J = 8.0 Hz, 1H), 7.39 (d, J = 1.5 Hz, 1H), 7.62 (d, J = 8.4 Hz, 2H); LC−MS m/z 570.5 $[MH]^+$, t_R 5.45 min. Anal. ($C_{31}H_{41}Cl_2N_5O \cdot {}^1/_3H_2O$) C, H, N.

References

(1) *The Melanocortin Receptors*; Cone, R. D., Ed.; Humana Press Inc.: Totwa, NJ, 2000.

(2) Chhajlani, V.; Wikberg, J. E. Molecular cloning and expression of the human melanocyte stimulating hormone receptor cDNA. *FEBS Lett.* **1992**, *309*, 417−420.

(3) (a) Hadley, M. E.; Heward, C. B.; Hruby, V. J.; Sawyer, T. K.; Yang, Y. C. Biological actions of melanocyte-stimulating hormone. *Ciba Found. Symp.* **1981**, *81*, 244−262. (b) Levine, N.; Lemus-Wilson, A.; Wood, S. H.; Abdel Malek, Z. A.; Al-Obeidi, F.; Hruby, V. J.; Hadley, M. E. Stimulation of follicular melanogenesis in the mouse by topical and injected melanotropins. *J. Invest. Dermatol.* **1987**, *89*, 269−273.

(4) (a) Desarnaud, F.; Labbe, O.; Eggerickx, D.; Vassart, G.; Parmentier, M.; Molecular cloning, functional expression and pharmacological characterization of a mouse melanocortin receptor gene. *Biochem. J.* **1994**, *299 Pt. 2*, 367−373. (b) Penhoat, A.; Naville, D.; El Mourabit, H.; Buronfosse, A.; Durand, E.; Begeot, M. Functional expression of the human ACTH receptor gene. *Endocr. Res.* **2000**, *26*, 549−557.

(5) (a) Gantz, I.; Konda, Y.; Tashiro, T.; Shimoto, Y.; Miwa, H.; Munzert, G.; Watson, S. J.; DelValle, J.; Yamada, T. Molecular cloning of a novel melanocortin receptor. *J. Biol. Chem.* **1993**, *268*, 8246−8250. (b) Roselli-Rehfuss, L.; Mountjoy, K. G.; Robbins, L. S.; Mortrud, M. T.; Low, M. J.; Tatro, J. B.; Entwistle, M. L.; Simerly, R. B.; Cone, R. D. Identification of a receptor for gamma melanotropin and other proopiomelanocortin peptides in the hypothalamus and limbic system. *Proc. Natl. Acad. Sci. U.S.A.* **1993**, *90*, 8856−8860.

(6) Gantz, I.; Miwa, H.; Konda, Y.; Shimoto, Y.; Tashiro, T.; Watson, S. J.; DelValle, J.; Yamada, T. Molecular cloning, expression, and gene localization of a fourth melanocortin receptor. *J. Biol. Chem.* **1993**, *268*, 15174−15179.

(7) Labbe, O.; Desarnaud, F.; Eggerickx, D.; Vassart, G.; Parmentier, M. Molecular cloning of a mouse melanocortin 5 receptor gene widely expressed in peripheral tissues. *Biochemistry* **1994**, *33*, 4543−4549.

(8) (a) Butler, A. A.; Kesterson, R. A.; Khong, K.; Cullen, M. J.; Pelleymounter, M. A.; Dekoning, J.; Baetscher, M.; Cone, R. D. A unique metabolic syndrome causes obesity in the melanocortin-3 receptor-deficient mouse. *Endocrinology* **2000**, *141*, 3518−3521. (b) 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.; Van der Ploeg, L. H. Inactivation of the mouse melanocortin-3 receptor results in increased fat mass and reduced lean body mass. *Nat. Genet.* **2000**, *26*, 97−102.

(9) (a) 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. (b) Heisler, L. K.; Cowley, M. A.; Tecott, L. H.; Fan, W.; Low, M. J.; Smart, J. L.; Rubinstein, M.; Tatro, J. B.; Marcus, J. M.; Holstege, H.; Lee,

C. E.; Cone, R. D.; Elmquist, J. K. Activation of central melanocortin pathways by fenfluramine. *Science* **2002**, *297*, 609−611. (c) Van der Ploeg, L. H.; 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. P.; Palyha, O. C.; Pong, S. S.; MacNeil, T.; Rosenblum, C.; Vongs, A.; Tang, R.; Yu, H.; Sailer, A. W.; Fong, T. M.; Huang, C.; Tota, M. R.; 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*, 11381−11386.

(10) (a) Wikberg, J. E. S.; Muceniece, R.; Mandrika, I.; Prusis, P.; Post, C.; Skottner, A. New aspects on the melanocortins and their receptors *Pharmacol. Res.* **2000**, *42*, 393−420. (b) Wikberg, J. E. S. Melanocortin receptors: Perspectives for novel drugs *Eur. J. Pharmacol.* **1999**, *375*, 295−310.

(11) (a) Chen, W.; Kelly, M. A.; Opitz-Araya, X.; Thomas, R. E.; Low, M. J.; Cone, R. D. Exocrine gland dysfunction in MC5−R-deficient mice: Evidence for coordinated regulation of exocrine gland function by melanocortin peptides. *Cell* **1997**, *91*, 789−798. (b) van der Kraan, M.; Adan, R. A.; Entwistle, M. L.; Gispen, W. H.; Burbach, J. P.; Tatro, J. B. Expression of melanocortin-5 receptor in secretory epithelia supports a functional role in exocrine and endocrine glands. *Endocrinology* **1998**, *139*, 2348−2355.

(12) Caldwell, H. K.; Lepri, J. J. Disruption of the fifth melanocortin receptor alters the urinary excretion of aggression-modifying pheromones in male house mice. *Chem. Senses* **2002**, *27*, 91−94.

(13) Morgan, C.; Thomas, R. E.; Cone, R. D. Melanocortin-5 receptor deficiency promotes defensive behavior in male mice. *Hormones Behav.* **2004**, *45*, 58−63.

(14) Goodfellow, V.; Saunders, J. The melanocortin system and its role in obesity and cachexia. *Curr. Topic Med. Chem.* **2003**, *3*, 855−883.

(15) Sebhat, I. K.; Martin, W. J.; Ye, Z.; Barakat, K.; Mosley, R. T.; Johnston, D. B. R.; Bakshi, R.; Palucki, B.; Weigberg, 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. T.; Patchet, A. A.; Nargund, R. P. 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.

(16) Herpin, T. F.; Yu, G.; Carlson, K. E.; Morton, G. C.; Wu, X.; Kang, L.; Tuerdi, H.; Khanna, A.; Tokarski, J. S.; Lawrence, R. M.; Macor, J. E. Discovery of Tyrosine-Based Potent and Selctive Melanocortin-1 Receptor Small-Molecule Agonists with Antiinflammatory Properties. *J. Med. Chem.* **2003**, *46*, 1123−1126.

(17) This series of compounds have been described as melanocrtin-4 ligands, see: Renhowe, P. A.; Chu, D.; Boyce, R.; Ni, Z.-J.; Duhl, D.; Tozzo, E. WO 0218327, 2002.

(18) Nickolls, S. A.; Cismowski, M. I.; Wang, X.; Wolff, M.; Conlon, P. J.; Maki, R. A. Molecular determinants of melanocortin 4 receptor ligand binding and MC4/MC3 receptor selectivity. *J. Pharmacol. Exp. Ther.* **2003**, *304*, 1217−1227.

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