

A potent and selective nonpeptide antagonist of the melanocortin-4 receptor induces food intake in satiated mice

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Received 20 January 2005; revised 10 March 2005; accepted 15 March 2005

Available online 9 April 2005

Abstract—Optimization on a series of piperazinebenzylamines resulted in analogues with low nanomolar binding at the human MC4 receptor but weak affinity ($K_i > 500$ nM) at the MC3 receptor. Compound **14c** was identified to be a potent MC4R antagonist ($K_i = 3.2$ nM) with a selectivity of 240-fold over MC3R. It proved to be an insurmountable antagonist in a cAMP assay. Compound **14c** potently stimulated food intake in satiated mice when given by intracerebroventricular administration.

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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 It has been well documented that nonselective peptide MC4 antagonists such as SHU9119 and AgRP have a profound effect in inducing feeding behavior in animals.² Selective peptide MC4R antagonists such as MBP10 and HS131 have been discovered and data from these peptides suggest the feeding effect is mediated by the MC4 receptor.³ In addition, recent studies have showed that MC4R also has a role in anxiety and pain.^{4,5} Thus, potent and selective MC4R antagonists may be useful in human diseases such as cachexia, anxiety, and neuropathic pain.

Several small molecule MC4R antagonists have been reported. MCL0129 (**1b**), which is structurally related to an earlier reported series as exemplified by **1a** (Fig. 1),⁶ inhibited the NDP-MSH binding to MC4R with a K_i value of 7.9 nM. This compound exhibits anxiolytic-like and antidepressant-like activity in several ani-

mal models.^{4a} In addition, a recent publication has shown that an MC4R antagonist **2** ($K_i = 160$ nM) can protect tumor-induced weight loss in mice following peripheral administration.⁷

We have reported substituted phenylpiperazines as MC4R-selective ligands which, interestingly, contain both agonists and antagonists having only subtly different chemical structures.⁸ For example, molecule **3** is a potent MC4R-selective agonist ($EC_{50} = 4.7$ nM, $IA = 100\%$),^{8b} whereas compound **4** ($K_i = 15$ nM) is a potent antagonist. The latter compound did, however, stimulate cAMP release in cells expressing the human MC4 receptor with 12% maximal level of α -MSH at high concentrations.^{8c,9} Recently we demonstrated that a 2,4-dichlorophenylalanine analogue of **5a** ($K_i = 21$ nM) possesses high affinity (**5b**, $K_i = 1.8$ nM) with inability to stimulate cAMP release ($IA < 3\%$).¹⁰ Here we report compounds, derived from this structure, as potent antagonists of the human melanocortin-4 receptor ($hMC4R$) with high selectivity over the melanocortin-3 receptor.¹¹ In addition, we demonstrate that one of these compounds, **14c**, is an insurmountable antagonist, and is able to stimulate food intake in satiated mice.

The synthesis of targeted compounds started from piperazinebenzaldehyde **6**, which was obtained from the

Keywords: Melanocortin-4; Antagonist; Piperazinebenzylamine; Food intake.

