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Melanocortin subtype 4 receptor agonists: Structure–activity relationships about the 4-alkyl piperidine core

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Abstract—SAR about the piperidine core in a series of MC4R agonists is described. A number of alkyl substituents that furnish compounds with good affinity and functional potency are reported.

Through interactions with the endogenous corticotropin and melanocortin ligands, the melanocortin family of G-protein-coupled receptors mediate a wide array of physiological functions. These range from the control of feeding and sexual behavior to skin pigmentation and neuroendocrine regulation. Five subtypes have been cloned and sequenced since the early 1990s.[1]

Melanocortin 4 receptor (MC4R) is expressed in the hypothalamus, brain stem, and many other brain regions. A significant amount of evidence points to the role of the receptor in feeding behavior.[2] The link between MC4R and feeding regulation is strengthened by knock-out studies of mice in which targeted deletion of the receptor results in obese mice with moderate levels of obesity in heterozygous mice.[3]

Efforts have been mounted by various research groups to identify suitable agonists of the receptor as possible treatments for obesity. In recent years, small molecule agonists have been reported in the literature.[4] Many of the more selective agonists have been based on the structure of compound **1**—reported by our laboratories in 2004.[4a] Pharmacological testing of compound **1** confirmed the food intake-lowering effects of MC4R agonism and provided evidence that the sexual effects exhibited by non-selective ligands may be mediated by MC4R[4a,b,c] (see Fig. 1).

The primary endogenous ligands for the five receptor subtypes are generated through cleavage of the 31–36 kDa protein pro-opio-melanocortin (POMC). This mechanism generates an array of biologically active peptides which share a His-Phe-Arg-Trp pharmacophoric core unit.[5] Recognizing the similarity of this pharmacophore to the active core of the growth hormone secretagogue peptide GHRP-6[6] and applying strategies that had yielded a clinical candidate in that program, we quickly identified compound **2** bearing a spirocyclic 'privileged structure' with a dipeptide cap.[7]

Lessons from the literature outlining the influence of the phenylalanine residue[8] allowed rapid movement into an agonist series exemplified by compound **3b**. Further optimization led to the development of a series of 4-substituted, 4-cyclohexylpiperidine-based structures. A very potent series emerged where the piperidine was substituted with a methylene bearing a heterocycle[4a] or with a *tert*-butyl amide.[4d] Replacement of the imidazole side chain of His with tetrahydroisoquinoline (TIC) or piperazine further enhanced potency and selectivity eventually leading to compounds **1** and **11a**.

Keywords: Obesity; Erectile dysfunction; Privileged structure; G-protein-coupled receptor; Apetite; Metabolic disorder; Peptidomimetic.
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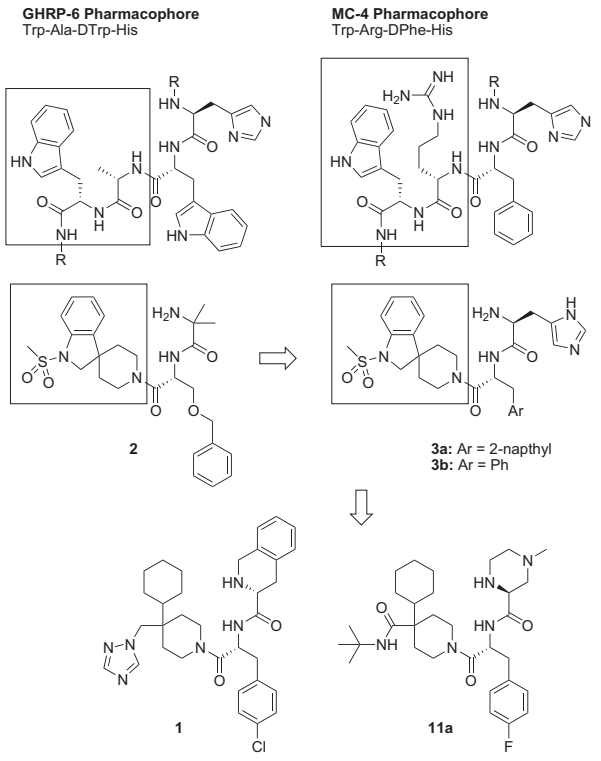

Figure 1. Lead discovery/optimization (a privileged structure approach).

Our group and others working in the area have reported some SAR about the core of these compounds.[4] While this work has described alterations to the dipeptide cap as well as the more polar piperidine substituent, there are no reports describing SAR of the lipophilic piperidine substituent. This paper seeks to outline SAR about this metabolically labile region and identifies a number of groups that allow for structural variation while maintaining good activity.

