Identification of 2-{2-[2-(5-Bromo-2-methoxyphenyl)-ethyl]-3-fluoro-phenyl}-4,5-dihydro-1*H*-imidazole (ML00253764), a Small Molecule Melanocortin 4 Receptor Antagonist That Effectively Reduces Tumor-Induced Weight Loss in a Mouse Model

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Abstract: The melanocortin 4 receptor (MC4R) plays an important role in body weight regulation and energy homeostasis. Administration of peptidic MC4R antagonists (usually by intracerebro ventricular injection) has been shown in the literature to increase body weight and/or food intake in several rodent models. We report here the identification of a novel nonpeptidic MC4R antagonist and its effects on tumor-induced weight loss in mice following peripheral administration.

The involuntary weight loss that occurs as a consequence of many diseases poses a serious medical problem. Sarcopenia,[1] cancer cachexia,[2] rheumatoid arthritis,[3] chronic obstructive pulmonary disease,[4] congestive heart failure,[5] and hip fractures are prevalent conditions that are often accompanied by involuntary weight loss. Patients suffering from these ailments experience increased catabolism of both lean and fat mass, which exacerbates the anorectic state, thereby negatively impacting overall health and quality of life.[6] Although estimates show that as many as 12 million individuals are affected by disease-associated involuntary weight loss, current treatment options remain limited. Agents that are used, such as dronabinol and magesterone acetate, provide only modest benefits and are associated with a variety of side effects.[7]

The melanocortin 4 receptor (MC4R) plays an important role in body weight regulation and energy homeostasis.[8] The signaling of this receptor is a tightly regulated process that involves activation by the proopiomelanocortin (POMC)-derived melanocyte stimulating hormones (α-, β-, and γ-MSH) and inactivation by agouti-related protein (AgRP). In the absence of MSH or upon disruption of receptor function (e.g., MC4R knock out), hyperphagia and significant weight gain result.[9] Both natural and synthetic MC4R antagonist peptides (e.g., AgRP,[10] HS014,[11] and SHU9119[12]) produce robust chronic effects on feeding and body weight when administered to rats via intracerebro ventricular (icv) infusion. While these studies provide important evidence for considering MC4R antagonism as a strategy to increase body weight, in humans a peripheral or oral agent is required.[13]

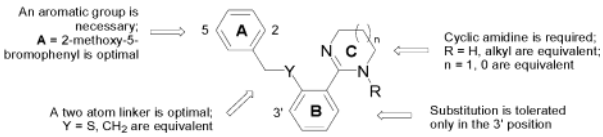
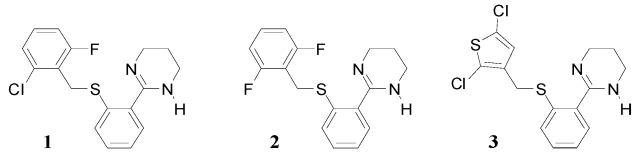


Figure 1. Elements of the benzamidine scaffold and generalized SAR results based on MC4R binding affinity (vide infra).

Chart 1. Benzamidines Identified via HTS and Their Associated MC4R Binding Affinities[15]



1 MC4 Ki = 2.7 μM

2 MC4 Ki = 15.8 μM

3 MC4 Ki = 2.2 μM

Our research focuses on the identification of novel MC4R modulators as possible therapeutics for the treatment of disease associated involuntary weight loss.[14] Herein, we describe the discovery of a novel small molecule MC4R antagonist that effectively reduces tumor-induced weight loss in mice after subcutaneous (sc) administration.

High-throughput screening (HTS) facilitated the identification of several benzamidines with moderate MC4R binding affinity (**1**−**3**, Chart 1). These compounds were selected as a starting point for a lead generation program. The framework of hits **1**−**3** was dissected into four components and each fragment was optimized (Figure 1). Areas of investigation included (1) substitution on the aromatic (A) ring, (2) the length and constitution of the linking moiety between rings A and B, (3) substituents on the B ring, and (4) the nature of the amidine ring (C). Compounds with submicromolar MC4R binding affinity that were determined to be MC4R antagonists (cAMP assay) were further evaluated for metabolic stability and CNS exposure following iv administration in order to identify candidates suitable for pharmacodynamic studies. A brief summary of the optimization effort is presented below and the biological data for select compounds is presented in Table 1.

