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Pyrrolidinones as potent functional antagonists of the human melanocortin-4 receptor

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Abstract—A series of pyrrolidinones derived from phenylalaninepiperazines were synthesized and characterized as potent and selective antagonists of the melanocortin-4 receptor. In addition to their high binding affinities, these compounds displayed high functional potencies. **12a** had a K_i of 0.94 nM in binding and IC_{50} of 21 nM in functional activity. **12a** also demonstrated efficacy in a mouse cachexia model.

The melanocortin-4 receptor (MC4R) is a member of the G-protein-coupled receptor (GPCR) superfamily, and plays an important role in regulating feeding behavior and other biological functions.[1] Several MC4R antagonists have been reported to reverse lean body mass loss as well as to increase food intake in animal models of cachexia, suggesting the potential to be used for the treatment of cancer cachexia.[2,3] For example, compound **1**, characterized as a MC4R inverse agonist, demonstrates efficacy in a mouse cachexia model.[4,5] In comparison, compound **2**, which is a potent and neutral antagonist, also shows positive effects in a similar model.[6,7] Interestingly, **2** is also a potent ghrelin agonist as demonstrated in vitro (EC_{50} = 18 nM, IA = 71%). We have recently demonstrated that **3**, a neutral MC4R antagonist with weak ghrelin agonist activity, is also efficacious in this animal model (Fig. 1).[8]

While **3** is characterized as a highly potent and selective MC4R ligand (K_i = 11 nM) in binding assays, its functional antagonism is only moderate as determined in dose-dependent inhibition of α-MSH-stimulated cAMP release (IC_{50} = 560 nM).[8] The cause for the discrepancy between the binding affinity and functional potency is unclear. To identify alternative compounds with improved functional antagonism, we conducted studies to introduce an *N*-side-chain bearing a polar group. Here, we report the structure–activity relationship and identification of compounds such as **12a** as potent functional MC4R antagonists.

The ethylenediamines **9**–**20** were synthesized from **4** to **8** using a reductive alkylation followed by deprotection (Scheme 1). The amides **21**–**30** were prepared by coupling reactions of **4**–**8** with various *N*-Boc-amino acids, and a TFA treatment. All final compounds were tested in a binding assay and the results are listed in Tables 1 and 2. The functional activity of selected compounds was tested using a cAMP assay as previously described,[9] and the results are listed in Table 3.

The primary benzylamines **4**–**8** displayed K_i values between 3 and 10 nM except the 4-fluoro compound **6** (K_i = 26 nM, Table 1). Interestingly, it was observed that the CYP3A4 inhibition of these compounds was reduced when a bulky group was introduced at the 4-position of the phenyl ring. Thus, the 4-methyl analog **7** possessed an over 10-fold higher IC_{50} value than the

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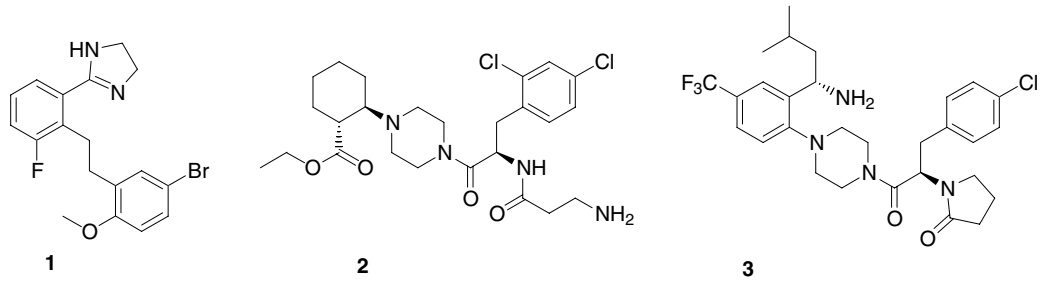
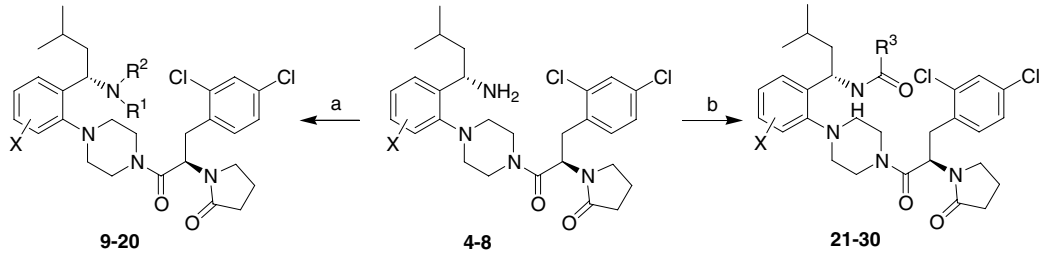


