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Optimization of privileged structures for selective and potent melanocortin subtype-4 receptor ligands

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ABSTRACT

Design, syntheses and structure–activity relationships of N-acetylated piperazine privileged structures containing MC4R agonist compounds were described. The most potent derivatives were low nM MC4R selective full agonists. Several compounds from the series had modest pharmacokinetic properties.

The melanocortin receptors are a family of seven-transmembrane G-protein-coupled receptors. Five different subtypes of melanocortin receptors (MC1R–MC5R) have been identified and cloned. They interact with their endogenous ligands (the corticotropins and melanocortins) to regulate diverse physiological functions such as skin pigmentation, steroidogenesis, energy metabolism, feeding and erectile activity, and exocrine secretion.[1] The melanocortin subtype-4 receptor (MC4R) is primarily expressed in the brain, and is involved in the regulation of energy balance including food intake, and is also associated with sexual behavior.[2] The pharmacological link between MC4R and feeding behavior comes from the studies with both agonists and the antagonists.[3] Treatment with MC4R antagonist SHU9119 and agouti protein (an MC1R and MC4R antagonist) increase food intake and body weight in rodents.[4] The non-selective peptide melanocortin agonist MTII reduces food intake and body weight in rodents.[4b] Studies of MC4R knock-out mice, which exhibit obesity, further confirm the relation between MC4R and feeding regulation.[5] These findings have attracted many medicinal chemists and biologists to seek potent MC4R agonists for the treatment of obesity.

In the past several years, small-molecule MC4 receptor agonists, both based on peptides and non-peptides, have been reported in the literature.[6] Earlier work from our laboratories and other research groups identified small-molecule MC4R agonists **1**–**5** (Fig. 1).[7] Both compounds **1** and **2** show significant reduction of food intake and body weight, and stimulation of erectile response in rodents.[7a,b] Compound **3** has good binding and functional MC4R activity and selectivity against the other MCR subtypes.[7c] Compound **4** also has good MC4R binding affinity.[7d] Compound **5** is a potent and selective MC4R agonist and is effective in rodent food-intake models.[7e]

Compared with the dipeptide analogs,[7a,b] non-peptide compounds had a better PK profile.[7e–g] We envisioned that a hybrid structure of *t*-butyl pyrrolidine acid (from **5**) and piperazine privileged structures (from **4**) might improve potency while maintaining

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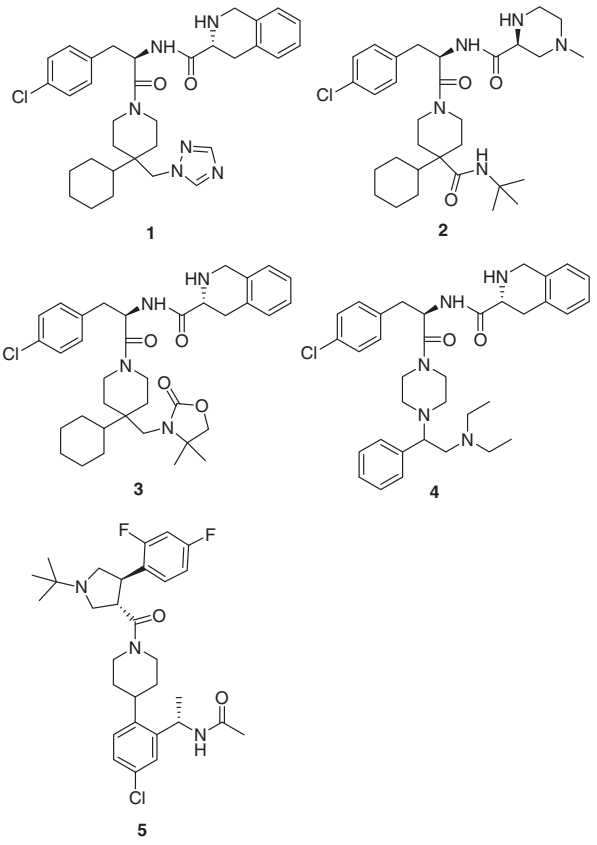


Figure 1. MC4R agonists.

a good PK profile. This Letter will discuss the discovery of novel piperazine derivatives **6** as MC4R agonists based on this design (Fig. 2). We will focus on the modification of the privileged structures while keeping the *t*-butyl-4-(2,4-difluorophenyl)pyrrolidine-3-carboxylate.

