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Synthesis and SAR of potent and orally bioavailable *tert*-butylpyrrolidine archetype derived melanocortin subtype-4 receptor modulators

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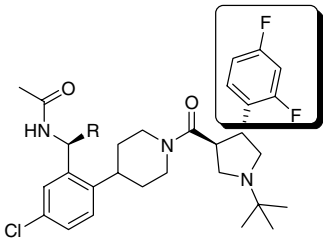
Abstract—Discovery of a series of *tert*-butyl pyrrolidine derived, potent and orally bioavailable melanocortin receptor subtype-4 (MC4R) selective modulators is disclosed.

The melanocortin receptors are known as a family of five seven-transmembrane G protein-coupled receptors. All the melanocortin receptor subtypes are activated by their endogenous ligands (the corticotropins and melanocortins), which are derived from the cleavage of pro-opiomelanocortin (POMC), to mediate a variety of physiological functions. Of these five subtypes, the melanocortin-4 receptor (MC4R) has been clearly linked to the regulation of energy homeostasis and feeding behavior, as well as sexual functions.[1] Since small molecules are more likely to be brain penetrable and orally bioavailable than peptide ligands, significant amount of effort has been spent in drug discovery to develop potent and selective non-peptide MC4R agonists for the potential treatment of human obesity.[2] A recent report from Merck research laboratories described the design and pharmacology of *tert*-butylpyrrolidine archetype derived MC4R agonist **1** (Fig. 1).[3] Here, we report the discovery of a series of interesting MC4R modulators by modifying the 2,4-difluorophenyl part in **2B**,[4] a close analog of **1**.

The general synthetic procedures for synthesizing 1-*tert*-butyl-4-arylpyrrolidine-3-carboxylic acid part structures and the MC4R modulators are summarized in Scheme 1.

Preparation of racemic *trans* methyl 1-*tert*-butyl-4-arylpyrrolidine-3-carboxylate (**3A**) as key intermediates in this study involved [3 + 2] cycloaddition of *trans* methyl arylacrylate and bis(trimethylsilylmethyl)-*tert*-butylamine in the presence of silver fluoride. The methyl aryacrylates were synthesized either from esterification of the corresponding cinnamic acids or from Wittig reactions of methyl(triphenylphosphoranylidene) acetate



1 R = Me
2B R = Et

Figure 1. MC4R modulators **1** and **2B**.

Keywords: G protein-coupled receptor; Melanocortin subtype 4 receptor agonist; Obesity; Oral bioavailability.
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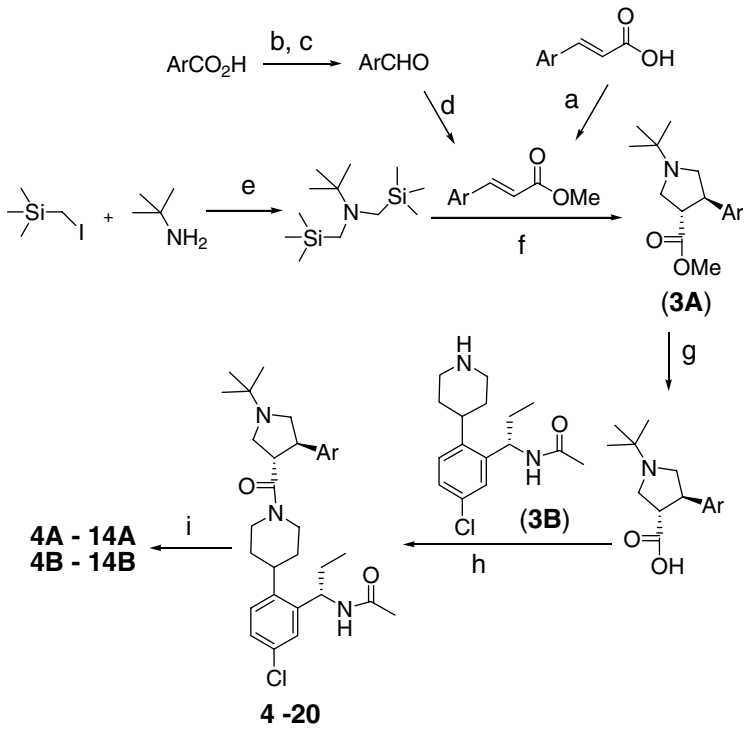


