Available online at www.sciencedirect.com
SCIENCE DIRECT®
Bioorganic & Medicinal Chemistry Letters
Bioorganic & Medicinal Chemistry Letters 15 (2005) 3434–3438

Structure–activity relationship of a series of cyclohexylpiperidines bearing an amide side chain as antagonists of the human melanocortin-4 receptor

Joseph A. Tran,[a] Joseph Pontillo,[a] Melissa Arellano,[a] Beth A. Fleck,[b] Fabio C. Tucci,[a] Dragan Marinkovic,[a] Caroline W. Chen,[a] John Saunders,[a] Alan C. Foster[c] and Chen Chen[a,*]

[a]*Department of Medicinal Chemistry, Neurocrine Biosciences, Inc., 12790 El Camino Real, San Diego, CA 92130, USA*
[b]*Department of Pharmacology, Neurocrine Biosciences, Inc., 12790 El Camino Real, San Diego, CA 92130, USA*
[c]*Department of Neuroscience, Neurocrine Biosciences, Inc., 12790 El Camino Real, San Diego, CA 92130, USA*

Received 25 March 2005; revised 29 April 2005; accepted 3 May 2005
Available online 9 June 2005

Abstract—A series of cyclohexylpiperazines was synthesized as potent and selective antagonists of the human MC4 receptor. Compound **14t** displayed binding affinity (K_i) of 4.2 and 1100 nM at MC4R and MC3R, respectively.

Among the five melanocortin receptors (MC1–5R), MC3R and MC4R are centrally expressed and are believed to play important roles in mediating feeding behavior, metabolism, and energy homeostasis.[1] While it has been well documented that peptide MC3R/MC4R antagonists such as SHU9119 and AgRP have a profound effect in inducing feeding in animals,[2] recent discovery of selective peptide MC4R antagonists such as MBP10 and HS131 has made it possible to define the biological role of the melanocortin-4 receptor.[3] More importantly, additional studies have showed that MC4R antagonists can protect weight loss in tumor-bearing mice.[4] Recent studies have also provided evidences that MC4R may have a role in anxiety[5] and pain.[6] Thus, a potent and selective MC4R antagonist could be useful in treatment of human diseases such as cachexia, anxiety, and neuropathic pain.

In addition to agonists such as **1**,[7] several small molecule antagonists for the human melanocortin-4 receptor (*h*MC4R) have been discovered. MCL0129 (**2**), which inhibits NDP-MSH binding to *h*MC4R with a K_i value of 7.9 nM, dose-dependently blocks α-MSH-stimulated cAMP release.[8] This compound also exhibits anxiolytic-like and antidepressant-like activity in several animal models. Another antagonist, compound **3** (K_i = 160 nM), has been demonstrated to be effective in protecting tumor-induced weight loss in mice following peripheral administration[9] (Fig. 1).

We have previously reported a series of piperazinebenzylamine derivatives as MC4R-selective ligands which, interestingly, possesses both agonistic and antagonistic activities with only subtly different chemical structures.[10] For example, molecule **4** exhibits a K_i of 1.8 nM in competitive binding and a pA_2 value of 7.9 in inhibition of MSH-stimulated cAMP release.[11,12] Here, we report the synthesis and structure–activity relationship of a series of cyclohexylpiperazines bearing an amine or amide side chain as potent antagonists of the human melanocortin-4 receptor.

The synthesis of the target compounds started from cyclohexanone **5**, which was converted to aminonitrile **6** using a Strecker reaction (*N*-Bn-piperizine/$Na_2S_2O_5$/KCN) in quantitative yield (Scheme 1). Reduction of the cyano group in **6** ($LiAlH_4$/Et_2O) at room temperature, followed by protection of the resulting primary

Keywords: Melanocortin-4 receptor; Cyclohexylpiperazine; Antagonist.
* Corresponding author. Tel.: +1 8586177634; fax: +1 8586177967; e-mail: cchen@neurocrine.com

