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Pyrrolidines as potent functional agonists of the human melanocortin-4 receptor

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Abstract—A series of pyrrolidine derivatives were synthesized and characterized as potent agonists of the human melanocortin-4 receptor. For example, **28c** had a K_i of 13 nM in binding affinity and EC_{50} of 6.9 nM in agonist potency with an intrinsic activity of 100% of the endogenous ligand α-MSH.

The melanocortin-4 receptor (MC4R) is a member of the G-protein-coupled receptor superfamily, and plays an important role in regulating feeding behavior and other biological functions.[1] Therefore, MC4R agonists have been extensively studied in an effort to discover small molecules for the treatment of obesity.[2] Several MC4R agonists from different chemical classes have been reported.[3] For example, compound **1** (Fig. 1),[4] characterized as a potent and selective MC4R agonist, has demonstrated efficacy in obesity models in rodents.[5] A series of phenylpiperazines **2**–**5** have been reported by several research groups. Richardson and coworkers have reported that compound **2** (EC_{50} = 71 nM) is moderately active, while its imidazole derivative **4** (EC_{50} = 14 nM) possesses potent agonist activity at *h*MC4R.[6] The methanesulfonamide **3** as an MC4R agonist has been reported by Dyck,[7] Richardson,[6] and Fotsch and coworkers.[8] In addition, a *N*,*N*-dimethylaminomethyl analog **5** (K_i = 60 nM, EC_{50} = 7 nM) exhibits good binding affinity and agonist potency.[6] We have also found that an additional amino group at this site improves the interaction of this series of compounds with the receptor.[9] For example, compound **6** (K_i = 6.4 nM, EC_{50} = 3.8 nM) is a 100-fold more potent agonist than the sulfonamide **3** (EC_{50} = 380 nM) in our assay.[7] Recently, we have discovered that benzylamine with a small alkyl group at the benzylic position increases the binding affinity of a series of antagonists such as **8**.[10]

Ujjainwalla has recently reported that a series of pyrrolidines are potent and selective MC4R agonists.[11] For example, compound **9** has an EC_{50} of 2 nM in a MC4R functional assay and an IC_{50} of 14 nM in a binding assay. One advantage of this compound is that it is less peptide-like than **1**. Here we report our exploratory work on the combination of the pyrrolidine moiety in **9** with the piperazinbenzylamine functionality in our MC4R antagonist template **8** to synthesize potent MC4R agonists.

The *trans*-*N*-isopropylpyrrolidine carboxylic amides **14**–**19** were synthesized by the reaction of *trans*-1-*tert*-butoxycarbonyl-4-(4-chlorophenyl)pyrrolidine-3-carboxylic acid **11** with a variety of phenylpiperazines **12a**– **f** under coupling conditions, followed by deprotection of the Boc-group of **13** and reductive alkylation with acetone as shown in Scheme 1. For compounds **17**–**19**,[12] an HCl/MeOH treatment was required to remove the *tert*-butylsulfinyl group before purification (Scheme 1).

Reduction of benzonitrile **15** with sodium borohydride catalyzed with nickel chloride afforded the benzylamine **20a**, which was converted to the corresponding methanesulfonamide **20b**. Reductive alkylation of **20a** with isobutyraldehyde provided the secondary amine

