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Piperazinebenzylamines as potent and selective antagonists of the human melanocortin-4 receptor

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Abstract—SAR studies of a series of piperazinebenzylamines resulted in the discovery of potent antagonists of the human melanocortin-4 receptor. Compounds **11c**, **11d**, and **11l**, which had K_i values of 21, 14, and 15 nM, respectively, possessed low efficacy in cAMP stimulation (~15% of α-MSH maximal level) mediated by MC4R, and functioned as antagonists in inhibition of α-MSH-stimulated cAMP release in a dose-dependent manner (**11l**, IC_{50} = 36 nM).

1. Introduction

Five subtypes of melanocortin receptors, MC1-5R, members of the class A G-protein-coupled receptor (GPCR) superfamily,[1] are associated with various biological activities.[2] The melanocortin peptides, the natural ligands for the MCRs, consist of the melanotropins α-MSH, β-MSH, and γ-MSH, and the adrenocorticotropin ACTH. In addition to these agonists, two endogenous antagonists, agouti protein, and agouti related protein (AgRP) have been identified. Because of the importance of the MC4 receptor in feeding behavior, metabolism, and energy homeostasis, selective MC4R agonists or antagonists may be useful in treatment of human diseases including obesity and cachexia.[3] While many synthetic peptide ligands have been known for decades,[4] small molecule agonists[5] and antagonists[6] of the melanocortin-4 receptor have been discovered more recently. Compound **1**, first reported to be an inhibitor of AgRP binding to the MC4 receptor (IC_{50} = 52 nM), also exhibits moderate inhibition (IC_{50} = 220 nM) of NDP-MSH binding and reduces α-MSH-stimulated cAMP production at high concentration.[6a] MCL0129 (**2**) binds to the human MC4 receptor with high affinity (K_i = 7.9 nM), exhibiting both anxiolytic-like and antidepressant-like activity in several animal models.[6b] A recent publication demonstrated that an MC4R antagonist, compound **3** (K_i = 160 nM), protected against tumor-induced weight loss in mice following peripheral administration.[6c] SAR studies conducted on a related series (compound **4**, K_i = 180 nM) have also been reported (Fig. 1).[6d]

Previously we described a series of substituted phenylpiperazines (e.g., **5c**, EC_{50} = 3.8 nM),[7] derived from an earlier lead that acts as a potent MC4R agonist (**5a**, EC_{50} = 80 nM).[5e] Herein we report our continued efforts on the optimization of this series of compounds, with the goal of developing potent and selective antagonists of the human MC4 receptor.

2. Chemistry

The synthesis (Scheme 1) started from piperazinebenzaldehyde **6**, which was subjected to a reductive amination with 2-thienylethylamine and sodium triacetoxyborohydride. The resulting secondary amine was protected with a Boc-group, generating intermediate **8** with an overall yield of 82%. Deprotection of **8** by palladium-catalyzed hydrogenation provided a free amine, which was

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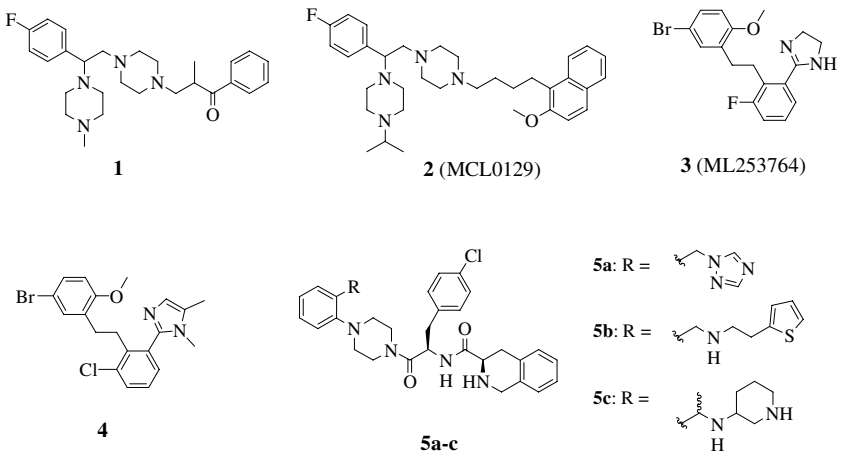
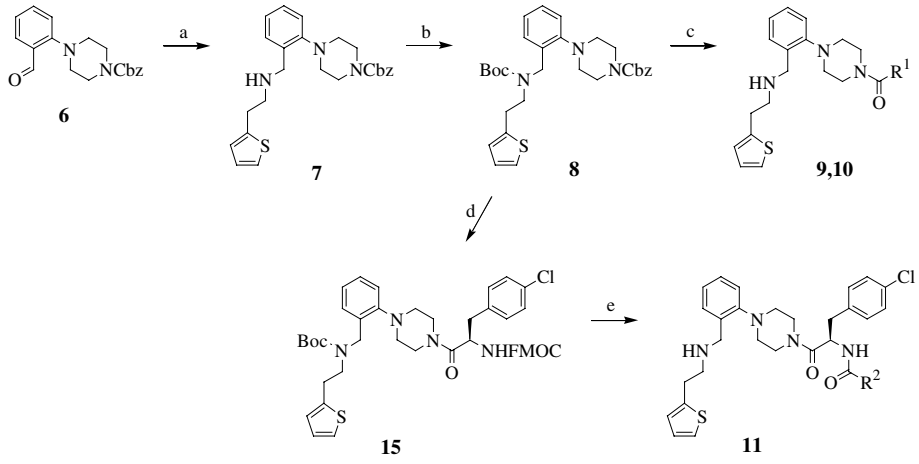


