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Discovery of a piperazine urea based compound as a potent, selective, orally bioavailable melanocortin subtype-4 receptor partial agonist

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ABSTRACT

We report the discovery of piperazine urea based compound **1**, a potent, selective, orally bioavailable melanocortin subtype-4 receptor partial agonist. Compound **1** shows anti-obesity efficacy without potentiating erectile activity in the rodent models.

The melanocortin receptors are a family of seven-transmembrane G-protein coupled receptors. The five known subtypes interact with the endogenous ligands, namely the melanocortins and corticotropins, to mediate a wide range of physiologic functions including feeding behavior and body weight homeostasis, skin pigmentation, steroid production, sexual behaviors, energy metabolism and exocrine gland secretion.[1–3]

The melanocortin subtype-4 receptor (MC4R), primarily expressed in the brain, is implicated in the regulation of feeding behavior and sexual function.[4–11] In the past decade, there have been intensive studies on identifying small molecular MC4 receptor agonists based on both peptides and non-peptides.[12–18] Previous efforts at Merck have led to the identification of **THIQ** and **MB243** as potent and selective MC4R full agonists (Fig. 1).[19,20] Compound **THIQ** is the first selective small-molecule agonist of MC4R receptor with submicromolar potency (EC_{50} = 2.1 nM, >100-fold functional selectivity over the other MC subtypes) to be disclosed in the literature.[19] It was shown to stimulate erectile activity as well as significantly reduce food intake in rats. These effects of **THIQ** were mediated by MC4R since none of these effects was found in MC4R/3R knockout mice.[19,35] **THIQ** served as an important tool for studying MC4R pharmacology, but its limited oral bioavailability and off-target activity prevented further development of this compound.[12,19] Compound **MB243** (MC4R EC_{50} = 11 nM) also displayed proerectile activity and food intake reduction in rodent models. However, undesirable properties such as high covalent protein-binding activity in vitro prompted further optimization of this series.[20] Recently a series of reports from Merck Research Laboratories and others described the discovery of potent and selective non-peptide MC4R full agonists for potential treatment obesity and dysfunction.[21–28] For example, potent, selective, oral bioavailable MC4R full agonist **MK0493** showed good food intake reduction and proerectile activity in rodents.[21] In clinical trial, **MK0493** was associated with modest weight reduction from baseline but had only small, statistically insignificant effects relative to placebo after 12 weeks trial and this compound did not provide clinically meaningful effects in proerectile effects (PE) study.[29,30] Recently, researchers from Pfizer reported MC4R agonist **2**[31] that showed clinical erectogenic activity. Compound

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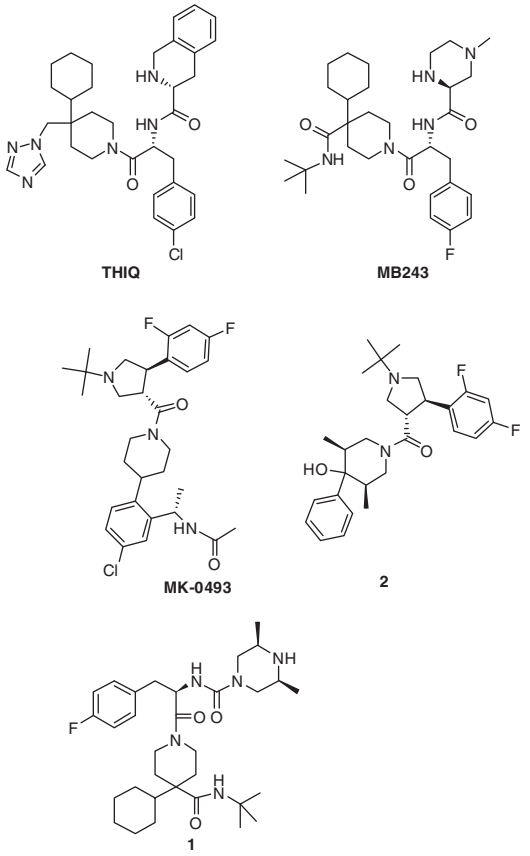


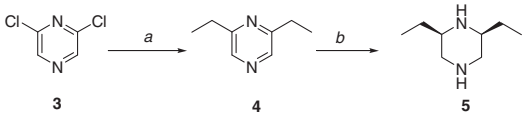
Figure 1. MC4R selective agonists.

