Studies on the Structure-Activity Relationship of the Basic Amine of Phenylpiperazines as Melanocortin-4 Receptor Antagonists

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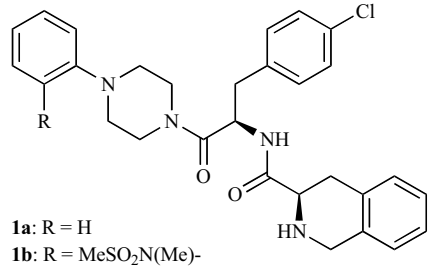
Abstract: A series of piperazinephenethylamines were synthesized to study the contribution of a basic amine to binding affinity at the melanocortin-4 receptor. Several potent compounds from this series possessed subnanomolar K_i values in a competition binding assay.

Key Words: Melanocortin-4 receptor, antagonist, piperazinephenethylamine, synthesis.

INTRODUCTION

The melanocortin-4 receptor (MC4R) is a member of the G-protein-coupled receptor (GPCR) superfamily and has been shown to play an important role in regulating feeding behavior and other biological functions [1]. Several MC4R antagonists have been reported to reverse lean body mass loss and to increase food intake in animal models of cachexia, suggesting their potential use in the treatment of cancer cachexia [2,3].

Assisted by receptor modeling, we have been able to optimize a series of phenylpiperazines by incorporating a basic benzylamine with an alkyl side-chain. Thus, while adding a methylsulfonamide at the *ortho*-position of the phenyl ring of **1a** (K_i = 4,800 nM [4]) results in a compound **1b** (K_i = 220 nM [5]) with 20-fold better affinity, a simple aminomethyl group at this position also achieves a similar result (**1c**, K_i = 380 nM) [6]. The binding affinity of **1c** is further improved by 34-fold when an alkyl side-chain is attached (**1d**, K_i = 11 nM [6]). These results indicate that, in addition to an aromatic side-chain, the basic nitrogen of the benzylamine is important in receptor binding. Receptor modeling suggests this amine may interact with an acidic residue(s) such as Asp122 of the MC4R [6,7]. While we have been able to optimize the affinity of compounds **1a-d**, they have been characterized as MC4R agonists with full efficacy and various potencies [4,5,6]. Based on the initial success in improving the binding affinity of these agonists, we synthesized several benzylamines such as **2a** and **2b** (Fig. **1**) by replacing the D-Tic-D-(4-Cl)Phe dipeptide with a β-Ala-D-(2,4-Cl)Phe moiety in our efforts to find an antagonist as a potential treatment for cachexia [8]. Resultantly, these compounds



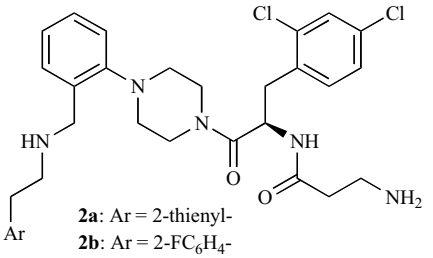


Fig. (1). Piperazinebenzylamines as MC4R agonists and antagonists.

behave as functional antagonists. For example, **2a** has K_i of 1.8 nM in binding affinity and dose-dependently inhibits α-MSH-stimulated cAMP release in cells expressing melanocortin-4 receptor. To further explore the role of this basic nitrogen, we synthesized phenethylamines to extend the amine from the phenylpiperazine template **2**. Herein, we report the synthesis and structure-activity relationship (SAR) of these compounds in their binding to the melanocortin-4 receptor.

