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Synthesis and characterization of pyrrolidine derivatives as potent agonists of the human melanocortin-4 receptor

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Abstract—A series of *trans*-4-phenylpyrrolidine-3-carboxamides were synthesized and characterized as potent ligands of the human melanocortin-4 receptor. Interestingly, a pair of diastereoisomers **20f-1** and **20f-2** displayed potent functional agonist and antagonist activity, respectively. Thus, the 3*S*,4*R*-compound **20f-1** possessed a K_i of 11 nM and an EC_{50} of 24 nM, while its 3*R*,4*S*-isomer **20f-2** exhibited a K_i of 8.6 and an IC_{50} of 65 nM. Both compounds were highly selective over other melanocortin receptor subtypes. The MC4R agonist **20f-1** also demonstrated efficacy in diet-induced obese rats.

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] Therefore, MC4R agonists have been extensively studied in efforts to discover small molecules for the treatment of obesity.[2] Several MC4R agonists from different chemical classes have been reported.[3] Recently, a series of pyrrolidines exemplified by **1** (Fig. 1) has been characterized as potent and selective MC4R agonists.[4] In our efforts to find orally active small molecule MC4R antagonists, we have discovered a series of piperazinebenzylamines attaching a 3-phenylpropionyl group (**2**) as potent and selective MC4R antagonists.[5] Introducing a small group next to the carbonyl moiety increases the binding affinity of **2a** (K_i = 74 nM). Thus the *R*-methyl derivative **2b** displayed a K_i of 26 nM, while the pyrrolidinone **2c** (K_i = 4.5 nM) had over 15-fold improvement over **2a**, demonstrating a role of a small group at this site. To further reduce flexibility of the molecule, we synthesized a *trans*-pyrrolidine **3** (K_i = 610 nM), which exhibited only moderate binding affinity. We then embarked upon an SAR study around this core structure, and here we report the characterization of this series of compounds as potent agonists and antagonists of the human MC4 receptor.

A set of close analogs of **3** were synthesized as shown in Scheme 1. Coupling reactions of the *trans*-*N*-benzylpyrrolidinecarboxylic acids **5** with the benzylamine **4**[6] afforded the amides **6**. Selective deprotection of **6b** using HCl in methanol gave the Boc-derivative **8**. Treatment of **6** with trifluoroacetic acid selectively removed the Boc-group to afford the key intermediates **7**. Acylation of **7b** provided, after a HCl/MeOH treatment, the amides **9a**–**c**, while treatment of **7b** with methanesulfonyl chloride in the presence of triethylamine gave the sulfonamide **10**. Reductive alkylations of **7** with carbonyl compounds afforded the tertiary amines **3**, **11a**–**c**, and **12**, after removing the sulfinyl protecting group. The aniline **11d** was obtained by the reaction of **7b** with bromobenzene under palladium-catalyzed conditions,[7] followed by HCl in methanol at room temperature for 1 h. For the compounds with a Boc-protected amine