2 was a potent full agonist of MC4R (EC_{50} = 12 nM) with good selectivity over other melanocortin receptor subtypes. MC4R agonist **2** at 200 mg dose showed efficacy similar to 100 mg of sildenafil in a pilot clinical study of male erectile dysfunction, although lower doses were not efficacious.[31]

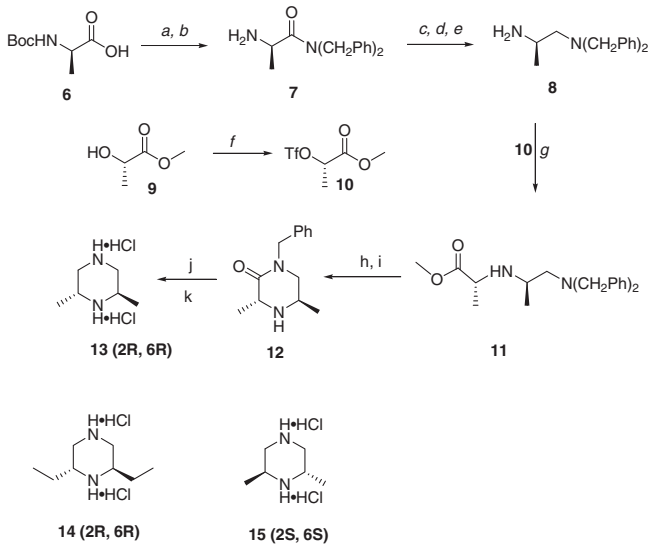
Most MC4R agonists previously reported are full agonists with activation larger than 90%. In this Letter, we describe a piperazine urea based MC4R partial agonist **1** (<60% activation) and difference between MC4R full agonists and partial agonists in animal penile erections (PE) model.

Synthetic effort of piperazine-based urea compounds **1, 19**–**24** focused on the modification of the substituted piperazine. *cis*-Diethyl piperazine **5** was synthesized via nickel-catalyzed Grignard coupling of dichloro pyrazine **3** followed by hydrogenation in the presence of platinum oxide (Scheme 1).

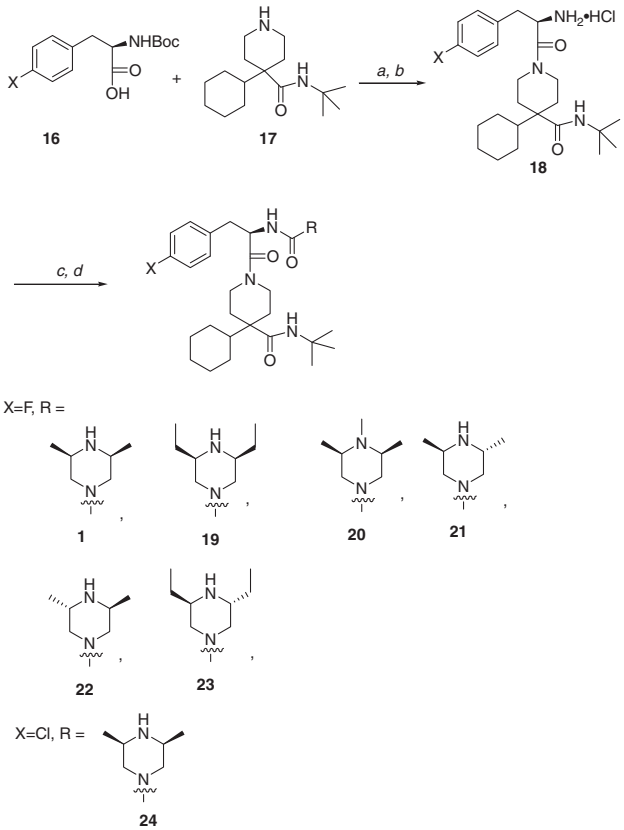
The synthesis of trans 2,6-dialkylpiperazine was shown in Scheme 2. A commercially available *N*-*t*-Boc-alanine **6** was coupled with dibenzyamine, followed by the removal of Boc group to give amide **7**. Borane-THF reduction provided diamine **8**. Alkylation of **8** with methyl(*S*)-2-[(trifluoromethanesulfonyl)oxy]propionate **10**, which was generated by the sequential treatment of methyl (*S*)-lactate **9** with trifluoromethanesulfonic anhydride and 2,6-lutidine, proceeded with inversion of stereo-chemistry to give the ester **11**. Hydrogenolysis in Pd $(OH)_2$/C of **11** resulted in monodebenzylation and partial cyclization. The completed cyclization



Scheme 1. Reagents: (a) [DPPP]$NiCl_2$, EtMgBr, THF; (b) H_2, PtO_2, AcOH.