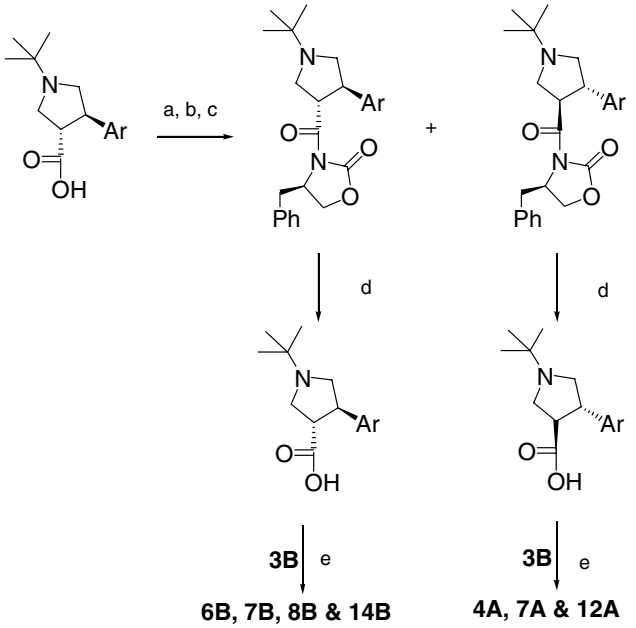
Scheme 1. Reagents and conditions: (a) MeOH, $SOCl_2$, reflux, 2 h; (b) BH_3, THF, rt, overnight; (c) NMO, TPAP; (d) $Ph_3P{=}CHCO_2Me$, THF; (e) K_2CO_3, CH_3CN, 80 °C, overnight; (f) AgF, CH_3CN, 1 h; (g) 3 N HCl, 85 °C, 24 h; (h) HOAt, HATU, DIEA, CH_2Cl_2; (i) Chiral HPLC resolution: ChiralCel OD column; mobile phase = 10% IPA/heptane; flow rate = 9 ml/min.

with the corresponding aryl aldehydes. The aryl aldehydes were either commercially available or made from aryl carboxylic acids by borane reduction followed by TPAP oxidation. Bis(trimethylsilylmethyl)-*tert*-butylamine was formed from reaction of (iodomethyl)trimethyl silane with *tert*-butylamine in the presence of potassium carbonate in acetonitrile. Acid hydrolysis of the ester functionality of **3A** provided the corresponding racemic *trans*-carboxylic acids, which were then coupled with 4-chlorophenyl piperidine part structure **3B**[4,5] to give the final compounds as a mixture of two diastereomers. While only marginal separations were achieved with racemic *trans*-*t*-butyl pyrrolidine methyl esters under a variety of conditions, most diastereomers were separated well with chiral HPLC using ChiralCel OD column (2 cm × 25 cm) and 10% isopropanol in heptane as mobile phase.

For scaled up synthesis of key compounds for in vivo studies, Evans auxiliary method was applied to resolve the racemic *tert*-butyl pyrrolidine carboxylic acids as shown in Scheme 2. The products were then confirmed with the authentic samples on chiral HPLC.

Compound **2B**, which was discovered through an earlier SAR effort, showed modest binding affinity and good functional activity as a full MC4R agonist (Table 1).[4]

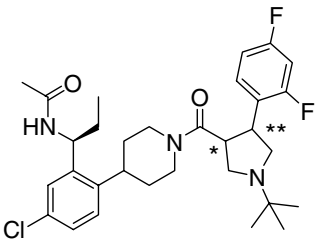
It was our intention to further improve the MC4R binding and functional activity. One of our approaches was replacing the 2,4-difluorophenyl part in compound **2B** with aromatics bearing various functional groups. The preliminary SAR studies were carried out with compounds as a mixture of two diastereomers, and the assay results are summarized in Table 2.



Scheme 2. Reagents and conditions: (a) trimethyl acetyl chloride, DIEA, THF, −10 °C, 2 h; (b) (R)-(+)-4-benzyl-2-oxazolidinone, LiCl, −10 °C to rt, overnight; (c) MPLC separation conditions: 20% EtOAc/hexane to 80% EtOAc/hexane gradient; (d) LiOH, THF, H_2O, rt, overnight; (e) DIEA, HOAt, HATU, CH_2Cl_2.