The initial compounds for SAR studies were synthesized from the piperazinebenzaldehyde **3**. Condensation of **3** with a nitroalkane under basic conditions gave the nitro-olefines **4** (for R^1 = H, **4a**: $MeNO_2/NH_4OAc/90^oC$, 2h, 55%), which were reduced with sodium borohydride in isopropanol with silica to afford the nitro-compound **5** in good yields (**5a**, 81%). Deprotection of **5** with TFA/CH_2Cl_2 at room temperature, followed by a standard peptide coupling reaction with

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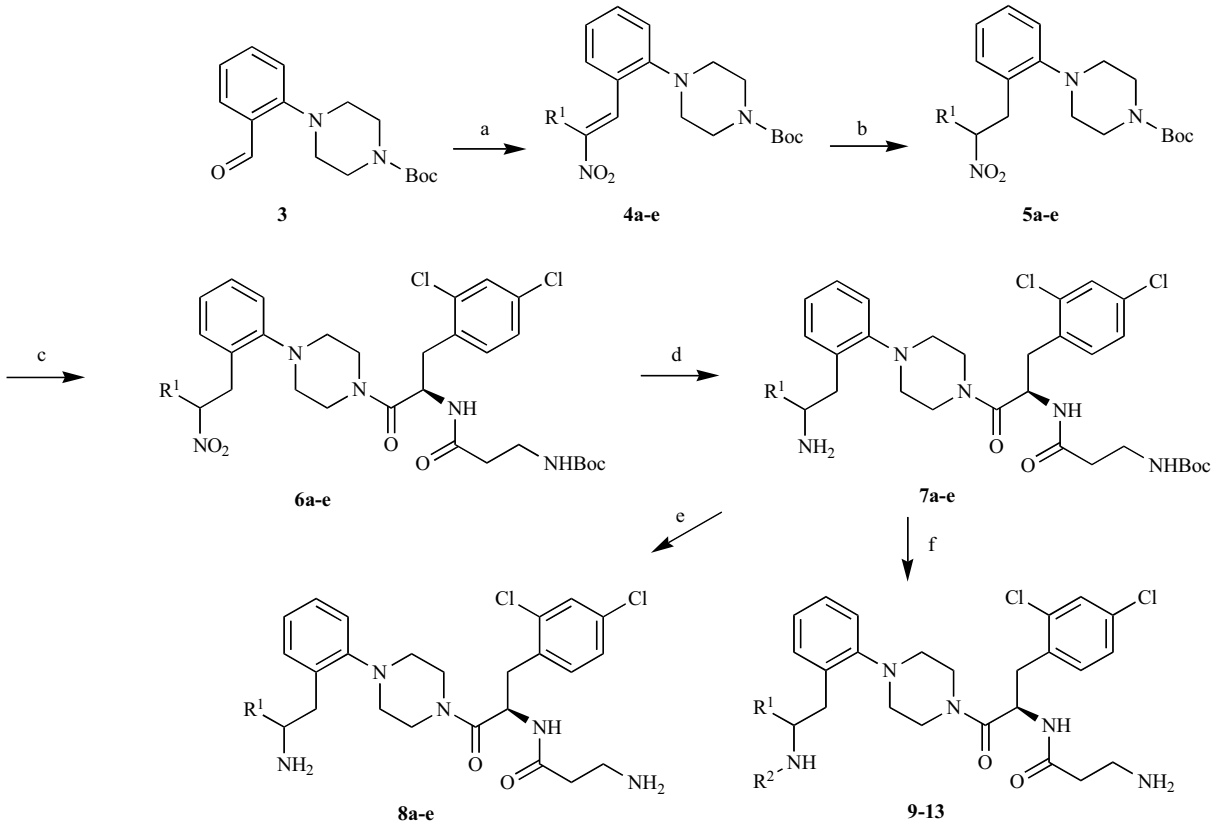


For **4-8**: **a**; R^1 = H; **b**: R^1 = Me; **c**: R^1 = Et; **d**: R^1 = nBu; **e**: R^1 = nPn

Scheme 1. (a) $R^1CH_2NO_2/NH_4OAc/90^oC$, 2h, 55% for **4a**; (b) $NaBH_4$, SiO_2, i-PrOH, 25°C, 1 h, 81% for **5a**; (c) i. TFA/CH_2Cl_2 (1:1); ii. (*R*)-*N*-(*N*-Boc-β-Ala)-(2,4-Cl)Phe-OH/HBTU/DIEA/DMF/r.t. 8h; (d) H_2 (50 psi) , Pd/C (10%w/w), EtOH, 12 h, r.t.; (e) TFA/CH_2Cl_2 (1:1); (f) Aldehyde/MeOH/$NaBH_4$ or Aldehyde/$NaBH(OAc)_3$/$ClCH_2CH_2Cl$.