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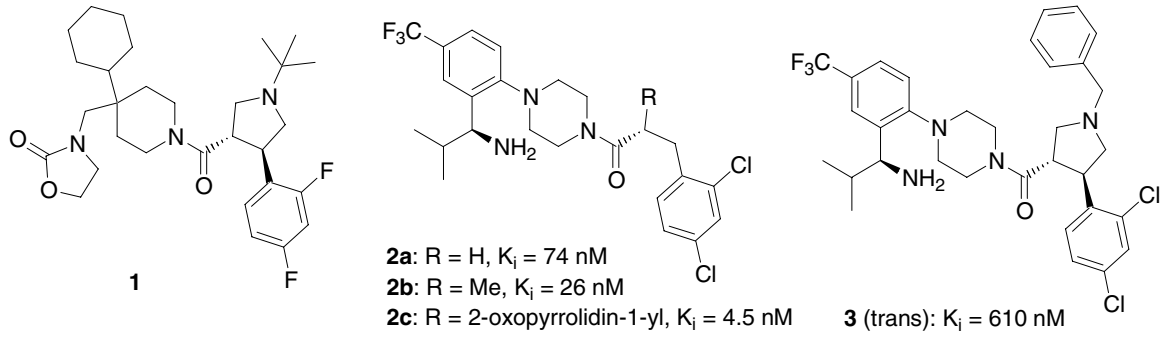
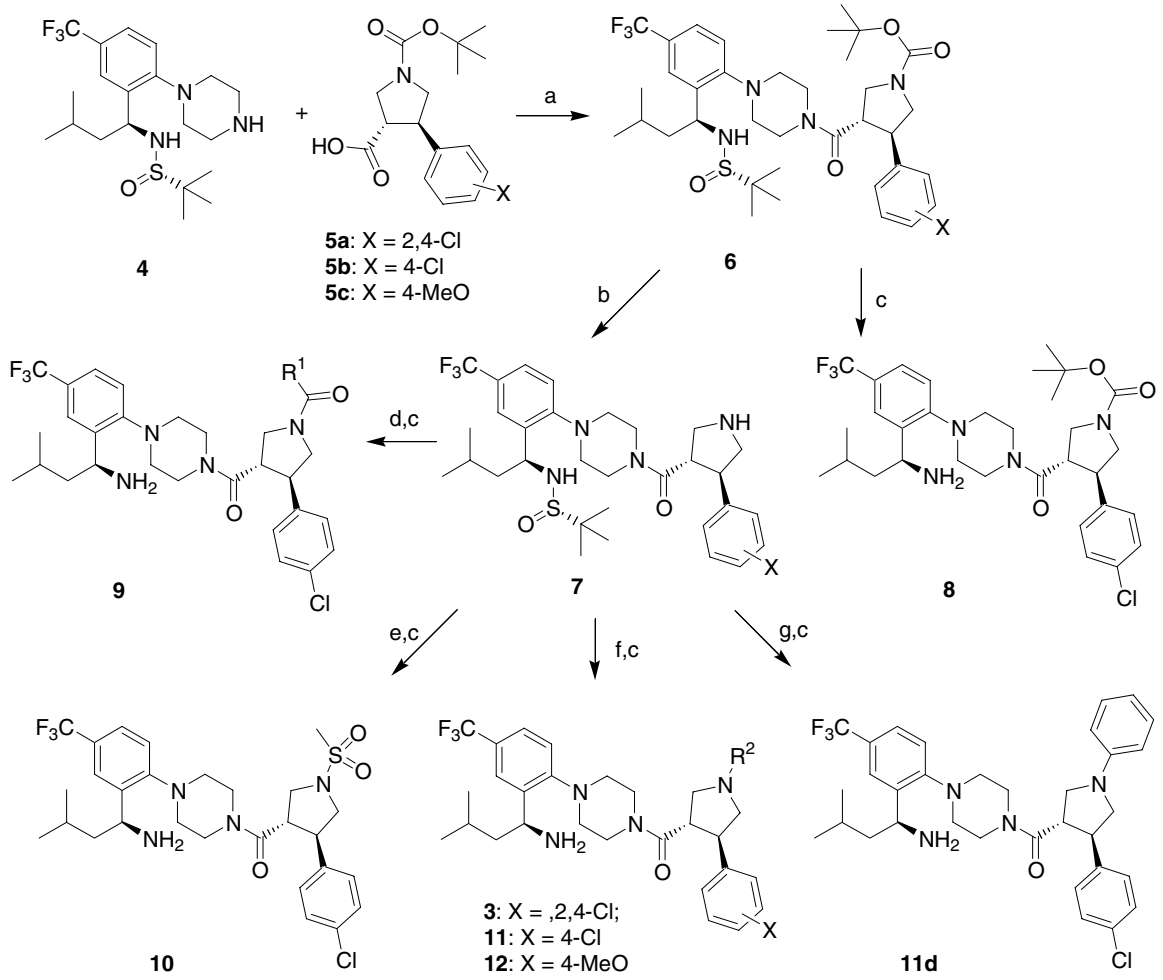