0960-894X/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2005.05.017

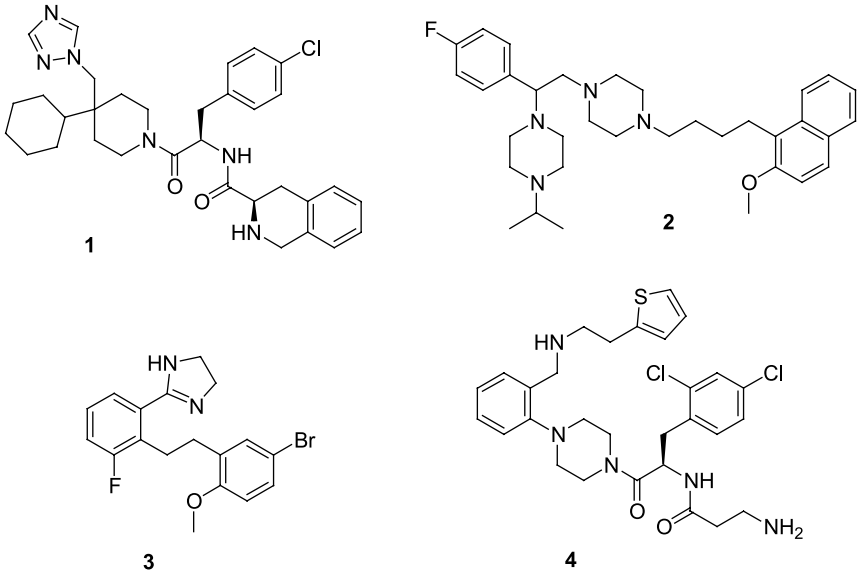
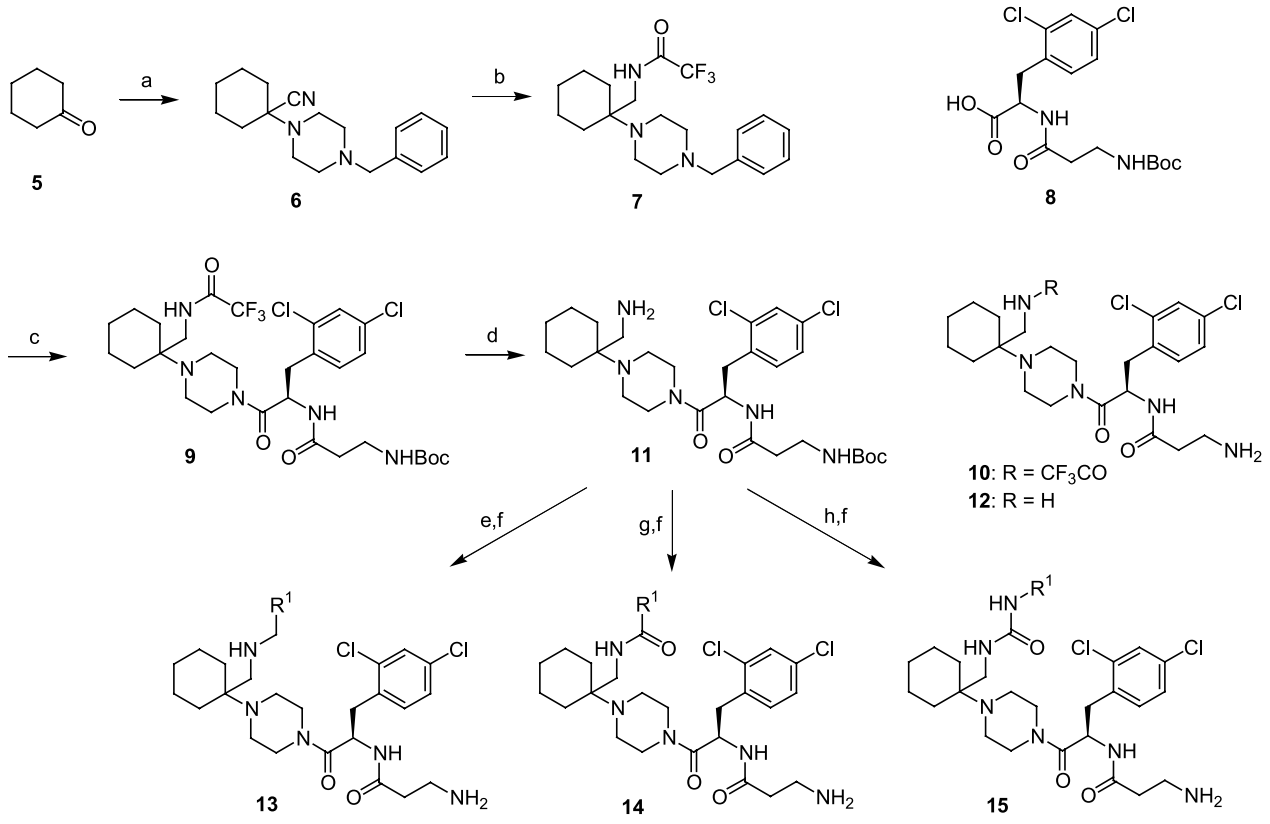


