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Synthesis and biological evaluation of imidazole-based small molecule antagonists of the melanocortin 4 receptor (MC4-R)

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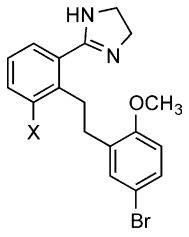
Abstract—A novel series of imidazole-based small molecule antagonists of the melanocortin 4 receptor (MC4-R) is reported. Members of this series have been identified, which exhibit sub-micromolar binding affinity for the MC4-R, functional potency <100 nM, and good oral exposure in rat. Antagonists of the MC4-R are potentially useful in the therapeutic treatment of involuntary weight loss due to advanced age or disease (e.g. cancer or AIDS), an area of large, unmet medical need.

The melanocortin receptors are a family of five G-protein coupled receptors, which have been shown to play physiological roles in sexual behavior, pigmentation, and body weight regulation.[1] Recently, the melanocortin 4 receptor (MC4-R) has been specifically identified as a key modulator of appetite, body weight, and energy homeostasis.[2–11] Initial proof of concept reports have demonstrated the reversal of anorectic conditions in rodents via intracerebroventricular (icv) injection of peptidic MC4-R antagonists.[12–15] Additionally, MC4-R knockout mice resist development of both LPS-induced and cancer cachexia.[16] Therefore, pharmacological antagonism of the MC4-R is a potential therapy for the treatment of involuntary weight loss.[17–19] The identification of MC4-R selective small-molecule ligands has been an area of intense pharmaceutical research in recent years,[20–27] as has been recently reviewed.[28–30] Consequently, we have initiated an effort to identify small molecule antagonists of the MC4-R with the goal of developing therapeutically useful agents for the treatment of involuntary weight loss due to advanced age or disease (e.g. cancer or AIDS).

Our initial MC4-R antagonist optimization effort examined a series of amidine-based small molecule antagonists, which were developed from lead compounds identified in a high throughput screen.[31] An antagonist identified in this effort (**1**, Fig. 1) exhibits 0.023 μM binding affinity and 0.30 μM functional activity. Although a representative member of this series (**2**, Fig. 1) efficiently penetrates the blood–brain barrier



1, X = Cl
2, X = F

Figure 1.

Keywords: MC4-R; Melanocortin receptor; Antagonist.
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after sc or iv dosing in mice,[31] **2** demonstrates low oral exposure when dosed in rat ($C_{max} = 31$ nM) and low iv plasma levels (AUC = 204 nM h) with rapid plasma clearance (Cl = 13.3 L/h/Kg).[32] The focus of the current study was to investigate a related series of compounds in which the previously examined amidine moiety was replaced by a series of imidazole ring systems. Initial metabolite identification studies indicate that the amidine ring system in the original antagonist series is metabolized to the corresponding imidazole (Fig. 2).[33] Therefore, the synthesis of a series of imidazole analogs enables us to identify and characterize the biological properties of proposed imidazole metabolites as well as circumvent this metabolic pathway. Furthermore, we hypothesized that reducing the basicity of the amidine ring system may improve pharmacokinetic properties, including oral exposure. The series of antagonists described herein (Fig. 3) incorporate the optimized central core phenyl ring (X = H, F, Cl), ethylene linker, and 2-methoxy-5-bromo phenyl southern aromatic ring as identified and described previously.[31] This SAR study of imidazole analogs examined the feasibility of replacing the parent imidazoline ring system as well as imidazole ring substituent effects.