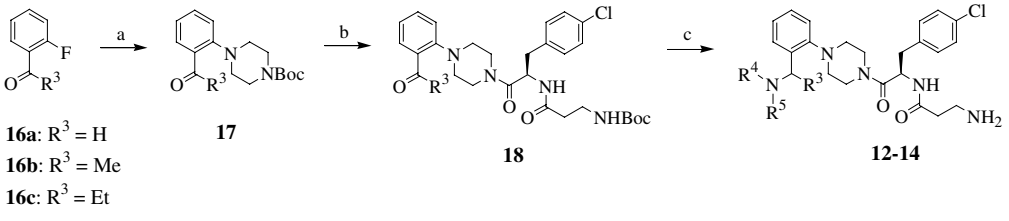
Figure 1. Some small molecule MC4 antagonists.



Scheme 1. Reagents and conditions: (a) 2-thienylethylamine/$NaBH(OAc)_3/CH_2Cl_2$; (b) $(Boc)_2O$, 82%, two steps; (c) i. H_2/Pd–C, 36%, ii. R^1COOH/EDC/HOBt, iii. TFA/CH_2Cl_2; (d) H_2/Pd–C, 36%, then N-FMOC-D-(4-Cl)Phe-OH/EDC/HOBt, 93%; (e) i. Et_2NH/CH_2Cl_2, 94%, ii. R^2COOH/EDC/HOBt, (iii) TFA/CH_2Cl_2.

coupled under the standard peptide coupling conditions (EDC/HOBt) with a various phenylacetic or phenylpropionic acids to afford the desired products **9** or **10**, after deprotection with TFA. Compound **8** was also coupled with N-Fmoc-D-(4-Cl)Phe-OH to give **15** in 93% yield. After deprotection with diethylamine, the free amine derived from **15** was coupled with various carboxylic acids, including N-Boc-amino acids to give the final compounds **11**, after TFA-deprotection.

The 2-piperazinephenyl aldehyde and ketones **17** (Scheme 2) were obtained from **16**. A coupling reaction of the free amine of **17** (obtained by TFA-deprotection) with the dipeptide **19** under standard conditions (EDC/HOBt) afforded the ketones **18**. Reductive aminations of **18** with various amines gave the desired products **12**–**14**. A pair of diastereoisomers could be detected during HPLC purification in some cases but were not separated.



Scheme 2. Reagents and conditions: (a) N-Boc-piperazine/DMF/120 °C; (b) i. TFA/CH_2Cl_2, ii. N-Boc-β-Ala-D-(4-Cl)Phe-OH (**19**)/EDC/HOBt/DCM; (c) i. $R^4R^5NH/NaBH(OAc)_3/CH_2Cl_2$ (for **18a**) or $R^4R^5NH/TiCl_4/NaBH_4/CH_2Cl_2$ (for **18b**,**c**), ii. TFA/CH_2Cl_2.