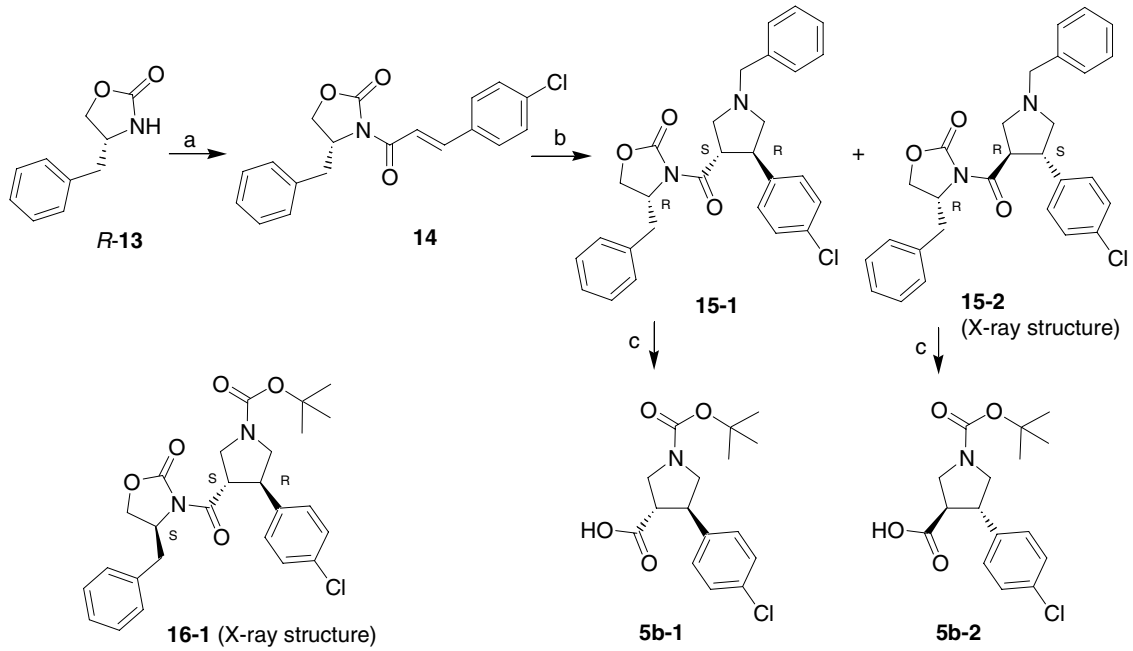
Figure 1. Chemical structures of pyrrolidine agonists and piperazinebenzylamine antagonists of the MC4 receptor.



Scheme 1. (a) EDC/HOBt/$NaHCO_3$/DMF/CH_2Cl_2/rt, 16 h, 87% for **6b**; (b) TFA/CH_2Cl_2/rt, 1 h, quantitative; (c) HCl/MeOH/rt, 1 h; (d) R^1COCl/Et_3N/THF/rt, 8 h, >30%, or $BocNHCH_2COOH$/EDC/HOBt/$NaHCO_3$/CH_2Cl_2/rt, 10 h, then TFA/CH_2Cl_2/rt, 1 h, 54% for **9c**; (e) $MeSO_2Cl$/Et_3N/THF/rt, 1 h; (f) carbonyl compound/$NaBH(OAc)_3$/HOAc/CH_2Cl_2/rt, 8 h, 17% for **11a**; (g) C_6H_5Br/$Pd(OAc)_2$/(±)BINAP/Cs_2CO_3/dioxane/100 °C, 24 h, 7.4%.

such as **9c** and **11c**, a TFA treatment was required before purification using an HPLC instrument.[8]

To obtain the two single stereoisomers of pyrrolidine **5b**, we first coupled (4*R*)-benzyl-2-oxazolidinone (*R*-**13**) with 4-chlorocinnamic acid to give the oxazoline **14**, which was cyclized with *N*-(methoxymethyl)-*N*-(trimethylsilylmethyl)benzylamine to provide the pyrrolidinecarboxamides **15**, which were separated by chromatography into the two diastereoisomers **15-1** and **15-2** (Scheme 2).[9] The absolute stereochemistry of **15–2** was resolved by X-ray crystal structure determination (Fig. 2a). The crystal structure of *S*-benzyloxazolinone **16-1** was also obtained using a similar process from (4*S*)-benzyl-2-oxazolidinone to confirm the stereochemistry of **15-1** (Fig. 2b).[10] Debenzylation of **15-1** or



Scheme 2. (a) i—4-chlorocinnamic acid/Me_3CCOCl/Et_3N/THF/−20 °C, 2 h; ii—LiCl/−20 °C, 16 h, 93%; (b) i—$Me_3SiCH_2N(Bn)(CH_2OMe)$/TFA/toluene/0 °C, 16 h; ii—Chromatography separation, 40% for **15-1** and 46% for **15-2**; (c) i—ACE-Cl/proton sponge/$ClCH_2CH_2Cl$/0 °C to reflux, 1 h, then MeOH/reflux, 0.5 h; ii—$(Boc)_2O/NaHCO_3/H_2O$/dioxane/rt, 16 h, 97%; iii—H_2O_2/LiOH//H_2O/0 °C, 2 h, 96% for **5b-1**.

