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Structure–activity relationship studies on a series of cyclohexylpiperazines bearing a phanylacetamide as ligands of the human melanocortin-4 receptor

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Abstract—Synthesis and structure–activity relationship studies of a series of cyclohexylpiperazines bearing an amide side chain as ligands of the MC4 receptor are discussed. Compounds such as **11i** from this series are potent agonists (EC_{50} = 33 nM, IA = 96%).

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 MC4R agonists[2] and antagonists[3] from different chemical classes have been discovered since the report of the first potent and selective agonist cyclohexylpiperidine **1** in 2002 (Fig. 1).[4] Very recently, cyclohexylpiperazines such as **2a** and **b**, with EC_{50} values of 1.4 and 1.0 μM, respectively,[5] have been reported as MC4R agonists.[6] These data suggest that the extra 1-triazolemethyl group of **2b**, which is designed to mimic that of compound **1**, has little effect in interaction with the receptor. The phenylpiperazine **2c**, however, has an EC_{50} value of only 30 μM in the same assay, suggesting the cyclohexyl group has stronger interaction with the receptor than does the phenyl ring, and that this interaction does not require the π-electron density of an aromatic ring.

Previously we reported that benzylamines such as **2d** (EC_{50} = 4.7 nM) and **3** (K_i = 1.8 nM) are potent and selective agonists and antagonists of the MC4R,[7,8] demonstrating that a basic side chain at the *ortho*-position of the phenyl group has profound impact on potency in comparison with **2c**. Based on some initial hypotheses and in combination with computational modeling, we were able to identify a phenylacetamido side chain on the cyclohexyl ring for improved binding affinity at the MC4R.[9] For example, **4** has a K_i value of 8.8 nM. We decided to investigate whether replacement of the triazole in cyclohexylpiperazine **2b** with a phenylacetamide moiety would result in improvement of its potency as a MC4 agonist.

The synthesis of the cyclohexylpiperazines **11** is described in Scheme 1. Reduction of the nitrile **5**, obtained from the corresponding cyclohexanone and piperazine via a Strecker reaction, with $LiAlH_4$ in ether, followed by acylation with phenylacetyl chloride under basic conditions (Et_3N/CH_2Cl_2) afforded the amide **6**. Debenzylation of **6** under hydrogenolysis conditions ($HCOONH_4$/Pd–C/MeOH/reflux) gave the amine **7**, which was coupled with *N*-Boc-(4-Cl)phenylalanine (EDC/HBTU/CH_2Cl_2) to provide the intermediate **8**. Deprotection of **8** with TFA afforded **9**, which was coupled with various carboxylic acids to give the desired compound **10**. When *N*-Boc-protected amino acids were used in the coupling, further deprotection with TFA afforded the final compound **11**. The carbamate **12** was prepared by reaction of **9** with isobutyl chloroformate in the presence of triethylamine. All final compounds were purified using an HPLC system equipped with a mass detector as

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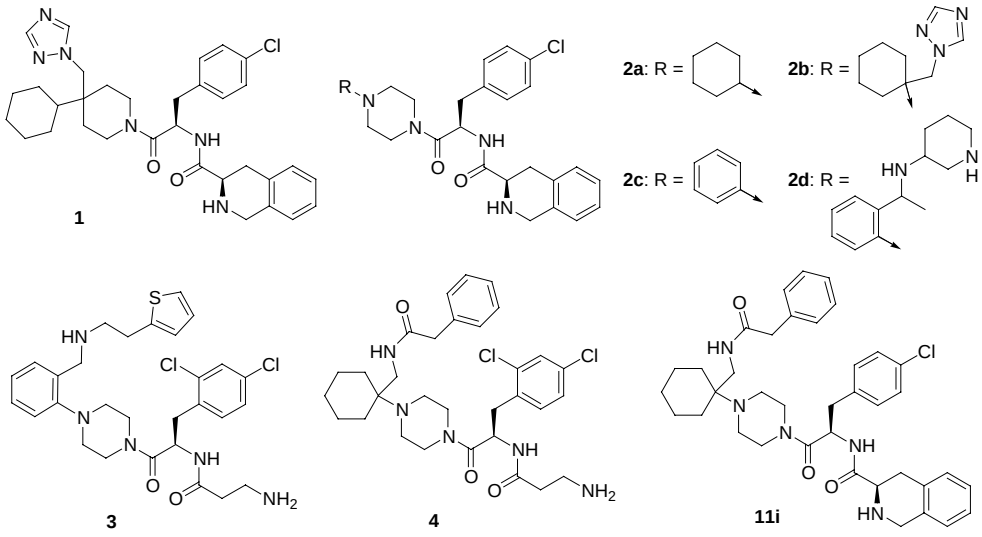
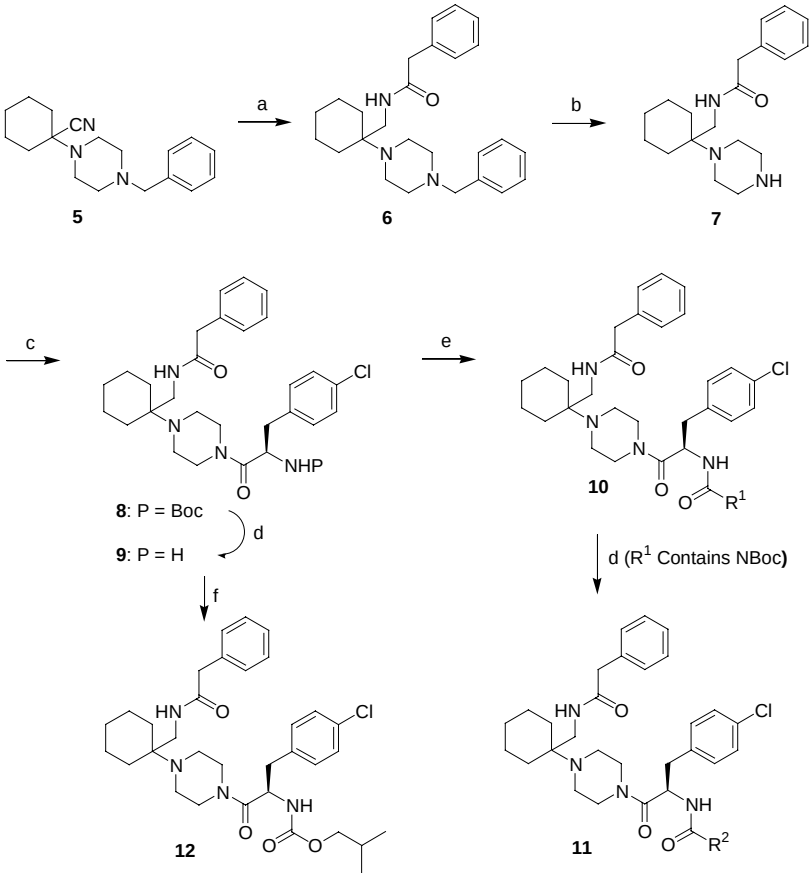