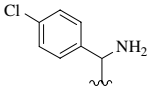
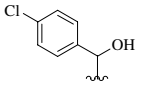
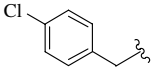
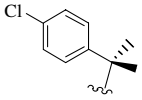
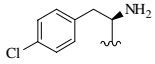
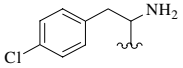
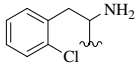
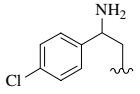
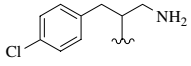
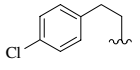
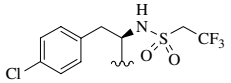
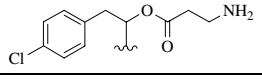
3. Results and discussion

The competition binding experiments, functional antagonist and agonist assays were performed using HEK293 cells stably transfected with the human melanocortin-4 receptor as previously described.[8] [^{125}I]-NDP-MSH was used as radiolabeled ligand in the binding assay. α-MSH was used to stimulate cAMP release in the functional antagonist assay, and as a standard for calculation of intrinsic activity in the agonist assay.

The observation that most reported MC4 ligands with good binding affinity possess a 4-chlorophenyl group led to the examination of chlorophenyl derivatives of compounds **9** and **10** (Table 1). The substituted 4-chlorophenylacetyl derivatives (**9a**–**d**) exhibited poor binding affinity (K_i > 1.8 μM). Deletion of the (*R*)-1,2,3,4-tetrahydroisoquinoline-3-carbonyl (Tic) group of **5b** resulted in 50-fold loss in binding (**10a**, K_i = 600 nM). All variations of **10a** gave analogs (**10b**–**h**) with similar or lower affinity. None of the phenylpropionyl analogs other than **10a** (IA = 85%, EC_{50} = 8.7 μM) were able to stimulate cAMP release to 50% of the α-MSH level at 10 μM concentration. While these results suggest the Tic group is very important for receptor binding, the role of its amide functionality is not clear at this point.

We then examined a series of amide derivatives of 4-chlorophenylalanine. The results are summarized in Table 2. The binding affinity of the glycine derivative **11a** (K_i = 44 nM) increased 15-fold from **10a**, and was comparable to **5b**. This compound still stimulated a significant level of cAMP production (EC_{50} = 580 nM, IA = 77%). The β-alanine analog **11c**, however, exhibited slightly better binding affinity (K_i = 21 nM) than **11a** but much lower level of cAMP stimulation (14% at 10 μM concentration). The γ-aminopropionyl analog

Table 1. SAR of the chlorophenyl carboxylic derivatives **9**–**10** at the *h*MC4R[a]

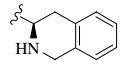
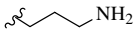
Compound	R^1	K_i (nM)[b]
5b		12
9a		1800
9b		>10,000
9c		>10,000
9d		3800
10a		600[c]
10b		3900
10c		1000
10d		5400
10e		6400
10f		1000
10g		410
10h		290

[a] Human melanocortin-4 receptor stably expressed in HEK 293 cells, and [^{125}I]-NDP-MSH as radioligand. Data are average of two or more independent measurements.

[b] Less than 45% of α-MSH-stimulated cAMP level at 10 μM concentration.

[c] EC_{50} = 8.7 μM, IA = 85%.

Table 2. SAR of amides of (*R*)-(4-chloro)phenylalanine **11**[a]

Compound	R^2	K_i (nM)[a]	EC_{50}, nM (IA, %)[b]
5b		12	320 (100)
11a		44	580 (77)
11b		38	1400 (45)
11c		21	(14)
11d		14	(15)
11e		66	1500 (80)
11f		30	2600 (45)
11g		40	1800 (57)
11h		46	(12)
11i		48	1627 (47)
11j		50	1900 (56)
11k		75	1100 (60)
11l		15	(12)
11m		26	(39)
11n		70	(16)
11o		41	(5)

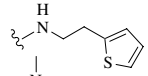
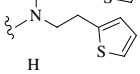
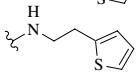
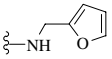
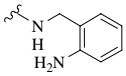
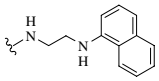
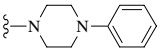
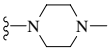
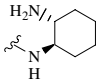
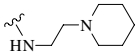
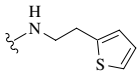
[a] Human melanocortin-4 receptor stably expressed in HEK 293 cells, and [^{125}I]-NDP-MSH as radioligand. Data are average of three or more independent measurements.

[b] Intrinsic activity: percentage of α-MSH-stimulated cAMP level at 10 μM concentration.