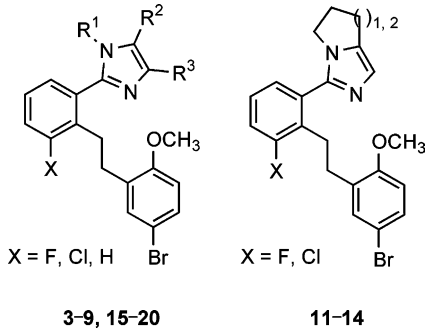
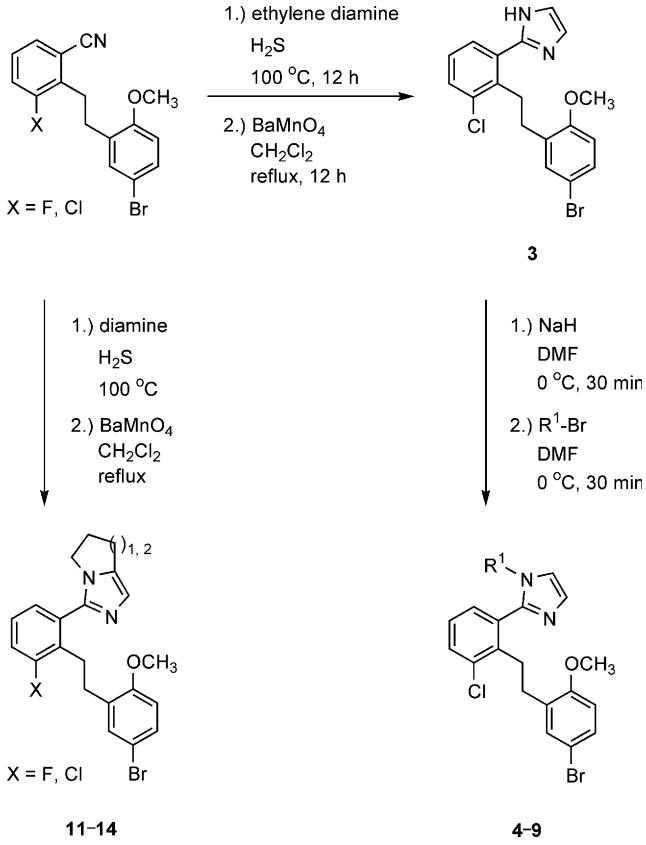


Figure 3.

The synthesis of a series of *N*-substituted imidazole analogs is shown in Scheme 1. The nitrile synthetic intermediate[31] was reacted with ethylene diamine in the presence of H_2S (neat, 100 °C, 12 h). The imidazoline produced was oxidized with $BaMnO_4$[34] to the corresponding imidazole **3** (CH_2Cl_2, reflux, 12 h). Imidazole **3** was alkylated with a series of alkyl halides under basic conditions (NaH, DMF, 0 °C) to generate analogs **4**–**9**. The syntheses of the fused *c*-pentyl and *c*-hexyl imidazoles (**11**–**14**) followed a similar synthetic strategy (Scheme 1). Commercially available 2-(aminomethyl) pyrrolidine and 2-(aminomethyl) piperidine were reacted with the appropriate nitrile synthetic intermediates in the presence of H_2S (neat, 100 °C, 1–3 d). The resulting imidazoline intermediates were oxidized with $BaMnO_4$ to the corresponding imidazoles (**11**–**14**, CH_2Cl_2, reflux, 1–3 d). The syntheses of imidazole analogs **15**–**20** required a different synthetic strategy, as shown in Scheme 2. *o*-Toluic acid was deprotonated (2.1 equiv *sec*-BuLi, 2 equiv TMEDA, anhyd THF, −78 °C, 1 h) followed by alkylation with 5-bromo-2-methoxy-benzyl bromide[31] (1 equiv, anhyd THF, −78 °C, 1 h). The resulting carboxylic acid intermediate was coupled with the appropriate amino alcohols[35] utilizing TFFH[36] to form the corresponding hydroxy amides. The hydroxy amide intermediates were cyclized to the corresponding *N*-methyl imidazolines via reaction with $SOCl_2$, followed by sequential reaction with



Scheme 1.