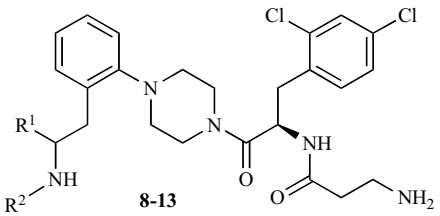
N-(*N*-Boc-β-Ala)-(2,4-Cl)Phe-OH (HBTU/EtN(iPr)$_2$/DMF), gave the amides **6**, which were subjected to catalytic reduction under hydrogen (H_2, 50 psi, Pd/C 10% w/w, EtOH) to provide the key intermediate amines **7**. Deprotection of the Boc-group of **7a-e** with TFA afforded the phenethylamines **8a-e** in good yields. Alternatively, **7** were subject to reductive alkylation with various aldehydes to give the final piperazinephenethylamine derivatives **9-13** (Scheme **1**) whose binding affinities on the human melanocortin-4 receptor were measured (Table **1**) using a binding assay as previously reported [9].

The 2-(2-thienyl)methylamine **9c** (K_i = 4.7 nM) was slightly less potent than the benzylamine **2a** (K_i = 1.8 nM) which has a 2-(2-thienyl)ethyl side-chain. Between these two regioisomers, the benzylamine **2a** was slightly favored. The primary phenethylamine **8a** displayed moderate binding affinity (K_i = 130 nM), while the *N*-benzyl analog **9a** was almost 30-fold better, suggesting the additional aromatic ring of the benzyl group is important. In comparison with the 2-fluorophenyl compound **9b**, the 2-thiazolyl **9d** and the 2-pyridyl **9e** analogs were less potent.

Introducing a methyl group at the carbon attached to the nitrogen of **8a** improved its potency. Thus, the propylamine **8b** had 3-fold better affinity than the ethylamine **8a**. Similarly, the benzyl **10o** and the 2-thienylmethyl **10r** (K_i = 1 nM) were about 3- and 5-fold better than the corresponding analogs **9a** and **9c**, respectively. The 2-(2-thienyl)ethyl **10a** (K_i = 58 nM) was substantially less potent than **10r**, suggesting the distance between the two aromatic rings is important. The phenethyl derivatives **10b-d** had K_i values of 6-11 nM, which were less potent than the benzylamine **10o**. While the *N*-alkyl amines **10e-h** exhibited moderate binding affinity (K_i = 23-76 nM), the corresponding *N*-alkoxyalkyl compounds **10i-m** displayed K_i values of 10-20 nM. Similarly, the 2-aminoethyl analog **10n** had a K_i of 20 nM. Among the arylmethyl derivatives **10o-u**, the 2-furan **10q** had a K_i value of 1.3 nM, while the 2-pyridyl **10u** (K_i = 33 nM) was much less potent.

The butylamine **8c** (K_i = 27 nM) was 2-fold better in binding affinity than the propyl analog **8b**, suggesting an increased lipophilic interaction from **8c**. However, *N*-Alkylation (**11a-d**) only improved potency by 3- to 5-fold. In comparison, **11a** displayed a similar K_i value to the propylamine **10j**, while **11c** was less potent than the ethylamine **9b**, suggesting a saturation of lipophilicity in contributing to binding affinity. Similar results were obtained for the hexylamine **8d** and its *N*-alkyl analogs **12a-e**. Finally, the heptylamine **8e** showed slightly lower potency than the hexyl **8d** and the propyl **8c**. Only the 2-methoxyethyl **13a** showed a 4-fold increase in binding affinity compared to its parent **8e**,

Table 1. Binding Affinity of Phenethylamines 8-13 at the *h*MC4R[a]



