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Optimization of piperazinebenzylamines with a *N*-(1-methoxy-2-propyl) side chain as potent and selective antagonists of the human melanocortin-4 receptor

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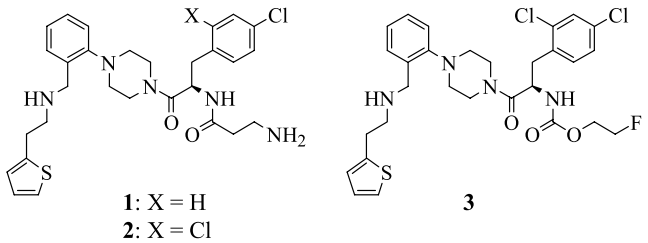
Abstract—Piperazinebenzylamines bearing a small *N*-(1-methoxy-2-propyl) side chain were found to be potent and selective antagonists of the human melanocortin-4 (MC4) receptor. Compound **7b**, having K_i values of 6.9 and 2800 nM at the human MC4 and MC3 receptors, respectively, has moderate oral bioavailability in mice, which is improved relative to the arylethyl analogues.

1. Introduction

The melanocortin-4 receptor (MC4R) is known to be a major mediator for feeding behavior, metabolism and energy homeostasis, and non-selective peptide antagonists such as SHU9119 and AgRP have been demonstrated, after central administration, to have a profound effect in stimulating food-intake in rodent.[1] Importantly, recent studies have shown that MC4R antagonists prevent tumor-bearing mice from weight loss.[2] Thus, potent and selective MC4R antagonists[3] may have potential utility in the treatment of cachexia.[4] We recently reported a series of substituted piperazinebenzylamines exemplified by **1**–**3** as potent and selective MC4R antagonists (Fig. 1).[5] For example, **2** possesses a K_i value of 1.8 nM in competitive binding with radiolabeled NDP-MSH and a pA_2 value of 7.9 in the inhibition of α-MSH-stimulated cAMP production. It alone does not stimulate cAMP release in cells expressing the human MC4R. We also demonstrated that the MC4R-selective antagonist **3** (K_i = 3.2 and 950 nM, respectively, at MC4R and MC3R) stimulates food intake in satiated mice when given by intracerebroventricular administration. Here, we report our efforts to identify potent and selective MC4R antagonist derivatives with improved metabolic stability and suitable physicochemical properties for possible peripheral administration.

The metabolic stabilities of **2** and **3** were initially determined in human liver microsomes in vitro. While **2** exhibits good stability (CL_{int} = 23 ml/min kg), **3** (CL_{int} = 870 ml/min kg) has high clearance in this assay. Following oral administration (10 mg/kg) in rats, plasma concentrations of **2** were low due to poor absorp-



1: X = H
2: X = Cl

3

Figure 1.

Keywords: Melanocortin-4; Antagonist; Piperazinebenzylamine; Synthesis.
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tion, probably associated with its high hydrophilicity (log D = 1.1) and dibasic structure. Incubation of **3** or its close analogues with liver microsomes resulted in rapid degradation, which appears to be related to the thi enylethylamino group. We postulated that the large and flexible nature of this side chain of **3** may contribute at least in part to the poor metabolic stability of lipophilic derivatives (calculated log D value of **3** was 4.0).[6]

4-Chlorophenylalanine derivatives of **4** were synthesized in a manner similar to that used for the preparation of **3**.[5a] In brief, piperazinebenzaldehyde **8**, after trifluoroacetic acid (TFA) deprotection, was coupled with *N*-Boc-*R*-(4-chlorophenyl)alanine under standard coupling conditions to give **9**. Reductive amination of **9** with a set of alkylamines in the presence of sodium triacetoxyborohydride afforded the desired products **4a**–**f** (Scheme 1).

Alternatively, the piperazinebenzaldehyde **8** was subjected to reductive amination with 1-methoxy-2-propylamine using sodium triacetoxyborohydride, followed by protection of the resulting secondary amine with an Fmoc group to give the protected diamine intermediate **10.** Selective deprotection with TFA in dichloromethane, followed by peptide coupling with *N*-Boc-2,4-dichlorophenylalanine using a standard protocol, gave, after TFA deprotection, the amide **11**. Reaction of **11** with alkyl chloroformates under basic conditions, followed by Fmoc-deprotection with diethylamine, provided carbamates **5a**–**d**. Similarly, reaction of **11** with isocyanates in dichloromethane, and then with diethylamine provided the ureas **6a**–**e**. Alternatively, the ureas **6f**–**h** were obtained from the reaction of **11** with phosgene in toluene, followed by the addition of alkylamines. Finally, coupling of **11** with various carboxylic acids gave the amides **7a**–**i** after Fmoc deprotection. Final products were purified by a HPLC equipped with a mass detector as previously described.[5b] Compounds were tested in the competitive binding experiments using [^{125}I]-NDP-MSH as the radiolabeled ligand and in the antagonist assay in HEK293 cells stably transfected with the human melanocortin-3 and -4 receptors as previously described.[7]

