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2-Piperazinecarboxamides as potent and selective melanocortin subtype-4 receptor agonists

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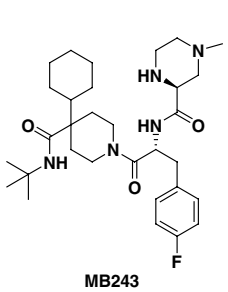
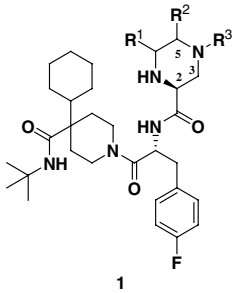
Abstract—We report the discovery and optimization of substituted 2-piperazinecarboxamides as potent and selective agonists of the melanocortin subtype-4 receptor. The 5- and 6-alkylated piperazine compounds exhibit low bioactivation potential as measured by covalent binding in microsome preparations.

The five cloned melanocortin receptor subtypes (MC1R–MC5R) are a 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] Over the past few years, research efforts in drug discovery groups are focusing on identifying novel MC4R agonists for the potential treatment of obesity and sexual dysfunction.[2]

Recent efforts at Merck have led to the identification of **MB243**, a potent and selective, small-molecule agonist of the MC4R. Indeed, **MB243** was shown to stimulate erectile activity as well as reduce food intake in rats.[3] Nonetheless, further development of this compound was discontinued due to the potential for bioactivation as seen in vitro through extensive covalent binding in rat and human liver microsomes.[4] NMR analysis of the isolated adducts suggested that bioactivation was occurring via initial oxidation at C-5 on the piperazine ring. Consequently, we speculated that blocking the site of activation via alkylation on the piperazine ring should reduce bioactivation. Herein, we report the synthesis and evaluation of alkyl-substituted 2-piperazinecarboxamides, which are potent and selective MC4R agonists with significantly reduced bioactivation.



MB243



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The 5-alkylated piperazine compounds were prepared in a linear fashion as illustrated in Scheme 1. Starting with commercially available methyl *N*-β-Boc-L-α,β-diaminopropionate hydrochloride, **2**, conversion to diamine **3** was achieved via alkylation with various substituted α-bromoketones.[5] Removal of the Boc group followed by intramolecular reductive amination provided

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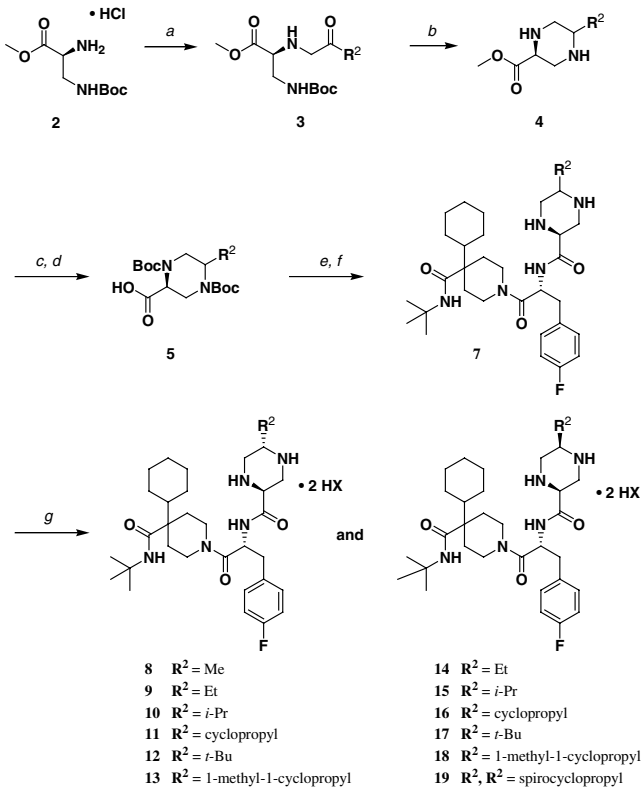
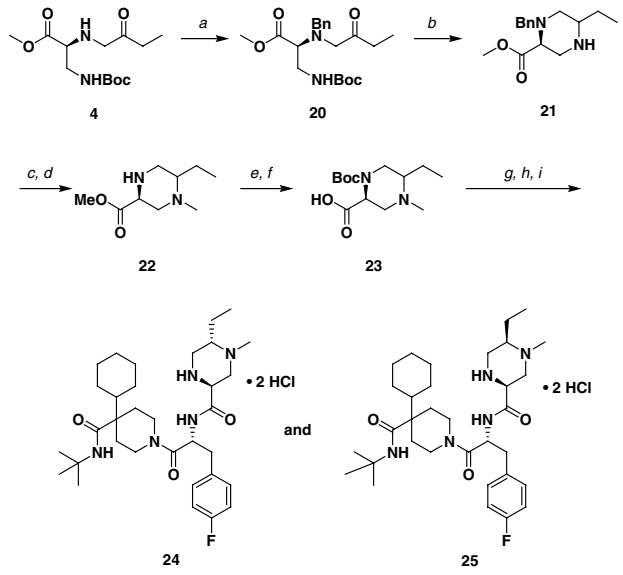


