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# Discovery of a novel melanin concentrating hormone receptor 1 (MCHR1) antagonist with reduced hERG inhibition

Jeffrey T. Mihalic [a,*], Pingchen Fan [a], Xiaoqi Chen [a], Xi Chen [a], Ying Fu [a], Alykhan Motani [b], Lingming Liang [b], Michelle Lindstrom [b], Liang Tang [c], Jin-Long Chen [b], Juan Jaen [a], Kang Dai [b], Leping Li [a]

[a] Department of Chemistry, Amgen San Francisco, 1120 Veterans Boulevard, South San Francisco, CA 94080, USA
[b] Department of Biology, Amgen San Francisco, USA
[c] Department of Pharmacokinetics, Amgen San Francisco, USA

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ABSTRACT

An initial SAR study resulted in the identification of the novel, potent MCHR1 antagonist **2**. After further profiling, compound **2** was discovered to be a potent inhibitor of the hERG potassium channel, which prevented its further development. Additional optimization of this structure resulted in the discovery of the potent MCHR1 antagonist **11** with a dramatically reduced hERG liability. The decrease in hERG activity was confirmed by several in vivo preclinical cardiovascular studies examining QT prolongation. This compound demonstrated good selectivity for MCHR1 and possessed good pharmacokinetic properties across preclinical species. Compound **11** was also efficacious in reducing body weight in two in vivo mouse models. This compound was selected for clinical evaluation and was given the code AMG 076.



Melanin concentrating hormone (MCH) is a cyclic 19 amino acid peptide found in the CNS of all vertebrates.[1] Recently, several studies have emerged demonstrating the significance of the MCH pathway in controlling body weight through modulating food intake and energy balance. When MCH is delivered by intracerebroventricular (ICV) injection to rodents, a dose dependent increase in food intake and body weight gain is observed.[2] In transgenic mouse studies, animals expressing twofold higher than normal MCH levels are mildly obese and are prone to weight gain and insulin resistance when fed a high fat diet.[3] In contrast, MCH knockout mice are healthy, lean, consume less food, and are hypermetabolic.[4]

There are two GPCR receptors for MCH, MCHR1 and MCHR2. MCHR1 is ubiquitous to all vertebrates whereas MCHR2 is present in dogs, ferrets, and primates including humans. Rodents lack a functional form of MCHR2. MCHR1 is believed to be involved in the neuronal regulation of food consumption. High levels of this receptor are found in the ventromedial hypothalamus, arcuate nucleus, and zona incerta.[5] These areas of the brain are thought to be responsible for taste, olfaction, and the positive reward aspects of feeding and satiety. Targeted disruption of the MCHR1 gene in mice causes resistance to diet-induced obesity, leanness, reduced food consumption, hyperactivity, and altered metabolism.[6] Little is known about the second receptor for MCH, MCHR2. It is expressed throughout the brain and is enriched in the cortical areas of the CNS.[7] The difference in the expression patterns of MCHR1 and MCHR2 suggests that MCHR2 might not be involved in feeding behaviors or neuroendocrine function.

The preceding evidence suggests that an MCHR1 antagonist might be useful as a treatment for obesity. Via a high throughput screen of our chemical library, we discovered a unique indole lead **1** (Fig. 1). Using a systematic lead optimization approach, a potent selective MCHR1 inhibitor was then developed (**2**, Fig. 1).[8] Compound **2** has a high affinity to MCHR1 ($K_i$ = 0.3 nM) and is a potent antagonist of MCH ($IC_{50}$ = 1 nM). This compound (**2**) also possesses good PK in several animal species and is highly CNS permeable. Pharmacologically, **2** was efficacious in reducing food consumption in an in vivo MCH cannulated rat model. However, it was later discovered that **2** was a potent inhibitor of the hERG (human *ether-a-go-go* gene) potassium channel which prevented its further development. The hERG potassium channel plays a central role in the repolarization of the cardiac action potential. This potassium channel is known to be susceptible to inhibition from a variety of drugs and has the potential to cause QT prolongation

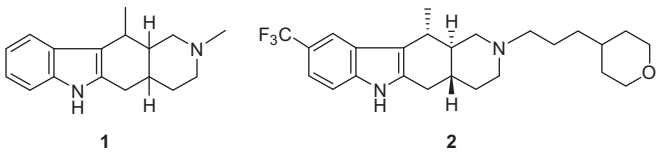


**Figure 1.** MCHR1 antagonist HTS lead and optimized structure.

* Corresponding author. Fax: +1 650 244 2015.
*E-mail address:* jmihalic@amgen.com (J.T. Mihalic).