11d had similar profile to **11c**. As a matter of fact, many amide derivatives with a small aminoalkyl group exhibited similar levels of affinities, however, α-amino amides possessed higher levels of cAMP stimulation (**11a**, **11e**, and **11k**, IA $\geqslant$ 60%) than other amide analogs (**11c** and **11l**, IA $\leqslant$ 14%). The piperizin-3-yl compound **11l** possessed a K_i value of 15 nM. These results suggest that the tetrahydroisoquinoline moiety of the Tic group is critical for receptor activation, but less important for receptor binding.

We also examined the effect of the side-chain at the benzylamino group while the β-Ala-D-(4-Cl)Phe-group was held constant (**12**–**14**, Table 3). Previously we found that incorporation of a methyl group at the α-position of the benzylamine of **5** increased binding affinity in most cases.[7] However, either N-methylation or α-alkylation of **11c** resulted in a decrease in affinity (**12**: K_i = 220 nM, **13a**: K_i = 110 nM). Many other alkylamine derivatives (**13d**–**i**) exhibited similar or lower binding affinities. Diamines such as **13h** from this set had poor cAMP stimulation. Interestingly, the 2-aminobenzyl analog **13c** (K_i = 120 nM, EC_{50} = 364 nM, IA = 100%) possessed high efficacy and moderate agonist potency in stimulation of cAMP release.

Table 3. SAR of the substitution at the benzylamines **12**–**14**[a]

Compound	R^3	R^4NR^5	K_i (nM)	EC_{50}, nM (IA, %)[b]
11c	H		21	(14)
12	H		220	(32)
13a	Me		110	(32)
13b	Me		140	1872 (99)
13c	Me		120	364 (100)
13d	Me		470	2717 (56)
13e	Me		290	(22)
13f	Me		430	(35)
13g	Me		86	471 (59)
13h	Me		150	590 (64)
13i	Me		210	(41)
14	Et		140	(29)

[a] Human melanocortin-4 receptor stably expressed in HEK 293 cells, and [^{125}I]-NDP-MSH as radioligand.
[b] Intrinsic activity: percentage of α-MSH-stimulated cAMP level at 10 μM concentration.

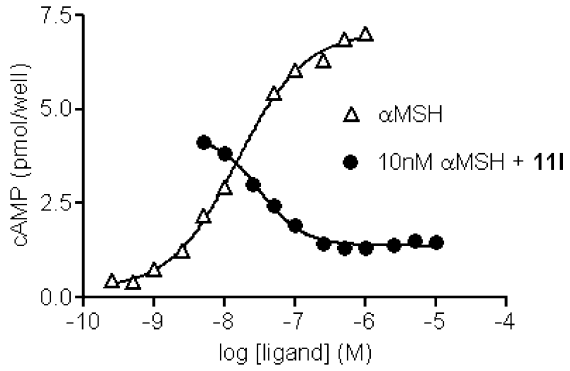


Figure 2. Dose response curves of α-MSH-stimulated cAMP production in HEK 293 cells expressing the human MC4 receptor, and inhibition by compound **11l**.

Table 4. Selectivity and functional antagonist potency of **11c**, **11d**, and **11l**[a]

Compound	Binding affinities (K_i, nM)				MC4 function[b]
	MC1	MC3	MC4	MC5	IC_{50} (nM)
11a	(50%)[c]	1900	44	990	[d]
11c	1300	1000	21	1000	90
11d	1200	1400	14	1000	66
11l	2300	1400	15	720	36
11o	(23%)[c]	1800	41	730	880

[a] Human melanocortin receptors stably expressed in HEK 293 cells, and [^{125}I]-NDP-MSH as radioligand. Data are average of two or more independent measurements.
[b] Inhibition of α-MSH-stimulated cAMP production.
[c] Percentage of inhibition at 10 μM concentration.
[d] Agonist with an EC_{50} value of 580 nM (IA = 77%).

Selected compounds were tested and found to be functional antagonists in dose-dependent inhibition of α-MSH-stimulated cAMP production in HEK 293 cells expressing the MC4 receptor. For example, **11l** had an IC_{50} value of 36 nM in this functional assay (Fig. 2). This compound also exhibited good selectivity over other melanocortin subtypes. It possessed K_i values of 2.3, 1.4, and 0.72 μM at the human MC1, MC3, and MC5 receptors, respectively. Selectivity and functional antagonism data of **11a**, **11c**–**d**, **11l**, and **11o** are summarized in Table 4.