Figure 1. Some small molecule MC4R ligands.



Scheme 1. Reagents and conditions: (a) 4-Bn-piperazine/$Na_2S_2O_5$/KCN/H_2O/rt, 16 h, quant.; (b) $LiAlH_4/Et_2O$/rt, 0.5 h, 94%, then TFAA/Et_3N/CH_2Cl_2/0 °C to rt, quant.; (c) NH_4COOH/Pd-C/MeOH/65 °C, 2 h, 86%; then *N*-(*N*-Boc-β-Ala)-(*R*)-(2,4-Cl)Phe-OH (**8**)/HBTU/EtN(iPr)$_2$/DMF/rt, 8 h, 54%; (d) K_2CO_3/MeOH-H_2O/65 °C, 8 h, 86%; (e) R^1CHO/$NaBH_4$/MeOH/rt, 1–8 h; (f) TFA/CH_2Cl_2/rt, 0.5 h; then HPLC purification; (g) R^1COCl/Et_3N/THF/rt, 8 h; (h) R^1NCO/Et_3N/THF/rt, 8 h.

amine with trifluoroacetic anhydride, afforded the protected triamine **7** in about 94% yield. Debenzylation of **7** under catalytic conditions ($HCOONH_4$/Pd-C), followed by a peptide coupling reaction of the free amine with *N*-(*N*-Boc-β-Ala)-(2,4-Cl)Phe-OH (**8**) under standard conditions (HBTU/DIEA/DMF), gave the amide **9** in 46% yield. The key intermediate **11** was obtained in 86% yield from deprotection of the trifluoroacetyl group in **9** with potassium carbonate in methanol at 65 °C. Deprotection of the Boc-group in **9** and **11** with TFA/CH_2Cl_2 afforded the amines **10** and **12**, respectively. Reductive alkylation of **11** with various aldehydes

($NaBH_4$/MeOH) and deprotection provided the secondary amines **13a**–**o** as the desired products. Acylation of **11** with a set of acyl chlorides (Et_3N/THF) gave the amides **14a**–**v**. And finally, the reaction of **11** with aryl isocyanates afforded the ureas **15a**–**f**.[13] In each series, the final deprotection was achieved in almost quantitative yield by exposure to TFA in dichloromethane. All final products were purified with a preparative HPLC instrument, as previously described, and the purity of these compounds was greater than 95%.[11]

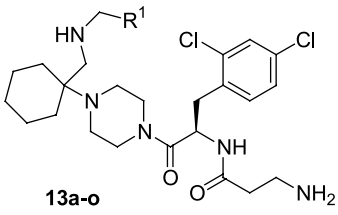
Competition binding experiments were performed using HEK293 cells stably transfected with the human melanocortin receptors, as previously described, using [^{125}I]-NDP-MSH as a radiolabeled ligand.[14] The functional antagonist activity of selected compounds was measured for their ability to inhibit α-MSH-stimulated cAMP production in the same cell lines.

Previously we have shown that the basic benzylamine functionality of **4** and its analogues is important for binding of this series of compounds to MC4R.[11] On the basis of our receptor model, the phenyl moiety of the benzylamine is seen to interact with several lipophilic residues such as Phe284, Leu288, Ile289, Ile291, and Met292 of transmembrane helix 7 (TM-7), while the basic nitrogen may pair up with Asp122 of TM-3. Since most of these amino acid residues of the top TM-7 projecting into the putative binding pocket are non-aromatic, replacing the phenyl ring of **4** with an aliphatic cyclohexyl moiety could retain or improve the interaction between the ligand and the receptor. A series of cyclohexylpiperazines bearing an alkylamine side chain **13** was, therefore, synthesized and tested in the MC4R binding assay.