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which can be associated with deadly cardiac arrhythmias known as torsades de pointes.[9] In fact, it is well known in the literature that many MCHR1 inhibitors also inhibit the hERG potassium channel.[10] Discovering ways to overcome hERG inhibition has become an important goal for drug discovery in general and specifically for the MCHR1 research area. In this Letter, we describe the efforts to remove the hERG liability of **2** while maintaining good potency towards MCHR1 and preserving good PK, in vivo efficacy, and selectivity.

The synthesis of analogs **7**, **10**, and **11** is outlined in Scheme 1. The synthesis began with the treatment of the corresponding ester **3** with LDA and allyl bromide to give intermediate **4**. This allyl intermediate **4** was then oxidized using Brown's hydroboration–oxidation conditions followed by a Swern oxidation to give aldehyde **5**.[11] Intermediate **5** was subsequently reductively aminated with amine **6** and hydrolyzed with lithium hydroxide to yield analog **7**. Amine **6** was synthesized according to published procedures.[12] The synthesis of analogs **10** and **11** began with the conversion of allyl intermediate **4** to aldehyde **8** with osmium tetraoxide and sodium periodate. Next, the ester group on **8** was cleaved with lithium hydroxide and then cyclized under acidic workup to give the oxaspiro intermediate **9**. Finally, reductive amination conditions were utilized to condense amine **6** with intermediate **9** to give analogs **10** and **11**.

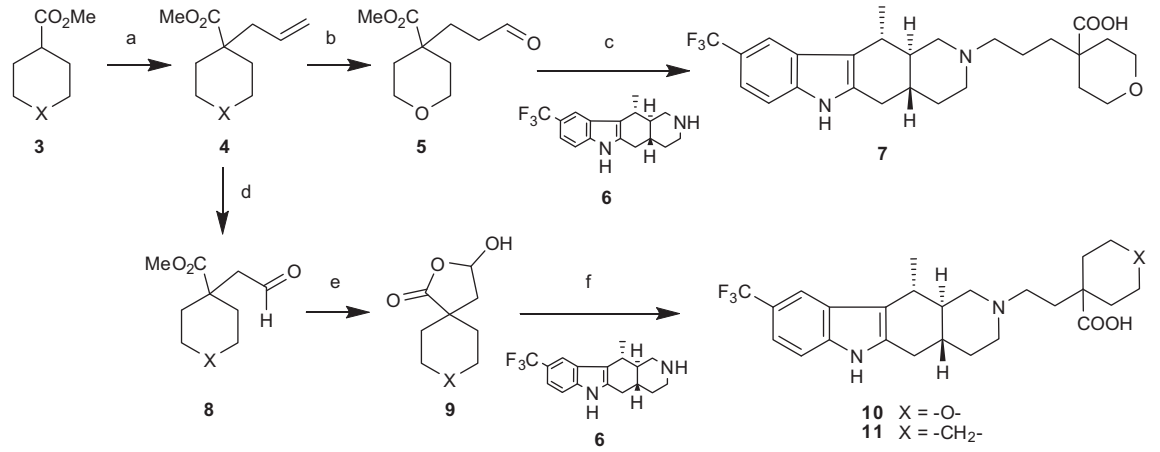
Two assays were initially used to rank order the molecules. The first assay was a MCH competitive binding assay, and the second was a hERG rubidium efflux assay.[13] MCHR1 antagonism was later determined by using an aequorin cellular assay measuring the blockade of intercellular $Ca^{2+}$ mobilization caused by MCH.[14]