4. Conclusion

In summary, a series of piperazinebenzylamines bearing the (*R*)-(4-Cl)Phe moiety were synthesized and tested as melanocortin-4 ligands. Structure–activity relationship studies of the (*R*)-(4-Cl)-Phe amides revealed several potent MC4 antagonists such as **11c** (K_i = 21 nM) and **11l** (K_i = 15 nM). The replacement of the Tic group of an early MC4 ligand **5b** with a small aminoalkyl carboxamide such as β-analine diminished its ability to stimulate cAMP production in cells expressing the human MC4 receptor, but retained binding affinity to this

receptor. Selected compounds exhibited potent antagonist activity at the MC4 receptor by inhibiting α-MSH-stimulated cAMP production. They also displayed good selectivity over other melanocortin subtypes.

References and notes

1. (a) Beck-Sickinger, A. G. *Drug Discovery Today* **1996**, *1*, 502–513; (b) Karnik, S. S.; Gogonea, C.; Patil, S.; Saad, Y.; Takezako, T. *Trends Endocrinol. Metab.* **2003**, *14*, 431.
2. (a) Wikberg, J. E. S.; Muceniece, R.; Mandrika, I.; Prusis, P.; Post, C.; Skottner, A. *Pharmacol. Res.* **2000**, *42*, 393; (b) Wikberg, J. E. S. *Eur. J. Pharmacol.* **1999**, *375*, 295; (c) Wikberg, J. E. S. *Exp. Opin. Ther. Pat.* **2001**, *11*, 61.
3. Goodfellow, V.; Saunders, J. *Curr. Top. Med. Chem.* **2003**, *3*, 855.
4. Holder, J. R.; Haskell-Luevano, C. *Med. Res. Rev.* **2004**, *24*, 325.
5. (a) 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.; Drisk, 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; (b) Pan, K.; Scott, M. K.; Lee, D. H. S.; Fitzpatrick, L. J.; Crooke, J. J.; Rivero, R. A.; Rosenthal, D. I.; Vaidya, A. H.; Zhao, B.; Reitz, A. B. *Bioorg. Med. Chem.* **2003**, *11*, 185; (c) 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. *J. Med. Chem.* **2003**, *46*, 1123; (d) Fotsch, C.; Smith, D. M.; Adams, J. A.; Cheetham, J.; Croghan, M.; Doherty, E. M.; Hale, C.; Jarosinki, M. A.; Kelly, M. G.; Norman, M. H.; Tamayo, N. A.; Xi, N.; Baumgartner, J. W. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 2337; (e) Dyck, B.; Parker, J.; Philips, T.; Carter, L.; Murphy, B.; Summers, R.; Hermann, J.; Baker, T.; Cismowski, M.; Saunders, J.; Goodfellow, V. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 3793; (f) Ruel, R.; Herpin, T. F.; Iben, L.; Luo, G.; Martel, A.; Mason, H.; Mattson, G.; Poirier, B.; Ruediger, E. H.; Shi, D.; Thibault, C.; Yu, G.; Zimanyi, I. A.; Poindexter, G. S.; Macor, J. E. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 4341; (g) Ujjainwalla, F.; Warner, D.; Walsh, T. F.; Wyvratt, M. J.; Zhou, C.; Yang, L.; Kalyani, R. N.; MacNeil, T.; Van der Ploeg, L. H. T.; Rosenblum, C. I.; Tang, R.; Vongs, A.; Weinberg, D. H.; Goulet, M. T. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 4431; (h) Richardson, T. I.; Ornstein, P. L.; Briner, K.; Fisher, M. J.; Backer, R. T.; Biggers, C. K.; Clay, M. P.; Emmerson, P. J.; Hertel, L. W.; Hsiung, H. M.; Husain, S.; Kahl, S. D.; Lee, J. A.; Lindstrom, T. D.; Martinelli, M. J.; Mayer, J. P.; Mullaney, J. T.; O'Brien, T. P.; Pawlak, J. M.; Revell, K. D.; Shah, J.; Zgombick, J. M.; Herr, R. J.; Melekhov, A.; Sampson, P. B.; King, C.-H. R. *J. Med. Chem.* **2004**, *47*, 744; (i) 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.
6. (a) 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; (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) 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; (d) 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.
7. 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. C.; Chen, C. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 4417.
8. Nickolls, S. A.; Cismowski, M. I.; Wang, X.; Wolff, M.; Conlon, P. J.; Maki, R. A. *J. Pharmacol. Exp. Ther.* **2003**, *304*, 1217.