The work was carried out in three distinct series bearing nitrile, tetrazole, and amide functional groups. In all the series, flexible methodologies were developed that allowed for facile variation of the alkyl subunit.

The synthesis of the nitrile and tetrazole analogs proceeded initially with the Knoevenagel condensation of *N*-Cbz-protected 4-piperidone with ethyl cyanoacetate. The resultant unsaturated cyanoacetate **4** was treated with a cuprate generated from an alkyl Grignard or lithium species. The 4-alkylpiperidine was then thermally decarboxylated to generate the nitrile **5**. Deprotection followed by sequential coupling to the dipeptide cap and final deprotection afforded the nitrile analogs **6**. Prior cycloaddition of azidotrimethylsilane in the presence of catalytic tin followed by methylation afforded the various tetrazole analogs **8** (see Fig. 2).

Synthesis of the amide analogs required three distinct routes to access compounds bearing secondary, tertiary, and quaternary or allylic alkyl centers. Each route led to protected acid **10**. This was converted to a *tert*-butyl amide, deprotected, and coupled to the dipeptide cap. Hydrogenation of the Cbz group and methylation prior to a final deprotection allowed access to the various agonists **11** (see Fig. 3).

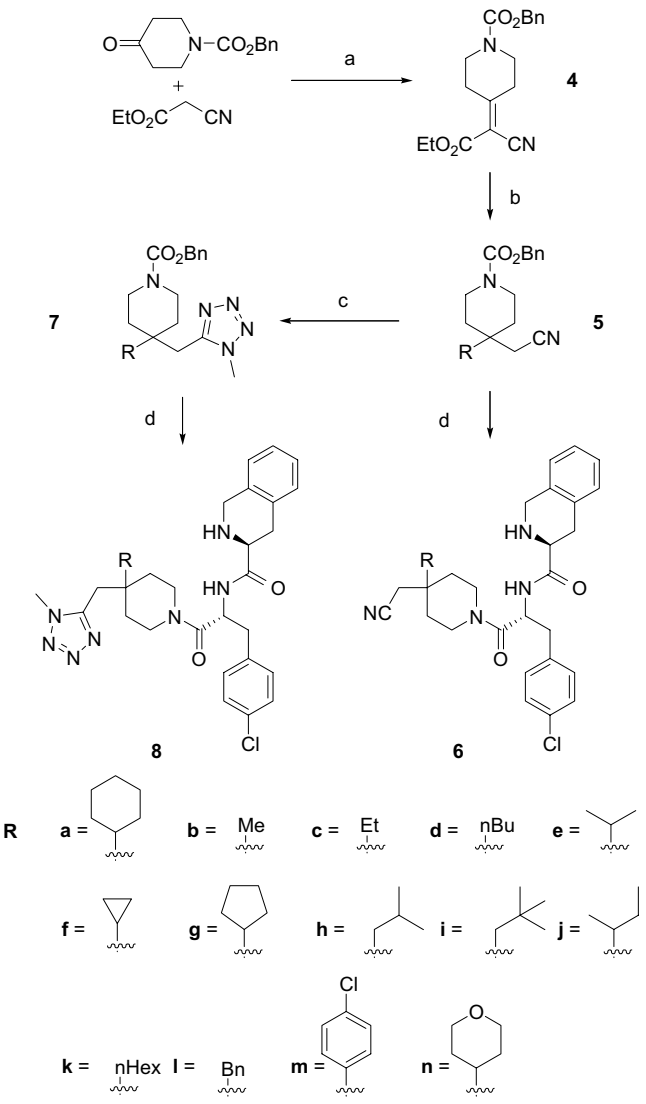

Figure 2. Synthesis of nitrile and tetrazole analogs. Reagents and conditions: (a) $NH_4Cl/AcOH/C_6H_6$, reflux ($-H_2O$); (b) i—RMgX/CuCN/THF; ii—$LiCl/H_2O$/DMSO, 160 °C; (c) i—$TMSN_3/Bu_2SnO$/toluene, reflux; ii—K_2CO_3/MeI/DMF; (d) i—$Pd(OH)_2/H_2$/HCl/MeOH; ii—Boc-D-Phe(p-Cl)-OH/EDC/HOBt/NMM/CH_2Cl_2; iii—HCl/CH_2Cl_2; iv—Boc-D-Tic-OH/EDC/HOBt/NMM/CH_2Cl_2; v—HCl/CH_2Cl_2.

Secondary alkyl substituents were generated in a straightforward fashion via deprotonation and alkylation of *N*-Boc-4-carbethoxy piperidine **9**. The ester was then saponified to afford acid **10**.