Figure 1. Some small molecule MC4R agonists and antagonists.



Scheme 1. Reagents and conditions: (a) i—$LiAlH_4/Et_2O$/rt, 0.5 h; ii—$PhCH_2COCl/Et_3N/CH_2Cl_2$, 0 °C to rt, 69%; (b) HCO_2NH_4/Pd–C/MeOH/reflux, 40 min, 100%; (c) (*R*)-*N*-Boc-(4-Cl)Phe-OH/EDC/HOBT/CH_2Cl_2/rt, 16 h, 93%; (d) TFA/CH_2Cl_2/rt, 20 min, 100%; (e) R^1COOH/EDC/HOBT/CH_2Cl_2/rt, 16 h; (f) *i*-BuOCOCl/Et_3N/CH_2Cl_2/0 °C to rt.

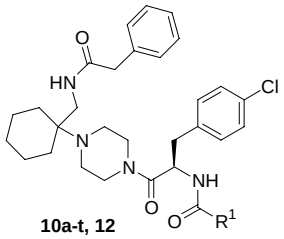
previously described, and tested in the competition binding experiments with HEK293 cells stably transfected with the human melanocortin-4 receptor, using [^{125}I]-NDP-MSH as radiolabeled ligand.[8] The agonist activity of these compounds were measured in the same cells, and α-MSH was used as a standard.[10]

N-Acyl phenylalanine derivatives with a small lipophilic R^1 group (methyl to cyclopentyl) displayed only moderate binding affinity (**10a**–**i**, K_i in the range of 110–300 nM) and poor stimulation of cAMP release at 10 μM concentration (10–30%). Larger and lipophilic groups such as 3-pentyl and neopentyl **10j**

(K_i = 1,000 nM) and **10k** (K_i = 750 nM) exhibited reduced affinity. Methoxy- and dimethylamino-methyl analogues **10l** (K_i = 170 nM) and **10m** (K_i = 100 nM) possessed similar potency in binding; however, **10m** had the ability to stimulate cAMP release to a significant level (63% of α-MSH) with an EC_{50} value of 300 nM, while **10l** exhibited poor efficacy (22%). Compounds with an aromatic group directly attached at this site showed similar binding affinity to derivatives having an aliphatic moiety, but lower cAMP stimulation (**10n–q**, K_i = 60–100 nM, IA = 2–6%). The 3-quinolinyl derivative **10t** exhibited a K_i value of 61 nM, which was one of the best binders in this subclass. Interestingly, a 3-quinolinyl analogue in the benzylamine series exhibits poor efficacy.[11] Finally, the carbamate **12** displayed moderate binding affinity and poor cAMP stimulation, similar to aliphatic analogues **10a–i**. Overall, only a basic nitrogen was noted to improve agonist activity as assessed by cAMP stimulation (**10m**) (Table 1).