Compared with F, Larger halogen atoms (Cl and Br) at para position resulted in improvement in binding and

Table 1. Binding affinity and functional activity of **2B** and its diastereomer **2A** at the human MC4R[6]

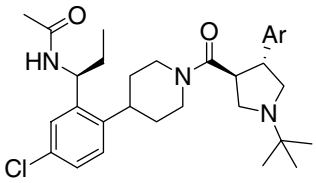


Compound	(*, **)	MC4R Binding IC_{50} (nM)	MC4R cAMP EC_{50} (nM) [% Max]
2A	(*R*, *S*)	640	565 [83%]
2B	(*S*, *R*)	70	17.9 [108%]

functional activity (**6** and **7**), and the larger the halogen, the greater the improvement in IC_{50} and EC_{50}. However, introducing a larger group, such as Cl, at ortho position decreased the activation (**5**). Replacing 2, 4-difluorophenyl with 2, 4-dichlorophenyl resulted in improvement in binding affinity but significant loss in functional activity (**4**). Introducing OCH_3 (**8**) or OCF_3(**13**) to the para position also provided modest improvement in binding and functional activities. CF_3 group at the para position improved binding affinity but decreased MC4R activation (**9** and **12**). Installing one more F at 5 position resulted in loss in binding affinity and functional activity (**11**). Incorporating 2-naphthyl group (**14**) showed modest binding affinity and much lowered activation at MC4R. Functional groups like NH_2, NMe_2 and NHAc at the para position all resulted in great loss in binding and functional activities (**17**, **19**, and **18**), suggested that the receptor does not favor basicity or polarity at this position.

After preliminary SAR studies with the mixture products, we then separated the two diastereomers of interesting compounds by means of chiral HPLC. The assay results of single compounds are summarized in Table 3. Generally the fast eluting isomers (A series) on ChiralCel OD column in isopropanol/heptane system are less potent in functional assay than the slow eluting isomers (B series). With 2,4-dichlorophenyl part, the fast eluting isomer (**4A**) showed higher MC4R affinity (IC_{50} = 2.8 nM), but showed no or little activation at MC4R. Its slow eluting isomer (**4B**) showed modest MC4R agonism. With 2-F-4-Cl, 2-F-4-Br or 2-F-4-OMe phenyl parts, all three slow eluting isomers (**6B**, **7B**, and **8B**) showed very strong MC4R potency with high activation of the receptor, nearly 3-fold improvement in EC_{50} and 5- to 10-fold improvement in IC_{50} compared with compound **2B**. Compounds incorporating 2-F-4-CF_3 phenyl or 4-CF_3 phenyl part (**9A** vs **12A**; **9B** vs **12B**) showed minimal difference in terms of MC4R potency, but compounds with 2-F-4-CF_3 phenyl part showed slightly better selectivity, suggesting that F group at ortho position is not crucial for MC4R receptor binding and functional activities. Replacing 2,4-difluorophenyl part with 2-naphthyl resulted in a modest agonist with partial activation (**14B**). Both **9A** and

Table 2. Binding affinity and functional activity of diastereomeric compounds at the human MC4R[6]



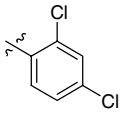
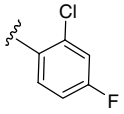
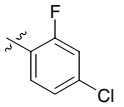
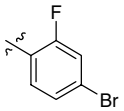
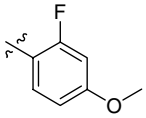
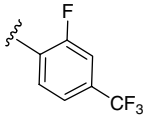
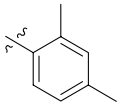
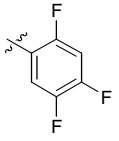
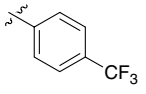
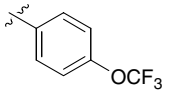
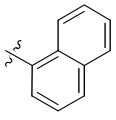
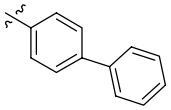
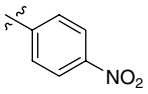
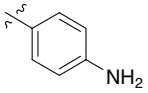
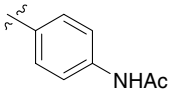
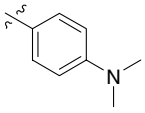
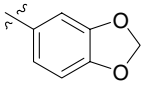
Compound[a]	Ar	MC4R Binding IC_{50} (nM)	MC4R cAMP EC_{50} (nM) (% Max)
4		11.4	184.9 (29%)
5		157.7	342.9 (77%)
6		10.1	12.7(117%)
7		4.3	4.7 (92%)
8		14.1	6 (98%)
9		26.7	190.7 (66%)
10		45.1	256.4 (45%)
11		180.5	320.4 (92%)
12		17.2	67.1 (37%)
13		22.9	177 (88%)
14		119.8	17% at 10 μM