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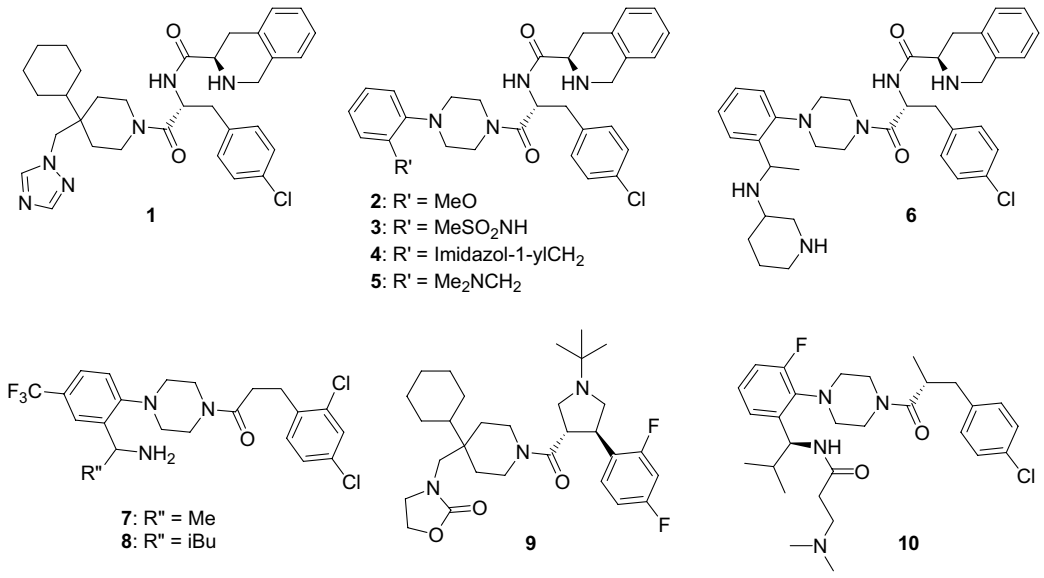
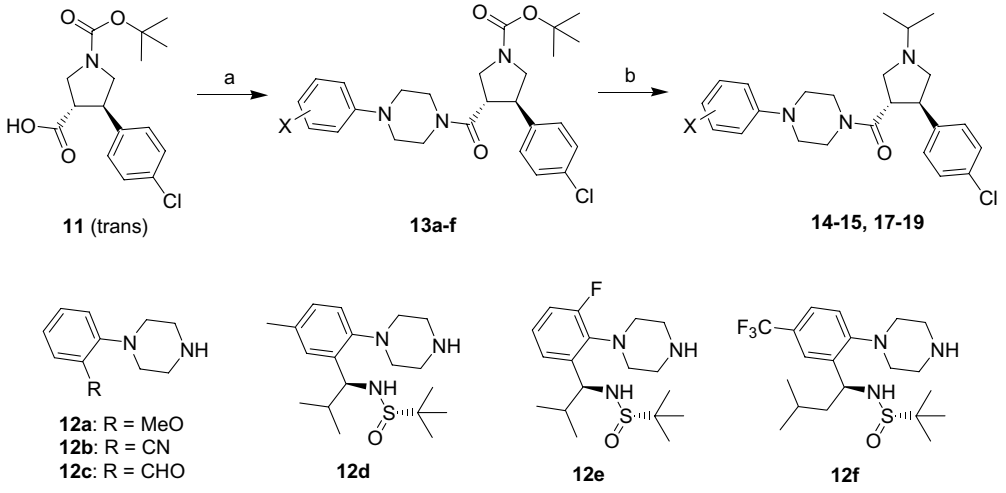


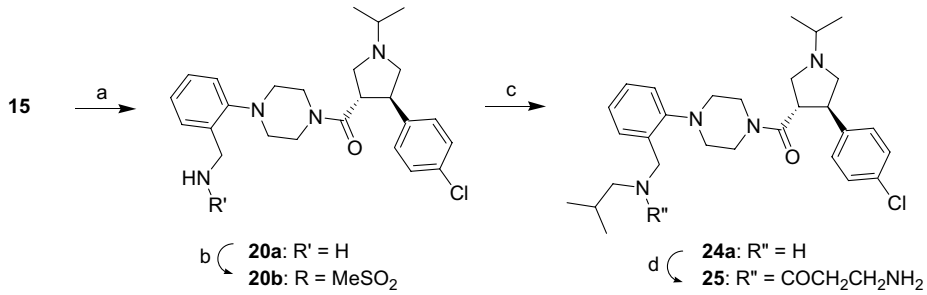
Figure 1. Small molecules as *h*MC4R ligands.