Following on this MC4R agonist lead, a series of amino acid derivatives **11** was studied (Table 2). The α-amino-benzyl and phenethyl compounds (**11a–h**) had similar binding affinity and agonist potency. However, both *R*- and *S*-configured cyclic isomers (**11i** and **j**) of the aminophenethyl compound **11e** exhibited much better activity in both binding and activation. There was no

Table 1. SAR of *N*-acyl-4-(Cl)phenylalanines at *h*MC4R

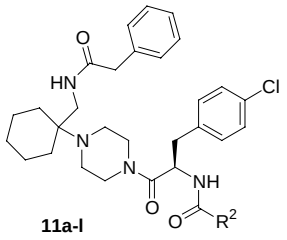


10a-t, 12

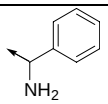
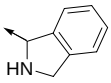
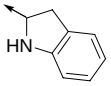
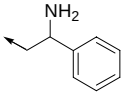
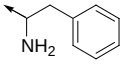
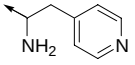
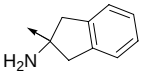
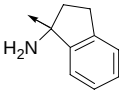
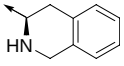
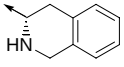
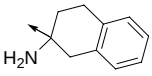
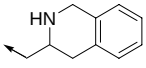
Compound	R^1	K_i (nM)[a]	Stimulation (%)[b]
10a	Me–	110	14
10b	Et–	160	12
10c	*c*-Pr–	130	11
10d	*n*-Pr–	210	18
10e	*i*-Pr–	300	23
10f	*c*-Bu–	140	14
10g	*n*-Bu–	150	22
10h	*i*-Bu–	270	30
10i	Cyclopentyl–	230	18
10j	3-Pentyl–	1000	30
10k	*t*-BuCH_2–	750	43[c]
10l	MeOCH_2–	170	22
10m	Me_2NCH_2–	100	63[d]
10n	Ph–	84	4
10o	4-FC_6H_4–	97	3
10p	2-Furanyl–	100	2
10q	3-Pyridinyl–	60	6
10r	2-F$C_6H_4CH_2$–	180	20
10s	2-ThienylCH_2–	80	12
10t	3-Quinolinyl–	61	11
12	*i*-BuO–	280	15

[a] Binding affinity at the human melanocortin-4 receptor stably transfected in HEK 293 cells, using [^{125}I]-NDP-MSH as the radiolabeled ligand. Key compounds were measured two or three times, and SEM of these measurements are less than 20% of the average.
[b] Percentage of cAMP stimulation at 10 μM concentration in comparison with E_{max} of α-MSH, otherwise indicated.
[c] % E_{max}, EC_{50} = 570 nM.
[d] % E_{max}, EC_{50} = 300 nM.

Table 2. SAR of 4-chlorophenylalanine derivatives at *h*MC4R



11a-l

Compound	R^{2a}	K_i (nM)[b]	EC_{50} (nM), E_{max}[c] (%)
11a		79	530, 26
11b		99	440, 43
11c		210	360, 22
11d		42	270, 61
11e		130	420, 62
11f		120	590, 54
11g		140	280, 49
11h		260	810, 51
11i		16	33, 96
11j		11	46, 76
11k		82	160, 87
11l		16	37, 96

[a] Mixture of stereoisomers other than indicated.
[b] Binding affinity at the human melanocortin-4 receptor stably transfected in HEK 293 cells, using [^{125}I]-NDP-MSH as the radiolabeled ligand. Key compounds were measured two or three times, and SEM of these measurements are less than 20% of the average.
[c] Percentage of cAMP stimulation in comparison with E_{max} of α-MSH.

significant difference between these two stereoisomers, although the *R*-isomer **11i** (K_i = 16 nM, EC_{50} = 33 nM, IA = 96%) had a slightly better E_{max} value. Finally, the homo-analogue of **11i** exhibited comparable properties (**11l**, K_i = 16 nM, EC_{50} = 37 nM, IA = 96%) while its isomeric tetraline **11k** displayed 4-fold reduction in both binding affinity and agonist potency. These detailed SAR confirm the tetrahydroisoquinolinecarbonyl (Tic) moiety is the optimal group at this region for several known MC4R agonists such as **1**.

Receptor models of the human MC4 receptor[12] indicate that a cavity lined by several lipophilic amino acids and two acidic residues (Asp122 and Asp126) of transmembrane-3 and -4 is unoccupied and could host a lipophilic side chain with hydrogen-bonding ability from the cyclohexane adjacent to the piperazine in **2a**.[9] Interestingly, the Tic-group, which is important for receptor activation, resides in an area near transmembrane-3, -4, and -5 based on this model, and the basic nitrogen of the Tic-group most likely interacts with the acidic residue Asp126 of the receptor. Further studies are required to understand the interaction of key residues of the MC4R with the lipophilic part of this Tic group.

In conclusion, a series of cyclohexylpiperazines bearing an amide side chain was synthesized and studied as ligands of the melanocortin-4 receptor. Compounds with an additional phenylacetamido group displayed improved potency in both binding and activation over **2a** or **b**. Structure–activity relationship studies revealed that the Tic-group is very important for receptor activation. Compound **11i** (EC_{50} = 33 nM, IA = 96%) was identified as a potent agonist of MC4R.

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