Earlier's work in our group showed that cyclohexyl ring (R in structure **6**) which had replaced a phenyl ring in the privileged

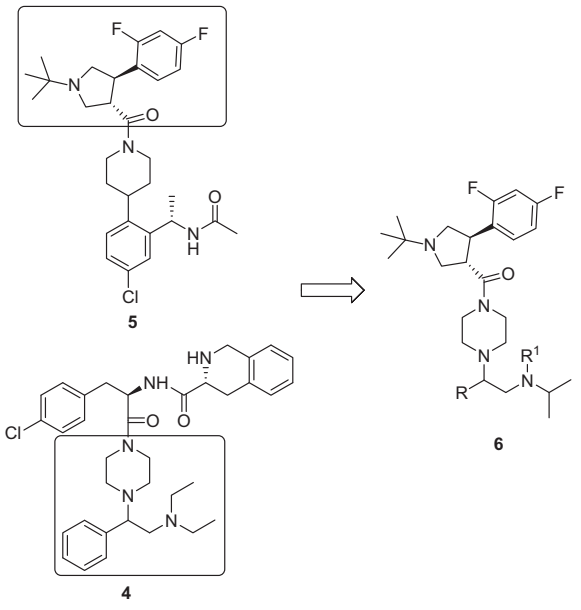


Figure 2. Design of MC4R agonists.

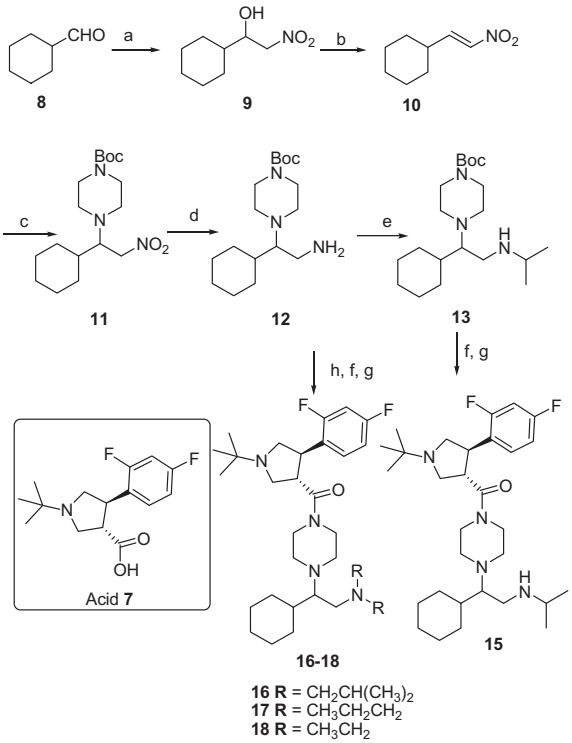
structure improved the MC4R potency. Initially, we decided to make the cyclohexyl-based amine privileged compounds.

The synthesis of the cyclohexyl-based amine privileged structure compounds, **15–18**, is shown in Scheme 1. Cyclohexanecarboldehyde **8** underwent potassium *tert*-butoxide catalyzed nitro-aldol condensation with nitromethane to afford nitro alcohol **9**. Dehydration with trifluoroacetic anhydride and triethylamine generated nitroalkene **10**. Reaction of **10** with Boc-piperazine in CH_2Cl_2 gave nitro compound **11**, which was reduced using $NaBH_4$ in the presence of $NiCl_2$ to generate amine **12**. Reductive amination of compound **12** with acetone to give amine **13**, which was followed by deprotection with HCl and coupled with *t*-butyl pyrrolidine acid **7** to generate **15**. Alkylation of intermediate **12** by reductive amination with isobutylaldehyde or reaction with appropriate bromide in DMF in the presence of potassium carbonate, followed by deprotection and coupling sequence gave compounds **16–18**. Compounds **15–18** are the mixtures of diastereomers since compound **12** is racemic.

