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Structure–activity relationships of piperazinebenzylamines as potent and selective agonists of the human melanocortin-4 receptor

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Abstract—SAR studies on a series of piperazinebenzenes directed toward the human melanocortin-4 receptor resulted in potent MC4R agonists. Replacement of the triazole moiety of an initial lead **4** by a basic nitrogen baring a lipophilic side-chain increased the binding affinities of these compounds. Analogs bearing an additional hetero-atom in the side-chain possessed good agonist potency. Thus, **11h** had a K_i of 11 nM, and **13g** exhibited an EC_{50} of 3.8 nM and a K_i of 6.4 nM.

1. Introduction

Five subtypes of melanocortin receptors, MC1-5R, have been identified and cloned, and they belong to the Class A G-protein-coupled receptor (GPCR) superfamily.[1] The MC1R regulates skin pigmentation and the immune system. The MC2R (ACTH receptor) controls steroid production. The MC3R and MC4R are involved in the regulation of central sexual behavior and the control of feeding behavior, and the MC5R has a role for regulating exocrine gland secretion.[2] The melanocortic peptides derived from a single prohormore, proopiomelanocortin (POMC), are the natural ligands for the melanocortin receptors. They consist of the melanotropins α-MSH, β-MSH, and γ-MSH, and the adrenocorticotropin ACTH. All these peptides have a His-Phe-Arg-Trp (HFRW) motif, which is crucial for activation of the melanocortin receptors. Because of the importance of the MC4R in feeding behavior, metabolism and energy homeostasis, selective MC4R agonists and antagonists may be useful in human diseases such as obesity and cachexia.[3] Small molecule MC4R agonists such as **1**–**6** (Fig. 1) have been disclosed from several laboratories.[5–10] Compound **1**, which has a (*R*)-Tic-(*R*)-(4-Cl)Phe-piperidine, is the first potent small molecule MC4R agonist reported (Fig. 1). It is designed and optimized based on the HFRW core of the melanotropin peptides by mimicking the Arg-Trp residues with a privileged structure for GPCRs.[4] **1** is also highly selective over other subtypes of the melanocortin receptors MC1R, MC3R, and MC5R.[5] Piperidine di-peptide compounds represented by **2** are reported as potent MC1R agonists (EC_{50} = 0.19 nM, 98% intrinsic activity), however, **2** also possesses high agonist potency at the MC4R (EC_{50} = 2.9 nM, 95% intrinsic activity).[6] This series of compounds have been further derived by replacement of the tetrahydroisoqinoline of **1** with a β-alanine moiety (compound **3**, EC_{50} = 40 nM, 98% intrinsic activity).[7] An early report from our laboratory has disclosed a series of piperazinebenzenes bearing a sulfonamide or a triazole functionality as MC4R-selective agonists (compound **4**, EC_{50} = 80 nM, 100% intrinsic activity).[8] Very recently, two independent publications, which

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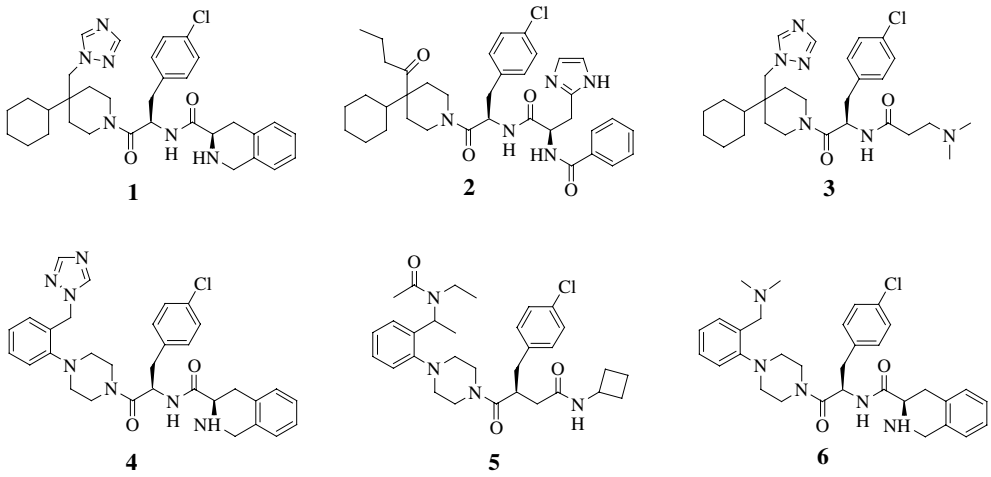