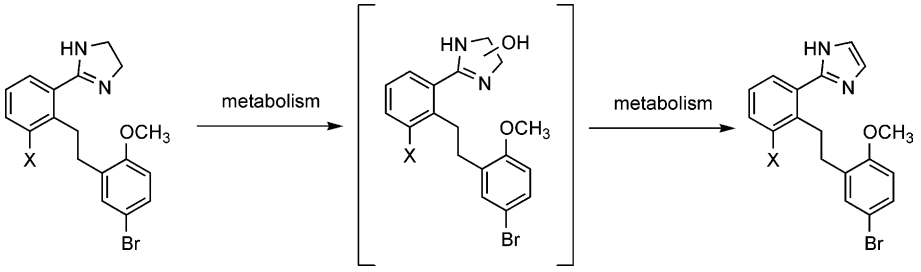
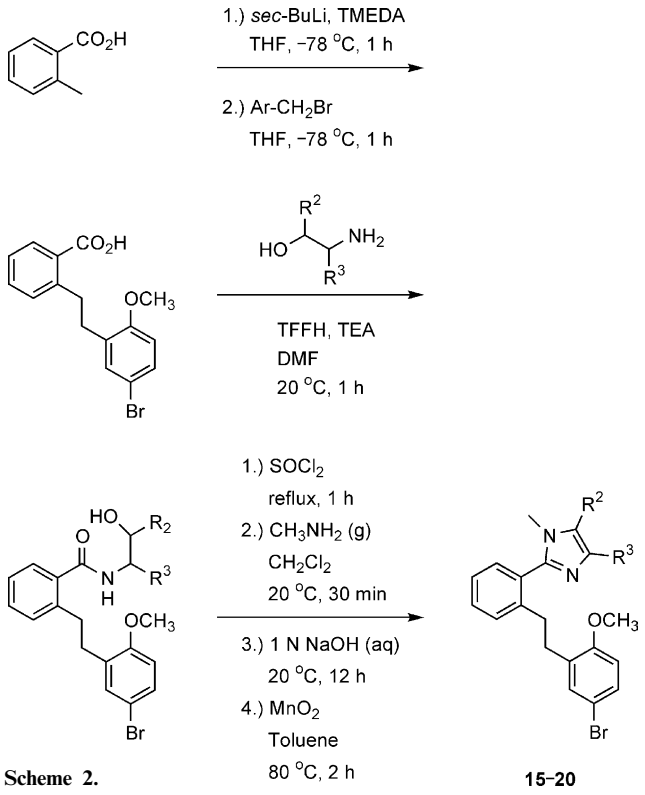


Figure 2.



Scheme 2.

methylamine (g) and 1 N NaOH (aq).[37] Finally, the imidazoline intermediates were oxidized with MnO_2 to the corresponding imidazoles (**15**–**20**, toluene, 80 °C, 2 h).

The binding affinities and functional activities of agents **3**–**20** against the MC4-R are shown in Table 1. In comparison with the parent imidazoline **1**, the corresponding imidazole **3** exhibits a ca. 65-fold decrease in MC4-R binding affinity (1.5 μM vs 0.023 μM). However, **3** retains similar (<2× difference) functional activity to that of imidazoline **1** (0.58 μM vs 0.30 μM).

Initially, the biological activities of a series of *N*-alkylated imidazoles (**4**–**9**) were examined. Small alkyl substitutents on the nitrogen (e.g. Me (**4**) and Et (**5**)) result in modest gains in MC4-R binding affinity (ca. 5×) compared to the unsubstituted imidazole **3**. The *N*-isopropyl substituted imidazole **6** exhibits a smaller enhancement of binding affinity (ca. 2×). Larger *N*-substituents (**7**–**9**) decrease binding affinity to micromolar levels, indicating potential steric constraints within this portion of the MC4-R binding pocket. All the *N*-substituted analogs discussed above exhibit modestly reduced functional activities (ca. 1–2 μM), compared to the parent imidazole (**3**).