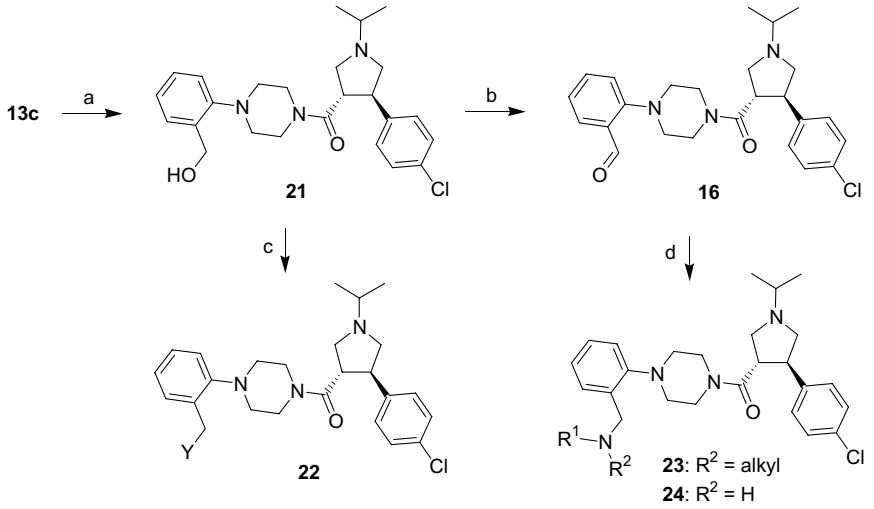
Scheme 1. Reagents and conditions: (a) HBTU/DIEA/DMF/rt, 4 h, ~80%; (b) i—TFA/CH_2Cl_2/rt, 1 h; ii—Me_2CO/$NaBH(OAc)_3$/AcOH/dichloroethane/rt, 8 h; for **17**–**19**: iii—HCl/MeOH/rt, 1 h.

24a, which was coupled with β-alanine to give the amide **25** (Scheme 2).

The benzaldehyde **13c** was derivatized as described in Scheme 3. Reductive alkylation of **13c**, after a TFA treatment to remove the Boc-group, with acetone afforded the benzylalcohol **21**, which was converted to the corresponding mesylate, followed by nucleophilic replacement with various imidazoles, triazoles, and pyrazoles to give compounds **22**. Oxidation of **13c** with



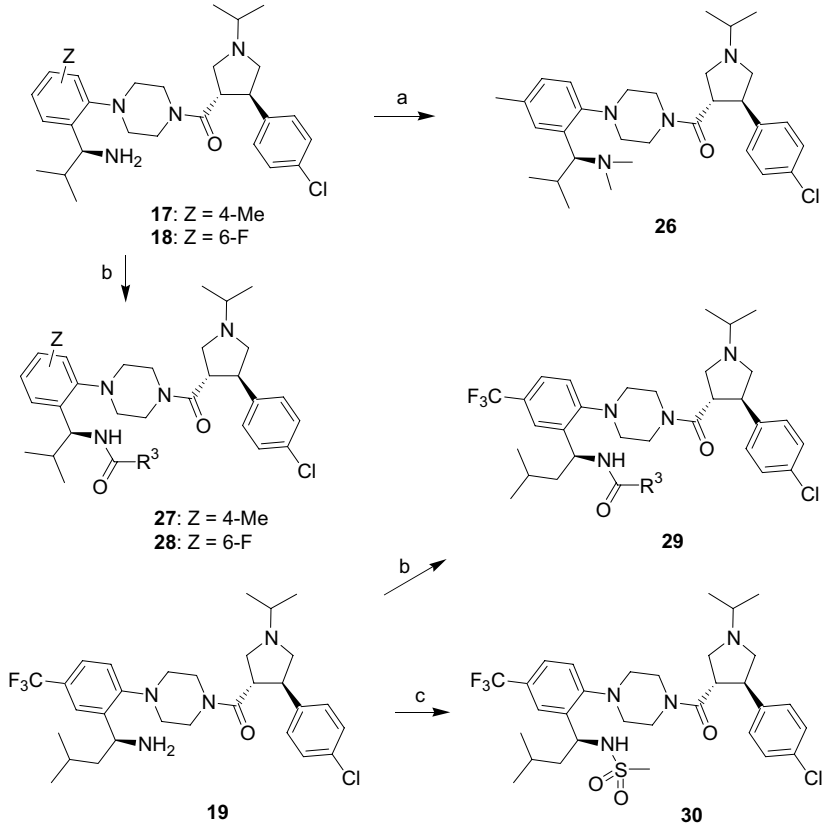
Scheme 2. Reagents and conditions: (a) $NiCl_2/NaBH_4$/EtOH/60 °C, 24 h; (b) $MeSO_2Cl/Et_3N/CH_2Cl_2$/rt, 1 h; (c) $Me_2CHCHO/NaBH(OAc)_3$/CH_2Cl_2/rt, 8 h; (d) i—*N*-Boc-β-Ala-OH/EDC/HOBt/Et_3N/CH_2Cl_2/rt, 2 h; ii—TFA/CH_2Cl_2/rt, 1 h.



