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Discovery of (2*S*)-*N*-[(1*R*)-2-[4-cyclohexyl-4-[[(1,1-dimethylethyl)-amino]carbonyl]-1-piperidinyl]-1-[(4-fluorophenyl)methyl]-2-oxoethyl]-4-methyl-2-piperazinecarboxamide (MB243), a potent and selective melanocortin subtype-4 receptor agonist

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Abstract—We report the discovery and optimization of substituted 2-piperazinecarboxamides as potent and selective agonists of the melanocortin subtype-4 receptor. Further in vivo development of lead agonist, **MB243**, is disclosed.

The five cloned melanocortin receptor subtypes (MC1R-MC5R) are part of a family of seven-transmembrane G-protein-coupled receptors. These receptors, which are located peripherally and centrally, interact with their endogenous ligands, the melanocortins and corticotropins, to regulate a diverse number of physiological functions, including the control of feeding and sexual behavior, as well as skin pigmentation, steroidogenesis, energy metabolism, and exocrine secretion.[1] The melanocortin subtype-4 receptor (MC4R) is expressed in the hypothalamus, and both rodent and human genetic and pharmacological data indicate that inactivation of this receptor results in obesity.[2,3] In addition, rodent and human pharmacological data indicates that activation of MC4R modulates erectile function and sexual behavior.[4,5] Consequently, over the past few years, research efforts in drug discovery groups have focused on identifying novel MC4R agonists for the potential treatment of obesity and sexual dysfunction.[6]

Numerous reports on the identification of structurally related small-molecule MC4R agonists have emerged in the literature[7,8] as well as structurally distinct MC4R agonists.[9–11] Recent efforts at Merck have lead to the identification of **1**, a potent and selective, small-molecule agonist of the MC4R. Indeed, **1** was shown to stimulate erectile activity as well as reduce food intake in rats.[12]

Lead compound **1** proved to be a useful tool for studying the pharmacology of an MC4R selective agonist; however, issues with off-target activity prevented further development of this compound. We speculated that the triazole and tetrahydroisoquinoline moieties were contributing to the undesirable off-target activity. Consequently, efforts were focused on identifying suitable chemical subunits, which can replace the triazole and tetrahydroisoquinoline moieties to minimize off-target

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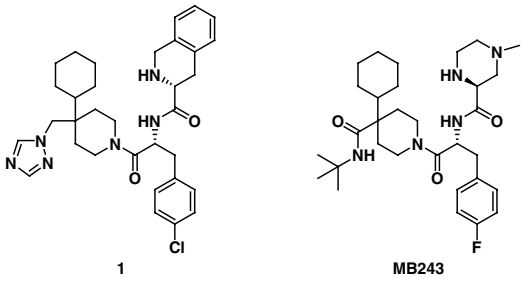
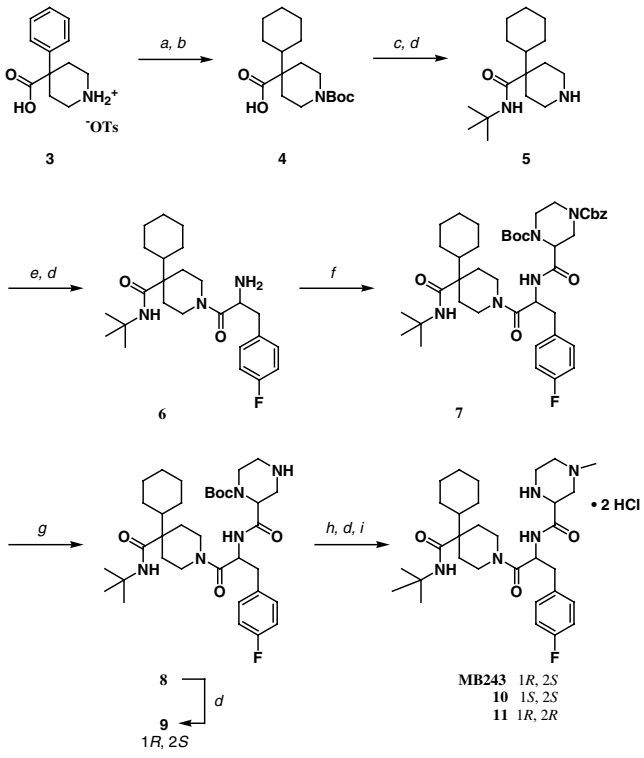
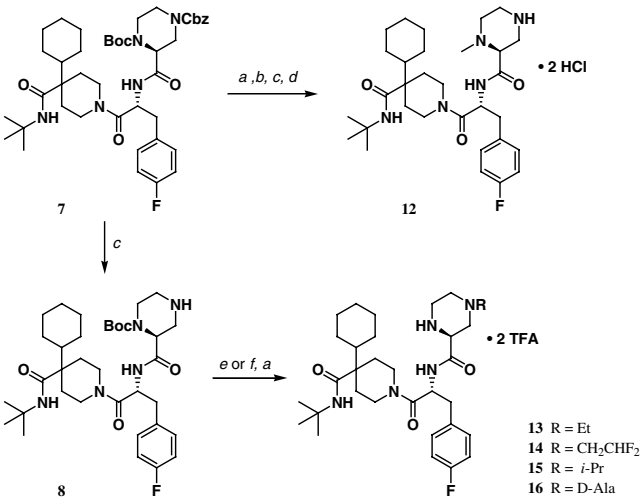
activity while maintaining potency. Structure–activity studies led to the discovery that a *t*-butylamide group can replace the triazole,[13] and a piperazine can substitute the tetrahydroisoquinoline side chain. In addition, replacement of the *p*-chloro-D-phenylalanine amino acid with a *p*-fluoro-D-phenylalanine amino acid led to improved functional potency at the MC4R.[14] Herein, we report the synthesis and evaluation of MC4R agonists containing a piperazine side chain as alternatives to the tetrahydroisoquinoline moiety. These efforts lead to the discovery of a potent and selective MC4R agonist, **MB243**, which is efficacious in our animal models for both food intake and erectile dysfunction.