8-13

Compound	R^1	R^2	K_i (nM)[b]
8a	H	H	130
9a	H	Bn	4.9
9b	H	2-$FC_6H_4CH_2$-	2.0
9c	H	2-thienylCH_2-	4.7
9d	H	2-thiazolylCH_2-	13
9e	H	2-PyCH_2-	18
8b	Me	H	52
10a	Me	2-thienylCH_2CH_2-	58
10b	Me	2-$MeOC_6H_4CH_2CH_2$-	8.4
10c	Me	2-$FC_6H_4CH_2CH_2$-	11
10d	Me	2,5-$MeOC_6H_3CH_2CH_2$-	6.3
10e	Me	cPr-	26
10f	Me	cPn-	23
10g	Me	cHx-	75
10h	Me	3-Pn-	26
10i	Me	$MeOCH_2CH_2$-	17
10j	Me	$EtOCH_2CH_2$-	12
10k	Me	$MeOCH_2CH_2CH_2$-	23
10l	Me	$MeOCH_2CH(Me)$-	11
10m	Me	$MeOCH_2CH(Et)$-	19
10n	Me	$NH_2CH_2CH_2$-	20
10o	Me	Bn-	1.6
10p	Me	1-NaphthylCH_2-	7.4
10q	Me	2-furanylCH_2-	1.3
10r	Me	2-thienylCH_2-	1.0
10s	Me	2-PyCH_2-	6.7
10t	Me	3-PyCH_2-	5.2

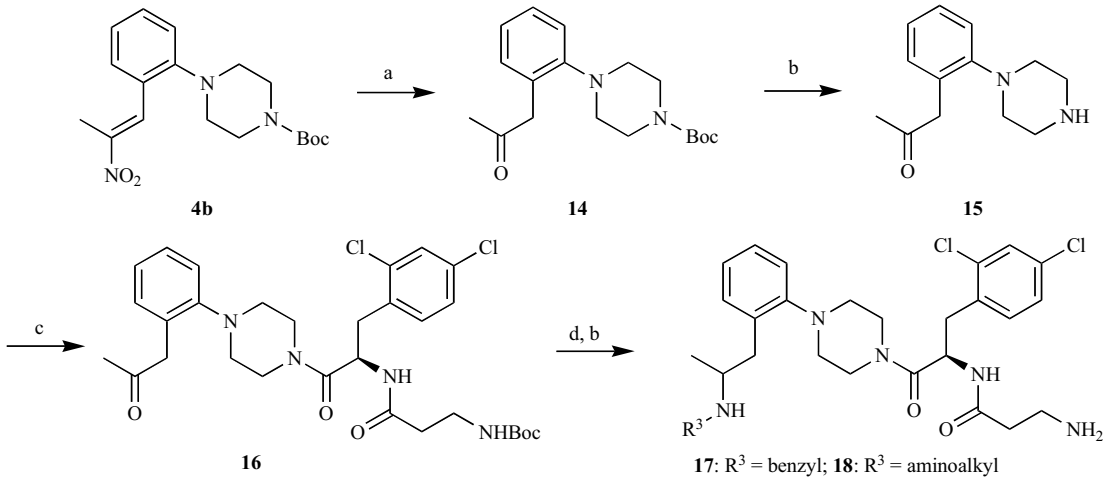
(Table 1. Contd….)

Compound	R^1	R^2	K_i (nM)[b]
10u	Me	4-$PyCH_2$-	33
8c	Et	H	27
11a	Et	$EtOCH_2CH_2$-	9.2
11b	Et	2-$MeOC_6H_4CH_2$-	12
11c	Et	2-$FC_6H_4CH_2$-	6.3
11d	Et	2,4-$FC_6H_4CH_2$-	5.5
8d	nBu	H	27
12a	nBu	$MeNHCH_2CH_2$-	9.2
12b	nBu	$MeOCH_2CH_2$-	8.1
12c	nBu	$MeOCH_2CH$(Et)-	23
12d	nBu	2-$FC_6H_4CH_2$-	41
12e	nBu	2,4-$FC_6H_3CH_2$-	46
8e	nPn	H	39
13a	nPn	$MeOCH_2CH_2$-	10
13b	nPn	$MeOCH_2CH$(Et)-	32
13c	nPn	2-$MeOC_6H_4CH_2$-	64
13d	nPn	2-$FC_6H_4CH_2$-	94
13e	nPn	2,4-$FC_6H_4CH_2$-	140

(a) Binding experiments were performed on HEK293 cells expressing human MC4 receptors using [^{125}I]-NDP-MSH as the radiolabeled ligand. (b) Data are average of two or more independent measurements.

while the other derivatives (**13c-e**) displayed decreased potency.