Figure 1. MC4R antagonists with anti-chechectic activity.



Scheme 1. (a) Aldehyde/$NaBH(OAc)_3$, 40–80%; (b) R^3COOH/EDC, then TFA/CH_2Cl_2, rt.

Table 1. SAR of *N*-alkylamine MC4R ligands **4**–**20**[a]

Compound	X	R^1NR^2	*h*MC4R K_i (nM)	CYP3A4 IC_{50} (nM)
4	H	NH_2	6.4	120
5	6-F	NH_2	9.7	170
6	4-F	NH_2	26	220
7	4-Me	NH_2	3.2	1900
8	4-CF_3	NH_2	4.5	49,000
9	6-F	NHEt	6.2	640
10	6-F	NEt_2	7.9	2700
11a	6-F	$NHCH_2CH_2OH$	6.4	480
11b	4-CF_3	$NHCH_2CH_2OH$	8.7	35,000
12a	6-F	$NHCH_2CH_2NH_2$	0.94	4900
12b	4-F	$NHCH_2CH_2NH_2$	60	8900
12c	4-CF_3	$NHCH_2CH_2NH_2$	1.0	6300
13	6-F	$N(Me)CH_2CH_2NH_2$	1.5	650
14a	H	$NHCH_2CH_2NHMe$	3.8	4800
14b	4-F	$NHCH_2CH_2NHMe$	49	11,000
15	4-F	$NHCH_2CH_2NMe_2$	120	32,000
16	4-F	$NHCH_2CH_2NEt_2$	56	37,000
17a	H	$NHCH_2CH_2CH_2NH_2$	0.95	3400
17b	6-F	$NHCH_2CH_2CH_2NH_2$	0.90	16,000
17c	4-CF_3	$NHCH_2CH_2CH_2NH_2$	1.4	15,000
18	4-F	$NHCH_2CONH_2$	140	720
19	4-CF_3	$NHCH_2COOH$	27	>50,000
20a	4-F	$NHCH_2CH_2CH_2COOH$	240	21,000
20b	4-CF_3	$NHCH_2CH_2CH_2COOH$	40	25,000

[a] Binding affinity data are average of two independent measurements.

Table 2. SAR of amide MC4R ligands **21**–**30**[a]

Compound	X	R^3	*h*MC4R K_i (nM)	CYP3A4 IC_{50} (nM)
21	4-F	CH_2NH_2	3.8	3600
22a	H	CH_2NHMe	2.4	2300
22b	4-F	CH_2NHMe	6.0	6500
22c	4-Me	CH_2NHMe	1.0	3600
23	4-F	CH_2NMe_2	23	15,000
24a	4-F	*R*-$CH(Me)NH_2$	7.0	5300
24b	4-Me	*R*-$CH(Me)NH_2$	0.71	2900
25	4-F	*S*-$CH(Me)NH_2$	26	8600
26	4-F	$C(Me)_2NH_2$	57	28,000
27a	H	$CH_2CH_2NH_2$	2.0	2900
27b	6-F	$CH_2CH_2NH_2$	1.9	6700
27c	4-F	$CH_2CH_2NH_2$	5.0	6400
27d	4-CF_3	$CH_2CH_2NH_2$	1.1	5400
28a	H	CH_2CH_2NHMe	2.6	1300
28b	4-Me	CH_2CH_2NHMe	1.4	3400
29a	H	$CH_2CH_2NMe_2$	1.5	6900
29b	4-Me	$CH_2CH_2NMe_2$	0.59	6000
30	4-F	$CH_2CH_2CH_2NH_2$	12	15,000

[a] Binding affinity data are average of two or more independent measurements.

