Structure−Activity Relationship of (1-Aryl-2-piperazinylethyl)piperazines: Antagonists for the AGRP/Melanocortin Receptor Binding

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Abstract: Agouti-related protein (AGRP) is an endogenous antagonist of the melanocortin action.[1] In the hypothalamus, melanocortin peptide agonists act as satiety-inducing factors that mediate their action through the melanocortin-4 receptor (MC4R) whereas AGRP is an opposing orexigenic agent. Novel inhibitors of the AGRP/MC4 binding based on (piperazinylethyl)piperazines were prepared, and their structure−activity relationship was established.

Introduction. Obesity is the most common and costly nutritional disorder in the industrialized world.[2] It is a chronic condition that leads to increased risk for diabetes and cardiovascular disease. A great deal of evidence suggests that the central melanocortin system may be a fundamental component in the regulation of food intake and body weight.[3] As part of our antiobesity program, we investigated agouti-related protein (AGRP), a 132 amino acid protein expressed in the feeding centers of the brain (e.g., hypothalamus). When AGRP binds to the melanocortin-4 (MC4) receptor, it stimulates food intake.[4] AGRP increases feeding in rodents when administered icv,[5] and mice that express AGRP ectopically are hyperphagic and obese.[4] On the other hand, melanocortin peptides (e.g., α-MSH) decrease feeding in rodents by agonizing the MC4 receptor.[6] Inhibition of the interaction between AGRP and the MC4R could lead to a new approach for the treatment of obesity. However, finding a small-molecule antagonist for the AGRP/MC4R interaction is particularly challenging for two reasons. First, the interaction between AGRP and the MCRs is a protein/protein interaction where contact may occur over a large area. A small molecule may not be able to disrupt the interactions between these two proteins.[7] Second, it is not clear, as Haskell-Luevano and co-workers[8] suggest, that AGRP and α-MSH share overlapping binding sites on the MCRs. Recent reports have established that some regions of the receptor that contribute to AGRP binding are not responsible for binding to α-MSH.[9] The present report describes efforts in the screening, design, and synthesis of AGRP/MC4 inhibitors. The selectivity of these compounds versus a potent agonist to the MCRs, [Nle^4,D-Phe^7]MSH (NDP-MSH), will also be discussed.

Chemistry. The dipiperazines (**7a**−**l**) were prepared following the general method described by Vejdelek[10] and are summarized in Scheme 1. The α-bromopropiophenones **1a**−**d** were reacted with a protected piperazine to provide intermediates **2a**−**d**. These intermediates were reduced to give alcohols **3a**−**d**, which were then converted to the corresponding chlorides **4a**−**d** by treatment with thionyl chloride. Treatment of **4a**−**d** with an N-substituted piperazine gave the dipiperazines **5a**−**g**. Compounds **6a**−**g** were obtained by deprotection with potassium hydroxide in methanol. A Mannich reaction was performed to convert **6a**−**g** to the corresponding dipiperazines **7a**−**l**.

The synthesis of compounds **8**−**10** is described in Scheme 2. Compounds **8** and **9** were prepared by treating the dipiperazine **6d** with 1-bromo-3-phenylpropane and β-chloropropiophenone, respectively. Compound **10** was prepared via Mannich reaction of **6b** with α-tetralone.

Results and Discussion

All analogues were evaluated in vitro for their ability to inhibit the binding of ^{125}I-AGRP to the human MC4R. The inhibition of the ^{125}I-NDP-MSH/MC4R binding was also measured as a secondary assay. The potency and the selectivity for AGRP vs NDP-MSH binding are reported in Table 1.

Screening of our internal compound collection for inhibitors of AGRP/MC4 resulted in the identification of the *N*-phenyldipiperazine **7f**. Although this compound was a weak antagonist of AGRP, it was 10-fold better inhibitor of the MSH/MC4 binding. To optimize the potency and selectivity, we prepared several analogues with different substituents at R_1. Analogues containing an alkyl group were much more potent and selective (e.g., **7d**,**e** and **g**). Incorporation of an electron-withdrawing group X at the meta and para positions of the phenyl substituent increased the AGRP potency by more than 10-fold, with para substitution being slightly preferred (c.f. **7a** to **7b**−**d**). On the other phenyl ring, unsubstitution, para chloro and para and meta dichloro substitutions were favored over para methoxy, para trifluoromethyl, and the para nitro groups (see **7d**,**h**−**l**). Analogues **8** and **9** exhibited no activity and minimal selectivity, indicating that the α-substituted ketone was required for activity. The cyclic analogue **10** was also less active. All compounds were also tested in a ^{125}I-AGRP/MC3R binding assay where the IC_{50} values were all >10μM. To determine if these compounds are truly inhibitors of a protein/protein interaction, we evaluated their ability to bind to AGRP in the absence of MC4R. Binding to AGRP could also lead to inhibition of the AGRP/MC4 interaction. The interaction of compound **7g** with des62 AGRP, a functionally active C terminal domain truncation of the full-length protein, was studied via HSQC[11] based NMR titration. A 100 μM solution of ^{15}N-labeled des62 AGRP was titrated from 500 μM to 2.5 mM **7g**, using 5 × 10 μL aliquots of 25 mM stock solution prepared in DMSO-d_6. The resulting HSQCs

