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Identification of agonists and antagonists of the human melanocortin-4 receptor from piperazinebenzylamines

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Abstract—SAR studies of a series of piperazinebenzylamines resulted in identification of potent agonists and antagonists of the human melanocortin-4 receptor. Thus, the 1,2,3,4-tetrahydroisoquinolin-1-ylacetyl compound **12e** and the quinolin-3-ylcarbonyl analogue **12l** possessed K_i values of 6.3 and 4.5 nM, respectively. Interestingly, **12e** was a full agonist with an EC_{50} value of 31 nM, and **12l** was a weak partial agonist (IA = 17%) and functioned as an antagonist (IC_{50} = 300 nM).

The melanocortin-4 receptor (MC4R) has been associated with regulation of food intake and energy homeostasis, therefore, MC4R agonists and antagonists could be potentially used for treatment of obesity and cachexia.[1] Several small molecule MC4 agonists[2] and antagonists[3] from different chemical classes have been discovered since the report of the first potent agonist **1a** in 2002.[4] In our previous paper we described that by replacing the triazole moiety of our initial lead Tic-(4-Cl)Phe dipeptide **2a** (K_i = 270 nM, EC_{50} = 80 nM)[5] with a basic nitrogen bearing an aliphatic side chain we were able to produce a series of piperazinebenzylamines exemplified by **2c** (K_i = 6.4 nM, EC_{50} = 4.7 nM) as potent and selective agonists of the human MC4 receptor.[6] Interestingly, when the triazole moiety of **2a** was replaced by a basic nitrogen bearing a small lipophilic side chain, the binding affinity of these compounds increased over 10-fold in many cases, however, their agonist potency was barely changed. For example, the K_i value (11 nM) of **2b** was almost 25-fold better than that of **2a**, but its EC_{50} value in stimulation of cAMP production was only 360 nM. By analyzing the initial data we concluded that when a polar group, such as a hydroxyl and an amine was incorporated into the side chain, agonist potency of these compounds was improved. For example, **2c** was a potent full agonist. To further understand the structure–activity relationships as agonists and antagonists of the melanocortin-4 receptor, a series of benzylamines bearing an additional polar moiety was synthesized. In addition, we surveyed a set of different (4-Cl)Phe-amides to explore the role of the 1,2,3,4-tetrahydroisoquinolin-3-ylcarbonyl (Tic) group of **2** in the activation of the MC4 receptor. Here we report our findings of the structure–activity relationships on this set of compounds (Fig. 1).

The synthesis of the piperazinebenzylamines **5** started from 2-fluorobenzaldehyde **4** with a similar procedure as previously described (Scheme 1).[6] Compounds **12** were prepared from 2′-fluoroacetophenone **7**. Thus, condensation of **7** with mono-Boc-protected piperazine gave the corresponding 2′-(piperazin-1-yl)acetophenone, which was deprotected with TFA and coupled with (*R*)-*N*-Boc-(4-Cl)Phe-OH (EDC/HOBt/DCM) to give the acylpiperazine **8**. After deprotection of **8**, the resultant free amine **9** was coupled with (*R*)-*N*-Boc-Tic-OH to give the dipeptide **10**. Reductive amination of **10** with an alkylamine afforded, after Boc-deprotection with TFA, the products **6a**,**b**. Compound **9** was also coupled with various carboxylic acids including *N*-Boc protected

Keywords: Melanocortin; MC4R; Agonists; Antagonist.
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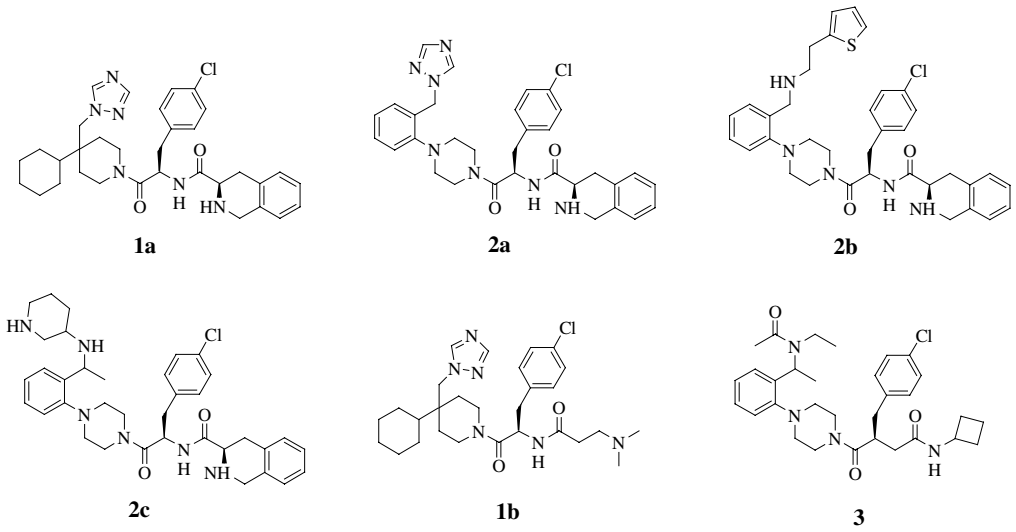
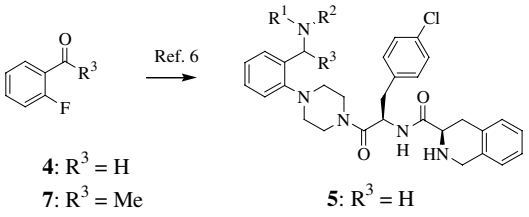