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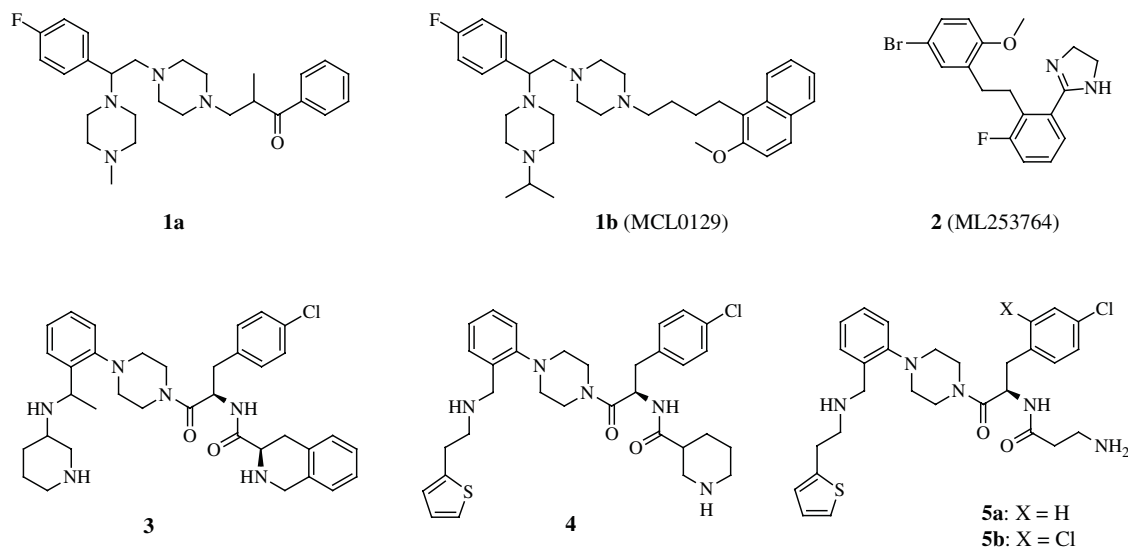


Figure 1. Example small molecule MC4R ligands.

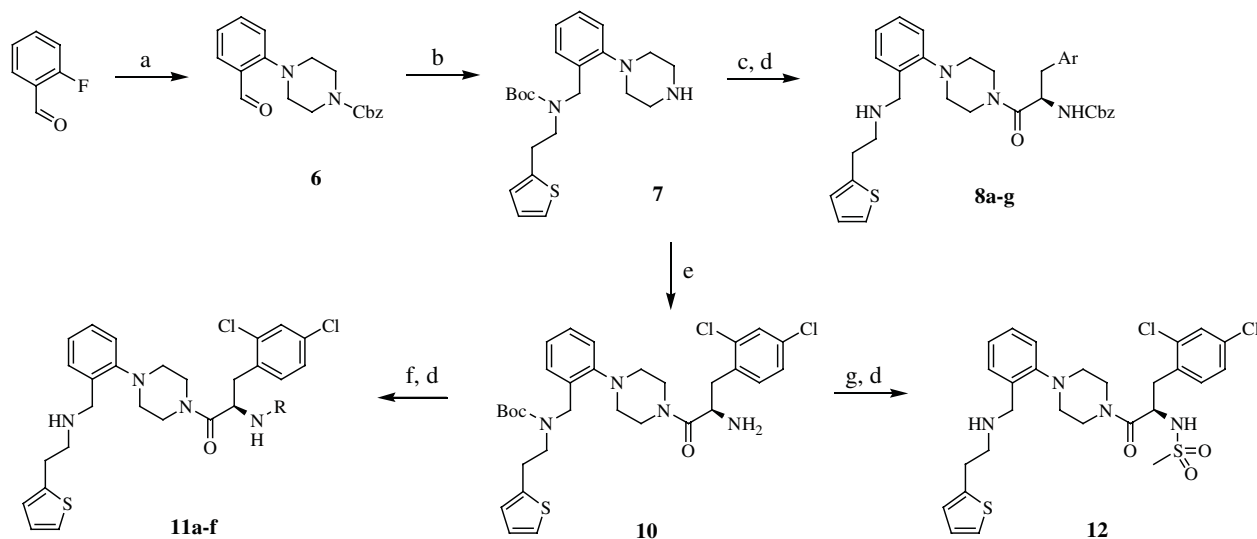
condensation of 2-fluorobenzaldehyde and *N*-Cbz-piperazine in DMSO at 120 °C (Scheme 1). Compound **6** was subjected to reductive amination with 2-(2-thienyl)ethylamine in the presence of sodium triacetoxyborohydride in dichloromethane, followed by protection of the resulting secondary amine with a Boc-group in 82% yield. The protected diamine intermediate was selectively deprotected with palladium-catalyzed hydrogenation in methanol to give the amine **7** in 35% yield. Coupling reaction of **7** with *N*-Cbz-(*R*)-arylalanines (**9**) under the standard peptide coupling conditions (EDC/HOBt/CH₂Cl₂), followed by Boc-deprotection with HCl/dioxane afforded the desired products **8a–g** in 60–80% yields (Table 1). Compound **7** was also coupled with *N*-Fmoc-(*R*)-(2,4-Cl)Phe-OH, followed by removal of the Fmoc-group with diethyl-