Scheme 3. Reagents and conditions: (a) i—TFA/CH_2Cl_2, rt, 1 h; ii—Me_2CO/$NaBH(OAc)_3$/CH_2Cl_2/rt, 8 h; (b) PCC/CH_2Cl_2/rt, 3 h, 55%; (c) i—$MeSO_2Cl$/Et_3N/CH_2Cl_2/rt, 3 h; ii—YH/NaI/DMF/80 °C, 16 h; (d) R^1R^2NH/$NaBH(OAc)_3$/Cl CH_2CH_2Cl/rt, 16 h.

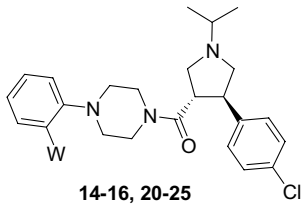
PCC gave the benzaldehyde **16**, which was subjected to reductive amination with secondary or primary amines to afford the tertiary or secondary amines **23** and **24**, respectively (Scheme 3).

Reductive alkylation of **17** with excess of formaldehyde afforded the *N*,*N*-dimethylamine **26**. Acetylation of **17** with acetic anhydride in the presence of DMAP gave the acetamide **27a**. Coupling reactions of **17**–**19** with a variety of Boc-protected amino acids under peptide coupling conditions provided the corresponding amides **27**, **28**, and **29**, respectively, after Boc-deprotection (Scheme 4). A sulfonamide **30** was also obtained by the reaction of **19** with methanesulfonyl chloride. All final compounds were tested in a binding assay using membranes from HEK293 cells transfected with the *h*MC4R and [^{125}I]-NDP-MSH as the radioligand, and the results are listed in Tables 1 and 2. The functional



Scheme 4. Reagents and conditions: (a) CH_2O/$NaBH(OAc)_3$/CH_2Cl_2/rt, 2 h, 50%; (b) R^3COOH/EDC/HOBt/*i*-Pr_2NEt/rt, 16 h; (c) $MeSO_2Cl$/Et_3N/CH_2Cl_2/rt, 2 h.

Table 1. Binding affinity of phenylpiperazines at *h*MC4R



14-16, 20-25

Compound	W	K_i (nM)
14	MeO	520
15	CN	4800
16	CHO	1150
20a	NH_2CH_2	1100
20b	$MeSO_2NHCH_2$	1700
22a	Imidazol-1-yl-CH_2	220[a]
22b	2-Methylimidazol-1-yl-CH_2	280
22c	5-Methylimidazol-1-yl-CH_2	250
22d	1,2,4-Triazol-4-yl-CH_2	580
22e	1,2,4-Triazol-1-yl-CH_2	320
22f	1,2,3-Triazol-1-yl-CH_2	420
22g	Pyrazol-1-yl-CH_2	490
23a	Me_2NCH_2	1200
23b	Et_2NCH_2	1200
23c	$(Me_2CHCH_2)_2NCH_2$	1100
23d	$MeOCH_2CH_2N(Me)CH_2$	320
23e	Pyrrolidin-yl-CH_2	550
23f	Piperidin-1-yl-CH_2	950
23g	Piperazin-1-yl-CH_2	360
23h	4-Methylpiperazin-1-yl-CH_2	840
23i	4-Aminopiperidin-1-yl-CH_2	92[b]
24a	$Me_2CHCH_2NHCH_2$	510
24b	$NH_2CH_2CH_2NHCH_2$	230
24c	$MeNHCH_2CH_2NHCH_2$	270
24d	Azetidin-3-yl-$NHCH_2$	520
24e	Azetidin-3-yl-$N(Me)CH_2$	240
24f	Piperidin-4-yl-CH_2	190
24g	Pyridin-3-yl-$NHCH_2$	1600
24h	$2\text{-}NH_2C_6H_4NHCH_2$	1400
25	$Me_2CHCH_2N(COCH_2CH_2NH_2)CH_2$	450