Incorporation of a small lipophilic alkyl group (compound **13a**–**c**) to the nitrogen of the primary amine **12** (K_i = 290 nM) had little effect on binding affinity, while a phenyl group seemed to have an impact (**13d**, K_i = 60 nM, Table 1). A study of the linker (**13d**–**f**) between the nitrogen and the phenyl ring revealed that the ethylene connection provided the optimal binding (**13e**, K_i = 11 nM), although the shorter methylene linkage maintained comparable activity (**13f**, K_i = 18 nM). A brief survey on the substitution of this phenyl ring with different functionality (**13g**–**l**) showed no obvious improvement. A heteroaromatic ring such as thiophene and pyridine exhibited a similar effect to the phenyl moiety in binding. Overall, despite the distinct advantage of an aromatic ring over a plain aliphatic side chain, the electronic preference of this aromatic group is unclear.

The above results are quite similar to those of the benzylamines exemplified by **4**, in which an N-side chain with an ethylene linker connecting an aromatic ring such as phenyl or thienyl moiety to the nitrogen is optimal for

Table 1. SAR of amines **13a**–**o** at *h*MC4R



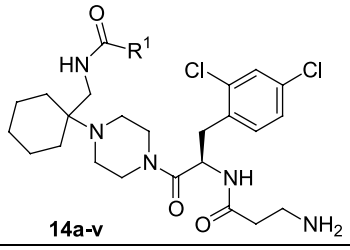
13a-o

Compound	R^1	K_i (nM)[a]	Stimulation (%)[b]
12		290	0.5
13a	*i*Pr-	180	0.7
13b	*t*-$BuCH_2$-	340	3.6
13c	Cyclohexyl-	220	5.1
13d	$PhCH_2CH_2$-	60	2.8
13e	$PhCH_2$-	11	0.4
13f	Ph-	18	5.1
13g	2-FC_6H_4-	12	4.7
13h	4-FC_6H_4-	61	6.4
13i	4-NCC_6H_4-	92	5.0
13j	4-$(Me_2N)C_6H_4$-	110	4.2
13k	4-$CF_3C_6H_4$-	45	4.1
13l	4-$CF_3OC_6H_4$-	22	2.4
13m	2-Thienyl-	12	8.1
13n	2-Thiazolyl-	49	4.9
13o	2-Pyridinyl-	36	3.0

[a] Inhibition of [^{125}I]-NDP-MSH binding to the human melanocortin-4 receptor stably expressed in HEK293 cells, and as radioligand in the competitive binding assay. Data are average of two to three independent measurements.

[b] Stimulation of cAMP levels in the same cell line at 10 μM ligand concentration, in comparison with that of α-MSH.

Table 2. SAR of amides **14a**–**v** at *h*MC4R[a]



14a-v

Compound	R^1	K_i (nM)	Stimulation (%)
10	CF_3-	490	0.9
14a	Ph-	220	5.1
14b	4-FC_6H_4-	190	7.9
14c	4-ClC_6H_4-	210	10
14d	4-BrC_6H_4-	240	12
14e	4-$MeOC_6H_4$-	250	7.7
14f	2-$MeOC_6H_4$-	350	6.5
14g	4-$CF_3OC_6H_4$-	400	8.9
14h	4-$NO_2C_6H_4$-	120	6.7
14i	4-MeC_6H_4-	270	7.8
14j	4-$CF_3C_6H_4$-	340	8.7
14k	4-*t*-BuC_6H_4-	520	8.2
14l	2-Furanyl-	260	4.5
14m	2-Thienyl-	230	5.1
14n	3-Pyridinyl-	220	4.3
14o	4-Pyridinyl-	150	4.4
14p	$C_6H_5CH_2$-	8.8	1.6
14q	4-$FC_6H_4CH_2$-	21	2.3
14r	4-$ClC_6H_4CH_2$-	23	1.4
14s	3-$MeOC_6H_4CH_2$-	7.6	1.8
14t	4-$MeOC_6H_4CH_2$-	4.2	0.6
14u	3,4-$(MeO)_2C_6H_3CH_2$-	18	2.7
14v	2-ThienylCH_2-	8.4	3.4