The compounds (**15–18**) with piperazine amine privileged structure were evaluated in a competitive binding assay and functional assay (Table 1). The binding assay was performed to assess the competitive binding of test compounds versus [^{129}I]-NDP-α-MSH. The functional assay was conducted by measuring the accumulated cAMP in CHO cells expressing the human receptors.[8]

As shown in Table 1, compounds (**15–18**) have modest MC4R binding and functional activity. The smaller alkyl group shows enhanced MC4R binding and functional activity. For example, the isopropyl amine compound **15** shows good functional activity in this series (IC_{50} = 310 nM, EC_{50} = 240 nM).

In order to further improve the MC4R binding and functional activity, we prepared the isopropyl amide and sulfonamide privileged compounds.



Scheme 1. Reagent and conditions: (a) CH_3NO_2, *t*-BuOK, *t*-BuOH, THF, 0 °C, 2 h, rt 16 h; (b) TFAA, Et_3N, CH_2Cl_2, −10 °C, 1 h; (c) Boc-piperizane, CH_2Cl_2, rt 16 h; (d) $NiCl_2$, $NaBH_4$, CH_3OH, 0 °C, 2 h; (e) CH_3COCH_3, AcOH, $NaBH(OAc)_3$, CH_2Cl_2, rt 16 h; (f) HCl, dioxane, rt 1 h; (g) EDC, HOBt, CH_2Cl_2, NMM, acid **7**, rt 16 h; (h) $(CH_3)_2CHCHO$, AcOH, $NaBH(OAc)_3$, CH_2Cl_2, rt 4 h; or RBr, K_2CO_3, DMF, rt 16 h.

Table 1
Binding affinity and functional activity of compounds at human melanocortin subtypes 4 receptors[a]

Compds	MC4R binding[b] IC_{50} (nM)	cAMP[c] EC_{50} (nM)	Activation[d] at 10 μM (%)
15	310 ± 30	240 ± 43	95
16	1900 ± 91[e]	6700 ± 3300[e]	10
17	200 ± 21[e]	710 ± 170[e]	97
18	260 ± 24	570 ± 98	101

[a] Values represent mean ± standard error. All data represent at least three determinations except for where indicated.
[b] Displacement of [^{125}I]-NDP-α-MSH from human receptors expressed in CHO cells.
[c] Concentration of compound at 50% maximum cAMP accumulation.
[d] Percentage of cAMP accumulation at 10 μM compound relative to α-MSH.
[e] Values (n = 2) with standard error.

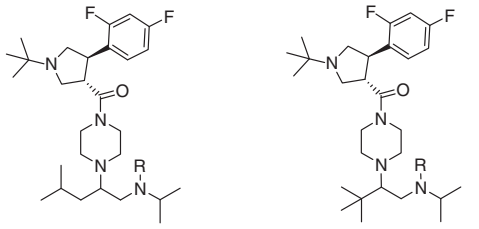
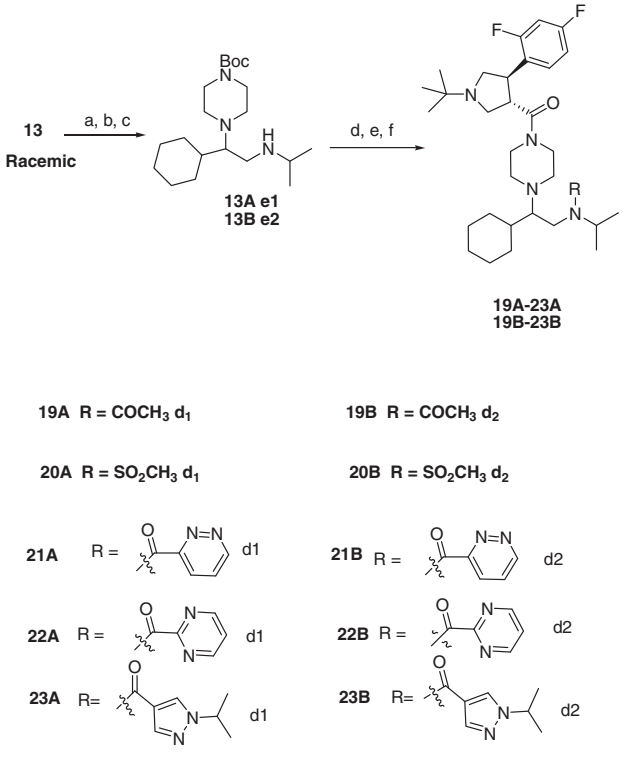


Figure 3. Structures of Compounds **24**–**27**.