Scheme 1. Reagents and conditions: (a) α-bromoketone, DIEA, DMF (12–60%); (b) TFA, CH_2Cl_2, then, $Na(OAc)_3BH$, AcOH, DCE; (c) $(Boc)_2O$, TEA, CH_2Cl_2 (45–74% for two steps); (d) NaOH, MeOH, H_2O (88–100%); (e) **6**, EDC, HOBt, DIEA, CH_2Cl_2 (46–93%); (f) TFA, CH_2Cl_2 (98–100%); (g) preparative HPLC.

piperazine **4**. Protection of both secondary amines of the piperazine ring followed by hydrolysis of the ester yielded the 5-alkylated-2-piperazinecarboxylic acids, **5**. Coupling of piperazine **5** with 1-[(2*R*)-2-amino-3-(4-fluorophenyl)-1-oxopropyl]-4-cyclohexyl-*N*-(1,1-dimethylethyl)-4-piperidine carboxamide (**6**)[6] and deprotection provided dipeptide **7** as a 2:1–10:1 mixture of *trans*:*cis* diastereomers. The diastereomers were separated using preparative reverse-phase HPLC to give the *trans*-piperazines **8**–**13**, and *cis*-piperazines **14**–**18**, as either the ditrifluoroacetic acid or dihydrochloride salts.[7] Piperazine **19** contains a spirocyclopropyl group at C-5, and consequently, preparative HPLC separation was not necessary.

Scheme 2 illustrates the synthesis of compounds **24** and **25**. Starting with diamine **4**, benzyl protection of the α-amine, Boc-deprotection, and intramolecular reductive amination provided piperazine **21**. Reductive amination of the piperazine amine with formaldehyde followed by hydrogenation yielded the methylated piperazine **22**. Final Boc-protection and hydrolysis provided piperazine acid **23**. Coupling of piperazine acid **23** with amine **6**,[6] deprotection, and separation via preparative reverse-phase HPLC provided the *trans*-piperazine **24** and *cis*-piperazine **25**.

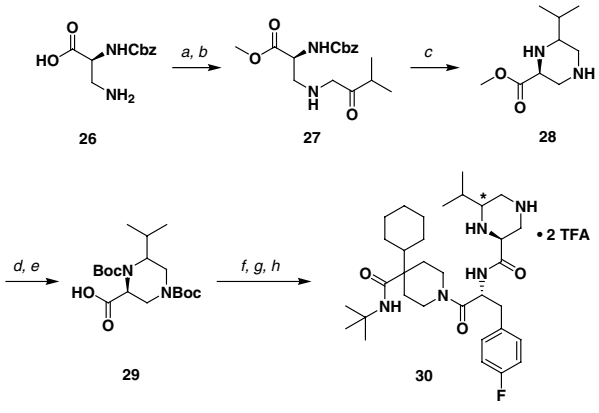
In an effort to further understand bioactivation of 2-piperazinecarboxamides, we also synthesized and examined



Scheme 2. Reagents and conditions: (a) PhCHO, $Na(OAc)_3BH$, DCE (100%); (b) TFA, CH_2Cl_2, then, $Na(OAc)_3BH$, AcOH, DCE (72%); (c) HCHO, $NaCNBH_3$, NaOAc, TFA, MeOH (71%); (d) H_2, Pd/C, HCl, EtOH (55%); (e) $(Boc)_2O$, TEA, CH_2Cl_2 (83%); (f) NaOH, MeOH, H_2O (100%); (g) **6**, EDC, HOBt, DIEA, CH_2Cl_2 (84%); (h) TFA, CH_2Cl_2 (98%); (i) preparative HPLC.

a 6-alkylated-2-piperazincarboxamide. The synthesis of piperazine **30**, is depicted in Scheme 3. The commercially available *N*-α-*Z*-L-2,3-diaminopropionic acid, **26**, was converted to the methyl ester and then alkylated with α-bromo-3-methyl-2-butanone[5] to give **27**. Deprotection via hydrogenation and concomitant intramolecular reductive amination provided piperazine ester **28**. Protection of both secondary amines of the piperazine ring and hydrolysis yielded acid **29**. Coupling, deprotection, and separation via preparative reverse-phase HPLC afforded piperazine **30** as a single diastereomer.[8]

The piperazine compounds (**8**–**19**, **24**–**35**, **30**) were evaluated in a competitive binding assay (Table 1) and



Scheme 3. Reagents and conditions: (a) TMSCl, MeOH (98%); (b) α-bromo-3-methyl-2-butanone, DIEA, DMF (49%); (c) H_2, Pd/C, EtOH (93%); (d) $(Boc)_2O$, TEA, CH_2Cl_2 (62%); (e) NaOH, MeOH, H_2O (53%); (f) **6**, EDC, HOBt, DIEA, CH_2Cl_2 (48%); (g) TFA, CH_2Cl_2 (97%); (h) preparative HPLC.