Figure 1. Some small molecule agonists of the human MC4 receptor.

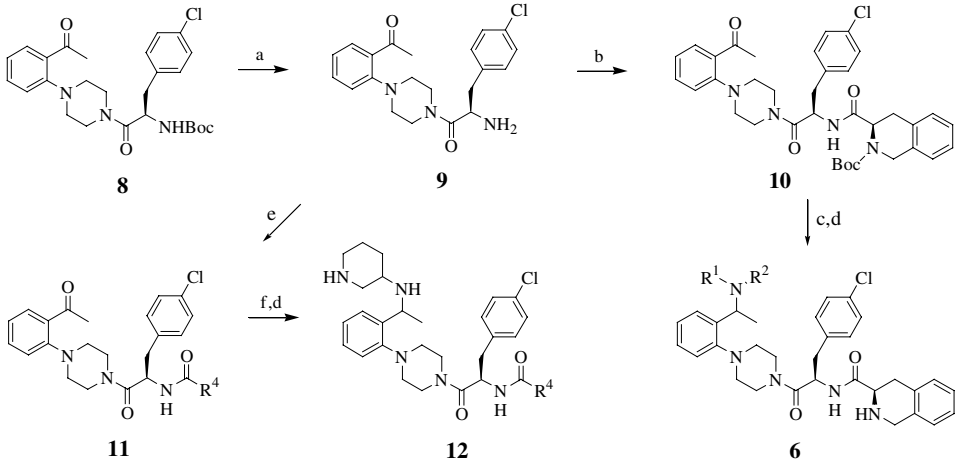


Scheme 1. Synthesis of compounds **5**.

amino acids to give the intermediates **11**, which were subjected to a reductive amination with 3-aminopiperidine, followed by Boc-deprotection where needed, to give the desired products **12** (Scheme 2). The final compounds **5**, **6**, and **12** were purified on a HPLC instrument equipped with a mass detector, and the possible diastereo-isomers of **6** and **12** were not separated by this purification method.

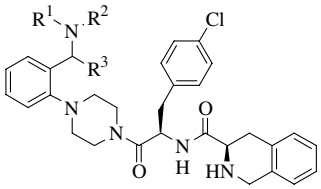
The competition binding experiments and the agonist assay were performed using HEK293 cells stably transfected with the human melanocortin-4 receptor as previously described.[7] In the binding assay [^{125}I]-NDP-MSH was used as the radiolabeled ligand. For the functional measurement, α-MSH was used to stimulate cAMP release in the antagonist assay, and as a standard for calculation of intrinsic activity in the agonist assay.

Initial data from our previous report on piperazinebenzylamines indicate that compounds with a N-alkyl group bearing a heteroatom such as nitrogen and oxygen display better agonist potency than those with a simple N-alkyl side chain. For example, the diamine **2c** (EC_{50} = 4.7 nM) is a much more potent agonist than the alkylamine **2b** (EC_{50} = 360 nM), while they possess similar K_i values of 6.4 and 11 nM, respectively. Despite the difference in potency, these two compounds display full efficacy with the intrinsic activity at the same level of α-MSH. These results prompted us to postulate that the diamine side chain of **2c** is a good mimic of the Arg-8 residue of the melanocortin peptides, which is critical for receptor activation.[8] Interestingly, while the

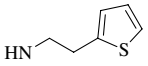
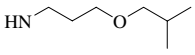
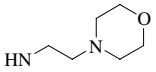
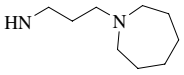
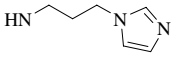
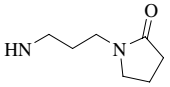
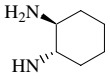
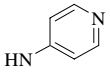
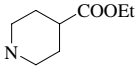
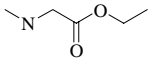
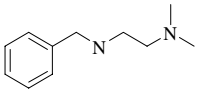
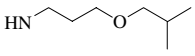
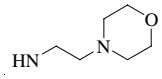


Scheme 2. Reagents: (a) TFA/CH_2Cl_2, 98%; (b) (*R*)-*N*-Boc-Tic-OH/EDC/HOBt/Et_3N; (c) R^1R^2NH/$NaBH(OAc)_3$; (d) TFA/CH_2Cl_2; (e) R^4COOH/EDC/HOBt/Et_3N; (f) 3-aminopiperidine/$NaBH(OAc)_3$.