Scheme 2. Reagents and conditions: (a) $NH(CH_2Ph)_2$, EDC, HOBt, NMM; (b) TFA, CH_2Cl_2; (c) BH_3·THF; (d) HCl; (e) KOH reflux; (f) Tf_2O, 2,6-lutidine, CH_2Cl_2, 0 °C, 20 min; (g) Et_3N, CH_2Cl_2, 0 °C, 2 h, rt, 3 h; (h) H_2, Pd/C, HCl; (i) *p*-TsOH, reflux; (j) BH_3·THF; (k) $Pd(OH)_2$, H_2, HCl, CH_3OH.



Scheme 3. Reagents: (a) EDC, HOBt, NMM, CH_2Cl_2; (b) HCl, dioxane, CH_2Cl_2; (c) triphosgene, CH_2Cl_2, $NaHCO_3$; (d) various piperzine, Et_3N.

product **12** was obtained by heating the reaction mixture in the presence of the catalytic *p*-toluene sulfonic acid. Borane reduction of **12** followed by hydrogenolysis of the benzyl group with palladium hydroxide catalyst in methanol and HCl gave (2*R*,6*R*)-dimethyl piperazine dihydrochloride salt **13.** (2*R*,6*R*)-diethylpiperazine **14** and (2*S*,6*S*)-dimethyl piperazine **15** were prepared analogously.

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

Compounds	Receptor	Binding[b] IC_{50} (nM)	cAMP[c] EC_{50} (nM)	Activation[d] at 10 μM (%)
1	hMC1R	5100 ± 950[e]	––	30
	hMC3R	650 ± 67	––	3.6
	hMC4R	4.9 ± 0.3	22 ± 4.4	59
	hMC5R	850 ± 75	>3000	22
19	hMC1R	2100 ± 190	420 ± 110	24
	hMC3R	230 ± 20	––	1.2
	hMC4R	4.7 ± 0.3	13 ± 3.3	41
	hMC5R	430 ± 120[e]	––	14
20	hMC1R	7800 ± 445[e]	910 ± 290[f]	14
	hMC3R	760 ± 70	––	0.6
	hMC4R	16 ± 1.3	60 ± 7.8	21
	hMC5R	1400[f]	––	14
21	hMC1R	2100 ± 172	260 ± 78	39
	hMC3R	970 ± 60	––	2.8
	hMC4R	11 ± 0.8	18 ± 2.6	66
	hMC5R	1400 ± 280	1000 ± 110	31
22	hMC1R	3300±500	250±20[e]	46
	hMC3R	740 ± 160	––	2
	hMC4R	12 ± 2.0	58 ± 15	58
	hMC5R	1400[f]	1400 ± 340	30
23	hMC1R	2800 ± 280	400 ± 87	31
	hMC3R	390 ± 65	––	0[f]
	hMC4R	5.1 ± 0.5	––	18
	hMC5R	1300 ± 510[f]	––	1[f]
24	hMC1R	1500 ± 88[e]	280 ± 80[f]	24
	hMC3R	33 ± 4.5	––	1.3
	hMC4R	0.78 ± 0.1	––	15[e]
	hMC5R	54[f]	210 ± 110	26

[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 general procedure for the synthesis of urea analogs included EDC coupling of Boc-D-fluoro-Phe or Boc-D-chloro-Phe **16** and amine **17**, followed by removal of Boc gave compound **18**. It was then treated with triphosgene in the presence of sodium bicarbonate to form isocyanate followed by addition with various substituted piperazine to give urea compounds **1**, **19**, and **21**–**24**. Alkylation of compound **1** gave compound **20** (Scheme 3).

Compounds **1, 19**–**24** were initially evaluated in a competitive binding assays and functional assays (Table 1). Binding assay was performed to assess the competitive binding of test compounds vs [^{129}I]-NDP-α-MSH. The functional assay was conducted by measuring the accumulated cAMP in CHO cells expressing the human receptors.[32,33]