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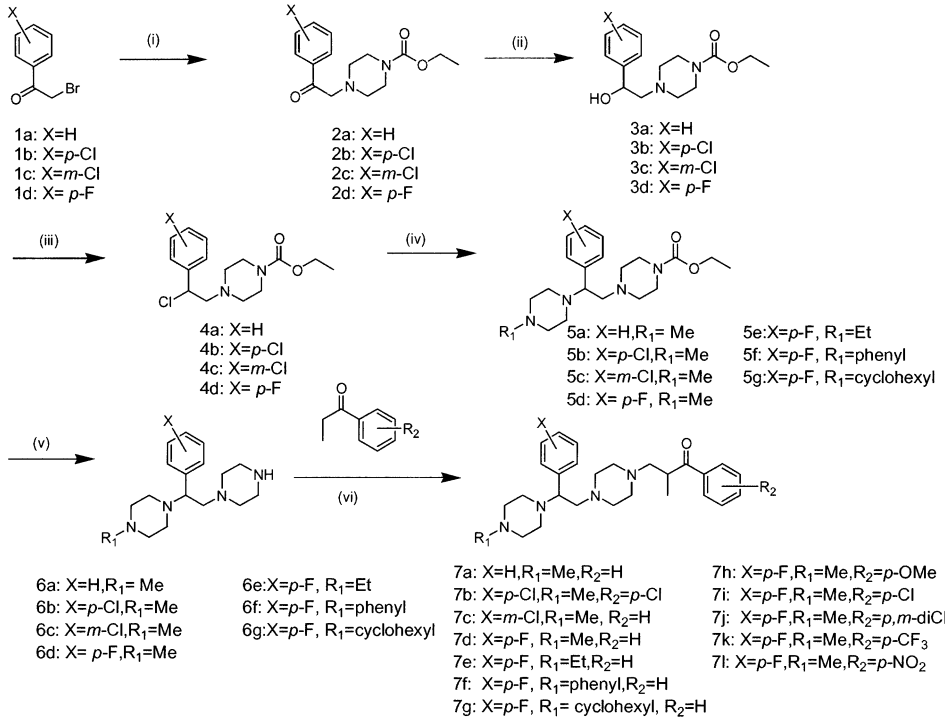
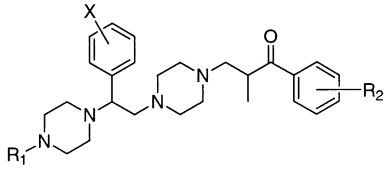
Scheme 1. Synthesis of Dipiperazine Analogues **7a**−**l**[a]



[a] Reagents: (i) 1-ethoxycarbonylpiperazine, TEA, CH_2Cl_2, 0−25 °C; (ii) $NaBH_4$, EtOH, 0−25 °C; (iii) $SOCl_2$, CH_2Cl_2, 0−25 °C; (iv) R_1-piperazine, benzene, 60 °C; (v) 10 equiv of KOH, EtOH, reflux; (vi) paraformaldehyde, concentrated HCl, EtOH, reflux.

Table 1. Biological Activity of the Piperazinylethylpiperazines Analogues at the Human Melanocortin Receptors