Following the initial success of the SAR study on different substituted ethylamine side chains, **10o** and **10n** were selected as lead compounds for detailed investigation based on their differences in potency and physicochemical properties. A series of substituted *N*-benzyl and *N*-aminoalkyl phenylpropylamines **17** and **18** were synthesized as shown in Scheme **2**. Thus, the nitro-olefin **4b** was partially reduced and then hydrolyzed to the corresponding ketone **14** (Raney-Ni/Na_2HPO_4/EtOH/50°C, 2h, 61%), which was deprotected with TFA/CH_2Cl_2 at room temperature for 20 minutes to give the amine **15**. **15** was converted to **16** by a standard peptide coupling reaction with *N*-(*N*-Boc-β-ala)-(2,4-Cl)Phe-OH (HBTU/EtN(iPr)$_2$/DMF/r.t., 8h, 80%), followed by reductive



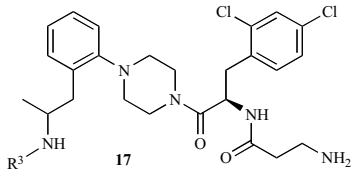
Scheme 2. (a) Ra-Ni/NH_4OOCH/NaH_2PO_4/H_2O/50°C, 2h, 61%; (b) TFA/CH_2Cl_2/r.t., 20 min., quant.; (c) (*R*)-*N*-(*N*-Boc-β-Ala)-(2,4-Cl)Phe-OH/HBTU/DIEA/DMF/r.t., 8h, 80%; (d) RNH_2/$NaBH(OAc)_3$/$ClCH_2CH_2Cl$/r.t., 8h.

amination with various benzylamines or *N*-Boc-aminoalkylamines to afford the final products **17** and **18** after the deprotection of the Boc group with trifluoacetic acid [10].

As summarized in Table **2**, the survey on the 2-substitution of the phenyl ring (**17a-h**) revealed that the 2-fluoro analog was the most potent compound (**17b**, K_i = 0.7 nM), while the 2-trifluoromethyl derivative **17f** was the least active. 3-Substitution (**17i-m**) had little impact on potency, except for the 3-nitro compound **17m** (K_i = 13 nM). Incorporating a functional group at the 4-position generally reduced binding affinity (**17n-r**) compared to the unsubstituted analog **10o**. Among the disubstituted benzyl analogs **17s-v**, the 2,6-difluoro compound **17t** (K_i = 0.7 nM) also possessed subnanomolar binding affinity. Overall, this study only generated a couple of analogs which were slightly more potent than the unsubstituted benzylamine **10o**.

Interestingly the *N*-aminoalkyl compounds **18a-q** (Table **3**) showed similar binding affinity regardless of the position and the size of the amino group.

Table 2. Binding Affinity of Substituted Benzylamines 17 at *h*MC4R[a]