Early exploratory SAR demonstrated that the core indole structure of **2** was essential for MCHR1 affinity and had a very low tolerance to structural change; therefore, modifications to this region to mitigate the hERG liability were not aggressively pursued. Attention mostly focused on the THP side chain as a possible site for modification. The SAR results can be found in Table 1. In the literature, hERG pharmacophore models suggest incorporation of polar substituents in the periphery or lipophilic binding pockets may reduce hERG potency.[15] Furthermore, there are several reports of successfully reducing hERG potency by adding a carboxyl group to the molecule of interest (Fig. 2).[16] This approach was utilized with our structure. The first molecule synthesized was acid **7**. The tertiary center on the THP side chain was chosen for the carboxylic acid placement. With this substitution, a 10-fold loss in hERG potency was obtained; however, MCHR1 affinity also fell 80-fold. If the linker is reduced from three to two carbons, as in analog **10**, most of the MCHR potency is regained and hERG activity decreases to greater than 5 μM. Finally, the replacement of the THP ring with a cyclohexyl ring (**11**) improves the MCHR1 affinity an additional 15-fold to 0.6 nM while maintaining the hERG potency at greater than 5 μM. This compound showed significantly reduced hERG inhibition over analog **2**, while maintaining excellent MCHR1 affinity.

Compound **11** is a selective MCHR1 antagonist. Affinity to other receptors and transporters was evaluated for **11** using Panlab's lead profile screen of 64 targets and in-house assays. A partial list of selectivity data for **11** can be found in Table 2. This list includes some known receptors which likely influence food intake and/or energy expenditure. When tested with each of these targets, compound **11** showed $K_i$ values > 2 μM with the exception of serotonin $5HT_{2C}$, which had a $K_i$ of 1.12 μM in the binding assay and an $IC_{50}$ of 1.14 μM in the functional aequorin assay. Functionally, compound **11** is a potent antagonist to human MCHR1 with an aequorin $IC_{50}$ of 1.2 nM. Compound **11** also showed potency across species with $IC_{50}$ values ranging from 0.6 nM in mouse to 0.8 nM in rat and rhesus monkey. No activity against MCHR2 was observed up to 10 μM.

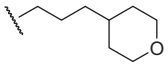
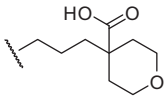
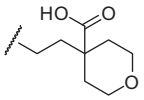
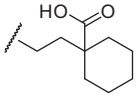
Analog **11** was further evaluated in mouse, rat, dog (beagle), and cynomolgus PK. The corresponding pharmacokinetic results can be found in Table 3. Overall, analog **11** showed low clearance across species and oral bioavailabilities ranging from 29 to 79%. Compound **11** was also evaluated in rat brain PK, since there was a concern that the carboxylic acid functionality on **11** might prevent penetration of the compound into the brain. It possessed the following concentration ratios after a 10 mg/kg oral dose: [blood]/[plasma] = 0.79, [brain]/[plasma] = 0.40, and [CSF]/[plasma] = 0.21. These data suggested that **11** could sufficiently cross the blood brain barrier. In general, compound **11** possessed a good pharmacokinetic profile.

Because hERG inhibition is a common mechanism for QTc prolongation, an extensive cardiac toxicity assessment measuring QTc prolongation was performed with compound **11** in several species. In an anesthetized rhesus monkey QT study, compound **11** was administered as a bolus, followed by an IV infusion for 45 min to a steady plasma concentration of ~3000 ng/mL (~6.5 μM). Throughout the duration of this study, no QTc change was observed. Similarly, no QTc change was observed after PO dosing of **11** at 30 mg/kg in cynomolgus monkey ($n$ = 3). In a 4 h dog study, no QTc change was observed after PO dosing at 300 mg/kg ($C_{max}$ 15,200 ng/mL, ~33 μM). Finally, in a chronic dog study where compound **11** was dosed at 100 mg/kg PO BID for 28 days, no QTc




**Scheme 1.** Reagents and conditions: (a) LDA, allyl bromide, THF; (b) (1) $BH_3$·THF complex, THF (2) $H_2O_2$, NaOH, $H_2O$; (3) oxalyl chloride, DMSO, $Et_3N$, DCM; (c) (1) $NaBH(OAc)_3$, amine **6**, DCM; (2) LiOH, EtOH, $H_2O$; (d) $OsO_4$, $NaIO_4$, 1,4-dioxane, MeOH; (e) (1) LiOH, THF, $H_2O$; (2)HCl; (f) $NaBH(OAc)_3$, amine **6**, DCM.