Table 1. SAR of benzylamines **5** and 1-phenethylamines **6** at the human MC4 receptor[a]



2b,5a-m,6a-b

Compound	R^1NR^2	R^3	K_i (nM)[b]	EC_{50} (nM)[c]
2b		H	11	360
5a		H	110	520
5b		H	120	570
5c		H	160	570
5d		H	370	640
5e		H	270	360
5f		H	89	390
5g		H	36	350
5h		H	110	750
5i		H	53	360
5j		H	240	720
5k		H	78	210
5l		H	140	210
5m		H	22	550
6a		Me	41	560
6b		Me	87	230

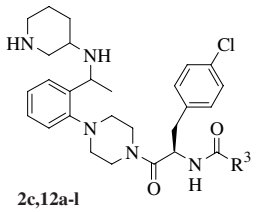
[a] The human melanocortin receptor was stably transfected in HEK 293 cells.

[b] In the binding assay, [^{125}I]NDP-MSH was used as the radiolabeled ligand. Key compounds (**5a**,**b**, **5d**, **5f**–**i**, **5k**, **5m**, **6a**,**b**, **12d**, and **12l**) were measured three times or more, and SEM of these measurements are less than 20% of the average.

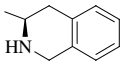
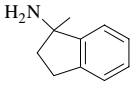
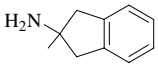
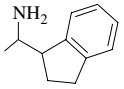
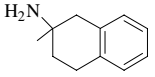
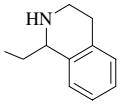
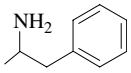
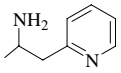
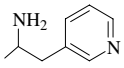
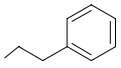
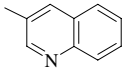
[c] All compounds displayed intrinsic activity >80% of α-MSH.

guanidine functionality is highly basic ($pK_a > 12$), and its interaction with the melanocortin receptors is largely believed to be a charge–charge attraction with a negatively charged cage formed by three acidic residues, that

Table 2. SAR of substituted acetamides **12** at the human MC4 receptor[a]



2c,12a-l

Compound	R^4	K_i (nM)	EC_{50} (nM)
2c		6.4	4.7
12a		47	(40%)
12b		24	440 (66%)
12c		120	630
12d		10	330
12e		6.3	31
12f		52	400 (70%)
12g		56	220 (66%)
12h		49	(40%)
12i		34	(22%)
12j		33	(31%)
12k		30	(15%)
12l		4.8	(17%)

[a] The human melanocortin receptor was stably transfected in HEK 293 cells.

is, Glu-90, Asp-122, and Asp-126 of the human melanocortin-4 receptor,[9] recent data suggest that the basicity of this guanidine is not essential for efficient interactions of peptide ligands, and an acylguanidine analogue, which is unlikely to be charged positively under physiological conditions, retains the agonist activity of the corresponding guanidine compound.[10] These results may indicate that the charge–charge attraction between the Arg-8 residue of a melanocortin peptide and the acidic residues of the receptor is replaceable by hydrogen-bonding interactions of a nonbasic functionality such as an acylguanidine, or the triazole of **1a** and **2a**. The acidic residues of the receptor such as Asp-122 and Asp-126, which reside very closely on transmembrane domain three of the *h*MC4R, most likely interact cooperatively with a ligand.

We first examined a set of alkylamines bearing a polar group such as amine, ether, amide, and ester, which are able to form hydrogen-bonding interaction, to explore the scope of the structure–activity relationship at the benzylamine site. Unexpectedly, while these compounds possessed moderate to good binding affinities (K_i's 22–370 nM) and high intrinsic activity in stimulation of cAMP release (>80%), none of the side chains we selected significantly improved agonist potency of these compounds (EC_{50}'s 210–750 nM) from **2b** (Table 1). Among them, the homopiperazine **5k** showed a K_i value of 78 nM and EC_{50} of 210 nM. The 4-pyridyl analogue **5g** had good binding affinity but only moderate agonist potency (K_i = 36 nM, EC_{50} = 365 nM). The diamine **5m** (K_i = 22 nM) exhibited the highest affinity of this set of compounds in Table 1. Introduction of a methyl group at the benzylic position of **5** only improved binding affinity slightly. Thus, in comparison with **5a** (K_i = 110 nM, EC_{50} = 520 nM), the α-methyl analogue **6a** possessed a K_i value of 41 nM and an EC_{50} value of 560 nM. Similarly, compound **6b** was slightly better in both binding affinity and agonist potency than the benzylamine analogue **5b**.