Table 1. SAR of arylalanines at hMC4R^a

Compound	Ar	K _i (nM)
8a	2,4-ClPh	23 ± 3
8b	1-Naphthyl	420 ± 280
8c	4-HOPh	5700 ± 490
8d	4-Imidazolyl	3600 ± 1100
8e	4-Thiazolyl	>10,000
8f	3-Indolyl	2300 ± 210
8g	3-Benzothienyl	1100 ± 190

^a Binding affinity at the human melanocortin-4 receptor stably transfected in HEK 293 cells, using [¹²⁵I]-NDP-MSH as the radiolabeled ligand.

amine to give **10** in 87% yield, which served as the key intermediate to be derivatized in several ways as follows.



Scheme 1. Reagents and conditions: (a) *N*-Cbz-piperazine/K₂CO₃/DMSO/120 °C, 19 h, 53%. (b) (i) 2-(2-Thienyl)ethylamine/NaBH(OAc)₃/CH₂Cl₂/rt, 16 h; (ii) (Boc)₂O/CH₂Cl₂/rt, 6 h, 82%; (iii) Pd/C–H₂ (40 psi)/NH₃/MeOH/rt, 23 h, 35%. (c) *N*-Cbz-(*R*)-(Ar)Ala-OH (**9**)/EDC/HOBt/CH₂Cl₂/rt, 18 h, 60–80%. (d) HCl/dioxane/rt, 2 h; (e) (i) *N*-Fmoc-(*R*)-(2,4-Cl)Phe-OH/EDC/HOBt/CH₂Cl₂/rt, 18 h; (ii) Et₂NH/CH₂Cl₂/rt, 3 h, 87%. (f) Aldehyde/NaBH(OAc)₃/CH₂Cl₂/rt. (g) MsCl/pyridine/rt.

Table 2. SAR on the *N*-alkylation of the 2,4-dichlorophenylalanine^a

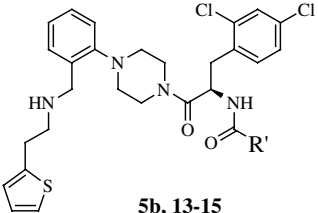
Compound	R	<i>K</i> _i (nM) ^b
11a	H	94 ± 18
11b	<i>i</i> -Bu	23 ± 6
11c	<i>c</i> -PrCH ₂	18 ± 3
11d	2-ThiazoleCH ₂	16 ± 4
11e	Bn	31 ± 9
11f	CH ₂ CH ₂ NH ₂	18 ± 5
12	SO ₂ Me	14 ± 6

^a Binding affinity at the human melanocortin-4 receptor stably transfected in HEK 293 cells, using [¹²⁵I]-NDP-MSH as the radiolabeled ligand.

^b Data are average of three or more independent measurements.

Compound **11a** (R = H) was obtained by deprotection of **10** with HCl/dioxane. Reductive alkylation of **10** with various aldehydes afforded, after HCl treatment, the diamines **11b–f** (Table 2). Compound **10** was also coupled with methanesulfonyl chloride under basic conditions to give the sulfonamide **12** after Boc-deprotection. Alternatively, coupling reactions of **10** with various carboxylic acids, including *N*-Boc-amino acids, gave the final compounds **13a–d** in 34–65% yields after Boc-deprotection (Scheme 2). Reaction of **10** with alkyl chloroformates under basic conditions, followed by HCl-deprotection provided carbamates **14a–d**, whilst reaction with isocyanates afforded the ureas **15a–e**. The ureas **15f–i** were obtained from the reaction of **10** with phosgene in toluene at 0 °C, followed by alkylamines at room temperature (Table 3). All final compounds were purified using a HPLC-MS system as previous described.¹⁰