Initially, MC4R activity was optimized by investigating the A ring. This work led to the identification of the 5-bromo-2-methoxyphenyl group as a moiety that enhances MC4R activity by 2 orders of magnitude relative to the initial HTS hits (e.g., **4**). While the sulfur-containing linker proved useful for rapid analogue synthesis, this group is readily oxidized in the presence of human liver microsomes (data not shown). Analogues

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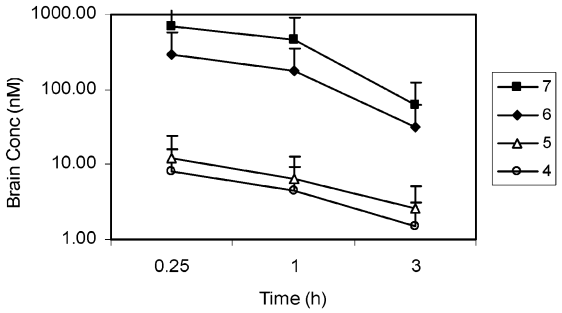
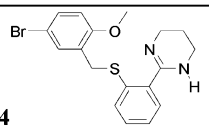
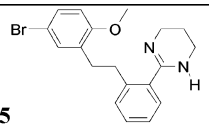
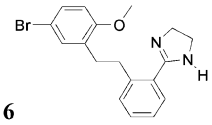
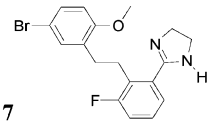


Figure 2. Brain concentration vs time profiles of compounds **4**−**7** after 0.2 mg/kg iv injection of mice. AUC 0−3 h (nM·h): **7**, 1160 ± 330; significantly different from **6**, 470 ± 130; **5**, 14.4 ± 1.6; and **4**, 9.8 ± 1.8 ($P < 0.05$).

Table 1. Benzamidines and Their Associated MC4R Activities[a]

Compound	Ki (μM)[b]	IC_{50} (μM)[c]
 **4**	0.067 ± 0.009	0.27 ± 0.090
 **5**	0.22 ± 0.21	0.28 ± 0.25
 **6**	0.039 ± 0.041	0.45 ± 0.18
 **7** (ML00253764)	0.16 ± 0.037	0.103 ± 0.041

[a] Measurements are ± SD and represent the average of at least three measurements. [b] Binding affinity measured in a membrane filtration assay using ^{125}I-[NDP]-α-MSH as radioligand. [c] Functional antagonism measured by inhibition of cAMP production induced by [NDP]-α-MSH in a whole cell assay.

of **4**, where the thiomethyl ether group is replaced by an ethylene unit, are approximately equipotent MC4R antagonists (cf., **4** and **5**) that show improved metabolic stability. For example, compounds **4** and **5** were incubated with human liver microsomes and after a 60 min exposure, 11% of **4** was detected, whereas 70% of **5** remained.[16]

The benzamidines **4**−**7** were administered iv to mice in order to evaluate their CNS exposure. All tetrahydropyrimidines dosed (e.g., **4** and **5**) were nearly undetectable in the brain (Figure 2).[17] Dihydroimidazole **6** had low but detectable brain exposure while the fluoro-substituted analogue **7** showed significantly enhanced[18] brain exposure with a concomitant increase in MC4R antagonism.[19]

Benzamidine **7** was also administered to mice sc at several doses (3, 10, or 30 mg/kg), and plasma and brain concentration profiles were evaluated (Figure 3). Since **7** achieved brain concentrations in excess of its functional MC4R IC_{50} for longer than 6 h following a sc dose of 30 mg/kg, this compound was chosen for evaluation in a mouse efficacy model.

A CT-26-derived mouse (BALB/c) xenograft model[20]

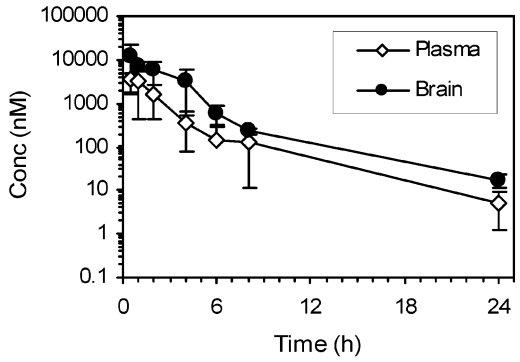


Figure 3. Brain and plasma concentration vs time profiles of **7** after 30 mg/kg sc administration to C57Bl/6 mice. AUC-(0−inf) (nM·h): brain, 29 900 ± 15 400; significantly different from plasma, 8800 ± 5300 ($P < 0.05$).[18]