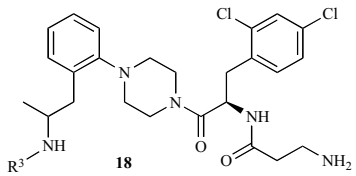


17

Compound	R^3	K_i (nM)[b]
10o	$C_6H_5CH_2$-	1.6
17a	2-$MeC_6H_4CH_2$-	2.9
17b	2-$FC_6H_4CH_2$-	0.7
17c	2-$MeOC_6H_4CH_2$-	3.6
17d	2-$ClC_6H_4CH_2$-	1.8
17e	2-$BrC_6H_4CH_2$-	6.1
17e	2-$NO_2C_6H_4CH_2$-	3.8
17f	2-$CF_3C_6H_4CH_2$-	15
17g	2-$NH_2C_6H_4CH_2$-	3.0
17h	2-$EtOC_6H_4CH_2$-	2.5
17i	3-$MeC_6H_4CH_2$-	2.5
17j	3-$MeOC_6H_4CH_2$-	2.4
17k	3-$ClC_6H_4CH_2$-	5.4
17l	3-$BrC_6H_4CH_2$-	4.8
17m	3-$NO_2C_6H_4CH_2$-	13
17n	4-$FC_6H_4CH_2$-	13
17o	4-$MeOC_6H_4CH_2$-	12
17p	4-$ClC_6H_4CH_2$-	6.5
17q	4-$BrC_6H_4CH_2$-	6.2
17r	4-$NO_2C_6H_4CH_2$-	13
17s	3,4-$CH_2O_2C_6H_4CH_2$-	4.9
17t	2,6-$FC_6H_4CH_2$-	0.7
17u	2,6-$MeOC_6H_4CH_2$-	4.2
17v	2,6-$ClC_6H_4CH_2$-	5.0

(a) Binding experiments were performed on HEK293 cells expressing human MC4 receptor using [^{125}I]-NDP-MSH as the radiolabeled ligand. (b) Data are average of two or more independent measurements.

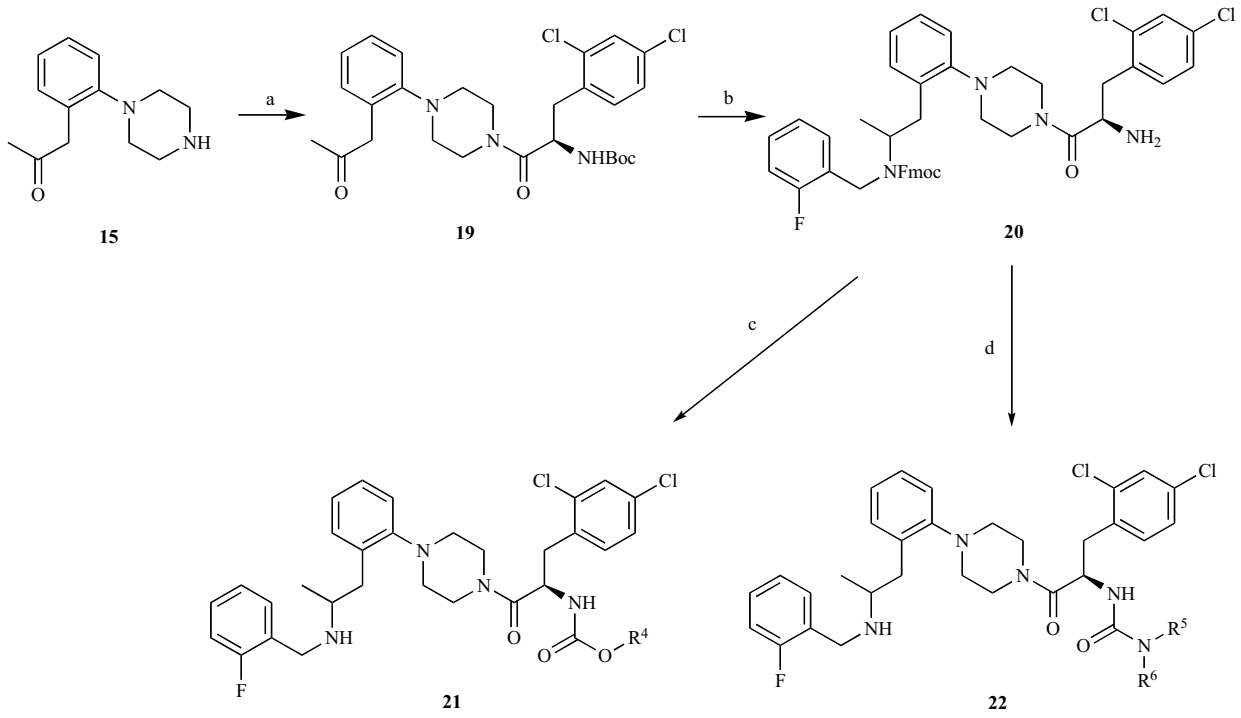
Table 3. Binding Affinity of the Aminoalkyl Derivatives 18 at *h*MC4R[a]