The competition binding experiments were performed using HEK293 cells stably transfected with the human melanocortin receptors as previously described, using [¹²⁵I]-NDP-MSH as the radiolabeled ligand.¹² The functional antagonist activity of selected compounds was

Table 3. Binding affinity (*K*_i, nM) of compounds **13–15** at the human MC4 receptor and selectivity over the human MC3 receptor^a


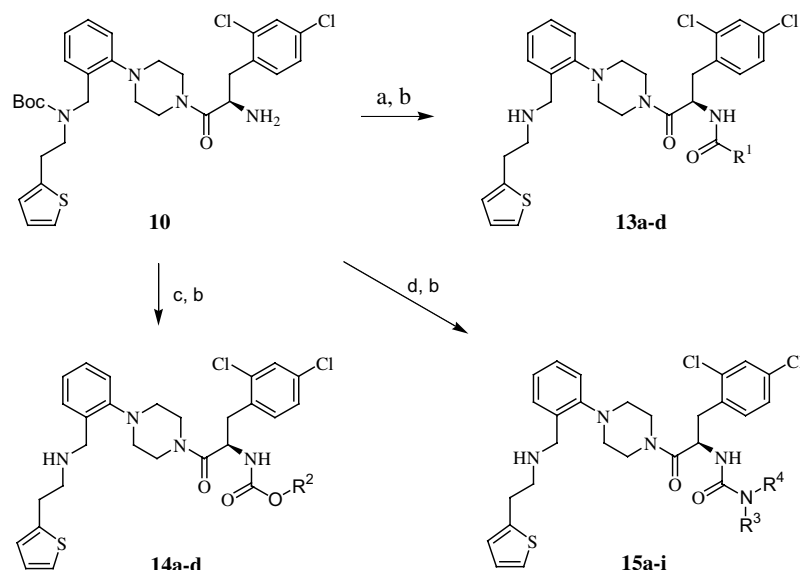
Compound	R'	MC4R ^b	MC3R ^b
5b	CH ₂ CH ₂ NH ₂	1.8 ± 0.2	640 ± 190
13a	Me	2.4 ± 0.4	570 ± 210
13b	Ph	3.1 ± 1.1	1600 ± 500
13c	CH ₂ CH ₂ CH ₂ NH ₂	2.0 ± 0.8	950 ± 500
13d	CH ₂ CH ₂ COOMe	2.8 ± 0.2	830 ± 150
14a	OMe	5.0 ± 0.9	810 ± 210
14b	OEt	4.6 ± 1.8	800 ± 10
14c	OCH ₂ CH ₂ F	3.2 ± 1.4	790 ± 120
14d	O(<i>i</i> -Pr)	4.5 ± 1.0	1300 ± 450
15a	NHEt	1.7 ± 1.0	790 ± 120
15b	NH(<i>c</i> -Pr)	2.1 ± 0.4	860 ± 150
15c	NH(<i>i</i> -Pr)	1.4 ± 0.1	450 ± 80
15d	NH(<i>t</i> -Bu)	2.4 ± 0.5	1000 ± 190
15e	NH(2-FBn)	5.0 ± 0.3	2300 ± 720
15f	NHCH ₂ CH ₂ OH	2.9 ± 0.3	390 ± 150
15g	NH(cyclopentyl)	1.3 ± 0.3	580 ± 100
15h	NMe ₂	2.0 ± 0.2	560 ± 41
15i	1-Pyrrolidinyl	1.5 ± 0.3	670 ± 41

^a Binding affinity at the human melanocortin-4 or melanocortin-3 receptor stably transfected in HEK 293 cells, using [¹²⁵I]-NDP-MSH as the radiolabeled ligand.

^b Data are average of three or more independent measurements.

measured for their ability to inhibit α-MSH-stimulated cAMP production in the same cell lines.