As shown in Table 1, compound **1** had potent MC4R binding activity (IC_{50} = 4.9 nM). In contrast to most MC4R compounds reported previously, compound **1** showed partial activation (59%) on MC4R with subnanomolar EC_{50} (22 nM). It also had excellent selectivity against other human sub-types receptors (over 100-fold selectivity). Diethyl piperazine analog **19** slightly improved the functional activity (EC_{50} = 13 nM), but reduced activation to 41% and diminished the MC1 selectivity (32-folds). Compound **20** with a methyl group on the nitrogen reduced both binding and functional activity by 2- to 3-folds and had low activation (21%) and it also reduced MC1 selectivity. *trans* (2*R*,6*R*)-Dimethyl piperazine compound **21** has potency similar to the corresponding *cis* compound **1**. (2*S*,6*S*)-Dimethyl piperazine compound **22** reduced functional activity and selectivity agonist MC1 and MC5. When the two methyl group on compound **21** was replaced by two ethyl group, *trans* (2*R*,6*R*)-diethyl piperazine compound **23** had good binding activity (IC_{50} = 5.1 nM), but no functional activity. Replacement of fluorine with chlorine in compound **1** gave compound **24,** which reduced the functional activity and became MC4R antagonist. Interestingly, unlikely piperazine compound **MB 243**, all those urea compounds had high binding potency, but weak agonism with activation below 60%.

Table 2
Pharmacokinetic data in rat[a]

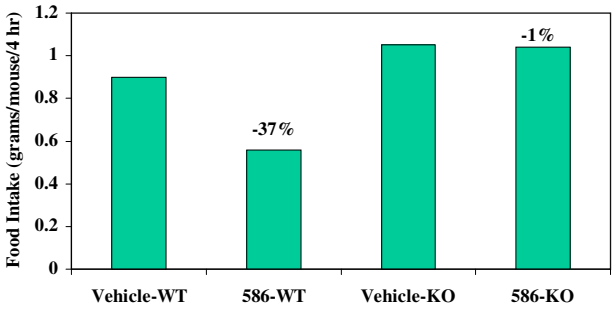
Compound	**1**	**19**	**21**	**23**
F (%)	28	46	1.9	18
Cl (mL min^{-1} kg^{-1})	35	36	43	62
V_{dss} (L kg^{-1})	4.7	4.7	9.2	8.2
$t_{1/2}$ (h)	2.0	1.7	4.2	1.8
AUCn (μM h/mpk)	0.24	0.37	0.02	0.09

[a] Compounds 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.

Table 3
Pharmacokinetic data for **1**

PK parameter	Rat[a]	Dog[b]	Monkey[c]
F (%)	28	118	20
Cl (mL min^{-1} kg^{-1})	35	5.4	5.2
V_{dss} (L kg^{-1})	4.7	1.8	1.0
$t_{1/2}$ (h)	2.0	5.1	5.3
$AUCN_{po}$ (μM h/mpk)	0.24	6.6	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] Compound dosed in beagles as a solution in EtOH/PEG400/water (10:40:50) at 0.5 mg/kg, iv and in 0.5% methylcellulose in water at 1.0 mg/kg, po.
[c] Compound dosed in rhesus as a solution in EtOH/PEG400/water (10:40:50) at 0.5 mg/kg, iv and in 0.5% methylcellulose in water at 1 mg/kg po.



Graph 1. Food intake of compound **1** on 4 h in wild type (WT) and MC3R/MC4R knockout (KO) mice.

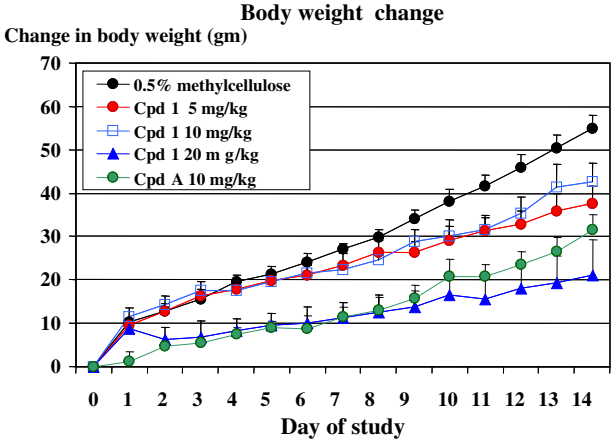
The pharmacokinetic (PK) properties of compounds **1, 19, 21** and **23** were further evaluated in the rat (Table 2). *cis*-Dimethyl-piperazine compound **1** had better oral bioavailability (28%) and plasma drug exposure (0.24 μM h/mpk) than *trans*-dimethyl piperazine compound **21** (F = 1.9% and AUCN = 0.02 μM h/mpk). *cis*-Diethyl piperazine compound **19** had improved oral bioavailability (F = 46%) in comparing to compound **1**. Similarly, the *trans* diethyl compound **23** also reduced oral bioavailability (F = 18%) and AUCN level (AUCN = 0.02 μM h/mpk).