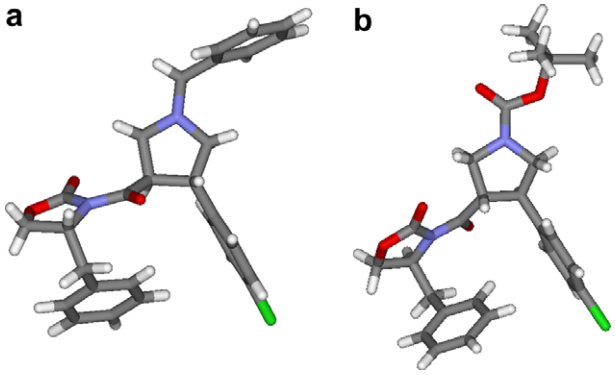


Figure 2. (a) (Left) and (b) (right). X-ray crystal structures of **15-2** and **16-1**.

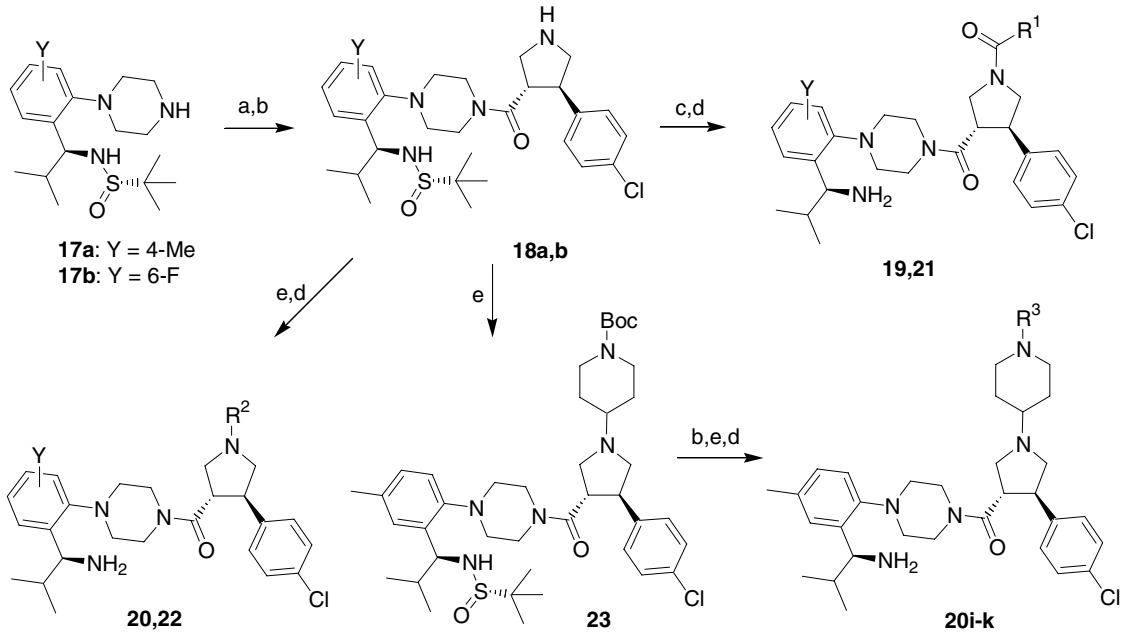
15-2 was achieved with ACE-Cl, and the resultant amine was protected with a Boc-group, which was hydrolyzed using H_2O_2/LiOH to give the single isomer **5b-1** or **5b-2**.

Coupling reactions of **5b** with α-isopropylbenzylamines **17**[4] gave the amides **18**. Acylations of **18a** and **18b** with various carboxylic acids provided the amides **19** and **21**, respectively, after a HCl/MeOH treatment. Reductive alkylations of **18a** and **18b** with a variety of aldehydes and ketones provided the tertiary amines **20** and **22**, respectively, after deprotections. Compounds **20f-1** and **20f-2** were synthesized using a similar procedure from **5b-1** and **5b-2**, respectively. The piperidine intermediate **23** was selectively deprotected with trifluoroacetic acid, followed by reductive alkylations to give **22i–k** after an HCl/MeOH treatment at room temperature (Scheme 3).