Previously we have showed that replacing the 4-chlorophenylalanine of **5a** (*K*_i = 21 nM, IA = 14%) with the



Scheme 2. Reagents and conditions: (a) R¹COOH/EDC/HOBt/CH₂Cl₂/rt, 16 h, 34–65%. (b) HCl/dioxane/rt, 2 h. (c) R²OCOCi/Et₃N/CH₂Cl₂/0 °C to rt. (d) R³NCO/CH₂Cl₂/rt; or (i) COCl₂/Et₃N/toluene/0 °C to rt; (ii) R³R⁴NH/THF, rt.

2,4-dichloro variant not only increases its binding affinity over 10-fold, but also diminishes the intrinsic activity in stimulating cAMP release of this compound (**5b**, $K_i = 1.8$ nM, IA < 3%).¹⁰ A brief survey on the phenylalanine moiety of **8** revealed that the highly lipophilic 2,4-dichlorophenyl compound (**8a**, $K_i = 23$ nM) was more potent in binding than other analogues including the 1-naphthyl variant (**8b**, $K_i = 420$ nM). The relatively polar 4-hydroxyphenyl derivative (**8c**, $K_i = 5.7$ μ M) was only weakly active. All heteroaromatic analogues **8d–g** exhibited poor binding affinity ($K_i > 1$ μ M, Table 1).

We also demonstrated in this series that the amide functionality of the 2,4-chlorophenylalaninyl nitrogen has a profound effect on both binding affinity and, more significantly, functional activity. Thus, the primary amine **11a** displayed a K_i value of 94 nM, which was over 50-fold reduction in affinity caused by the removal of the β -alanine of **5b**. Introduction of a 2-aminoethyl group on **11a** restored affinity by about 4-fold (**11f**, $K_i = 18$ nM). Incorporation of thiazolemethyl (**11d**, $K_i = 16$ nM) and a small lipophilic isobutyl group (**11b**, $K_i = 23$ nM), or sulfonylation (**12**, $K_i = 14$ nM) of **11a** resulted in compounds with affinity similar to **11f**. These results indicate that the 2-aminoethyl group of β -alanine **5b** has minimal effect on binding, and that the amide functionality (–CONH–) plays an important role either by direct interaction with MC4R, or by forcing a more favored binding conformation of the 2,4-dichlorophenylalanine moiety.

Amides (**13a–d**), carbamates (**14a–d**), and ureas (**15a–i**) derived from **10** displayed potent binding affinity at MC4R (Table 3). Since all amides (**13a–d**) had K_i values of 2.0–3.1 nM, irrespective of the acyl substituent, it is obvious that the only important element of the amide group is the –CONH– moiety. Interestingly, this region tolerated groups with different properties, such as phenyl (**13b**) and amino alkyl (**5b**, **13c**). Additionally, since the carbamates **14** and ureas **15** were also potent binders, this locus offers an opportunity for optimization of this series of compounds towards desirable physicochemical properties as potential CNS agents.

Since the MC3 receptor is also centrally localized and may also play a role in control of food intake and metabolism,¹ we tested compounds **13–15** in the MC3R binding assay, and found that these compounds showed weak binding affinity ($K_i \geq 530$ nM). For example, while compound **14c** displayed a K_i value of 3.2 nM at hMC4R, it possessed low binding affinity at hMC3R ($K_i = 790$ nM). Thus, **14c** exhibited a 240-fold selectivity of MC4R over the MC3 receptor subtype.

Selected compounds were also tested on other melanocortin receptor subtypes (MC1R and MC5R) and MC4R selectivity was demonstrated (Table 4). In addition, the compounds derived from 2,4-dichlorophenylalanine showed no significant stimulation of cAMP accumulation at 10 μ M concentration (<3% of α -MSH maximal levels, data not shown) in cells expressing MC4R. These data demonstrate that these compounds do not behave as functional MC4R agonists. Moreover,

Table 4. Binding affinity (K_i , nM) of selected compounds at the human melanocortin receptor subtypes^a