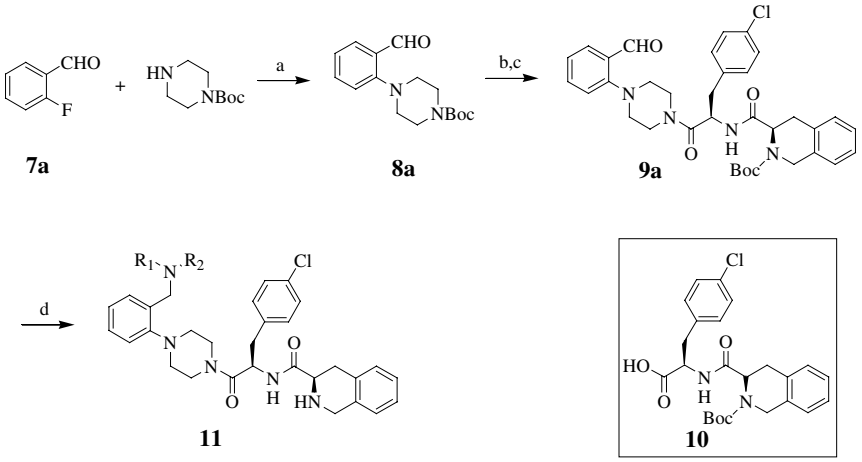
Figure 1. Small molecule MC4 agonists.

reveal the triazole of **4** can be replaced by an amide (compound **5**, $EC_{50} = 15\,nM$, 100% intrinsic activity)[9] or a dimethylamino group (compound **6**, $EC_{50} = 7.0\,nM$, intrinsic activity >80%),[10] further elaborate the structure–activity relationships of this piperazine series as MC4R agonists. Here we report our continued efforts on the optimization of these piperazinebenzenes toward potent and selective agonists of the human MC4 receptor.

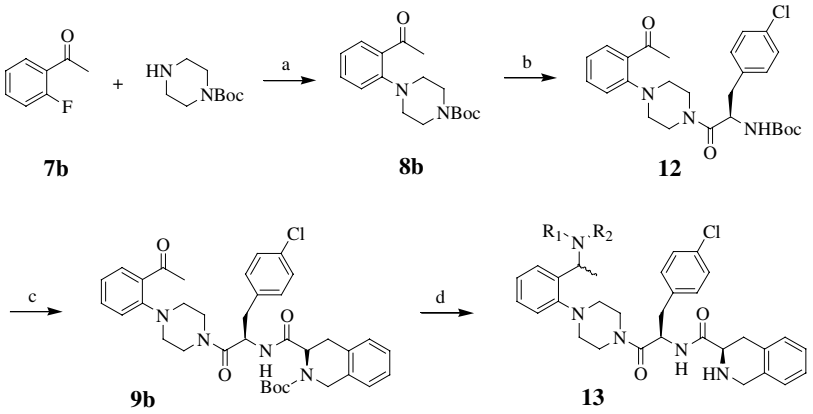
2. Chemistry

The synthesis of the piperazinebenzylamines **11a**–**u** started from 2-fluorobenzaldehyde **7a** (Scheme 1). Thus, condensation of **7a** with mono-Boc-protected piperazine in DMF at 120–160 °C gave the piperazinebenzaldehyde **8a**. After deprotection of **8a**, a coupling reaction of the free amine with (*R*)-*N*-Boc-Tic-(*R*)-(4-Cl)Phe-OH (**10**)[10] in the presence of HOBt and EDC in DCM afforded the dipeptidic derivative **9a**. Reductive amination of **9a** with a various primary or secondary amines, followed by deprotection of the Boc-group, resulted in the desired products **11** in 60–80% yields.[11]