Scheme 2. Reagent and conditions: (a) $NaHCO_3$, CH_2Cl_2, H_2O, Cbz-succinimide, rt 16 h; (b) chiral HPLC resolution: AD column; mobile phase 40% IPA/heptane; (c) $Pd(OH)_2$, H_2, HOAc, EtOH, rt 5 h; (d) $(CH_3CO)_2O$ or CH_3SO_2Cl, DIEA, DMAP, CH_2Cl_2; or RCOOH, EDC, HOBt, CH_2Cl_2; (e) HCl, dioxane; (f) EDC, HOBt, CH_2Cl_2, NMM, acid **7**.

Table 2
Binding affinity and functional activity of compounds at human melanocortin subtype-4 receptors[a]

Compds	MC4R binding[b] IC_{50} (nM)	cAMP[c] EC_{50} (nM)	Activation[d] at 10 μM (%)
19A	29 ± 5	50 ± 11	134
19B	28 ± 3	25 ± 5	123
20A	15 ± 2	25 ± 8	114
20B	13 ± 1	13 ± 2	117
21A	18 ± 4	5.4 ± 2	102
21B	64 ± 20	9.1 ± 1	99
22A	35 ± 4[e]	10 ± 1[e]	98
22B	130 ± 66[e]	15 ± 2.9[e]	88
23A	18 ± 3	6.4 ± 1	106
23B	62 ± 8	19 ± 3	81
24A	49 ± 4	29 ± 4	101
24B	23 ± 5	9.7 ± 1	113
25A	88 ± 4	47 ± 12	117
25B	27 ± 1	19 ± 6	100
26A	150 ± 16	180 ± 87	89
26B	150 ± 3.7	180 ± 81	94
27A	53 ± 3.1[e]	19 ± 2.5[e]	88
27B	46 ± 12	44 ± 10	102

[a] Values represent mean ± standard error. All data represent at least three determinations except for where indicated.
[b] Displacement of [^{125}I]-NDP-α-MSH from human receptors expressed in CHO cells.
[c] Concentration of compound at 50% maximum cAMP accumulation.
[d] Percentage of cAMP accumulation at 10 μM compound relative to α-MSH.
[e] Values (n = 2) with standard error.

Table 3
Binding affinity and functional activity of selected compounds at human melanocortin receptors[a]

Compds	Receptor	Binding[b] IC_{50} (nM)	cAMP[c] EC_{50} (nM)	Activation[d] at 10 μM (%)
19B	hMC1R	1800 ± 380	890 ± 180	61
	hMC3R	6500 ± 540	2000[f]	81
	hMC4R	28 ± 3	25 ± 5	123
	hMC5R	660[f]	2800[f]	64
20B	hMC1R	1000 ± 105	540 ± 150	63
	hMC3R	3600 ± 186	1800 ± 300	94
	hMC4R	13 ± 1	13 ± 2	117
	hMC5R	250[f]	>5000[f]	57
21A	hMC1R	1900 ± 720	1200 ± 450	58
	hMC3R	770 ± 170	90 ± 19	108
	hMC4R	18 ± 4	5.4 ± 2	102
	hMC5R	130[f]	490 ± 83	68
23A	hMC1R	730 ± 97	520 ± 212	60
	hMC3R	870 ± 49	79 ± 9	102
	hMC4R	18 ± 3	6.4 ± 1	106
	hMC5R	620[f]	1000 ± 143	62
24B	hMC1R	3600 ± 960	560 ± 115[e]	77
	hMC3R	4700 ± 1000	880 ± 215	109
	hMC4R	23 ± 5	9.7 ± 1	113
	hMC5R	1100[f]	>5000[f]	56
25B	hMC1R	5000 ± 160	>5000[f]	62
	hMC3R	4900 ± 350	2500 ± 0[e]	87
	hMC4R	27 ± 1	19 ± 6	100
	hMC5R	2800 ± 270	>5000[e]	76

[a] Values represent mean ± standard error. All data represent at least three determinations except for where indicated.
[b] Displacement of [^{125}I]-NDP-α-MSH from human receptors expressed in CHO cells.
[c] Concentration of compound at 50% maximum cAMP accumulation.
[d] Percentage of cAMP accumulation at 10 μM compound relative to α-MSH.
[e] Values (n = 2) with standard error.
[f] Values (n = 1).