Compound	MC1R ^b	MC3R	MC4R	MC5R
13a	(31%)	570 $\pm$ 210	2.4 $\pm$ 0.4	730 $\pm$ 90
14c	1000 $\pm$ 340	790 $\pm$ 120	3.2 $\pm$ 1.4	560 $\pm$ 110
14d	(9%)	1300 $\pm$ 450	4.5 $\pm$ 1.0	680 ^c
15a	(18%)	790 $\pm$ 120	1.7 $\pm$ 1.0	300 ^c
15f	(8%)	390 $\pm$ 150	2.9 $\pm$ 0.3	350 $\pm$ 42
15h	6800 $\pm$ 2200	560 $\pm$ 190	2.0 $\pm$ 0.2	350 $\pm$ 38
15i	6900 $\pm$ 1100	670 $\pm$ 41	1.5 $\pm$ 0.3	160 $\pm$ 16

^a Human melanocortin receptors stably expressed in HEK 293 cells.

^b Data in parenthesis are percentage of inhibition at 10 μ M concentration, and average of 2–3 measurements.

^c Single measurement.

selected compounds from this series dose-dependently inhibited α -MSH-stimulated cAMP production as exemplified by a representative **14c** (Fig. 2). Interestingly, unlike **5b** which is a competitive antagonist in the cAMP assay with a pA_2 of 7.9,¹⁰ compound **14c** was demonstrated by a Schild analysis to be an insurmountable antagonist on the inhibition of α -MSH-stimulated cAMP release (Fig. 3). Thus, the small structural

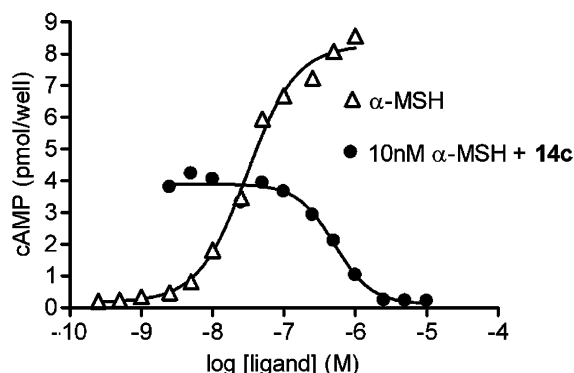


Figure 2. Dose-dependent inhibition of α -MSH-stimulated cAMP accumulation by **14c** in HEK 293 cells expressing the human melanocortin-4 receptor. Results are average of two independent experiments.

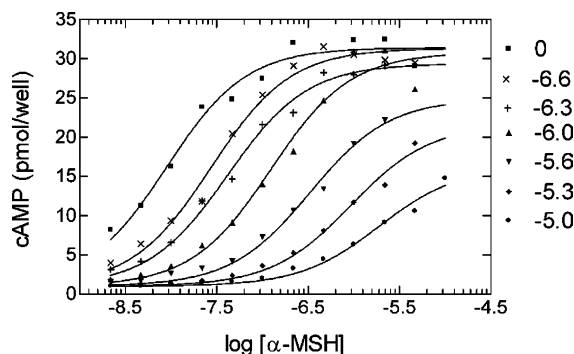


Figure 3. α -MSH-stimulated cAMP accumulation in the presence of different concentrations of the MC4R antagonist **14c**. Results are average of two independent experiments.

difference in the amide group between **5b** and **14c** causes profound pharmacological changes in these two compounds.

In addition to high affinity on human MC4R, compound **14c** bound to the mouse MC4R with a K_i value of 3.7 nM, demonstrating no selectivity between these two species. Thus, we were able to utilize compounds from this series to examine the ability of a small molecule MC4R antagonist to stimulate food intake in mice. Unfortunately, many of the potent MC4R antagonists listed in Table 3 suffered from either poor absorption associated with high hydrophilicity (**5b**, $\log D = 1.1$, $F = 1\%$), or metabolic instability in liver microsomes (**14c**, $Cl_{int} = 1750$ mL/min kg in mouse liver microsomes), thus precluding them from peripheral administration. Therefore, we measured the efficacy by directly delivering the compound into the mouse brain via intracerebroventricular (icv) administration. Female CD-1 mice were divided into four groups ($n = 10$ /group) and injected, with distilled water, 0.3, 3, or 10 nmol of **14c**, into the lateral/third cerebral ventricles using a free-hand injection method.¹³ Bonferroni-adjusted t -test post hoc comparisons indicated that compound **14c** significantly increased food consumption at the highest dose given at all the time points measured (all P s < 0.01) (Fig. 4).