All of the final compounds were tested in a binding assay using membranes from HEK293 cells expressing human MC4 receptor and [^{125}I]-NDP-MSH as the radiolabeled ligand. Compounds with good binding affinity were also tested in a whole cell functional agonist assay measuring accumulation of cAMP with competitive ELISA as previously reported.[11]

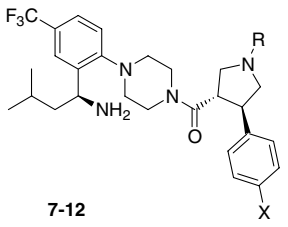
In the binding assay, the secondary amine **7** (K_i = 82 nM) was more potent than its *N*-Boc analog **8** (K_i = 390 nM). In comparison, the acetamide **9a** had better binding affinity than **8**, while the benzoyl analog **9b** slightly improved potency, and the glycine derivative **9c** and the methanesulfonamide **10** had similar K_i values to **7**. Therefore, except for **8**, there was no clear SAR at this site of the molecule. In the functional assay, only the acetamide **9a** (EC_{50} = 1,900 nM, IA = 72%) had comparable intrinsic activity (IA) to α-MSH, which was used as a standard, but with much lower potency. The other amides, as well as the parent amine **7**, had low intrinsic activity (IA < 35%). Introducing an *N*-isopropyl group to **7** not only improved its binding affinity but also significantly increased its intrinsic activity (**11a**, K_i = 26 nM, EC_{50} = 1,100 nM, IA = 94%). The *N*-benzyl compound **11b** was less potent in both binding and functional assays than **11a**, while the *N*-phenyl analog **11d** had very low intrinsic activity, suggesting that the basic nitrogen of the pyrrolidine plays a role in receptor activation. However, an additional amine in **11c** did not increase efficacy. Finally, the 4-methoxy compound **12** (K_i = 67 nM) was less potent than its 4-chloro analog **11a**, which was the most efficacious among the compounds in Table 1.

These initial results prompted us to expand the SAR study, which is summarized in Table 2. The acetamides **19a** and **21a** had moderate binding affinity which was slightly less potent than **9a**, although **19a** had lower efficacy than **9a**. Other amides **19b–g** and **21c–g** did not



Scheme 3. (a) **5b**/EDC/HOBt/$NaHCO_3$/DMF/CH_2Cl_2/rt, 16 h, 88% for **18a**; (b) TFA/CH_2Cl_2/rt, 1 h, 98%; (c) R^1COOH/EDC/HOBt/$NaHCO_3$/DMF/CH_2Cl_2/rt, 16 h; (d) HCl/MeOH/rt, 1 h; (e) carbonyl compound/$NaBH(OAc)_3$/CH_2Cl_2/rt, 1–16 h.

Table 1. SAR of pyrrolidine derivatives **7**–**12** at MC4R[a]



Compound	X	R	K_i (nM)	EC_{50} (nM)[b]
7	Cl	H	82	(34%)
8	Cl	Boc	390	(32%)
9a	Cl	MeCO	66	1900 (72%)
9b	Cl	PhCO	30	(3%)
9c	Cl	$COCH_2NH_2$	96	(12%)
10	Cl	$MeSO_2$	95	(34%)
11a	Cl	*i*-Pr	26	1100 (94%)
11b	Cl	Bn	120	7300 (52%)
11c	Cl	$CH_2CH_2NH_2$	180	(37%)
11d	Cl	Ph	260	(24%)
12	MeO	*i*-Pr	67	450 (67%)

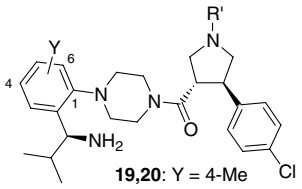
[a] Average of two or more independent measurements.
[b] Intrinsic activity (100% of the endogenous ligand α-MSH) is indicated in parentheses.