unsubstituted **4**, while the CF_3 group (**8**) led to a further 20-fold reduction in CYP3A4 enzyme inhibition.[10] *N*-Ethylation (**9**) of **5** had a minimal effect, while the *N*-diethyl analog (**10**) improved CYP3A4 profile by 15-fold, which might be caused by a steric effect.[11] Like the *N*-ethylation, the *N*-hydroxyethyl analogs **11a**–**b** possessed affinities at the MC4 receptor almost identical to their parents **5** and **8**, respectively. However, the *N*-(aminoethyl) substitution had different effects. Thus, **12a** was 10-fold more potent than its parent **5** at the MC4R but it was almost 30-fold weaker as an inhibitor of the CYP3A4 enzyme; in comparison, **12b** had slightly decreased MC4R binding from **6**, but its CYP3A4 inhibition reduced by over 40-fold; **12c** possessed increased affinity for both the MC4R and CYP3A4 enzyme over **8**. *N*-Methylation of **12a** had little effect on MC4R binding (**13**, K_i = 1.5 nM), but increased its CYP3A4 inhibition. Incorporating an *N*-(methylaminoethyl) group (**14a**–**b**) on the primary amines **4** and **6** had negligible impact on the MC4 receptor binding, but improved the CYP3A4 inhibition profile. *N*,*N*-Dimethyl and

Table 3. Functional activity of selected compounds at the *h*MC4R[a]

Compound	K_i (nM)	IC_{50} (nM)	*c*log *D*[b]
4	6.4	220	2.2
8	4.5	360	3.7
14a	3.8	48	2.0
22a	2.4	53	3.1
27a	2.0	94	2.4
28a	2.6	35	1.9
29a	1.5	80	3.4
12a	0.94	21	2.4
13	1.5	99	3.0
17b	0.90	44	1.7
27b	1.9	480	2.8
28b	1.4	52	2.3
29b	0.59	19	3.8
19	27	460	3.7
20b	40	340	3.8

[a] Data are average of two or more independent measurements.
[b] Calculated using ACD software.

N,*N*-diethyl analogs (**15** and **16**) of **12b** further reduced CYP3A4 inhibition, demonstrating a steric effect on a basic amine. Like the aminoethyl group, the *N*-aminopropyl moiety (**17a**–**c**) provided improved MC4R potency and CYP3A4 profile except for **17c**, which had a slightly decreased IC_{50} value from **8** on the CYP3A4 enzyme.

The polar acetamide (**18**) decreased the MC4R binding of **6** by over 5-fold, but only slightly improved the CYP3A4 inhibition profile, while the *N*-butyric acid (**20a**) further decreased the MC4R potency. It is worth noting that the CYP3A4 inhibition of **20a** was almost abolished ($IC_{50} > 20\ \mu M$), suggesting the polar acid function greatly decreases CYP3A4 enzyme interactions. The acetic acid **19** had a similar effect when compared to **8**.

To further explore the relationship of MC4R binding and CYP3A4 inhibition, we incorporated several amino acids onto the primary amines **4**–**8**. Similarly to the glycine derivative **21**, the sarcosine **22a**–**c** improved both the MC4R binding and CYP3A4 inhibition profile over their parents (Table 2). The *N*,*N*-dimethylglycine **23** further reduced CYP3A4 inhibition of **6**, but had little improvement in the MC4R binding. The *R*-alanine **24a** had better MC4R potency than its *S*-isomer **25**. The α-methylalanine **26** had low CYP3A4 inhibition possibly due to a steric effect near the basic amine. The β-alanines (**27a**–**d**, **28a**–**b**, and **29a**–**b**) had good in vitro profiles in general, as did the 4-aminobutyric amide **30**.

In the cAMP assay, the *N*-(methylaminoethyl) compound **14a** ($IC_{50} = 48$ nM) increased the potency by 5-fold over its parent **4** ($IC_{50} = 220$ nM, Table 3). Similar results were obtained from sarcosine **22a** and β-alanines **27a**, **28a**, and **29a** (Table 3). **12a**, **17b**, and **29b** were also potent in this functional assay. Interestingly, the acids **19** ($IC_{50} = 460$ nM) and **20b** ($IC_{50} = 340$ nM) possessed similar IC_{50} values to their parent **8** ($IC_{50} = 360$ nM), despite being much less potent than **8** in the binding assay.