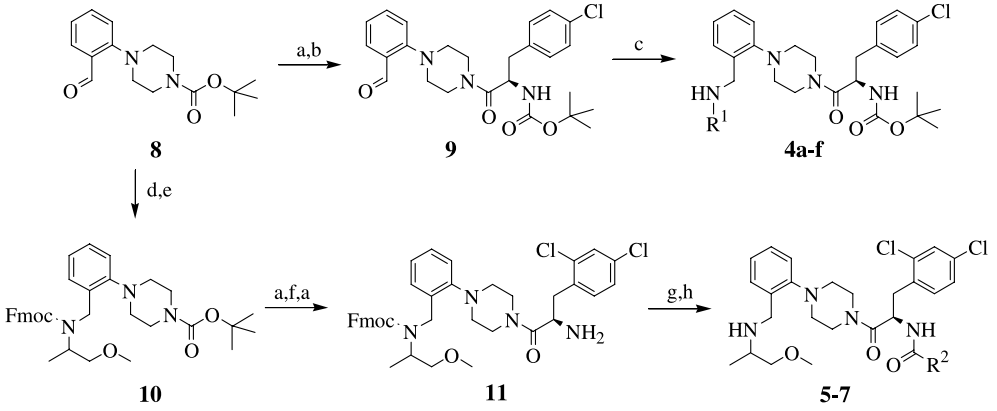
Previous structure–activity studies on the *N*-side chain of the benzylamines have shown that a diamine such as 3-aminopiperazine provides compounds with high binding affinity.[8] However, compounds bearing a diamine side chain possess high residual agonistic activity. Alternatively, 2-thienylethylamine was found to be a side chain at the basic nitrogen for compounds with high binding affinity but low cAMP stimulation. On the basis of these early results, a study was conducted to find a replacement for the lipophilic and flexible 2-thienylethyl moiety of **1**–**3**. In addition to small alkyls such as the cyclopropyl group, a set of alkoxyalkyls were evaluated because of their lower lipophilicity, which led to the identification of potent analogue **4a** (K_i = 60 nM) (Table 1). The non-branched methoxyethyl analogue **4b** (K_i = 190 nM) displayed a 3-fold reduction in binding. Larger isopropoxyethyl (**4c**) and methoxy-2-butyl (**4e**) derivatives were slightly less potent. The additional methoxy group of **4d** (K_i = 280 nM) resulted in further loss of potency.

We have previously shown that the 2,4-dichlorophenylalanine derivative **2** is about 10-times more potent than the monochloro analogue **1** (K_i = 21 nM, IC_{50} = 90 nM).[5] As expected, replacing the 4-chlorophenylalanine moiety of **4a** with a 2,4-dichloro analogue increased the binding affinity by 10-fold (**5a**, K_i = 5.4 nM) (Table 2). To further optimize this compound, a number of carbamates, ureas, and amides were prepared in the 1-methoxy-2-propylamine series. As shown in Table 2,

Table 1. SAR of *N*-alkoxyalkyl benzylamines **4a**–**f** at MC4R

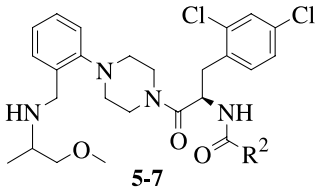
Compound	R^1	K_i (nM)[a]
4a	$CH(Me)CH_2OMe$	60
4b	CH_2CH_2OMe	190
4c	$CH_2CH_2{}^iOPr$	110
4d	$CH_2CH(OMe)_2$	280
4e	$CH(Et)CH_2OMe$	120
4f	*S*-$CH(Bn)CH_2OMe$	210

[a] Binding affinity at the human melanocortin-4 receptor (MC4R) stably transfected in HEK 293 cells, using [^{125}I]-NDP-MSH as the radiolabeled ligand.