The piperazine compounds were prepared in a linear fashion as illustrated in Schemes 1 and 2. Starting with commercially available 4-phenyl-4-piperidine carboxylic acid 4-methylbenzenesulfonate, **3**, Boc-protection of the secondary amine followed by reduction using Rh/Al_2O_3 as catalyst provided piperidine **4**. The carboxylic acid was then converted to the *tert*-butylamide followed by deprotection of the amine to yield piperidine **5**. Piperidine **5** was coupled to *N*-Boc-*p*-fluoro-(D or L)-phenylalanine by standard procedures. Removal of the Boc-protecting group provided the free amine, which was then coupled with 1-Boc-4-Cbz-piperazine-2-(*S* or *R*)-carboxylic acid to afford dipeptide **7**. Sequential amine deprotections yielded piperazine **9**. Reductive amination of piperazine **8** and final deprotection with conversion to the hydrochloride salt, afforded **MB243** and its stereoisomers, **10** and **11**.



Scheme 1. Reagents and conditions: (a) $(Boc)_2O$, NaOH, dioxane; (b) Rh/Al_2O_3, 70 psi H_2, MeOH, 70 °C; (c) $(COCl)_2$, DMF, CH_2Cl_2, then, *t*-$BuNH_2$, CH_2Cl_2; (d) TFA, CH_2Cl_2; (e) *N*-Boc-*p*-fluoro-(D or L)-Phe, EDC, HOBt, DIEA, CH_2Cl_2; (f) 1-Boc-4-Cbz-piperazine-2-(*S* or *R*)-carboxylic acid, EDC, HOBt, DIEA, CH_2Cl_2; (g) H_2, Pd/C, EtOH; (h) HCHO, $NaBH_3CN$, TFA, NaOAc, MeOH; (i) HCl/Et_2O, CH_2Cl_2.



Scheme 2. Reagents and conditions: (a) TFA, CH_2Cl_2; (b) HCHO, $NaBH_3CN$, NaOAc, MeOH; (c) H_2, Pd/C, EtOH; (d) HCl/Et_2O, CH_2Cl_2; (e) acetaldehyde, or difluoroacetaldehyde ethyl hemiacetal, or acetone, $NaBH_3CN$, TFA, NaOAc, MeOH; (f) *N*-Boc-D-Ala, EDC, HOBt, DIEA, CH_2Cl_2.

As illustrated in Scheme 2, various substituted piperazine compounds were synthesized from dipeptide **7**. Reversal of the deprotection sequence on the piperazine ring and reductive amination provided piperazine **12**. Piperazines **13**, **14**, and **15** were made in a similar fashion to **MB243** using the requisite aldehyde precursors for reductive amination. Likewise, piperazine **16** was synthesized from intermediate **8** by coupling with *N*-Boc-D-alanine and final deprotection.