The synthesis of the piperazine-α-methylbenzylamine compounds **13a**–**i** started from 2′-fluoroacetophenone **7b** as showed in Scheme 2. Thus, condensation of **7b** with mono-Boc-protected piperazine gave the corresponding piperazineacetophenone **8b** similarly as described above. Next we used a sequential peptide coupling reaction using the conditions with minimal possibility of racemerization of the two *R*-amino acids. After deprotection of **8b**, the resulting free amine was coupled with (*R*)-*N*-Boc-(4-Cl)Phe-OH in the presence of EDC and HOBt in DCM to afford the amide **12**, which was deprotected with TFA and subjected to a second coupling reaction with (*R*)-*N*-Boc-Tic-OH to give the dipeptidic derivative **9b**. Reductive amination of **9b** were accomplished with various amines using a standard protocol, followed by deprotecting the Boc-moiety of the Tic-group, to give the desired products



Scheme 1. Reagents and conditions: (a) $DMF/K_2CO_3/\Delta$, 80%; (b) 50% TFA/CH_2Cl_2, quant.; (c) **10**/EDC/HOBt/Et_3N, 90%; (d) $R^1R^2NH/NaBH(OAc)_3$; then TFA/CH_2Cl_2, 60–80%.



Scheme 2. Reagents and conditions: (a) DMF/Δ, 85%; (b) TFA/CH_2Cl_2, then (*R*)-Boc-(4-Cl)Phe-OH/EDC/HOBt/Et_3N, 90%; (c) TFA/CH_2Cl_2, then (*R*)-Boc-Tic-OH/EDC/HOBt/Et_3N, 85%; (d) R^1R^2NH/$NaBH(OAc)_3$, then 50% TFA/CH_2Cl_2, 25–90%.

13 in 25–80% yields.[11] These compounds were presumably mixtures of diastereoisomers although we were not able to see separation in the purity characterization by HPLC on a C-18 column.

3. Results and discussions

The competition binding experiments were performed using HEK293 cells stably transfected with the human melanocortin-4 receptor as described before,[12] using [^{125}I]-NDP-MSH as the radiolabeled ligand in the binding assay. The agonist activities of these compounds were tested for their stimulation of cAMP in CHO cells stably expressed the human melanocortin-4 receptor and α-MSH was used as a standard.[13] The K_i and EC_{50} values reported in Tables 1 and 2 are the average of at least three independent measurements.

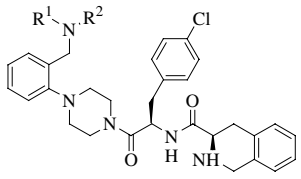
The primary benzylamine (**11a**) showed modest binding affinity (K_i = 380 nM) and agonist potency (EC_{50} = 274 nM) at the human MC4R, which were comparable to that of the triazole **4** (K_i = 270 nM, EC_{50} = 110 nM). Incorporation of a small aliphatic side chain such as cyclopropyl and cyclopentyl group on the benzylamine nitrogen of **11a** resulted in a 4–6-fold increase in binding affinity (**11b** and **11c**). The 2-thiophenylethyl analog **11h** had a K_i of 11 nM, which was a 35-fold increase over **11a**. These results suggest that an additional aromatic ring is favored for high receptor binding. For fluorine-substituted phenethyl side-chain (**11e–g**), the 2- or 3-position seemed to be favored and the binding affinities of **11e** and **11f** were over 5-fold better than that of the corresponding 4-fluoro-analog **11g**. However, while a small lipophlic side-chain at the basic nitrogen of **11a** enhanced the binding affinity, it had little effect on the agonist potency of these compounds (EC_{50} 164–910 nM). Furthermore, the intrinsic activities (maximal levels of cAMP stimulation) of **11e** and **11f** (47% and 37%, respectively) were somewhat reduced.