have significant improvement on binding affinity except the cyclopropylcarboxamide **21b** (K_i = 31 nM) which was about 7-fold better than **21a**. The *N*-isopropyl amines **20a** (K_i = 12 nM, EC_{50} = 105 nM, IA = 99%) and **22a** (K_i = 21 nM, EC_{50} = 270 nM, IA = 86%) possessed good binding affinity and moderate agonist potency with full efficacy. Similar to **11b**, the *N*-benzyl analogs **20g** and **22i** had low intrinsic activity. Among the tertiary amines **20b**–**g** and **22b**–**i**, the *N*-cyclohexyl compounds **20d** (K_i = 6.6 nM) and **22h** (K_i = 9.3 nM) exhibited good binding affinity, however, their intrinsic activity was low (21% and 49%, respectively). An *N*-cyclopentyl analog **22g** (K_i = 26 nM, EC_{50} = 400 nM, IA = 80%) had high efficacy but moderate potency in the cAMP assay. A series of *N*-piperidin-4-yl derivatives **20h**–**k** possessed high binding affinities ($K_i \leqslant 20$ nM), especially **20k** (K_i = 5.4 nM) with an *N*-isobutylpiperidinyl group, but these compounds exhibited negligible intrinsic activity.

While the *N*-tetrahydropyran-4-yl derivative **20f** (K_i = 12 nM) as a mixture of *trans*-isomers exhibited high binding affinity and moderate intrinsic activity (IA = 36%), the two single stereoisomers **20f-1** and **20f-2** (Fig. 3), prepared separately from the pyrrolidinecarboxylic acids **5b-1** and **5b-2**, displayed quite interesting results. While these two stereoisomers possessed similar binding affinity (K_i of 11 and 8.6 nM, respectively, for **20f-1** and **20f-2**), **20f-1** was a potent and full agonist with an EC_{50} of 24 nM, and **20f-2** had very low intrinsic activity (18% cAMP stimulation up to 10 μM concentrations). Instead, in a functional antagonist assay, **20f-2** dose-dependently inhibited α-MSH-stimulation of cAMP production with an IC_{50} value of 65 nM (Fig. 3), demonstrating that **20f-2** was a functional antagonist.[12] In comparison, both single isomers (**22i-1** and **22i-2**) of the *trans*-*N*-benzylpyrrolidine **22i** had moderate binding affinity and low intrinsic activity, suggesting the *N*-substituent of the pyrrolidine plays an important role in receptor binding as well as activation.

Compounds **20f-1** and **20f-2** were also found to be highly selective at the MC4 receptor over the other receptor subtypes (Table 3). This potent and selective

Table 2. SAR of 1-substituent of pyrrolidines **19**–**22** at *h*MC4R[a]



Compound	Y	R′	K_i (nM)	EC_{50} (nM)[b]
19a	4-Me	MeCO	110	(35%)
19b	4-Me	EtCO	59	(31%)
19c	4-Me	*n*-PrCO	68	(14%)
19d	4-Me	cBuCO	78	(22%)
19e	4-Me	$Ph(CH_2)_2CO$	75	(0)
19f	4-Me	$Ph(CH_2)_3CO$	100	(0)
19g	4-Me	$Ph(CH_2)_4CO$	65	(0)
21a	6-F	MeCO	220	
21b	6-F	cPrCO	31	
21c	6-F	*n*-PrCO	260	
21d	6-F	*i*-PrCO	250	
21e	6-F	*t*-BuCO	540	
21f	6-F	cHxCO	350	
21g	6-F	PhCO	160	
20a	4-Me	*i*-Pr	12	105 (99%)
20b	4-Me	$MeOCH_2CH(Me)$	64	(36%)
20c	4-Me	$(MeOCH_2)_2CH$	200	(27%)
20d	4-Me	cHx	6.6	(21%)
20f	4-Me	4-THP	12	(36%)
20f-1	4-Me	*S*,*S*,*R*	11	24 (105%)
20f-2	4-Me	*S*,*R*,*S*	8.6	(18%)[c]
20g	4-Me	Bn	36	(52%)
20h	4-Me	*t*-Boc	15	(0)
20i	4-Me	1-Ethyl-4-piperidinyl	18	(3%)
20j	4-Me	1-Isopropyl-4-piperidinyl	18	(1%)
20k	4-Me	1-Isobutyl-4-piperidinyl	5.4	(1%)[d]
22a	6-F	*i*-Pr	21	270 (86%)
22b	6-F	Me	81	
22c	6-F	$HOCH_2CH_2$	580	
22d	6-F	$CF_3CH_2CH_2$	205	
22e	6-F	*i*-Bu	43	1400 (52%)
22f	6-F	cBu	32	
22g	6-F	cPn	26	400 (80%)
22h	6-F	cHx	9.3	(49%)
22i	6-F	Bn	94	(19%)
22i-1	6-F	*S*,*S*,*R*	340	(22%)
22i-2	6-F	*S*,*R*,*S*	98	(3%)