The single compounds **19A–23A** and **19B–23B** were prepared from intermediate **13**. In order to resolve the enantiomers, amine

Table 4
Pharmacokinetic data in rat[a]

Compounds	19B	20B	22A	18
F (%)	12	21	26	22
Cl (mL min^{-1} kg^{-1})	42	25	31	7.3
V_{dss} (L kg^{-1})	6.8	6.0	3.8	19
$t_{1/2}$ (h)	2.2	2.7	1.8	34
AUCN (μM h/mpk)[b]	0.10	0.26	0.24	1.3

[a] Compound dosed in Sprague-Dawley rats as a solution in EtOH:PEG400:water (10:40:50) at 1 mg/kg, iv and 4 mg/kg, po.
[b] AUCN from oral dosing.

13 was converted to Cbz-protected amine by treatment of Cbz-succinimide in the presence of sodium bicarbonate. The racemic compound was resolved on chiral HPLC to give separated enantiomers $\mathbf{e_1}$ and $\mathbf{e_2}$, followed by Cbz-deprotection gave **13A** and **13B**. The first elution designated as $\mathbf{e_1}$ and second elution as $\mathbf{e_2}$. Acylation, sulfonation or EDC coupling reaction of compounds **13A** and **13B**, respectively, followed by Boc-deprotection with HCl and coupled with *t*-butyl pyrrolidine acid **7** generated compounds **19A–23A** and **19B–23B**.

In order to further explore the SAR of this series, we also replaced the cyclohexyl group with isobutyl and *t*-butyl group and synthesis of compounds **24A–27A** and **24B–27B** (Fig. 3) using the same methods for making cyclohexyl compounds **19A–19B** and **20A–20B** in Schemes 1 and 2.

The piperazine compounds (**19A–27A** and **19B–27B**) were evaluated in a competitive binding assay and functional assay (Table 2). As illustrated in Table 2, converting amine compound **15** to acetyl amide privileged compound **19B** largely increased the MC4R binding potency (from 310 nM to 28 nM) and functional activity (from 240 nM to 25 nM). Replacing the acetyl amide privileged structure with sulfonamide, compound **20B** further improved the binding (IC_{50} = 13 nM) and functional activity (EC_{50} = 13 nM). *N*-Isopropyl heterocyclic amide (isopropyl pyridazine) and pyrazole privileged structure compounds (**21A** and **23A**) were more potent than isopropyl sulfonamide compounds (**20A–B**) in functional assay. Further SAR studies showed that the cyclohexyl group in the privileged structure could be replaced with an isobutyl group (**24–25**) without loss of potency. However, more hindered groups such as *t*-butyl group compounds (**26–27**) reduced potency.

The MC4R binding affinity and functional activity at the human receptor for several potent compounds is presented in Table 3. As shown in Table 3 these compounds were at least 20-fold selective in binding and functional activity versus the human MC1R and MC5R except **21A**. Most of these compounds were over 100-fold selective in functional activity versus the human MC5R. Compounds **21A** and **23A** showed the most potent MC3R activity, EC_{50} <100 nM.

The pharmacokinetic (PK) properties of compounds **18**, **19B**, **20B** and **22A** were further evaluated in the rat (Table 4). These compounds all had moderate bioavailability (12–26%), and most had a half life of about 2 h, except **18** which had a long $t_{1/2}$ of 34 h.

In summary, we report the design, synthesis and SAR and pharmacokinetic of a new novel N-acetylated piperazine compounds as MC4R agonists. SAR study of privileged structure led to the discovery of potent, selective and orally bioavailable analogs with isopropyl sulfonamide and amide piperazine as privileged structure for MC4R agonists. Replacement of methyl group by heterocyclic group in isopropyl amide piperazine privileged structures further improved the MC4R binding and functional potency.

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