Incorporation of a side-chain bearing an additional polar group at the basic nitrogen of **11a** had no significant impact on the binding affinity for **11i–l**, however, it improved agonist potency of these analogs. For example, **11j** with a 3-hydroxypropyl side-chain had a K_i of 120 nM and an EC_{50} of 36 nM. While the binding affinity of this compound was only 3-fold better than that of **11a**, its agonist potency improved about 8-fold.

The successful improvement in both binding affinity and agonist potency over the parent primary amine **11a** by introduction of a side-chain at the basic nitrogen prompted us to examine *N*,*N*-dialkylated derivatives (**11m–u**, Table 1). The diethylamino derivative **11m** had a K_i value of 52 nM, which was 7-fold better than **11a**, and an EC_{50} of 240 nM.[14] Similarly, other tertiary amine analogs **11n–r** had K_i values ranged from 35 nM (**11r**) to 84 nM (**11p**). Once again, compounds bearing a polar group at the side-chain (**11n**, **11o**, and **11q**) exhibited improved agonist potency. For example, **11o** had a K_i of 49 nM and an EC_{50} of 51 nM. Among the cyclic amines (**11s–u**), **11s** possessed a K_i value of 24 nM, however, its EC_{50} value was only 146 nM. In comparison, the *cis*-2,5-dimethylpiperazine **11u** exhibited similar binding affinity (K_i = 19 nM) to **11s**, however, it had an EC_{50} value of 18 nM, which was about 8-fold better than **11s**. Although these tertiary amines **11m–u** had in general better binding affinities than **11a**, the agonist potency (EC_{50} values) of these compounds exhibited little improvement other than compounds with a side-chain bearing a hetero-atom.

To further optimize this series of compounds toward better binding and agonist potency at the MC4R, we hypothesized that, since a relatively long and flexible alkylamino group, such as 2-thiophenylethyl, was used in this series, restriction of the free rotation of this side-chain could improve affinity if favorable low-energy conformers could be found. One simple way to achieve this objective is to introduce a methyl group at the benzylic position, and therefore, to reduce the flexibility of the side-chain. Among these α-methylbenzylamines, the *N*-cyclopropyl derivative **13a** had a K_i value of 23 nM, about 4-fold better than the corresponding benzylamino analog **11b** (K_i = 93 nM), and its EC_{50} value (174 nM) was, however, only slightly better than that of **11b**. The α-methyl compound **13b** was 6-fold better

Table 1. SAR of *N*-alkylpiperazinebenzylamines at the *h*MC4 receptor



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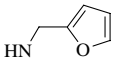
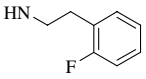
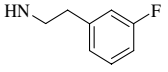
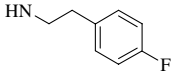
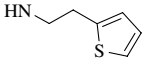
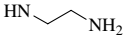
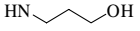
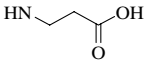
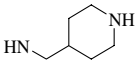
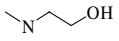
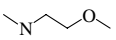
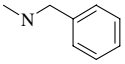
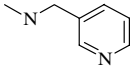
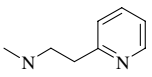
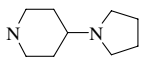
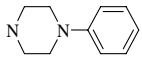
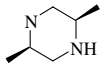
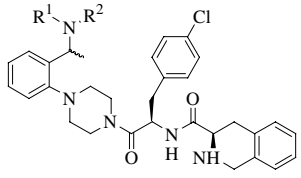
Compd	R^1NR^2	*h*MC4[a] K_i (nM)[b]	cAMP Stimulation[c]	
			EC_{50} (nM)[d]	IA (%)[e]
1		33 ± 11	5.7[a]	100[a]
4		270 ± 65	110 ± 61[a]	100[a]
11a	NH_2	380 ± 34	274 ± 122	74 ± 19
11b		90 ± 26	253 ± 82	85 ± 22
11c		66 ± 20	164 ± 190	89 ± 11
11d		53 ± 16	274 ± 205	91 ± 24
11e		27 ± 9	910 ± 878	81 ± 23
11f		14 ± 3	460 ± 160	47 ± 10
11g		81 ± 44	487 ± 125	37 ± 8
11h		11 ± 8	357 ± 312	105 ± 22
11i		127 ± 26	91 ± 28	69 ± 7
11j		120 ± 35	36 ± 19	83 ± 25
11k		490 ± 125	90 ± 31	77 ± 13
11l		102 ± 11	208 ± 94	75 ± 20
11m		52 ± 18	240 ± 17	55 ± 8
11n		55 ± 10	40 ± 9	78 ± 16
11o		49 ± 14	51 ± 25	82 ± 14
11p		84 ± 23	632 ± 374	71 ± 13
11q		77 ± 28	94 ± 30	70 ± 12
11r		35 ± 18	190 ± 100	57 ± 16