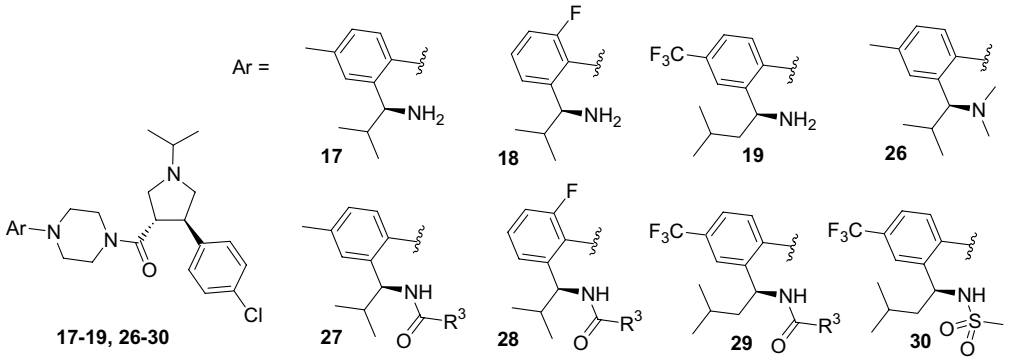
[a] This compound exhibited partial agonist activity in a functional assay (EC_{50} = 320, IA = 52%).
[b] Partial agonist (EC_{50} = 390, IA = 40%).

activity of selected compounds was tested using a cAMP assay as previously described,[13] and the results are listed in Table 2.

The 2-methoxy compound **14** displayed a moderate binding affinity (K_i = 520 nM), while the 2-cyano analog **15** and 2-carboxaldehyde **16** possessed lower affinity. In comparison, the 2-methoxyphenylpiperazine with Tic-(4-Cl)Phe dipeptide **2** (Fig. 1) has been reported by Richardson to have a K_i of 1100 nM.[6] The 2-aminomethyl compound **20a** displayed similar binding affinity to its methanesulfonamide analog **20b** (K_i = 1700 nM), which was less potent than its dipeptide counterpart (**3**, K_i = 210 nM reported, 250 nM in our assay).[6] However, the imidazole **22a** (K_i = 220 nM) possessed a K_i value similar to its dipeptide analog **4** (K_i = 110 nM, reported). The variation of this functional group (triazole and pyrazole) resulted in compounds with similar affinity (**22b–g**, K_i = 280–580 nM). In our functional assay, **22a** only exhibited partial agonism with moderate potency (EC_{50} = 320 nM, IA = 52%). In comparison, the dipeptide **4** (EC_{50} = 14 nM) is a full agonist with high potency as reported.[6] Similarly, the *N*,*N*-dimethylaminomethyl **23a** (K_i = 1200 nM) was also much less potent than its dipeptide analog **5** (K_i = 60 nM, reported),[6] and this trend was further confirmed by **23b** and cyclic amines, such as pyrrolidine **23e** and piperidine **23f** (K_i's 34, 24, and 27 nM, and EC_{50}'s 14, 39, and 37 nM, respectively, have been reported for the dipeptide analogs of **23b**, **23e**, and **23f**).[6] The current series of compounds possessed low binding affinity and no clear structure–activity relationship at this site of the molecule (Table 1), which is somewhat different from the Tic-(4-Cl)Phe compounds **2** and analogs.

Previously we have found from the SAR study of the Tic-(4-Cl)Phe dipeptide series that an additional amine on the benzylamine side chain increases potency. For example, compound **6** possesses a K_i of 6.4 nM and an EC_{50} of 3.8 nM. We tried a set of diamines on the current series (**23h–i** and **24b–h**), and the most potent binder

Table 2. Binding affinity and agonist potency of phenylpiperazines at *h*MC4R[a]



17-19, 26-30

Compound	R^3	K_i (nM)	EC_{50} (nM)	E_{max} (%)
17		12	105	99
18		21	270	68
19		26	1100	94
26		13	[b]	44

Table 2 (*continued*)