[a] See footnotes of Table 1.

binding,[10c] and appear to indicate that the phenethylamino side chain in these two series binds to the same site of the receptor. Therefore, the role of cyclohexyl moiety of **13** is similar to that of the phenyl group of **4**, even though **13** exhibited about 10-fold reduced affinity. We have shown that the basic nitrogen of the benzylamine is very important for high affinity binding, and this seems to agree with the results from a series of benzoylamides **14a–k** (Table 2). Thus, the benzoyl analogue **14a** (K_i = 220 nM), having a similar binding affinity to the TFA intermediate **10** (K_i = 490 nM), was much less potent than the corresponding benzyl derivative **13f** (K_i = 18 nM). While these data might suggest an important role of the basic nitrogen, the 12-fold reduction in binding for the amide **14a** could be the result of a conformational change induced by the carbonyl group, since substitution on the phenyl ring had little effect in binding (**14b–k**), and heteroaromatic derivatives exhibited similar potency (**14l–o**). These results actually point out that this aromatic ring is no longer in place to interact with the receptor. Replacing the benzoyl moiety with a phenylacetyl group, however, resulted in over a 20-fold increase in potency (**14p**, K_i = 8.8 nM). Based on the limited data, the substitution at the phenyl ring had no significant effect (**14q–u**). Thus, thienylacetyl compound **14v** had a K_i value of 8.4 nM, and the 4-methoxyphenylacetyl compound (**14t**) possessed a K_i of 4.2 nM.

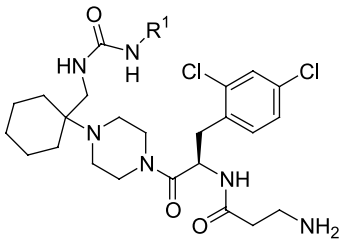
Replacement of the phenylacetyl amide with a phenylurea resulted in reduction of potency for these compounds (**15a–f**, Table 3). Thus, the urea **15a** (K_i = 210 nM) was about 10-fold less potent than the acetyl analogue **14q** (K_i = 21 nM). This set of data clearly showed that an electron-donating substituent at the phenyl ring was favored. Thus, the 4-methoxyphenyl compound **15c** (K_i = 36 nM) was about 15-fold better than the 4-nitrophenyl analogue **15e** (K_i = 540 nM) in binding affinity.

In this study, we demonstrated that, for this series of cyclohexylpiperidines bearing a functional side chain, an aromatic group and the linker connecting the nitrogen to this aromatic ring are important for receptor binding. The basic nitrogen could be replaced by an amide moiety, which presumably forms hydrogen-bonding interaction with an acidic residue of the MC4 receptor.

Further evaluation of selected compounds from this series demonstrated that these compounds were MC4R-selective. They were at least 80-fold selective over MC1R, MC3R, and MC5R (Table 4). For example, **14t** possessed K_i values of 5700, 1100, 4.2, and 540 nM at the MC1R, MC3R, MC4R, and MC5R, respectively. These compounds displayed similar affinity in displacement of both NDP-MSH and AgRP binding (K_i = 2.8 nM for **14t**) to the MC4 receptor (Table 4). None of the compounds were able to stimulate significant level of cAMP release at 10 μM concentration (less than 12% of the α-MSH level), suggesting they were not efficacious agonists. Instead, selected compounds were demonstrated to be potent antagonists in dose-dependent inhibition of α-MSH-stimulated cAMP release (Table 4). For example, **14t** exhibited an IC_{50} value of 150 nM in this cAMP assay.

In conclusion, a series of cyclohexylpiperidines bearing an amine or amide side chain was synthesized and found to possess potent antagonist activity at the human melanocortin-4 receptor. Results from this study indicate that the phenyl ring of the benzylamine moiety of **4**

Table 3. SAR of ureas **15a–f** at *h*MC4R[a]



15a-f

Compound	R^1	K_i (nM)	Stimulation (%)
15a	4-FC_6H_4-	210	5.5
15b	4-ClC_6H_4-	420	5.9
15c	4-$MeOC_6H_4$-	36	1.0
15d	4-$(Me_2N)C_6H_4$-	62	2.7
15e	4-$NO_2C_6H_4$-	540	11
15f	4-(MeOCO)C_6H_4-	260	12

[a] See footnotes of Table 1.