Table 1 (*continued*)

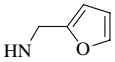
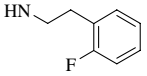
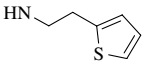
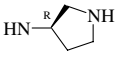
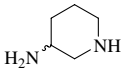
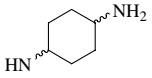
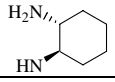
Compd	R^1NR^2	*h*MC4[a] K_i (nM)[b]	cAMP Stimulation[c]	
			EC_{50} (nM)[d]	IA (%)[e]
11s		24 ± 3	146 ± 50	87 ± 22
11t		145 ± 67	300 ± 34	61 ± 2
11u		19 ± 4	18 ± 3	64 ± 16

[a] Human melanocortin-4 receptor stably expressed in HEK 293 cells.
[b] [^{125}I]-NDP-MSH used as radiolabeled ligand.
[c] Human melanocortin-4 receptor stably expressed in CHO cells.
[d] EC_{50} determined by compound concentration at 50% maximum cAMP release.
[e] Intrinsic activity, percentage of α-MSH-stimulated cAMP level.

Table 2. SAR of *N*-alkylpiperazine-α-methylbenzylamines at the *h*MC4 receptor



13

Compd	R^1NR^2	*h*MC4[a] K_i (nM)[b]	cAMP Stimulation[c]	
			EC_{50} (nM)[d]	IA (%)[e]
13a		23 ± 3	174 ± 80	75 ± 1
13b		11 ± 2	72 ± 28	76 ± 13
13c		12 ± 2	138 ± 76	80 ± 20
13d		33 ± 8	2060 ± 1550	50 ± 23
13e		24 ± 6	577 ± 111	52 ± 17
13f		13 ± 3	12 ± 2	66 ± 7
13g		6.4 ± 4.4	3.8 ± 2.6[f]	84 ± 15[f]
13h		25 ± 13	34 ± 15	79 ± 13
13i		13 ± 3	15 ± 6	93 ± 30

[a] Human melanocortin-4 receptor stably expressed in HEK 293 cells.
[b] [^{125}I]-NDP-MSH as the radiolabeled ligand.
[c] Human melanocortin-4 receptor stably expressed in CHO cells.
[d] EC_{50} determined by compound concentration at 50% maximum cAMP release.
[e] Intrinsic activity, percentage of α-MSH-stimulated cAMP level.
[f] EC_{50} value of this compound at the *h*MC4R in HEK 293 cells was 4.7 nM with IA of 100%.

Table 3. Binding affinities at the human MC subtypes[a]

Compound	K_i (nM)[b]			
	*h*MC1R[c]	*h*MC3R[c]	*h*MC4R	*h*MC5R
1	1500	2300	33	860
4	(36%)	(24%)	270	1200
11a	(27%)	(33%)	380	2300
11e	(42%)	2300	27	260
11h	6000	1800	11	240
13f	2100	3600	13	590
13g	1500	2000	6.4	270

[a] Human melanocortin receptors stably expressed in HEK 293 cells.
[b] [^{125}I]-NDP-MSH as the radiolabled ligand.
[c] Data in parenthesis are percentage of inhibition at 10 μM concentration.