Compounds **12a**, **27b**, and **29b** were also highly selective over the other human melanocortin receptors in binding assays. In addition, **12a** bound to the mouse and rat melanocortin-4 receptors with high affinity, and it was about 200-fold selective over the melanocortin-3 receptors (Table 4). Compound **12a** was also characterized for its pharmacokinetic properties in mice. After an intravenous injection of 5 mg/kg, **12a** exhibited a moderate plasma clearance (CL = 15 mL/min kg) and a high volume of distribution ($V_d = 13$ L/kg), resulting in a long half-life ($t_{1/2} = 10$ h) in this species. The high V_d might be associated with its dibasic structure, although the second nitrogen of the ethylenediamine should only be weakly basic (calculated pK_a was about 6). At 1- and 4-h post-dosing, the whole brain concentrations were 63 and 53 ng/g, respectively, resulting in brain/plasma ratios of 0.14 and 0.23. After an oral dose of 10 mg/kg, **12a** reached a maximal plasma concentration of 552 ng/mL at 2 h to give an area under the curve (AUC) of 1950 ng/mL h, resulting in an absolute bioavailability of 19.5% (Table 5).

The measured log *D* of **12a** using a shake-flask method was 1.8, which is ideal for CNS penetration. The low brain penetration of **12a** might be associated with an efflux mechanism. In an in vitro Caco-2 assay, **12a** displayed low permeability ($P_{app} = 2 \times 10^{-6}$ cm/s). The basolateral to apical direction permeation was faster (6.7×10^{-6} cm/s), suggesting the involvement of P-glycoprotein.

Compound **12a** was further studied in a mouse LLC tumor model as previously described.[7] When administered

Table 4. Selectivity profiles (K_i, nM) of **12a**, **27b**, and **29b**[a]

Compound	MC1R	MC3R	MC4R	MC5R
12a	(38%)[b]	750	0.94	850
Mouse	nd[c]	180	0.71	nd
Rat	nd	240	1.0	nd
27b	(22%)	1100	1.9	1300
29b	(44%)	820	0.59	520

[a] Data are average of two independent measurements.
[b] Percentage inhibition at 10 μM concentration.
[c] nd—not determined.

Table 5. Pharmacokinetic parameters of compound **12a** in mice[a]

iv dose (mg/kg)	5
CL (mL/min kg)	15
V_d (L/kg)	13
$t_{1/2}$ (h)	10
AUC (ng/mL h)	9976
C_{brain} (ng/g) at 1, 4 h	63, 53
C_{brain}/C_{plasma}	0.14, 0.23[b]
po dose (mg/kg)	10
C_{max} (ng/mL)	552
T_{max} (h)	2
AUC (ng/mL h)	1950
F (%)	19.5

[a] Average of three animals.
[b] The brain concentration was 81 ng/g 1 h after an iv dose of 5 mg/kg to rats, which reflected a brain/plasma ratio of 0.47.

twice daily (3 mg/kg sc) for 4 days to C57BL6 mice bearing subcutaneous Lewis lung carcinoma tumors, **12a** stimulated food intake by 82% relative to vehicle-treated controls. The lean body mass of the tumor-bearing mice treated with **12a** (−0.5%) increased 9% over that of tumor-vehicle treated animals (−9.5%), demonstrating a positive effect in this cachexia model. It is worth noting that **12a** was a moderately potent full agonist of the ghrelin receptor, with an EC_{50} value of 79 nM (107% intrinsic activity). The extent to which the ghrelin component contributes to the efficacy is unclear.[12]

In conclusion, a series of pyrrolidinones derived from phenylalaninepiperazines were synthesized as potent antagonists of the melanocortin-4 receptor. Potent functional antagonists were identified. **12a** was characterized as a potent antagonist of the human melanocortin-4 receptor (K_i = 0.94 nM, IC_{50} = 21 nM). It was also potent at the mouse and rat MC4 receptors. **12a** displayed suitable pharmacokinetic properties in mice and was efficacious in a LLC mouse cachexia model despite its low brain concentration.

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