18

Compound	R^3	K_i (nM)[b]
10n	$NH_2CH_2CH_2$-	20
18a	$NH_2C(Me)_2CH_2$-	52
18b	Pyrrolidin-3-yl-	13
18c	1-Benzylpyrrolidin-3-yl-	13
18d	Piperidin-3-yl-	20
18e	*cis*-2-Aminocyclohexyl-	23
18f	*trans*-2-Aminocyclohexyl-	8.2
18g	$NH_2CH_2CH_2CH_2$-	13
18h	$MeNHCH_2CH_2CH_2$-	14
18i	$Me_2NCH_2CH_2CH_2$-	17
18j	Morpholin-1-yl$(CH_2)_3$-	24
18k	2-Methylpiperidin-1-yl$(CH_2)_3$-	19
18l	Piperidin-4-yl-	23
18m	1-Benzylpiperidin-4-yl-	31
18n	Piperidin-2-yl(CH2)2-	16
18o	$NH_2(CH_2)_4$-	10
18p	Piperidin-4-ylCH_2-	14
18q	$NH_2(CH_2)_5$-	11

(a) Binding experiments were performed on HEK293 cells expressing human MC4 receptors using [^{125}I]-NDP-MSH as the radiolabeled ligand. (b) Data are average of two or more independent measurements.



Scheme 3. (a) (*R*)-*N*-Boc-(2,4-Cl)Phe-OH/HBTU/DIEA/DNF/r.t., 8h, 55%; (b) i. 2-$FC_6H_4CH_2NH_2$/$NaBH(OAc)_3$/$ClCH_2CH_2Cl$/HOAc/r.t., 8h; ii. FmocCl/Et_3N/THF/0°C to r.t., 1h; iii. TFA/CH_2Cl_2/r.t., 20 min. 66%; (c) i. $COCl_2$/$NaHCO_3$/CH_2Cl_2/H_2O/0°C, 0.5h; ii. R^4OH/Et_3N/THF/r.t., 8h; iii. Et_2NH/MeCN/r.t., 1h; (d) i. $COCl_2$/$NaHCO_3$/CH_2Cl_2/H_2O/0°C, 0.5h; ii. R^5R^6NH/Et_3N/THF/r.t., 8h; iii. Et_2NH/MeCN/r.t., 1h.

Based on the above results, the 2-fluorobenzyl side chain was selected for further optimization at the amide group on the 2,4-dichlorophenylalanine. Various carbamates **21** and ureas **22** were synthesized as shown in Scheme **3**. Phenylpiperazine **15** was converted to the amide **19** by a coupling reaction with *N*-Boc-(2,4-Cl)Phe-OH. Reductive alkylation of **19** with 2-fluorobenzylamine ($NaBH(OAc)_3$/$ClCH_2CH_2$ Cl/HOAc, r.t.) provided the target amine which was protected with a Fmoc group, followed by Boc-deprotection to afford **20**. Reaction of **20** with various alkyl chloroformates gave the carbamates **21** after deprotection of the Fmoc group with diethylamine in acetonitrile. Alternatively, **21** were synthesized by the reaction of the amine **20** with phosgene in dichloromethane, followed by an alcohol under basic conditions, and subsequent deprotection. The ureas **22** were obtained using the same reaction scheme while the alcohol was replaced with an amine.

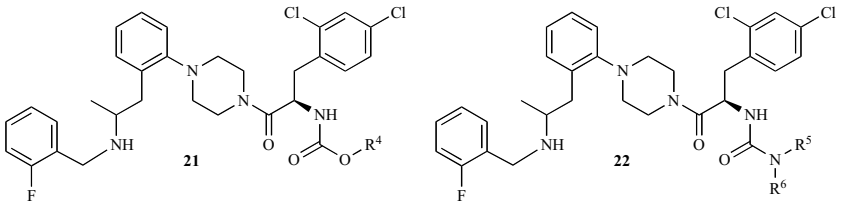
The carbamates **21a-f** possessed single digit nanomolar affinity, while the larger alkyl analogs **21g-n** displayed slightly lower potency than the small alkyl analogs **21a-c** (Table **4**). Among them, the sec-butyl compound **21f** showed a K_i of 0.7 nM, which was 10-fold better than its n-Bu analog **21e**. The three ureas **22a-c** possessed high potency (K_i = 0.6-2.1 nM), and the *N*'-isopropylurea **22a** had 5-fold better binding affinity than the O-isopropylcarbamate **21d**.