than **11b** in binding affinity, but only 2-fold in agonist potency (Table 2). This approach did not work well for **11h** (K_i = 11 nM, EC_{50} = 357 nM), and the α-methylated analog **13e** had a K_i value of 24 nM and an EC_{50} value of 577 nM. However, incorporation of an additional basic nitrogen at the side-chain improved agonist potency of these analogs. Thus, **13f** with a 3*R*-pyrrolidineamino group had a K_i of 13 nM and an EC_{50} of 12 nM. The agonist potency **13f** was much better than **13b** (K_i = 11 nM and EC_{50} = 72 nM), which had a cyclopentyl group instead. The most potent MC4R agonist of this series was **13g**, which had an EC_{50} of 3.8 nM (K_i = 6.4 nM). The other two diamine analogs **13h** (EC_{50} = 34 nM) and **13i** (EC_{50} = 15 nM) also exhibited good binding and agonist potency.

Selected compounds from this series were also tested and found to be selective in binding to the MC4R over the other melanocortin receptors. For example, **11h** showed K_i values of 6000, 1800, and 240 nM, respectively, at the human melanocortin-1, -3, and -5 receptors, which were over 20-fold selective (Table 3). The potent MC4R agonist **13g** possessed K_i values of 1500, 2000, 6.4, and 270 nM, respectively, at the MC1R, MC3R, MC4R, and MC5R. Thus, **13g** exhibited more than 300-fold selectivity in binding over the MC3R, which is the relevant receptor in control of feeding behavior. **11e** and **13f** were also proved to be selective and the data are summarized in Table 3.

The increase of binding affinity of this series of compounds over the triazole **4** could be contributed to a strong charge–charge interaction between the basic benzylamine of **11** and **13** (the calculated pK_a value for **11h** was 9.0),[15] instead of the weakly basic triazole moiety of **4** (calculated pK_a of 2.7),[15] and an acidic residue of the MC4 receptor. Mutagenesis studies[16] of the MC4 receptor have demonstrated that two aspartic residues, Asp-122 and Asp-126, are important for peptide ligand binding and activation. Recently receptor modeling based on the rhodopsin template[17] shows these two residues are located at the top part of the putative binding pocket. The fact that the Asp122Ala MC4 mutant can still be activated by peptide and small molecule agonists such as **1**[13] might suggest that the Asp-122 residue is not critical for receptor function. In addition, many benzylamine analogs with a small lipophilic side-chain from the current series had improved binding affinity, but not agonistic potency. Based on these facts and receptor modeling,[18] we speculated the Asp-122 was the acidic residue, which interacts with the basic benzylamine.

4. Conclusion

A series of piperazinebenzylamines bearing the (*R*)-Tic-(*R*)-(4-Cl)Phe dipeptide was synthesized and tested as melanocortin-4 receptor ligands. Replacement of the triazole of **4** by a basic amine, in combination with a small lilophilic side-chain, improved the binding affinity of this series of compounds. Structure–activity relationship studies around the benzylamine revealed that an additional basic nitrogen at the side-chain increased agonistic potency. Thus, **11h** exhibited a K_i value of 11 nM, and **13g** had an EC_{50} of 3.8 nM (K_i = 6.4 nM). In addition, these compounds displayed high MC4R-selectivity.

References and notes

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12. MC4 receptor binding assay. Crude membranes were prepared by differential centrifugation from HEK293 cells transfected with human MC4 receptor. These membranes were incunated for 90 min with a fixed concentration of [^{125}I]-NDP-MSH and varying concentrations of competing ligand. Reactions were terminated by rapid vacuum filtration using a Packard 96-well cell harvester over PEI (1%) soaked GF/C filter plates (Packard). Filter plates were then washed with 600 mL PBS (0.01% Triton-X100). Microscint scintillation fluid (Packard) was added to each well before monitoring [^{125}I]-NDP-MSH in a TopCount-NXT (Packard) microplate scintillation counter. Binding data were analyzed by GraphPad Prism software program. See Ref. 13 for details.
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