Scheme 1. Reagents and conditions: (a) TFA/CH_2Cl_2; (b) *N*-Boc-(*R*)-(4-Cl)Phe-OH/EDC/HOBt/DCM/rt, 80%; (c) R^1NH_2/$NaBH(OAc)_3$/DCM/rt; (d) $CH_3CH(NH_2)CH_2OCH_3$/$NaBH(OAc)_3$/DCM; (e) Fmoc-Cl, 82%; (f) *N*-Boc-(*R*)-(2,4-Cl)Phe-OH/EDC/HOBt/DCM, 80%; (g) ROCOCl/Et_3N/CH_2Cl_2/rt, or RNC/ CH_2Cl_2/rt, or $COCl_2$/THF/rt then RNH_2, or R^2COOH/EDC/HOBt/DCM; (h) Et_2NH/DCM.

Table 2. Binding affinity (K_i, nM) of compounds **5**–**7** at MC3R and MC4R[a]



5-7

Compound	R^2	MC4R[b]	MC3R
5a	O^{t}Bu	5.4	2800
5b	O^{i}Bu	7.7	2600
5c	OEt	17	—
5d	OMe	19	—
6a	NH^{i}Pr	4.9	2600
6b	NH^{s}Bu	4.8	2900
6d	NHPh	5.5	6600
6e	$NHCH_2CH_2OH$	4.7	1400
6e	NHBn	3.5	1600
6f	1-Piperidinyl	5.3	3000
6g	1-Pyrrolidinyl	4.8	3100
6h	NMe_2	7.2	3100
7a	H	27	—
7b	Et	6.9	2800
7c	^{n}Bu	5.3	3700
7d	Cyclohexyl	3.1	3700
7e	CH_2-2-thienyl	2.2	1900
7f	2-Thienyl	1.2	890
7g	$CH_2CH_2NH_2$	8.8	4900
R-**7g**	$CH_2CH_2NH_2$	5.6	3400
S-**7g**	$CH_2CH_2NH_2$	88	—
2		1.8	640

[a] Binding affinity at the human melanocortin-4 (MC4) or melanocortin-3 (MC3) receptor stably transfected in HEK 293 cells, using [^{125}I]-NDP-MSH as the radiolabeled ligand.

[b] Data are average of three or more independent measurements and SEM is <25% of the average.

carbamates with a smaller alkyl group exhibited slightly reduced potency (**5c**–**d**) from the *tert*- and *iso*-butyl analogues **5a**–**b**. Ureas **6a**–**g** possessed K_i values of 3.5–7.2 nM regardless of the size of the *N*-alkyl group. Various amides **7a**–**g** also displayed high binding affinity. Among them, the formyl **7a** (K_i = 27 nM) was the least potent, and the 2-thienyl analogue **7f** had the best K_i value of 1.2 nM, suggesting a lipophilic amide is preferred. The compound derived from β-alanine **7g** exhibited a K_i of 8.8 nM, which had similar binding affinity to the des-amino analogue **7b** (K_i = 6.9 nM).

Next, we determined the potency of the individual diastereomer of *N*-(1-methoxy-2-propyl) **7g**. The two diastereoisomers (*R*-**7g** and *S*-**7g**) were separated using HPLC. The stereochemistry of *S*-**7g** was established by chemical synthesis from commercially available *S*-1-methoxy-2-propylamine using the procedure described in Scheme 1. The *R*-isomer *R*-**7g** possessed a K_i value of 5.6 nM, which was much better than the corresponding *S*-**7g** (K_i = 88 nM). In comparison with the more lipophilic 2-thienylethyl analogue **2**, *R*-**7g** is only 3-fold less potent.

Since the MC3 receptor is also centrally localized and may also play a role in the control of food intake and

Table 3. Binding affinity (K_i, nM) at the melanocortin receptor subtypes of selected compounds[a]

Compound	MC1R	MC3R	MC4R	MC5R	IC_{50}(nM)[d]
6a	—	2600	4.9	1400	ND[c]
6b	—	2900	4.8	800	ND[c]
6e	(9%)[b]	1400	4.7	620	150
6g	(13%)[b]	3100	4.8	1100	220
7b	(16%)[b]	2800	6.9	(49%)[b]	170
7f	(36%)[b]	890	1.2	400	180
7g	200	4900	8.8	580	120

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

[b] Percentage of inhibition at 10 μM concentration.

[c] ND, not determined.