The piperazine compounds (**MB243**, **9**–**16**) were evaluated in a competitive binding assay (Table 1) and functional assay (Table 2).[15] **MB243** is the most promising compound in terms of overall binding affinity, functional potency, and receptor subtype selectivity. Stereochemistry about the *p*-flurorophenylalanine and piperazine groups is also very important for binding and functional activity and selectivity. The 1-(*S*)-*p*-fluorophenylalanine analog, **10**, shows poor binding affinity at MC4R, and cAMP levels from the functional assay suggest that it is only a partial agonist (29% activation). The 2-(*R*)-piperazine analog, **11**, shows decreased binding affinity and functional activity compared with the

Table 1. Binding affinity and selectivity of compounds for the human melanocortin subtype-4 receptor[a]

Compds	IC_{50}[b] (nM)		
	MC4R	MC3R/4R	MC5R/4R
α-MSH	19 ± 2	1	6
MB243	16 ± 1	101	147
9	18 ± 4	57	63
10	6500 ± 1900[c]	3	3
11	52 ± 44[c]	98	76
12	87 ± 37[c]	84	63
13	20 ± 3[c]	141	43
14	13 ± 3	58	54
15	8 ± 1	46	36
16	30 ± 8	43	42

[a] Values represent mean ± standard error except where indicated. 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] Values ($n = 2$) represent mean ± standard deviation.

Table 2. Functional activity of compounds at human melanocortin receptors

Compds	EC_{50}[a] (nM) [% max][b]		
	MC4R[c]	MC3R[d]	MC5R[c]
α-MSH	1.9 ± 0.1 [100]	1.1 ± 0.1 [102]	17 ± 1 [113]
MB243	11 ± 1 [87]	[11 ± 5]	1900 ± 180 [32]
9	13 ± 2 [99]	[10 ± 4]	1800 ± 320 [43]
10	[29 ± 18][d]	[1 ± 3]	[3 ± 3][d]
11	82 ± 14 [92]	[7 ± 4]	2700 ± 600 [39]
12	29 ± 11 [97]	2900 ± 650 [19][c]	3300 ± 800 [19]
13	12 ± 3 [90]	[3 ± 1]	840 ± 140 [20]
14	52 ± 11 [50]	[6 ± 4]	510 ± 120 [19]
15	34 ± 11 [55]	[4 ± 3]	340 ± 53 [22]
16	270 ± 82 [64]	[12 ± 4]	2000 ± 640 [27]

[a] Concentration of compound at 50% maximum cAMP accumulation.
[b] Percentage of cAMP accumulation at 10 μM compound relative to α-MSH.
[c] Values represent mean ± standard error except where indicated. All data represent at least three determinations.
[d] Values represent mean ± standard deviation except where indicated. All data represent at least three determinations.

2-(*S*)-piperazine analog, **MB243**, but more importantly selectivity versus the MC5R is diminished. Likewise, methylation at the alternate amine of the piperazine ring reduces binding affinity at MC4R and subtype selectivity (**12**).

Amine substitution on the piperazine ring revealed a tight structure–activity relationship. As illustrated in Tables 1 and 2, the non-substituted piperazine analog, **9**, exhibits similar binding affinity and functional potency as **MB243**, but with decreased binding subtype selectivity. Likewise, size and electronic effects of the alkyl substituent on the piperazine ring (**13**, **14**, and **15**) has little impact on binding affinity and functional activity, yet imparts a significant effect on MC5R selectivity. Finally, reducing the basicity of the amine on the piperazine ring through coupling with D-alanine dramatically reduces both binding and functional potency and selectivity (**16**).

MB243 was further evaluated in vitro on human, rat, and mouse melanocortin receptors. The data outlined in Table 3, shows this compound is >100-fold selective versus the subtype receptors in binding affinity in human, rat, and mouse cells. In the functional assays, **MB243** is >32-fold selective versus the other subtype receptors. Nonetheless, this compound shows good subtype selectivity on rat and mouse receptors.

MB243 was further characterized in pharmacokinetic (PK) studies with rats, dogs, and monkeys (Table 4). The oral bioavailability across all three species was low and was attributed, in part, to incomplete absorption. Nonetheless, stability data from liver microsome incubations with **MB243** predicted a low clearance in humans, and the potential for higher oral bioavailability than in rats, dogs, or monkeys.[16]

The efficacy of **MB243** was investigated in our in vivo rat obesity model. **MB243** was administered at 20 mg/kg p.o. bid for four days with vehicle and dexfenfluramine (3 mg/kg, bid) as controls, to Sprague–Dawley rats raised on an energy dense diet. Indeed, the body weights of the rats treated with **MB243** showed an overall decrease of -7 ± 5 g over the four days ($p < 0.01$ relative to vehicle; two tail *t*-test). The body weights of the