**Table 1**
SAR results of the THP sidechain[a]

| Compound | R | MCHR1 $IC_{50}$ (μm) $^{125}$I-MCH displ. | hERG est. $IC_{50}$ (μm) $Rb^+$ efflux |
|---|---|---|---|
| **2** |  | 0.0005 | 0.03 |
| **7** |  | 0.04 | 0.3 |
| **10** |  | 0.009 | >5 |
| **11** |  | 0.0006 | >5 |

[a] Values are means of at least two experiments.

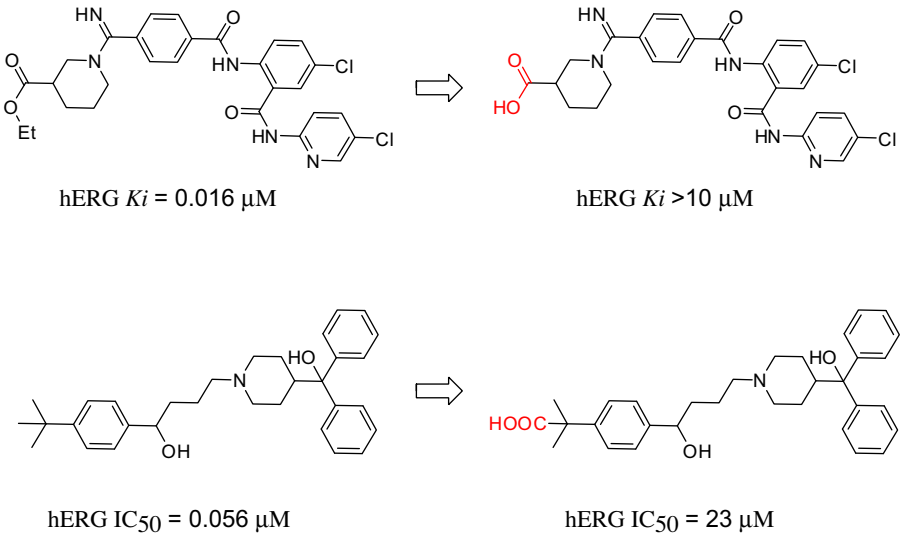



**Figure 2.** Literature examples of the inhibitory effect of adding a carboxylic acid group on hERG binding and activity.

**Table 2**
Selectivity profile for compound **11**[a]

| Receptor/Transporter | Binding $K_i$ (μM) | $Ca^{2+}$ mobl. $IC_{50}$ (μM) |
|---|---|---|
| MCHR1 (human) | 0.0006 | 0.0012 |
| MCHR1 (mouse) | | 0.0006 |
| MCHR1 (rat) | | 0.0008 |
| MCHR1 (rhesus monkey) | | 0.0008 |
| MCHR2 (human) | | >10 |
| Serotonin $5HT_{1A}$ | >2 | |
| Serotonin $5HT_{2A}$ | >2 | |
| Serotonin $5HT_{2C}$ | 1.12 | 1.14 |
| Dopamine D1 | >2 | |
| Dopamine D2 | 3.16 | |
| Adrenergic $\alpha_{2A}$ | >2 | |
| Adrenergic $\alpha_{2C}$ | >2 | |
| Opioid μ | >2 | |
| Muscarinic M2 | >2 | |
| Muscarinic M3 | >2 | |
| Norepinephrine transporter | >2 | |
| Serotonin transporter | >2 | |

[a] Experiments were conducted at MDS-Panlabs with the exception of MCHR1 and MCHR2 experiments. These assays were performed in-house.

changes were observed. Overall, these results suggest a minimal cardiovascular risk due to hERG inhibition with compound **11**.