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
8	6 ± 1	86	87
9	3 ± 2[c]	152	126
10	4 ± 1	140	116
11	8 ± 1[c]	119	59
12	5 ± 1	116	39
13	5 ± 1	99	40
14	40 ± 5	59	75
15	20 ± 6	61	49
16	25 ± 10	42	65
17	29 ± 9	48	26
18	24 ± 5	49	23
19	17 ± 3	42	61
24	3 ± 1[c]	116	192
25	46 ± 5	36	49
30	11 ± 1[c]	81	105

[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.

functional assay (Table 2).[9] The *trans*-piperazine compounds (**8**–**13**, **24**, **30**) are the best compounds in terms of overall binding affinity, functional potency, and receptor subtype selectivity. In addition, *N*-methylation on the piperazine ring, does not affect potency or selectivity (**24** vs **9**). The spirocyclopropylpiperazine compound, **19**, shows a 5-fold loss in functional potency in comparison to the *trans*-methyl analog, **8**, with reduced receptor subtype selectivity. Interestingly, the 6-isopropylpiperazine compound, **30**, is a potent and selective MC4R agonist with very little activation on the MC5R subtype (10%).

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][c]	17 ± 1 [113]
8	8 ± 2 [79]	[6 ± 3]	880 ± 100 [42]
9	5 ± 1 [89]	[9 ± 4]	710 ± 270 [43]
10	3 ± 1 [82]	[8 ± 2]	340 ± 29 [49]
11	5 ± 1 [102]	[9 ± 4]	760 ± 200 [44]
12	7 ± 2 [99]	[11 ± 3]	360 ± 58 [46]
13	7 ± 2 [95]	[10 ± 3]	300 ± 48 [62]
14	150 ± 73 [69]	[6 ± 2]	[45 ± 4][d]
15	42 ± 10 [77]	[6 ± 2]	1400 ± 210 [41]
16	36 ± 10 [67]	[4 ± 3]	1400 ± 280 [33]
17	91 ± 25 [77]	[5 ± 3]	1000 ± 170 [54]
18	93 ± 23 [69]	[5 ± 4]	920 ± 210 [56]
19	41 ± 16 [53]	[6 ± 4]	1900 ± 220 [36]
24	2 ± 1 [85]	150 ± 21 [29][c]	450 ± 51 [56]
25	330 ± 170 [58]	[8 ± 2]	2400 ± 1000 [36]
30	18 ± 2 [92]	[4 ± 1]	[10 ± 1][d]

[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.

Table 3. Covalent binding to rat liver microsomal proteins[a]

Compound	+ NADPH (pmol equiv/mg protein)
MB243	2153 ± 266[b]
8	50
10	107
11	103
30	216

[a] The numbers represent values for covalent binding after 30 min incubations of [^{3}H]-compound[11] with rat liver microsomes in the presence of NADPH.
[b] Mean ± standard deviation (n = 3).

A representative number of the alkylated piperazine compounds were evaluated for potential bioactivation.[10] Indeed, alkyl substitution on C-5 of the piperazine ring dramatically reduced covalent binding in comparison to **MB243** (Table 3). Likewise, the 6-isopropylpiperazine analog, **30**, also afforded a 10-fold decrease in covalent binding as compared to **MB243**. Thus, as predicted, alkylation at either C-5 or C-6 of the piperazine ring blocks oxidation and further bioactivation.

In summary, we report the synthesis and evaluation of MC4R agonists containing a 5- or 6-alkylated piperazine side chain. Structure–activity studies show the *trans*-5-alkylpiperazine diastereomers are potent and selective MC4R agonists, while the corresponding *cis*-isomers exhibit moderate potency and receptor subtype selectivity. More importantly, the 5- and 6-alkylated piperazine side-chain analogs show significantly reduced covalent binding in comparison to **MB243**.

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5. The α-bromoketones were either commercially available or prepared via bromination of ketone using bromine in methanol.
6. See Ref. 3 for the synthesis of this intermediate.
7. NMR studies were carried out to determine the stereochemistry of the piperazine ring, and the major isomer was assigned *trans* based on the observed coupling constants.
8. Absolute stereochemistry was not determined and minor diastereomer could not be isolated.
9. For a detailed description of the assay protocols, see: (a) Bednarek, M. A.; MacNeil, T.; Kalyani, R. N.; Tang, R.; Van der Ploeg, L. H. T.; Weinberg, D. H. *J. Med. Chem.* **2001**, *44*, 3665; (b) Bednarek, M. A.; Siva, M. V.; Arison, B.; MacNeil, T.; Kalyani, R. N.; Huang, R.-R. C.; Weinberg, D. H. *Peptides* **1999**, *20*, 401.
10. For a detailed description of the assay protocols, see Ref. 4.
11. The tritium group is on the *p*-fluorophenyl ring of each compound.