Table 4. Binding affinity (K_i, nM) at the melanocortin receptor subtypes and functional activity at MC4R of selected compounds[a]

Compound	MC1R	MC3R	MC5R	MC4R	MC4R[b]	IC_{50} (nM)[c]
13e	(44%)[d]	3200	1200	11	ND[e]	430
13g	(45%)[d]	2300	1400	10	13	760
13m	4500	2400	1900	12	ND[e]	ND[e]
14p	(47%)[d]	710	920	8.8	6.4	260
14s	2800	650	470	7.6	ND[e]	ND[e]
14t	5700	1100	540	4.2	2.8	150
14v	4600	1100	480	8.4	ND[e]	ND[e]

[a] Human melanocortin-1, -3, -4, and -5 receptors stably expressed in HEK293 cells, and [^{125}I]-NDP-MSH as radioligand in the competitive binding assay.
[b] [^{125}I]-AgRP(83–132) as radioligand in the competitive binding assay.
[c] Dose-dependent inhibition of cAMP production stimulated by 10 nM α-MSH in the same cell line.
[d] Percentage of inhibition at 10 μM concentration.
[e] ND—not determined.

might interact with lipophilic residues of the receptor since it is replaceable by a non-aromatic cyclohexyl moiety, which resides in an area near TM-7 based on a receptor model. These compounds are highly selective over the human melanocortin-3 receptor, as well as other melanocortin receptor subtypes.