Tertiary alkyl-substituted structures were generated in an analogous fashion to the nitrile/tetrazole-bearing compounds utilizing a Henry reaction. The requisite nitro olefin **12** was generated via addition of nitromethane to *N*-Cbz-4-piperidone followed by dehydration. Addition of a Grignard reagent then furnished nitrile oxide

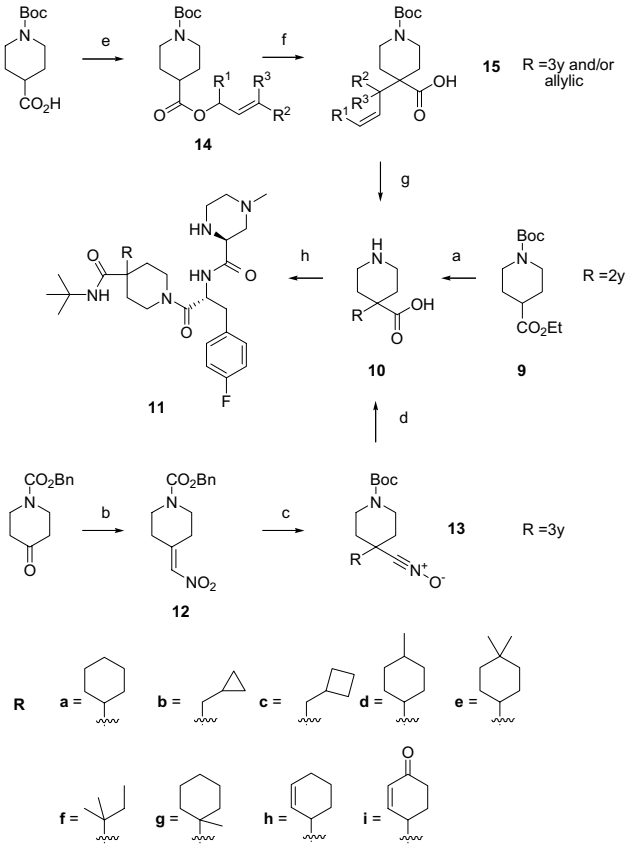

Figure 3. Synthesis of amide analogs. Reagents and conditions: (a) i—LDA/THF; ii—RCH_2X; (b) i—$MeNO_2$/NaOMe/MeOH; ii—Ac_2O/DMSO; (c) i—RMgX/THF; ii—H_2SO_4; (d) $NaNO_2$/AcOH/DMSO; (e) ROH/EDC/DMAP/HOBt/CH_2Cl_2; (f) LDA/TMSCl/THF; (g) HCl/CH_2Cl_2; (h) i—Boc-D-Phe(p-Cl)-OH/EDC/HOBt/NMM/CH_2Cl_2; ii—HCl/ CH_2Cl_2; iii—(2*S*)-1-Boc-4-methyl-piperazine-2-carboxylic acid/EDC/HOBt/NMM/CH_2Cl_2; iv—HCl/CH_2Cl_2.

13—a versatile intermediate that, for these purposes, was oxidized to generate **10**.

Finally quaternary and allylic alkyl substituents were accessed via Claisen rearrangement. *N*-Boc piperidine-4-carboxylic acid was coupled with an allylic alcohol to furnish the Claisen precursor allylic ester **14**. Warming the silyl enol ether generated on treatment with base and TMSCl then effected the rearrangement to allylic-substituted acid **15**. This was either hydrogenated to the saturated analog or maintained as the alkene.

Given the ease of generating the nitrile compounds (**6**), we used this series to rapidly determine the suitability of a number of alkyl groups of varying size. The cyclohexyl subunit (**6a**) emerged as the most potent (IC_{50} = 7 nM; EC_{50} = 189 nM at hMC4R) with 4% maximal activation of hMC3R and 3-fold selectivity over hMC5R. Methyl, ethyl, isopropyl, and cyclopropyl analogs (**6b**, **6c**, **6e**, and **6f**) caused over 10-fold reduction in potency. Use of a cyclopentane or *n*-butyl substituent (**6g** and **6d**) resulted in a smaller shift (2- and 3-fold, respectively) suggesting our efforts should focus on groups with larger steric bulk. Functional activity of the series at the hMC4 receptor (maximal stimulation of cAMP) did not exceed 52%, even in the case of compounds that had good affinity for the receptor. We have hypothesized that the nitrile group does not form optimal interactions with the receptor. This is possibly due to the lack of an additional lipophilic interaction that the other functional groups are capable of forming. In further investigating alkyl group SAR, we turned to the more active tetrazole series (**8**). Again, the cyclohexyl analog was potent and selective: **8a** maintained an EC_{50} of 3 nM at hMC4R with 36- and 69-fold selectivity over hMC3R and hMC5R, respectively. While *n*-hexyl and benzyl analogs **8k** and **8l** exhibited large reductions in potency, the neopentyl analog **8i** shifted 7-fold and the branched butyl- and chlorophenyl-substituted compounds **8h**, **8j**, and **8m** had <5-fold reductions in functional potency. The THP analog **8n** is the least potent in the series, suggesting that heteroatoms are not well tolerated in the region (Table 1).