[d] Inhibition of α-MSH-stimulated cAMP production in CHO cells expressing the human melanocortin-4 receptor.

metabolism, we tested all of the potent MC4R compounds in the MC3R binding assay. None of these compounds possessed high binding affinity at this receptor (K_i > 900 nM, Table 2), demonstrating high MC4R selectivity of these compounds. For example, **7b** displayed 400-fold selectivity with K_i values of 6.9 and 2800 nM, respectively, at MC4R and MC3R. High selectivity was also demonstrated at the other melanocortin receptor subtypes for compounds **6a**, **b**, **e**, **g**, **7b**, **f**, and **g** (Table 3).

All compounds derived from 2,4-dichlorophenylalanine were unable to stimulate significant levels of cAMP accumulation at 10 μM concentration (<3% of α-MSH maximal levels, data not shown) in cells expressing the human MC4 receptor, demonstrating that these compounds were not functional MC4 agonists. Moreover, selected compounds (**6e**, **g**, **7b**, **f**; and **g**, Table 3) were shown to dose dependently inhibit α-MSH-stimulated cAMP production. For example, **7b** and **g** exhibited IC_{50} values of 170 and 120 nM, respectively, in this assay.

Compound **7b** had a measured log *D* value of 1.8, which is a very desirable value for a CNS agent.[9] Therefore, this compound was further studied in rats for oral bioavailability and brain penetration (Table 4). After intravenous administration of 5 mg/kg, as an aqueous solution of the hydrochloride salt, **7b** displayed moderate volume of distribution (V_d = 6.2 l/kg)

Table 4. Pharmacokinetic profile of **7b** in rats (N = 3)

iv (5 mg/kg)	
CL (ml/min kg)	52
V_d (l/kg)	6.2
$t_{1/2}$ (h)	1.4
C_{brain} at 1 h (ng/g)	280
C_{brain}/C_{plasma} at 1 h	0.5
po (10 mg/kg)	
T_{max} (h)	6.0
C_{max} (ng/ml)	47
AUC_{0-8} (ng/ml h)	250
F (%)	8

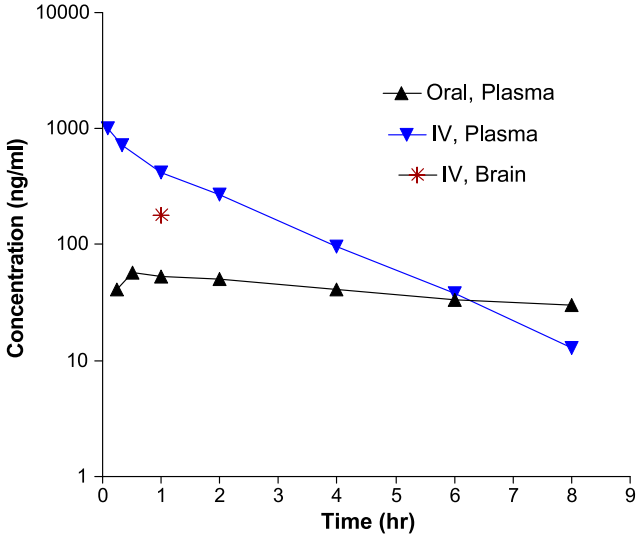


Figure 2. Pharmacokinetic profile of compound **7b** in rats (5 mg/kg, iv, 10 mg/kg, po, $N = 3$).

and high clearance (Cl = 52 ml/min kg), which resulted in a short half-life ($t_{1/2}$ = 1.4 h). At the 1-h time point, the concentration of **7b** in the brain was 280 ng/g, which reflected a brain/plasma ratio of 0.5. Oral dose at 10 mg/kg resulted in a maximal concentration (C_{max}) of 47 ng/ml at the 6-h time point (T_{max}), and an area under curve (AUC_{0-8}) value of 250 ng/ml.h. The absolute bioavailability (F) thus was calculated to be 8% in this species (Fig. 2). Although the absolute bioavailability of compound **7b** in rats was moderate based on this 8-h PK study, it displayed reasonably good brain penetration.

In conclusion, we have identified several piperazinebenzylamines bearing an *N*-(1-methoxy-2-propyl) group as potent and selective antagonists of the human MC4 receptor. In addition, many compounds possessed suitable lipophilicity for potential oral administration. Compound **7b**, having a desirable log D value of 1.8, exhibited moderate oral bioavailability and blood–brain barrier penetration in rats.

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