References and notes

1. (a) Gantz, I.; Fong, T. M. *Am. J. Physiol. Endocrinol. Metab.* **2003**, *284*, E468; (b) Goodfellow, V.; Saunders, J. *Curr. Top Med. Chem.* **2003**, *3*, 855.
2. Adage, T.; Scheurink, J. W.; de Boer, S. F.; de Vries, K.; Konsman, J. P.; Kuipers, F.; Adan, R. A. H.; Baskin, D. G.; Schartz, M. W.; Van Dijk, G. *Neuroreport* **2001**, *12*, 1281.
3. (a) Foster, A. C.; Joppa, M.; Markinson, S.; Gogas, K. R.; Fleck, B. A.; Murphy, B. J.; Wolff, M.; Cismowski, M. J.; Ling, N.; Goodfellow, V. S.; Chen, C.; Saunders, J.; Conlon, P. J. *Ann. NY Acad. Sci.* **2003**, *994*, 103; (b) Schioth, H. B.; Kask, A.; Mutulis, F.; Muceniece, R.; Mutule, I.; Mutule, I.; Mandrika, I.; Wikberg, J. E. S. *Biochem. Biophys. Res. Commun.* **2003**, *301*, 399.
4. (a) Marks, D. L.; Ling, N.; Cone, R. *Cancer Res.* **2001**, *61*, 1432; (b) Wisse, B. E.; Frayo, R. S.; Schwartz, M. W.; Cummings, D. E. *Endocrinology* **2001**, *142*, 3292.
5. (a) Chaki, S.; Ogawa, S.; Todaa, Y.; Funakoshi, T.; Okuyama, S. *Eur. J. Pharmacol.* **2003**, *474*, 95; (b) Chaki, S.; Hirota, S.; Funakoshi, T.; Suzuki, Y.; Suetake, S.; Okubo, T.; Ishii, T.; Nakazato, A.; Okuyama, S. *J. Pharmacol. Exp. Ther.* **2003**, *304*, 818; (c) Nakazato, A., Ishii, T., Nozawa, S., Tomisawa, K., Okubo, T., Ueki, T., Okuyama, S., Chaki, S. *Book of Abstracts*, 228th American Chemical Society National Meeting, August 22–26, 2004, Philadelphia, PA; MEDI055.
6. (a) Bellasio, S.; Nicolussi, E.; Bertorelli, R.; Reggiani, A. *Eur. J. Pharmacol.* **2003**, *482*, 127; (b) Beltramo, M.; Campanella, M.; Tarozzo, G.; Fredduzzi, S.; Corradini, L.; Forlani, A.; Bertorelli, R.; Reggiani, A. *Mol. Brain Res.* **2003**, *118*, 111; (c) Bertorelli, R.; Fredduzzi, S.; Tarozzo, G.; Campanella, M.; Grundy, R.; Beltramo, M.; Reggiani, A. *Behav. Brain Res.* **2005**, *157*, 55.
7. Sebhat, I. K.; Martin, W. J.; Ye, Z.; Barakat, K.; Mosley, R. T.; Johnston, D. B. R.; Bakshi, R.; Palucki, B.; Weinberg, D. H.; MacNeil, T.; Kalyani, R. N.; Tang, R.; Stearns, R. A.; Miller, R. R.; Tamvakopoulos, C.; Strack, A. M.; McGowan, E.; Cashen, D. E.; Drisko, J. E.; Hom, G. J.; Howard, A. D.; MacIntyre, D. E.; van der Ploeg, L. H. T.; Patchett, A. A.; Nargund, R. P. *J. Med. Chem.* **2002**, *45*, 4589.
8. Arasasingham, P. A.; Fotsch, C.; Ouyang, X.; Norman, M. H.; Kelly, M. G.; Stark, K. L.; Karbon, B.; Hale, C.; Baumgartner, J. W.; Zambrano, M.; Cheetham, J.; Tamayo, N. A. *J. Med. Chem.* **2003**, *46*, 9.
9. (a) Vos, T. J.; Caracoti, A.; Che, J.; Dai, M.; Farrer, C. A.; Forsyth, N. E.; Drabic, S. V.; Horlick, R. A.; Lamppu, D.; Yowe, D. L.; Balani, S.; Li, P.; Zeng, H.; Joseph, I. B. J. K.; Rodriguez, L. E.; Claiborne, C. F. *J. Med. Chem.* **2004**, *47*, 1602; (b) Marsilje, T. H.; Roses, J. B.; Calderwood, E. F.; Stroud, S. G.; Forsyth, N. E.; Blackburn, C.; Yowe, D. L.; Miao, W.; Drabic, S. V.; Bohane, M. D.; Daniels, J. S.; Li, P.; Wu, L.; Patanea, M. A.; Claiborne, C. F. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 3721.
10. (a) Dyck, B.; Parker, J.; Phillips, T.; Carter, L.; Murphy, B.; Summers, R.; Hermann, J.; Baker, T.; Cismowski, M.; Saunders, J.; Goodfellow, V. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 3793; (b) Pontillo, J.; Tran, J. A.; Arellano, M.; Fleck, B. A.; Huntley, R.; Marinkovic, D.; Lanier, M.; Nelson, J.; Parker, J.; Saunders, J.; Tucci, F. C.; Jiang, W.; Chen, C. W.; White, N. S.; Foster, A.; Chen, C. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 4417; (c) Pontillo, J.; Tran, J. A.; Fleck, B. A.; Marinkovic, D.; Arellano, M.; Tucci, F. C.; Lanier, M.; Nelson, J.; Parker, J.; Sauders, J.; Murphy, B.; Foster, A. C.; Chen, C. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 5605.
11. Chen, C.; Pontillo, J.; Fleck, B. A.; Gao, Y.; Wen, J.; Tran, J. A.; Tucci, F. C.; Marinkovic, D.; Foster, A. C.; Saunders, J. *J. Med. Chem.* **2005**, *47*, 6821.
12. Several phenylpiperazines bearing an amide side chain have been reported to be potent and selective antagonists of the melanocortin-4 receptor. See: Xi, N.; Hale, C.; Kelly, M. G.; Norman, M. H.; Stec, M.; Xu, S.; Baumgartner, J. W.; Fotsch, C. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 377.
13. For experimental details, see: Dyck, B. P., Goodfellow, V., Phillips, T., Parker, J., Zhang, X., Chen, C., Tran, J. A., Pontillo, J., Tucci, F. C. WO 03/031410, 2003.
14. Nickolls, S. A.; Cismowski, M. I.; Wang, X.; Wolff, M.; Conlon, P. J.; Maki, R. A. *J. Pharmacol. Exp. Ther.* **2003**, *304*, 1217.