Table 2. (*continued*)

Compound[a]	Ar	MC4R Binding IC_{50} (nM)	MC4R cAMP EC_{50} (nM) (% Max)
15		74.6	198.3 (59%)
16		99.4	261.4 (80%)
17		1274	1363 (69%)
18		10,780	27% at 10 μM
19		686.1	6129 (38%)
20		257.9	200.4 (94%)

[a] All compounds contain two *trans* isomers at 3,4 positions on the pyrrolidine ring.

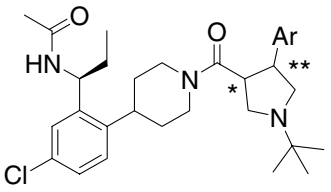
12A showed strong MC4R binding affinity with very low activation.

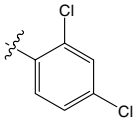
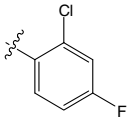
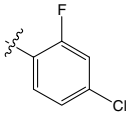
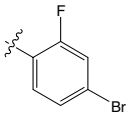
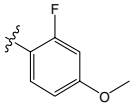
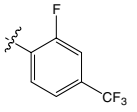
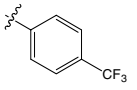
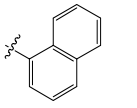
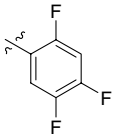
The complete MCR activity profiles and oral bioavailability for potent MC4R modulators are shown below in Table 4.

These data indicate that **6B** shows great MC4 receptor subtype selectivity with high activation, while **7B** and **8B** only show modest MC4R selectivity. Compound **6B** is over 100-fold functionally selective towards human MC4R and shows excellent selectivity versus human MC1b, MC3R, and MC5R. Following oral dosing to rats at 4 mpk, good PK profiles were observed with both **6B** (AUCN = 0.72 μM h, CL_p = 11.4 ml/min/kg) and **7B** (AUCN = 1.18 μM h, CL_p = 7.3 ml/min/kg), incorporating 2-F-4-Cl and 2-F-4-Br phenyl parts, respectively. However, compound incorporating 2-F-4-methoxy phenyl part (**8B**) showed poor PK profile with relatively high clearance (CL_p = 66.4 ml/min/kg).

The more detailed MCR activity profiles of **7A** and **14B** (Table 4) showed that both compounds are modest MC4R agonists with weak binding and functional activities at other receptors. While **7A** showed decent oral bioavailability with good drug level (AUCN = 1.2 μM h) and fairly low plasma clearance (CL_p = 9.12 ml/min/kg), compound **14B**, incorporating 2-naphthyl part, showed only modest oral bioavailability with a relatively high clearance rate (CL_p = 37.6 ml/min/kg).

Table 3. Binding affinity and functional activity of single compounds at the human MC4R[6]



Ar	Compound (*,**)[a]	MC4R binding IC_{50} (nM)	MC4R cAMP EC50 (nM) [% Max]
	**4A** (R,S)	3.3	1% at 10 μM
	4B (*S*, *R*)	17.9	155.6 (48%)
	**5A** (R,S)	82.2	10% at 10 μM
	5B (*S*, *R*)	216.2	214.5 (84%)
	**6A** (R,S)	13.23	110.8 (45%)
	6B (*S*, *R*)	6.2	2.8 (97%)
	**7A** (R,S)	4.1	13.4 (33%)
	7B (*S*, *R*)	4	2.1 (100%)
	**8A** (R,S)	37.2	244.7 (34%)
	8B (*S*, *R*)	11.2	4.6 (100%)
	**9A** (R,S)	9.8	5% at 10 μM
	9B (*S*, *R*)	22.7	88.8 (99%)
	**12A** (R,S)	7.8	1% at 10 μM
	12B (*S*, *R*)	28.1	67.2 (90%)
	**14A** (R,S)	528	1922 (57%)
	14B (*S*, *R*)	76.2	120.5 (47%)
	11A (R,S)	101.1	3702 (50%)
	11B (*S*, *R*)	127.6	130.3 (93%)