Compound	R^3	K_i (nM)	EC_{50} (nM)	E_{max} (%)
27a	Me	47	[b]	35
27b	CH_2NMe_2	7.1	170	100
27c	$CH_2CH_2NMe_2$	4.7	16	102
28a	CH_2NHMe	11	16	114
28b	*R*-CH(Me)NH_2	4.4	9.0	70
28c	$CH_2CH_2NH_2$	13	6.9	100
29a	CH_2NH_2	8.9	44	115
29b	CH_2NHMe	7.7	67	63
29c	CH_2NMe_2	17.3	52	99
29d	*R*-CH(Me)NH_2	4.9	31	103
29e	*S*-CH(Me)NH_2	47	210	104
29f	$CH_2CH_2NH_2$	8.7	4.6	95
29g	$CH_2CH_2NMe_2$	10	91	76
29h	Azetidin-3-yl	18	6.6	80
30		87	450	96

[a] Data are average of two or more independent measurements. The affinity measurements for each compound differed by less than 3-fold, resulting in an average coefficient of variance of 25% for the binding assay K_i values and 32% for the functional assay EC_{50} values.
[b] Not determined.

in this series was the 4-aminopiperidine **23g** (K_i = 94 nM), which possessed only moderate potency and partial agonism (EC_{50} = 390 nM, IA = 40%).

We then focused our efforts on the benzylamines with a small aliphatic group such as isopropyl or isobutyl moiety which has been demonstrated to improve binding affinity for a series of MC4R antagonists. For example, compound **7** has a K_i of 490 nM in the binding assay, while its isobutyl analog as an *S*-configure isomer **8** possesses a K_i of 74 nM, about 7-fold improvement. Thus, compound **17** had a K_i of 12 nM in binding affinity, which was almost 100-fold better than the simple benzylamine **20a** (K_i = 1100 nM, Table 1).[14] More importantly, **17** possessed good agonist activity (EC_{50} = 105 nM, IA = 99%). The 6-fluoro-analog **18** was somewhat less potent compared to **17**. Though the α-isobutyl compound **19** was less potent in the functional assay (K_i = 26 nM, EC_{50} = 1100 nM), its binding affinity was much better than the *N*-isobutylamine **24a** (K_i = 510 nM).[15]

We have also demonstrated that adding an amino acid to the benzylic amine increases binding affinity for a series of MC4R antagonists. For example, compound **10** possesses a K_i of 7.5 nM.[16] Thus, while the *N*,*N*-dimethylglycine **27b** had a minimal effect on both binding affinity and agonist potency over its parent **17**, the *N*,*N*-dimethyl-β-alanine **27c** (EC_{50} = 16 nM) greatly improved its potency. In comparison, the acetyl derivative of **17** reduced its binding affinity (**27a**, K_i = 47 nM). Like **27a** (IA = 35%), *N*,*N*-dimethylation of **17** also reduced agonist efficacy (**26**. IA = 44%). The β-alanine derivative of **18** increased its potency about 40-fold (**28c**, EC_{50} = 6.9 nM), and sarcosine and D-alanine derivatives (**28a**–**b**) were also potent MC4R agonists. Another β-alanine analog **29f** (K_i = 8.7 nM, EC_{50} = 4.6 nM) derived from **19** displayed high potency, which was over 200-fold better than its parent **19** in agonist potency and 50-fold better in binding affinity than the tertiary amide **25** (K_i = 450 nM).[15] For a pair of alanine stereoisomers, the *R*-configured **29d** (EC_{50} = 31 nM) was about 7-fold more potent than its *S*-isomer **29e**, demonstrating a stereo-effect and suggesting specific interaction of this amino group with the receptor. Finally, the methanesulfonamide **30** was less potent in binding affinity than its parent **19**, and this result was similar to that from the pair of **20a** and **20b**, further demonstrating the importance of an amine group in receptor binding.

In conclusion, a series of pyrrolidines derived from piperazinebenzylamines were synthesized, and potent agonists of the human melanocortin-4 receptor were identified. While derivatives from the simple benzylamine **20a** did not yield a potent MC4R agonist, compounds with an additional benzylic isopropyl or isobutyl group exhibited improved agonist activity (**17**–**19**). Further increases in potency were achieved by introducing an amino acid such as β-alanine. Thus, **28c**, **29f**, and **29h** possessed EC_{50} values of less than 10 nM.

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