A series of 1,5-fused *c*-pentyl and *c*-hexyl imidazoles (**11**–**14**) were examined. The MC4-R binding affinities of these bicyclic analogs are similar to the *N*-methyl, *N*-ethyl, and *N*-isopropyl analogs discussed above (0.18–0.62 μM). This indicates that the fusion of additional methylene subunits to the imidazole ring are sterically accommodated, but do not affect binding affinity to the MC4-R relative to *N*-substitution alone. However, these fused imidazoles do exhibit slightly enhanced functional potency (0.39–0.71 μM) as compared to the imidazole analogs (**4**–**9**), which were substituted only on the nitrogen atom of the imidazole ring. Within this series of imidazoles, the halogen substituent on the central core phenyl ring (Cl vs F, analogs **11**–**14**) was determined to have little effect (<2×) upon both MC4-R binding affinity and functional potency.

Finally, a series of *N*-methyl imidazoles (**15**–**20**) were examined, which contain alkyl substituents in the 4 and/or 5 positions of the imidazole ring. These analogs are devoid of core phenyl ring halogens since these substituents have negligible affects on MC4-R binding affinity or functional potency.[33] The addition of a methyl (**15**) or ethyl (**16**) substituent in the 5-position (R^2) of the imidazole ring causes no change (<2×) in MC4-R binding, as compared to the parent *N*-methyl imidazole **4**. However, the addition of these alkyl substituents significantly increases the functional potency of these analogs by ca. 5–20×, compared to compound **4**. The 1,5-dimethyl compound, **15**, is the most potent imidazole antagonist identified in this study, with an $IC_{50} = 0.051$ μM. Similarly, the addition of a methyl substituent at the 4-position (R^3) of the imidazole ring (**17**) enhances functional activity ca. 5-fold ($IC_{50} = 0.24$ μM) without changing MC4-R binding affinity. The addition of an ethyl substituent in the 4-position of the imidazole ring (**18**) significantly lowers binding affinity and functional activity as compared to the corresponding methyl-substituted compound **17**. This loss in binding affinity and functional potency is even more significant in the case of the corresponding 5-isopropyl analog **19** (K_i and IC_{50} both >1 μM), indicating that potential steric limitations exist at this portion of the MC4-R binding pocket. The tri-methyl substituted imidazole, **20**, exhibits 0.14 μM MC4-R binding affinity and functional activity (0.20 μM), which is within the standard deviation of the di-substituted imidazole analogs (**15**–**17**).

The importance of the aromatic methyl ether in the southern hemisphere to both MC4-R binding affinity and functional activity is apparent via the comparison of compounds **4** and **10** (Table 1). Phenol **10**, obtained from methyl ether **4** by means of reaction with BBr_3, exhibits a ca 30× loss in binding affinity (9.3 μM) and ca 9× loss in functional potency (10.2 μM) as compared to **4**.

Improving the poor pharmacokinetics of the amidine lead compound **2** was one of the motivations for examining a series of imidazole replacements for the amidine ring system. We hypothesized that this replacement would circumvent a primary metabolic pathway identified by initial metabolic studies (heterocyclic ring hydroxylation followed by elimination). In addition, we hypothesized that reducing the basicity of the parent compound may improve oral exposure. Upon iv dosing in rats (1 mg/kg), amidine **2** shows low plasma levels and high clearance (AUC = 204 nM h, Cl = 13.3 L/h/Kg). In comparison, a representative imidazole (**12**) demonstrates increased iv plasma levels

Table 1. In vitro MC4-R binding affinity and functional potency[a]