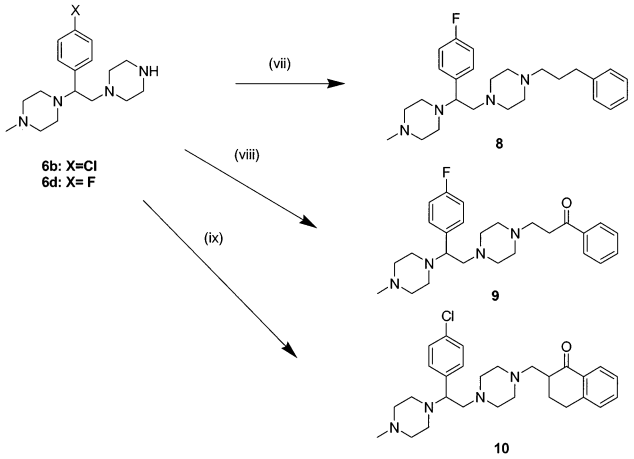


compd	X	R_1	R_2	AGRP/MC4 IC_{50} (nM)	αMSH/MC4 IC_{50} (nM)	fold selectivity AGRP/MC4R
7a	H	Me	H	>5000	>10000	NA
7b	*p*-Cl	Me	*p*-Cl	118 ± 66	150 (n = 1)	0.8
7c	*m*-Cl	Me	H	336 ± 49	598 ± 61	1.8
7d	*p*-F	Me	H	52 ± 47	217 ± 19	4
7e	*p*-F	Et	H	321 ± 65	193 ± 39	1.6
7f	*p*-F	phenyl	H	2862 ± 919	249 ± 17	0.1
7g	*p*-F	cyclohexyl	H	152 ± 59	164 ± 34	1
7h	*p*-F	Me	*p*-OMe	2146 ± 424	597 ± 64	0.3
7i	*p*-F	Me	*p*-Cl	266 ± 32	178 ± 15	0.7
7j	*p*-F	Me	*p*,*m*-diCl	364 ± 21	188 ± 19	0.5
7k	*p*-F	Me	*p*-CF_3	820 ± 158	469 ± 78	0.5
7l	*p*-F	Me	*p*-NO_2	588 ± 49	1500 ± 160	2.5
8	*p*-F	Me	Scheme 2	>10000	3829 ± 1258	NA
9	*p*-F	Me	Scheme 2	>10000	>10000	NA
10	*p*-Cl	Me	Scheme 2	>10000	3226 ± 1258	NA

at each point in the titration were compared with control data sets collected using DMSO-d_6 only (no compound present). Analysis of these data showed no evidence of direct small-molecule binding to the protein. This experiment suggests that the compounds are inhibiting the AGRP/MC4 interaction by binding to the receptor.

Through SAR, we were able to identify moderately potent and selective AGRP/MC4 antagonists that act as protein/protein inhibitors. In a functional assay, we tested to see if the compounds were able to inhibit the AGRP/MC4 binding without disrupting the MSH function. The percentage stimulation of the MC4R, using a fixed concentration of α-MSH and inhibitor, was measured while gradually increasing the concentration of AGRP. If the compounds were to selectively disrupt the AGRP/MC4R interaction, we would observe a rightward shift in the curve because more AGRP would be needed to decrease the percentage of α-MSH stimulation. However, a downward shift of the curve was observed, indicating that addition of compound **7d** is simply adding to the antagonistic effect of AGRP. This occurs because the dipiperazine **7d** is still a moderate inhibitor of the binding between NDP-MSH and MC4 (IC_{50} = 220 nM). Apparently, this activity is enough to make it appear as if **7d** is simply a functional antagonist. Similar functional results were obtained for other active compounds. In conclusion, given the fact that AGRP and α-MSH both bind to the MC4 receptor, we wanted to

Scheme 2. Synthesis of Dipiperazine Analogues **8**−**10**[a]



[a] Reagents: (vii) 1-bromo-3-phenylpropane, CH_2Cl_2; (viii) β-chloropropiophenone, acetone; (ix) α-tetralone, paraformaldehyde, concentrated HCl, EtOH.

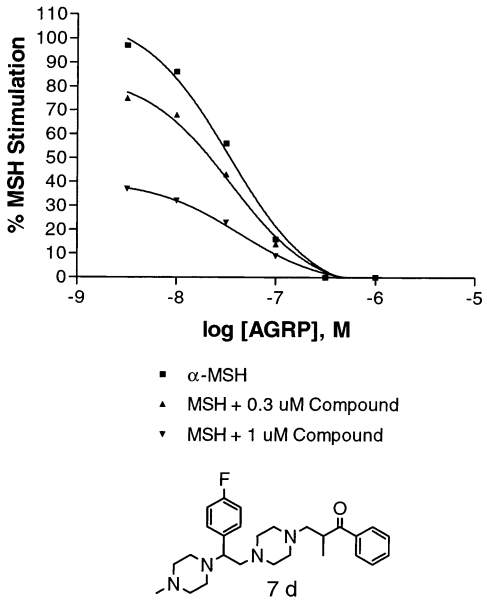


Figure 1. Functional activity of **7d**: measurement of compound activity against dose-dependent AGRP inhibition of α-MSH (50 nM) induced cAMP production.

identify compounds that solely inhibited AGRP binding and did not affect α-MSH binding. Compounds that demonstrated some binding selectivity (4-fold) for AGRP vs α-MSH were selected for testing in a functional assay to assess whether the compounds were acting to antagonize AGRP's effects on the MC4 receptor. Since all of the compounds that inhibit the binding of AGRP to MC4R are also inhibitors of the NDP-αMSH/MC4 interaction, we were unable to demonstrate clear evidence for AGRP antagonism. All of the observed effects corresponded to antagonism of α-MSH. These results are consistent with the hypothesis that the binding sites for AGRP and α-MSH are overlapping or very close.

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