Overall, compound **1** was the most promising compound in the series. As a partial agonist, it exhibited excellent binding affinity (IC_{50} of 4.9 nM) and functional activity (EC_{50} of 22 nM) at the human MC4 receptor and excellent selectivity agonist other MCR subtype (over 100-folds). It also had good oral bioavailability in rat. Compound **1** was further characterized in pharmacokinetics studies with other species. It showed an outstanding pharmacokinetic profile with excellent bioavailability, low clearance, and high plasma drug exposure, especially in dog (Table 3).

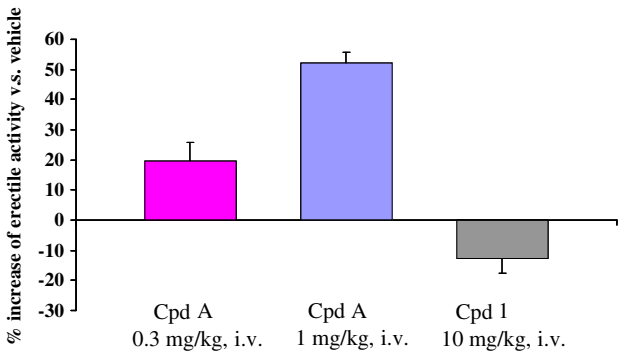
Compound **1** was tested in off-target activity screenings. It was very clean in Panlabs against hundreds of receptors, enzymes, and transporters. (>10 μM). It was also selective against ion channels (MK-499 >10 μM, Ca^{+2} 9.6 μM, Na^{+} 6.8 μM)

Compound **1** was evaluated on food intake in rodent in vivo obesity models (Graph 1). It showed mechanism based acute food-intake reduction in DIO mice (Graph 1). It reduced food intake 37% (P <0.05) in wild type mouse comparing to the vehicle and had no effects in MC3R/MC4R knockout mouse at 20 mpk dose at 4 h post dosing.

The efficacy of **1** was investigated in vivo rat obesity model. Compound **1** was administered orally at 5, 10, 20 mpk bid for fourteen days with vehicle and MC4R full agonist compound A[23]



Graph 2. Compound **1** body weight change study in Bid dosing to DIO rats using MC4R full agonist compound A as comparison.



Graph 3. Erectile activity study in CN-stimulated mouse of compound **1** using MC4R full agonist compound A as comparison.

(10 mpk dose) as controls. As illustrated in Graph 2, compound **1** reduced body weigh at 5, 10, 20 mpk in comparison with the vehicle. It was also comparable to MC4R full agonist **A** in reducing the body weight.

Finally, erectogenic activity of compound **1** was also evaluated using a conscious rat model in which the occurrence of spontaneous erections was quantified in the absence of an external stimulus.[1,34,35] This study was performed using MC4R full agonist **A**[23] as comparison (Graph 3). Consistent with previously reported MC4R full agonists, full agonist **A** had significant increase the erectile activity at 10 mpk, even enhanced the erectile activity in 0.3 mpk. However, when compound **1** was dosed at 10 mpk there was no effect on erectile activity. After 15 min administration, compound **1** had excellent plasma exposure (4.7 μM dosed at 10 mpk) compared with compound **A** (47 nM dosed at 0.3 mpk). Therefore, Compound **1** lacks PE activity in CN-stimulated mouse.

Earlier studies showed that dipeptide and non-peptide selective MC4R full agonists had both food intake reduction and proerectile activity in rodent models.[9–12,19,20] The effect of MC4R agonists on sexual functions represents a complication in the development of the compounds for obesity. It is unclear whether these effects will be a manageable side-effect or will outweigh any benefits of MC4R in the treatment of obesity in human.[10] Therefore, for the treatment of obesity purposes, increased erectile activity could be an undesirable adverse effect. This finding provides the potential for developing MC4R partial agonists for obesity without modulation of sexual behavior.

In summary, we report the synthesis and evaluation of MC4R partial agonists containing piperazine substructure. This study led to the discovery of MC4R partial agonist compound **1** with high in vitro potency and excellent pharmacokinetic profiles. Compound **1** also exhibits an excellent off-target activity profile. Finally, the efficacy of MC4R partial agonist compound **1** is comparable to MC4R full agonist **A** in our animal models for both food intake and body weigh study, while it has no effect on erectile activity. Therefore, MC4R partial agonists could be utilized to treat anti-obesity without potentiating erectile activity.

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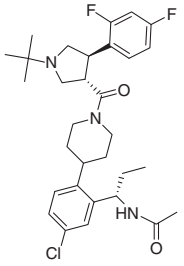
Structure of compound A:



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