[a] The stereochemistry was assigned based on the analogy of **2B**.[7]

The complete MCR activity profiles of **4A** and **12A** (Table 4) indicate that both compounds are potent and selective MC4R modulators with very low activation on all the human melanocortin receptors. Compound

Table 4. MCR activity profiles and oral bioavailability data of selected MC4R modulators

Compound	Receptor	Binding IC_{50} (nM)	cAMP EC50 (nM)	% acti. at 10 μM[a]	$F\%$[b]
6B	hMC1b	1339	905	57	24.3
	hMC3	1492	249.9	92	
	hMC4	6.2	2.7	97	
	hMC5	129.4	372	98	
	rMC4	—	0.9	106	
7B	hMC1b	695	273.6	57	24.7
	hMC3	1050	97.4	88	
	hMC4	4.0	2.1	100	
	hMC5	65.3	101.6	88	
	rMC4	1.7	0.5	94	
8B	hMC1b	311.6	—	—	3.8
	hMC3	2958	156.7	91	
	hMC4	11.2	4.6	100	
	hMC5	205	166.7	101	
	rMC4	—	—	—	
14B	hMC1b	6586	—	10	15.0
	hMC3	12,240	—	16	
	hMC4	76.2	120.5	47	
	hMC5	675	1233	35	
	rMC4	—	—	—	
7A	hMC1b	173,500	1065	33	34.3
	hMC3	2951	917.5	38	
	hMC4	4.1	13.4	33	
	hMC5	280.3	455	30	
	rMC4	1.5	10.3	44	
12A	hMC1b	16,370	—	5	28.0
	hMC3	3777	—	8	
	hMC4	7.8	—	1	
	hMC5	450	—	11	
	rMC4	1.0	—	15	
4A	hMC1b	20,000	—	3	30.3
	hMC3	4293	—	5	
	hMC4	3.3	—	1	
	hMC5	245.4	1600	10	
	rMC4	0.5	—	6	

[a] Percentage of cAMP accumulation at 10 μM relative to α–MSH.
[b] Compounds were dosed in Sprague–Dawley rats as a solution in EtOH/PEG/saline (1:4:5) at 1 mg/kg, iv ($n = 2$) and 4 mg/kg, po ($n = 3$).

4A exhibits excellent selectivity against MC1bR (>6000-fold) and MC3R (>1000-fold). Both compounds showed good PK profiles in rats with good drug level and low plasma clearance rate (**4A**, AUCN = 0.70 μM h; CL_p =11.4 ml/min/kg; **12A**, AUCN = 1.9 μM h; CL_p = 5.6 ml/min/kg).

In conclusion, we have described the synthesis, SAR and pharmacokinetics of a new class of non-peptidyl melanocortin subtype-4 receptor modulators derived from a *tert*-butyl pyrrolidine core structure. A number of potent, MC4R selective and orally bioavailable agonists have been discovered. The in vivo efficacy studies in terms of lowering food intake and body weight and showing erectile activity for MC4R agonists will be discussed in future reports from this laboratory.

References and notes

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5. Intermediate **3B** was synthesized in a similar way as described in Ref. 3 for compound **1**.
6. (a) MC4R binding IC_{50} was defined as the concentration of compound that can inhibit binding of [^{125}I]NDP-α-MSH by 50% from membranes prepared from CHO cells expressing human MC4R. Agonist potency was deter-

mined in cAMP release assays using CHO cells expressing the relevant receptors. MC4R cAMP EC_{50} was defined as the inflection point of the cAMP dose–response curve for any given compounds. Maxi percentage activation [% Max] is the percentage of cAMP accumulation at 10 μM of compound relative to α-MSH; For details about assay protocols see: (b) 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; (c) Bednarek, M. A.; Silva, M. V.; Arison, B.; MacNeil, T.; Kalyani, R. N.; Huang, R. R. C.; Weinberg, D. H. *Peptides* **1999**, *20*, 401.

7. The stereochemistry of 1-*tert*-butyl-4-aryl pyrrolidine-3-carboxylic acid substructure in **2B** is same as in **1** and defined as shown in Ref. 3. The slow eluting isomers discussed in this paper were assumed to have the same stereochemistry as **2B**, which is also the slow eluting isomer, under the same chiral HPLC conditions.