[a] Data are average of two or more independent measurements.
[b] Intrinsic activity is indicated in parentheses.
[c] Dose-dependent inhibition of α-MSH-stimulated cAMP release with an IC_{50} of 65 nM (Fig. 3, right).
[d] IC_{50} = 1800 nM.

MC4R agonist **20f-1** was further characterized for its pharmacokinetic properties in rats. After an intravenous injection at 2.5 mg/kg, **20f-1** displayed a very high plasma clearance of 118 mL/min kg, which was associated with its extremely high tissue distribution (V_d = 270 L/kg), resulting in a long half life of 27 h in this species. The high volume of distribution could be the result of its dibasic structure. However, the brain distribution of this compound was low. Thus the whole brain concentrations were only 5 and 32 ng/g at 1 and 4 h post-dosing, respectively, resulting in a brain to plasma ratio of 0.7–1.3 during this time frame. **20f-1** had a log *D* value of 1.8 measured by a shake–flake method, and the low brain penetration most likely is associated with its transporter activity. In an in vitro Caco-2 assay, **20f-1** displayed a P_{app} of 0.8×10^{-6}cm/s from apical (a) to basolateral (b) direction, which resulted in a (*b* to *a*/*a* to *b*) ratio of 25, demonstrating strong *P*-glycoprotein activity.

After oral administration of **20f-1** at a 10 mg/kg dose, the C_{max} was only 16 ng/mL, which appeared at about the 3 h time point, and the AUC was 154 ng/mL h. The oral bioavailability of **20f-1** was moderate (22%).

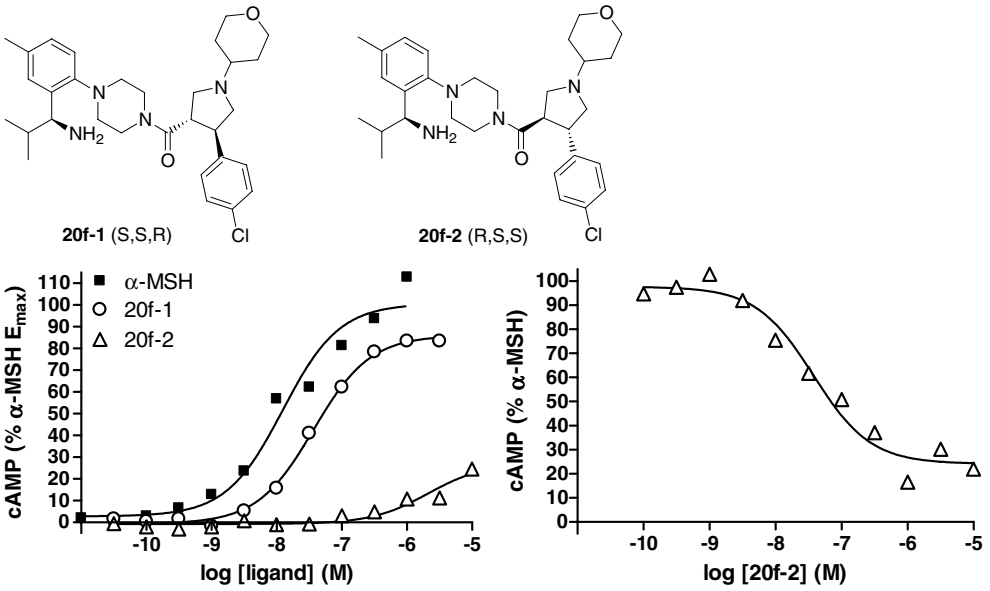


Figure 3. Chemical structures of **20f-1** and **20f-2** with absolute stereochemistry and functional agonist activity of **20f-1** and **20f-2** (left) and functional antagonist activity of **20f-2** (right).