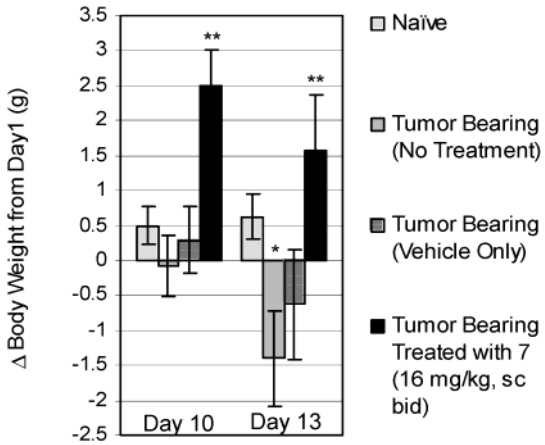


Figure 4. Effect of **7** on body weight changes in CT-26 tumor-bearing BALB/c mice. Dosing of **7** began on day 2. Average body weight of the naïve group was 19.2 g (day 1), 19.7 g (day 10), and 19.9 g (day 13). *Statistically significant weight loss of tumor bearing vs naïve groups ($P = 0.01$). **Statistically significant weight gain of tumor bearing animals treated with **7** vs vehicle ($P < 0.001$).

was chosen to evaluate the effects of the MC4R antagonist **7** on body weight. Disease-induced weight loss in this cancer model is readily measurable before tumor load becomes a complicating factor. The results of this study are shown in Figure 4. Two days post CT-26 inoculation, mice ($n = 10$/group) were dosed sc with **7** on a b.i.d. (16 mg/kg per dose) schedule for the duration of the study. Weight loss in tumor-bearing (TB) mice vs naïve controls was first noted on day 10 (0.5 g) and became statistically significant ($P = 0.01$)[21] by day 13.[22] Interestingly, the TB animals receiving drug weighed 2.5 g more than the untreated vehicle-dosed group ($P = 0.0003$) and 2 g more than naïve controls on day 10. The drug-treated TB animals on day 13 were 2 g heavier than the untreated TB vehicle control group ($P < 0.0001$) and 1 g heavier than the naïve mice. These results suggest that the MC4R antagonist **7** is effective in reducing the magnitude of cancer-induced weight loss in this model. A more comprehensive understanding of the effects of compound in naïve mice, the source of weight loss protection (fat vs lean body mass), and potential changes in food intake are needed but were not within the scope of this initial plan.

In summary, a novel class of small molecule MC4R antagonists has been identified and optimized for potency, metabolic stability, and CNS penetration fol-

lowing peripheral dosing. Subcutaneous administration of **7** effectively reduced tumor-induced weight loss in a mouse model. This represents the first report of a nonpeptidic MC4R antagonist showing protection against tumor-induced body weight loss upon chronic, peripheral dosing. Further optimization studies of compounds in this series and the evaluation of analogues in other cytokine-mediated and tumor-bearing models will be reported in due course.

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Supporting Information Available: Experimental procedures and characterization data for compounds **4**−**7**; methods for animal studies described in Figures 2−4. This material is available free of charge via the Internet at http://pubs.acs.org.

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(15) The high-throughput screen was based on a scintillation-proximity assay (SPA) format. Subsequent lower throughput investigations of MC4R binding affinity were carried out using a membrane-filtration binding assay format. Binding affinities in the two assays were generally in the same range but did not always give good correlation. Membrane-filtration binding assay results are generally in good agreement with functional activity as measured in the whole cell cAMP assay.

(16) Compound **4** was separately incubated with rat hepatocytes and LCMS of the mixture after 1 h was used to characterize the metabolites. The masses of the major metabolites corresponded to mono-oxidation (two different peaks). Bis-oxidation and demethylation occurred to a smaller extent. That oxidation of sulfur was occurring was confirmed by synthesis of an authentic sample of the corresponding sulfoxide and comparison of HPLC retention time.

(17) Plasma was also analyzed and levels of **4** and **5** were extremely low.

(18) Statistical analyses were performed using a *t* test (two-tailed, paired).

(19) We speculate that the enhanced brain concentration of **7** vs **6** may be in part due to the difference in basicity of the two compounds (experimentally determined pK_a **7** = 9.6, pK_a **6** = 10.4).

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(21) Statistical analyses were performed using a *t* test (two-tailed, unpaired, equal variance).

(22) Weight gains were independent of the size or weight of the tumors. Tumors were measured twice a week and tumor size did not vary between tumor-bearing untreated control, vehicle, or seven treated mice. Tumor volumes were calculated according to the equation: tumor volume (mm^3) = (length × $width^2$)/2.

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