Table 3. Binding affinity and functional activity of **MB243** at human, rat, and mouse melanocortin receptors[a]

Receptor	Binding[b] IC_{50} (nM) [% inh.]	cAMP[c] EC_{50} (nM) [% max]	Activation[d] at 10 μM (%)
hMC1R	1700 ± 320	350 ± 88 [85]	
hMC3R	1600 ± 300		11 ± 5
hMC4R	16 ± 1	11 ± 1 [87]	
hMC5R	2400 ± 130	1900 ± 180 [32]	
rMC3R	2300 ± 130	990 ± 180 [21]	
rMC4R	8 ± 1	6 ± 1 [76]	
rMC5R	13,000 ± 1200		4 ± 1
mMC3R	1100 ± 100		7 ± 1
mMC4R	7 ± 1	16 ± 11 [66]	

[a] All data represent at least three determinations.
[b] Displacement of [^{125}I]-NDP-α-MSH receptors expressed in CHO cells. Values represent mean ± standard error.
[c] Concentration of compound at 50% maximum cAMP accumulation. Values represent mean ± standard error.
[d] Percentage of cAMP accumulation at 10 μM compound relative to α-MSH. Values represent mean ± standard deviation.

Table 4. Pharmacokinetic data for **MB243**

PK parameter	Rat[a]	Dog[b]	Monkey[c]
F (%)	9	17	11
Cl ($mL\,min^{-1}\,kg^{-1}$)	48.7	5.6	15.7
V_{dss} ($L\,kg^{-1}$)	5.4	1.2	1.2
$t_{1/2}$ (h)	1.6	4.1	1.3
T_{max} (h)	1.54	0.75	5.0

[a] Compound dosed in Sprague–Dawley rats as a solution in water at 1 mg/kg, iv and 5 mg/kg, p.o.
[b] Compound dosed in beagles as a solution in water at 0.5 mg/kg, iv and 2.0 mg/kg, p.o.
[c] Compound dosed in rhesus as a solution in water at 1 mg/kg, iv and 2 mg/kg, p.o.

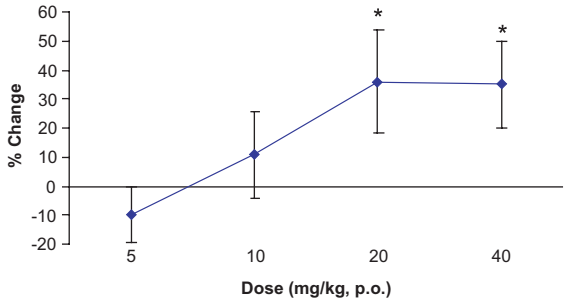


Figure 1. Penile erections in conscious rat ex copula model. **MB243** dose dependently increased the number of penile erections. Values represent mean ± SEM. $*p < 0.05$, compared to vehicle treatment (paired *t*-test). $N = 10$/dose.

control rats also changed during the four days with a decrease of −16 ± 3 g in the rats treated with dexfenfluramine ($p < 0.01$), and an increase of +15 ± 2 g in vehicle treated rats.[17]

Further in vivo studies were performed to evaluate the erectogenic activity of **MB243** using an established rodent model.[4c,d] The mean number of erections elicited over a fifteen-minute time period, determined by a direct visual count, in vehicle treated rats was 21.6 ± 1.2, $n = 40$. Erectogenic effects were investigated using a within animal design in which each rat served as its own control where the effect of compound was compared to that produced by vehicle. Sixty minutes following p.o. administration of **MB243** (5–40 mg/kg) adose-dependent increase in erections was observed (Fig. 1). The maximal increase in the number of erections (~35%) was detected at 20 and 40 mg/kg, p.o. In addition, iv administration of **MB243** (3 mg/kg) also produced statistically significant increases in erectile responses with a mean increase of 21.2 ± 7.9% ($p < 0.01$; $n = 10$). The magnitude of the MC4R agonist-induced erectile activity compared favorably to apomorphine (30.9 ± 8.2%, $p = 0.02$, @ 0.025 mg/kg, sc), a known central initiator of erectile activity.[18]

In summary, we report the synthesis and evaluation of MC4R agonists containing a piperazine side chain as alternatives to the tetrahydroisoquinoline moiety. Structure–activity studies around the piperazine side chain lead to the discovery of a potent and selective MC4R agonist, **MB243**, which exhibits an excellent off-target activity profile (IC_{50} or $K_i > 1000$ nM) based on broad screening performed at Panlabs versus >100 receptors, enzymes, and ion channels.[19] Finally, **MB243** is efficacious in our animal models for both food intake and erectile dysfunction.

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