Compound[a]	R^1	R^2	R^3	X	Ar	MC4-R Binding K_i (μM)[b]	MC4-R cAMP IC_{50} (μM)[c]
3	H	H	H	Cl	5-Br-2-OCH_3 phenyl	1.5 ± 0.3[d]	0.58 ± 0.02
4	Me	H	H	Cl	5-Br-2-OCH_3 phenyl	0.30 ± 0.17	1.1 ± 0.3
5	Et	H	H	Cl	5-Br-2-OCH_3 phenyl	0.31 ± 0.10	1.8 ± 0.3
6	*i*-Propyl	H	H	Cl	5-Br-2-OCH_3 phenyl	0.67 ± 0.06	1.4 ± 0.0
7	*n*-Propyl	H	H	Cl	5-Br-2-OCH_3 phenyl	1.3 ± 0.1	1.9 ± 0.1
8	*sec*-Butyl	H	H	Cl	5-Br-2-OCH_3 phenyl	2.5 ± 0.0	2.1 ± 0.4
9	CH_2-*c*-propyl	H	H	Cl	5-Br-2-OCH_3 phenyl	2.0 ± 0.1	1.7 ± 0.4
10	Me	H	H	Cl	5-Br-2-OH phenyl	9.3 ± 1.5	10.2 ± 2.6
11	Fused *c*-pentyl	Fused *c*-pentyl	H	Cl	5-Br-2-OCH_3 phenyl	0.18 ± 0.08	0.71 ± 0.28
12	Fused *c*-hexyl	Fused *c*-hexyl	H	Cl	5-Br-2-OCH_3 phenyl	0.34 ± 0.05	0.59 ± 0.11
13	Fused *c*-pentyl	Fused *c*-pentyl	H	F	5-Br-2-OCH_3 phenyl	0.32 ± 0.14	0.39 ± 0.00
14	Fused *c*-hexyl	Fused *c*-hexyl	H	F	5-Br-2-OCH_3 phenyl	0.62 ± 0.02	0.48 ± 0.12
15	Me	Me	H	H	5-Br-2-OCH_3 phenyl	0.18 ± 0.01	0.051 ± 0.003
16	Me	Et	H	H	5-Br-2-OCH_3 phenyl	0.32 ± 0.01	0.17 ± 0.10
17	Me	H	Me	H	5-Br-2-OCH_3 phenyl	0.27 ± 0.01	0.24 ± 0.17
18	Me	H	Et	H	5-Br-2-OCH_3 phenyl	0.69 ± 0.17	0.82 ± 0.00
19	Me	H	*i*-Propyl	H	5-Br-2-OCH_3 phenyl	1.6 ± 0.0	1.2 ± 0.3
20	Me	Me	Me	H	5-Br-2-OCH_3 phenyl	0.14 ± 0.00	0.20 ± 0.15

[a] All compounds demonstrated satisfactory LC–MS and ^{1}H NMR characterization.
[b] MC4-R membrane filtration binding assay.
[c] Functional IC_{50} values were calculated by the compounds ability to decrease the intracellular cAMP level induced by 1 nM NDP-MSH in HEK293/hMC4 cells.
[d] Standard deviation ($n \geqslant 2$).

(AUC = 1998 nM h) and dramatically reduced plasma clearance (Cl = 1.1 L/h/Kg). In addition, upon po dosing in rats (5 mg/kg), imidazole **12** shows significantly improved absolute oral exposure (C_{max} = 6454 nM) in comparison to amidine **2** (C_{max} = 31 nM). Additional in vivo profiling of the disclosed imidazole series is necessary in order to determine if the initial observation of dramatically improved pharmacokinetics over the original amidine series continues to be demonstrated.

In conclusion, a novel series of imidazole-based small molecule antagonists of the MC4-R is reported. Members of this class of compounds exhibit sub-micromolar binding affinity and potently antagonize the MC4-R with functional IC_{50} values <0.100 μM. The most potent agent identified (**15**) antagonizes the MC4-R with a functional IC_{50} = 0.051 μM. Representative members of the imidazole series were assayed against the MC1, MC3, and MC5 receptors and they offer 1–2 orders of magnitude selectivity for the MC4 receptor.[33] As hypothesized, a representative imidazole **12** demonstrates improved pharmacokinetics and oral exposure as compared to the earlier examined amidine **2**.

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