Table 1. Binding and functional activity of compounds **6a**–**6g** and **8a**–**8m** at human melanocortin receptors

Compound	MC4R (IC_{50}, nM)[a]	EC_{50} (nM)[a] [% max]		
		MC4R	MC3R	MC5R
6a	6.8 (±2)	189 (±38) [51]	na [4]	640 (±113) [42]
6b	1490 (±331)	na [7]	na [0]	na [3]
6c	759 (±312)	na [16]	na [1]	na [8]
6d	22.2 (±4)	281 (±66) [40]	na [1]	480 (±57) [21]
6e	63.0 (±3)	827 (±107) [32]	na [1]	1100 (±265) [20]
6f	173 (±37)	na [12]	na [0]	na [10]
6g	16.4 (±4)	196 (±51) [52]	na [2]	630 (±78) [40]
8a	0.85 (±0)	3.2 (±1) [96]	115 (±22) [50]	222 (±37) [57]
8h	6.0 (±1)	12.5 (±4) [98]	680 (±115) [31]	662 (±242) [46]
8i	3.6 (±0)	21.0 (±7) [76]	na [9]	380 (±50) [27]
8j	3.7 (±1)	3.9 (±0) [107]	263 (±60) [33]	250 (±55) [39]
8k	4.0 (±1)	28.7 (±7) [84]	1001 (±230) [34]	684 (±32) [15]
8l	52.3 (±14)	111 (±28) [70]	840 (±100) [17]	1760 (±596) [20]
8m	113 (±55)	4.9 (±2) [100]	434 (±126) [92]	527 (±167) [17]
8n	32 (±19)	157 (±55) [90]	2950 (±850) [18]	2950 (±132) [18]

[a] Values are means of at least three experiments, standard error of the mean is given in parentheses (na, not active).

Table 2. Binding and functional activity of compounds **11a**–**11z** at human melanocortin receptors

Compound	MC4R (IC_{50}, nM)[a]	EC_{50} (nM)[a] [% max]		
		MC4R	MC3R	MC5R
11a	13.6 (±4)	6.5 (±1) [98]	na [14]	977 (±123) [31]
11b	840 (±149)	2174 (±732) [54]	na [2]	na [5]
11c	177 (±13)	563 (±146) [55]	na [4]	na [8]
11d	6.8 (±1)	10.0 (±4) [81]	230 (±58) [37]	888 (±93) [27]
11e	162 (±81)	546 (±312) [87]	241 (±76) [42]	1117 (±95) [19]
11f	66.2 (±16)	151 (±54) [55]	na [2]	na [32]
11g	36.6 (±9)	57.8 (±26) [79]	na [5]	1980 (±186) [56]
11h	64.0 (±6)	84.6 (±15) [76]	na [8]	na [12]
11i	343 (±98)	1769 (±725) [59]	na [3]	na [2]

[a] Values are means of at least three experiments, standard error of the mean is given in parentheses (na, not active).

The most potent compounds in the first two series had maintained either a cyclohexane, *sec*-butyl or *iso*-butyl substituent. The amide series (**11**) allowed us to investigate further substitutions about these core-structures. While unsaturation (**11h**), substitution of the branch point (**11f** and **11g**), 4,4-di-substitution (**11e**) or cyclization (**11b** and **11c**) all reduced potency compared to their respective parents, 4-position mono-methylation (**11d**) maintained very similar activity (see Table 2).

In conclusion, the three sets of MC4 receptor agonists (nitriles, tetrazoles, and amides) allowed us to identify suitable alkyl substituents of the piperidine core in this lead series. The low potency of compounds bearing small alkyl groups suggests that this region of the molecule interacts with a large lipophilic pocket in the hMC4 receptor. A number of larger groups, in particular cyclopentyl, *n*-butyl, neo-pentyl, *iso*-butyl, *sec*-butyl, and cyclohexyl derivatives, furnished compounds with good potency at hMC4R. This variety of substituents may prove useful in tempering the in vivo and physical characteristics of the lead series.

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