**Table 3**
Pharmacokinetic results for analog **11**[a,b]

| Species | IV | | PO | |
|---|---|---|---|---|
| | Cl (L/h/kg) | $V_{ss}$ (L/kg) | MRT (h) | %F |
| Mouse | 0.64 | 3.6 | 5.8 | 79 |
| Rat | 0.55 | 3.8 | 7.9 | 70 |
| Dog (beagle) | 0.53 | 2.2 | 4.2 | 40 |
| Cyno | 0.22 | 2.3 | 11 | 29 |

[a] $n = 3$ animals per study.
[b] Dosed at 0.5 mg/kg IV and 3 mg/kg PO.

Compound **11** was further evaluated in two established mouse models for obesity.[17] In the first study, C57BL/6 mice were weaned at 4–5 weeks of age and maintained on a high-fat diet for more than 24 weeks before the start of dosing. Four groups of mice were dosed daily PO with either vehicle, Sibutramine (10 mg/kg), or compound **11** (3 mg/kg or 10 mg/kg dosed as the HCl salt), and the effect on body weight was measured for 137 days as the mean of $n = 6$–8 mice per group. Sibutramine (Meridia®), a serotonin and norepinephrine reuptake inhibitor, was chosen as a positive control since it was reported to show efficacy in rodent models of obesity and was prescribed as an appetite suppressant until its

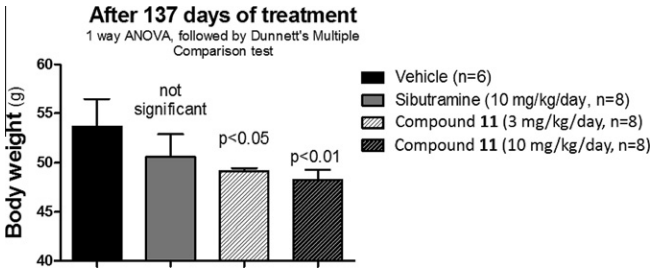



**Figure 3.** Changes in body weight in C57BL/6 mice fed high-fat diets. C57BL/6 male mice were fed a high fat diet (60 kCal% fat, Research Diets Inc. D12492i) for 24 weeks prior to start of treatment and throughout the course of treatment. Dosing was performed daily by bolus gavage.

removal from the market in 2010.[18] Within the graph in Figure 3, which plots body weight versus dose, a statically significant reduction in body weight ($p < 0.01–0.05$ vs vehicle) was observed in the compound **11** treated groups. Both the 3 mg/kg and 10 mg/kg doses of **11** showed a greater loss in body weight when compared to Sibutramine. Sibutramine did show a reduction in body weight when compared to vehicle; however, this reduction was not considered statistically significant. Food intake measurements were also obtained within the experiment and dose-related reduction in food intake in response to **11** was observed throughout the course of the experiment (data not shown).

The second in vivo mouse experiment was designed to confirm that the weight-loss promoting effects of compound **11** were indeed mediated by antagonism of MCHR1 ( Fig. 4). Within this experiment, two groups of female mice, MCHR1-expressing wild type and MCHR1 knockout mice, were utilized. Mice maintained on high-fat diets were dosed either with vehicle or compound **11** (HCl salt form 100 mg/kg/day ad libitum in the high fat diet) and their body weights were monitored for 34 weeks. At the end of the study, compound **11** treated animals showed a statistically significant ($p < 0.01$ vs vehicle) decrease in body weight gain only in the wild-type mice but not in the MCHR1 knockout mice. This result suggests that **11** is likely reducing food intake by antagonizing MCHR1.

In summary, we discovered compound **11**, a novel, potent MCHR1 antagonist with a diminished hERG activity. The decrease in hERG activity was accompanied by a cleaner cardiovascular safety profile, as seen in several preclinical QTc studies examining cardiovascular risk. This compound was selective for MCHR1 and possessed good PK across several species. Compound **11** was also efficacious in reducing body weight gain in two in vivo mouse models. These results suggest that **11** could be a useful treatment for obesity. Compound **11** was advanced for further clinical evaluation and was given the code AMG 076. A complete in vivo characterization of compound **11** will be the subject of a future publication.