While the Tic-group seems to be essential for agonist activity for small molecule MC4R ligands, limited information of structure–activity relationship on this moiety has been reported. A very recent publication shows that the Tic-group of **1a** can be replaced by other functionalities. For example, the *N*,*N*-dimethyl-β-alanine piperidine **1b** is a potent agonist (EC_{50} = 40 nM).[11] Similarly, the phenylpiperazine **3** without the Tic-group has an EC_{50} value of 15 nM.[12] A series of piperizinebenzylamines with different amides including α- and β-amino amides to replace the Tic-group was synthesized and tested to explore the structure–activity relationship (**12**, Table 2). While most of these compounds possessed good binding affinity (K_i < 50 nM), their agonist potency was lower than that of the Tic-analogue **2c**. Especially, many analogues were not able to stimulate cAMP production to the maximal level of α-MSH at high doses. The 1,2,3,4-tetrahydroisoquinolin-1-ylacetyl compound **12e**, however, possessed a K_i value of 6.3 nM and an EC_{50} of 31 nM with the maximal cAMP stimulation at the same level of α-MSH (Fig. 2).

On the other hand, **12l** with a quinolin-3-ylcarbonyl moiety also exhibited high binding affinity (K_i = 4.8 nM), but stimulated very low level of cAMP release at high concentrations (maximal 17% of α-MSH at a concentration of 10 μM). Moreover, **12l** was demonstrated to be a functional antagonist. Thus, it dose-dependently inhibited α-MSH-stimulated cAMP production with an IC_{50} value of 300 nM (Fig. 3).

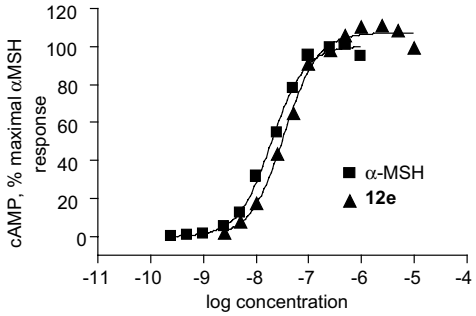


Figure 2. Dose–response curves of cAMP production stimulated by compound **12e** and α-MSH in HEK 293 cells stably transfected with the human melanocortin-4 receptor.

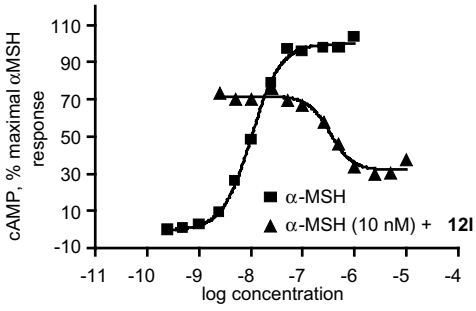


Figure 3. Dose–response curves of α-MSH-stimulated cAMP production and inhibition by compound **12l**.

Compound **12l** was also tested at the other human melanocortin subtypes and found to be very selective. Thus, **12l** possessed K_i values of 630 and 600 nM, respectively, at the *h*MC3R and the *h*MC5R, and it only showed 38% inhibition of α-MSH-stimulated cAMP production at 10 μM concentration at the *h*MC1R. Similarly, **12e** also had weak binding affinity at the human MC3 receptor (K_i = 1.9 μM).

In conclusion, we have designed and synthesized a series of piperazinebenzylamines to study the structure–activity relationship as agonists of the melanocortin-4 receptor. Attempting to mimic the functionality of the diamine side chain of **2c** by incorporating a heteroatom in the N-alkyl group to enhance the possibility of hydrogen-bonding interactions with the receptor was not very successful. However, we were able to find a replacement of the Tic-group of **2c**, thus, **12e** with the 1,2,3,4-tetrahydro-isoquinolin-1-ylacetyl moiety displayed good agonist potency (EC_{50} = 31 nM). In addition, the quinolin-3-ylcarbonyl derivative **12l** was found to be a weak partial agonist,[13] but it functioned as an antagonist (IC_{50} = 300 nM) in inhibition of α-MSH-stimulated cAMP release. These results demonstrate that the interaction of the Tic- or Tic-like group of these piperazinebenzylamine compounds with the MC4 receptor is crucial for receptor activation. All compounds in Table 1 with the Tic-group were able to fully activate the MC4 receptor, at least at a high concentration (10 μM), but many compounds without the Tic-moiety in Table 2, such as **12l**, only stimulated cAMP production to a minimal level (17% of α-MSH for **12l**).

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