Table 3. Binding affinity (K_i, nM) of **20f-1** and **20f-2** at the human melanocortin receptors[a]

Compound	MC1	MC3	MC4	MC5
20f-1	3350	1600	11	240
20f-2	(33%)[b]	1400	8.6	380

[a] Data are average of two and more independent measurements.
[b] Percentage inhibition at 10 μM concentration.

The low oral plasma exposure is due to its high tissue distribution coupled with its low oral bioavailability.

This MC4R agonist **20f-1** was further characterized in vivo to examine the effects on deprivation-induced food intake in diet-induced obese (DIO) rats ($n = 8$ per group). Both the 30 mg/kg dose (ip) of **20f-1** and the positive control fenfluramine significantly decreased cumulative food intake at all time points tested. By the 24 h time point all doses of **20f-1** significantly and dose-dependently reduced feeding ($p < 0.05$; Fig. 4).[13]

In conclusion, a series of 3-phenylpyrrolidine-3-carboxamides were synthesized and studied as MC4 receptor ligands. While the initial 2,4-dichlorophenylpyrrolidine **3** displayed lower binding affinity than the corresponding phenylpropionyl analogs **2**, potent functional antagonists such as **20f-2** were identified. More interestingly, its diastereoisomer **20f-1** possessed potent agonist activity. Thus, **20f-1** had an EC_{50} of 24 nM in a cAMP assay and was highly selective over other melanocortin receptor subtypes in binding affinity. This compound also demonstrated efficacy in an acute DIO model.

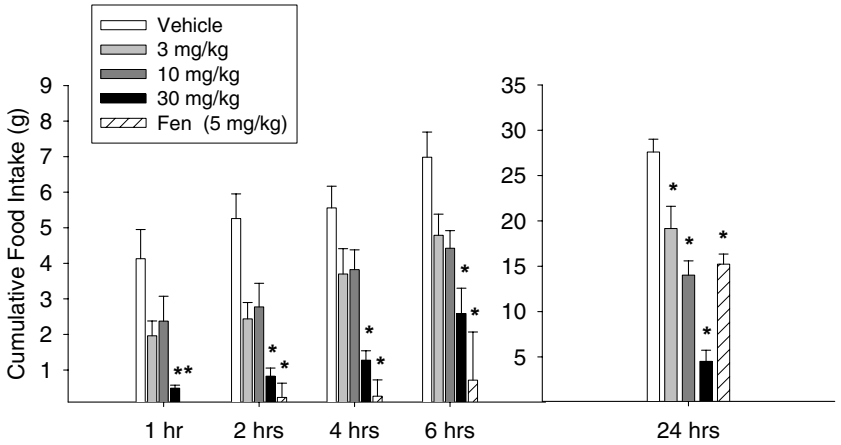


Figure 4. Effect of **20f-1** (3, 10, 30 mg/kg, ip) on deprivation-induced food intake in diet-induced obese rats. Cumulative food intake was significantly decreased at all time points for fenfluramine and the 30 mg/kg dose of **20f-1** and for all treatments at the 24 h time point ($^*p < 0.05$). Values are means ± SEM.

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12. Functional efficacy of **20f-1** (agonist) and **20f-2** (antagonist): Accumulation of cAMP production in CHO cells transfected with *h*MC4R was measured and analyzed using an ELISA. Data are from representative experiments performed 2–8 times with similar results. (Fig. 3, left): **20f-1**, **20f-2**, and α-MSH stimulation of cAMP production, where 100% cAMP is defined as the E_{max} of α-MSH stimulated cAMP production. (Fig. 3, right): **20f-2** antagonism of α-MSH stimulated cAMP production, where 100% cAMP is defined as stimulation of cAMP production by 10 nM α-MSH ($\sim EC_{50}$) in the absence of antagonist.
13. Male CD rats (Charles River) were fed a high fat diet from weaning until 12 weeks of age. Food was removed 24 h prior to testing. On the test day rats were injected ip with vehicle (sterile water), 5 mg/kg fenfluramine (Sigma), or 3, 10, or 30 mg/kg of **20f-1**. Rats were then placed into individual test cages with water available. Fifteen minutes after dosing, pre-measured test diet was presented to the rats and food intake was measured 1, 2, 4, 6, and 24 h later.