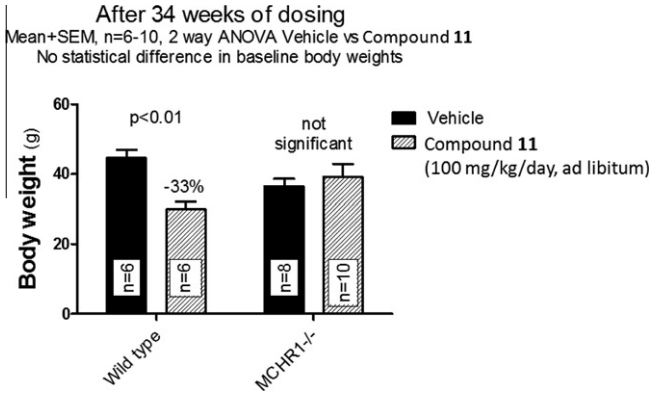



**Figure 4.** Effects of **11** on body weight wild-type versus knockout mice. C57BL/6 female mice received compound prepared in a high fat diet (60% kCal fat, Research Diets Inc. D12492i).

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13. (a) MCHR1 binding assay description: material and solutions: Binding-buffer: 50 mM Tris pH 7.4, 10 mM $MgCl_2$, 2.5 mM EDTA; 0.2% BSA (w/v). Washing-buffer: 25 mM Hepes pH 7.4, 5 mM $MgCl_2$, 1 mM $CaCl_2$, 0.5% BSA (w/v), 0.5 M NaCl. Mulitiscreen®-FB plates (Cat# MAFBN0B50, Millipore USA) were, prior to use, treated with 100 μl of 0.5% BSA (w/v)/$H_20$ solution. After 2 h at room temperature the solution was removed by vacuum filtration and the wells were washed two times with 100 μl of binding-buffer. Methods: 50 μl of compound-dilution, made in binding-buffer, was added to 50 μl of binding buffer containing 1 unit MCHR1 membrane preparation (Cat# ES-370M, Euroscreen; Belgium) and 0.02 μCi $^{125}$I-MCH (2200Ci (81.4TBq)/mmol; 100 μCi/mL, PerkinElmer, USA). The 100 μl reaction mixture was incubated in a Corning 96-well plate (Cat# 3363, Corning USA) at 37 °C for 2 h on a shaking platform. Bound and unbound ligand was separated by vacuum filtration by transferring 80 μl of the reaction mixture into a corresponding well of the Mulitiscreen®-FB plates. After filtration the wells were washed 4 times with 100 μl of washing-buffer before 100 μl of Scintillation cocktail (PerkinElmer, USA) was added. Plates were read on a MicroBeta® Trilux (PerkinElmer, USA). (b) Rubidium ($Rb^+$) efflux inhibition assay was carried out as described by Cheng, C.; Alderman, D.; Kwash, J.; Dessaint, J.; Patel, R.; Kay, M.; Lescoe, M. K.; Kinrade, M. B.; Yu, W. *Informa*, **2002**, *28*, 177. Briefly, HEK293 cells expressing hERG were plated in poly-D-lysine coated 96-well plates. On the next day, the cells were washed with hERG buffer (150 mM NaCl, 2 mM $CaCl_2$, 0.8 mM $NaH_2PO_4$, 1 mM $MgCl_2$, 5 mM Glucose, 12 mM HEPES, pH 7.4).

200 μL of $Rb^+$ loading buffer (5.4 mM RbCl in hERG buffer) was added. After 4 h incubation at 37 °C, compounds were added and the cells incubated at 37 °C for 30 min. The cells were then washed with washing buffer (5 mM KCl in hERG buffer). 200 μL of 50 mM KCl buffer (50 mM KCl in hERG buffer) was added and to activate the hERG channel for 5 min at rt. The supernatant was transferred to a new 96-well plate and the cells lysed with lysis buffer (1% Triton X-100 in hERG buffer). $Rb^+$ concentrations in the supernatant and the cells were determined by using an atomic absorbance spectrometer. $Rb^+$ efflux rate (RE) of compounds was calculated: $Rb^+$ efflux rate (RE): [$Rb^+$ supernatant]/[total $Rb^+$]; $Rb^+$ efflux efficiency (EE): (RE sample − RE negative control)/(RE positive control − RE negative control).

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