In conclusion, we have synthesized a series of (*R*)-2,4-dichlorophenylalanine derivatives as potent antagonists of MC4R. These compounds are highly selective over MC3R, as well as other human melanocortin receptor subtypes. One compound from the series, **14c**, had K_i values of 3.2 and 790 nM, respectively, at the melanocortin-4 and -3 receptors. This compound was also demonstrated to be an insurmountable antagonist in the inhibition of α -MSH-stimulated cAMP release by a Schild analysis, possibly due to slow dissociation rate.¹⁴ Compound **14c** potentially stimulated food intake in satiated mice when given intracerebroventricularly.

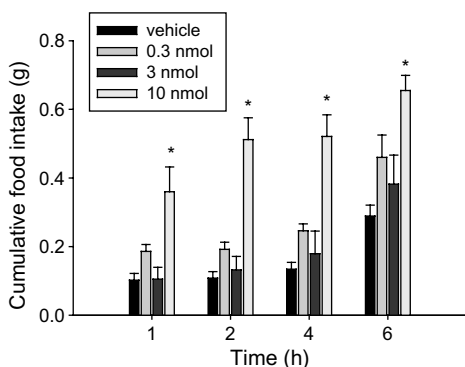


Figure 4. Effect of **14c** on cumulative food intake in satiated mice. Mice ($n = 10$ /group) were given vehicle (distilled water) or the MC4R antagonist, **14c**, via icv injection and food intake was measured over the following 6 h. Cumulative food intake was significantly increased by the 10 nmol dose of **14c** relative to vehicle at all time points ($*P < 0.01$).

References and notes

- (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.
- 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.
- (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. N.Y. 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.
- (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.
- (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.
- 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.
- (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.; Patane, M. A.; Claiborne, C. F. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 3721.
- (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.; Saunders, J.; Murphy, B.; Foster, A. C.; Chen, C. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 5605.
- A series of phenylpiperazines bearing a 3-(4-chlorophenyl)propionyl group has been reported without significant cAMP stimulation, 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.
- 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.* **2004**, *47*, 6821.
- Several phenylpiperazines bearing an amide side-chain have been reported to be potent and selective antagonists of the melanocortin-4 receptor, see Ref. 9.
- Nickolls, S. A.; Cismowski, M. I.; Wang, X.; Wolff, M.; Conlon, P. J.; Maki, R. A. *J. Pharmacol. Exp. Ther.* **2003**, *304*, 1217.
- Briefly, following a short anesthetization with isoflurane gas, a 30-gauge needle modified to a length of 4 mm was

placed at the apex of a triangle between the eyes and back of head and 5 μ L of vehicle or compound was injected over 30 s. Fifteen minutes after the injection, food was put in the cage and intake was measured 1, 2, 4, and 6 h later. A two-way repeated measures analysis of variance revealed significant main effects of dose ($F(3,36) = 13.64$, $P < 0.001$) and time ($F(3,108) = 79.31$, $P < 0.001$) as well

as an interaction ($F(9,159) = 2.04$, $P < 0.041$). Pellemounter, M. A.; Joppa, M.; Carmouche, M.; Cullen, M. J.; Brown, B.; Murphy, B.; Grigoriadis, D. E.; Ling, N.; Foster, A. C. *J. Pharmacol. Exp. Ther.* **2000**, 293, 799.

14. Vauquelin, G.; Van Liefde, I.; Vanderheyden, P. *Trends Pharmacol. Sci.* **2002**, 23, 514.