Several potent MC4R ligands were tested for selectivity over other subtypes of melanocortin receptors and were found to be highly selective for MC4R (Table **5**). For example, compound **17t** had K_i values of 820 and 360 nM at the MC3 and MC5 receptors, respectively, and only displayed 40% inhibition at a 10 μM concentration at the MC1 receptor but had a K_i of 0.7 nM at the MC4 receptor. These compounds also dose-dependently inhibited α-MSH-stimulated cAMP production in HEK293 cells stably expressing the human melanocortin-4 receptor. For example, **17t** possessed an IC_{50} value of 170 nM in this assay.

The pharmacokinetic profile of **9b** was assessed in rats (N = 3). This compound exhibited limited plasma exposure when given orally (<1% at 10 mg/kg dose). This was most likely caused by poor intestinal absorption that could be associated with the dibasic structure of this compound, although it had moderate hydrophilicity (measured logD value of 1.5 at pH 7.4). After an intravenous administration at 5 mg/kg, this compound displayed a plasma clearance of 26 mL/min.kg, a high volume of distribution of 14 L/kg, and a long half-life of 7.5 h in this species. At the 1-hour time point, the brain concentration of **9b** was 278 ng/g, which was calculated to be 120% of the plasma concentration (256 ng/mL) at the same time point, indicating that **9b** was able to penetrate into the brain.

In summary, a series of substituted phenethylamines were synthesized and studied for their structure-activity relationships at the human melanocortin-4 receptor. With the combination of a benzyl side chain, this series of compounds possessed high binding affinity and were very selective against other melanocortin receptor subtypes.

Table 4. Binding Affinity of Carbamates 21 and Ureas 22 at *h*MC4R[a]



Compound	R^4O or R^5R^6N	K_i (nM)[b]
10o		1.6
21a	EtO-	3.0
21b	FCH_2CH_2O-	2.1
21c	nPrO-	4.6
21d	iPrO	4.2
21e	nBuO-	7.0
21f	sBuO-	0.7
21g	iBuO-	12
21h	$cPrCH_2O$-	5.6
21i	$cBuCH_2O$-	17
21j	$cPnCH_2O$-	21
21k	$cHxCH_2O$-	22
21l	cPnO-	11
21m	cHxO-	13
21n	BnO-	11
22a	iPrNH-	0.9
22b	cPnNH-	2.1
22c	Et_2N-	0.6

(a) Binding experiments were performed on HEK293 cells expressing human MC4 receptor using [^{125}I]-NDP-MSH as the radiolabeled ligand. (b) Data are average of two or more independent measurements.

Table 5. Selectivity and Functional Profiles at the Human Melanocortin Receptor Subtypes

Compound	MC1[a]	MC3	MC5	MC4	IC_{50}(nM)[b]
9a	(41%)	1,100	430	4.9	430
9b	(43%)	1,000	310	2.0	690
9d	(50%)	1,400	610	13	710
9e	(44%)	2,100	760	18	660
10i	(50%)	1,700	970	19	330
11a	(56%)	1,600	1,100	9.2	380
12b	(32%)	2,200	840	8.1	840
17b	(54%)	500	260	0.7	140
17t	(40%)	820	360	0.7	170
21a	(14%)	810	236	3.0	550
22a	(20%)	250	92	0.9	270
22c	(32%)	430	130	0.6	390

(a) Percentage of inhibition at 10 μM concentration. (b) Dose-response inhibition of α-MSH-stimulated cAMP production at the human MC4 receptor.

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