Nonpeptide, Orally Bioavailable ACTH Antagonists: Suppression of ACTH-induced Corticosterone Secretion and Adrenal Hypertrophy in Rats

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Cushing's disease is most commonly the result of a microadenoma derived from pituitary corticotrophic cells that secretes excess adrenocorticotropic hormone (ACTH). ACTH is an important modulator of steroidal hormone synthesis and secretion from the adrenal gland, and its selective activity at the melanocortin type 2 receptor (MC2) dictates the synthesis and secretion of cortisol (corticosterone in rats). The resulting hypercortisolemia in Cushing's patients presents in a myriad of symptoms that include growth of fat pads, excessive sweating, dilation of capillaries, thinning of the skin, muscle weakness, hirsutism, depression/anxiety, hypertension, osteoporosis, insulin resistance, hyperglycemia, and heart disease, among others that result in high morbidity. We hypothesize that blocking ACTH action directly via a selective MC2 receptor antagonist may provide an important new therapeutic mechanism to help better manage Cushing's disease in patients.

To test this hypothesis, we launched an iterative medicinal chemistry program to identify potent and selective nonpeptide MC2 receptor antagonists with pharmaceutical and safety characteristics suitable for evaluation in human clinical trials. Unlike most other G protein-coupled receptors, MC2R requires the presence of an accessory protein (MRAP) for cell surface expression and recognition of ACTH. Our medicinal chemistry effort led to small molecule nonpeptides with antagonist activity in CHO-K cells stably expressing the MC2R-MRAP complex. Iterative optimization led rapidly to the discovery of multiple chemical classes of highly potent, nonpeptide MC2R selective antagonist leads, which were then further optimized for drug-like characteristics. We have identified multiple compounds that exhibit high potency for human and rat MC2 receptors (hMC2R K_B < 1 nM) while having little activity at the MC1, MC3, MC4, or MC5 receptors. In rat and dog pharmacokinetic studies, many of these selective MC2R antagonists exhibit good oral bioavailability. Probing their efficacy, these selective MC2R antagonists acutely suppress corticosterone secretion in an ACTH-challenge model in male Sprague-Dawley rats, and the degree of suppression is proportional to their activity at the rat MC2 receptor. In a 7-day hypercortisolemia model in which rats receive an implanted minipump that continually secretes ACTH, corticosterone levels were decreased, and body weight loss and adrenal hypertrophy were prevented. To our knowledge, these compounds represent the first potent nonpeptide MC2 receptor antagonists to demonstrate in vitro potency and in vivo efficacy, and we are actively pursuing preclinical safety and toxicology studies to select the optimal molecule(s) suitable for evaluation in human clinical trials.

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MC2R Antagonist Assay Cascade

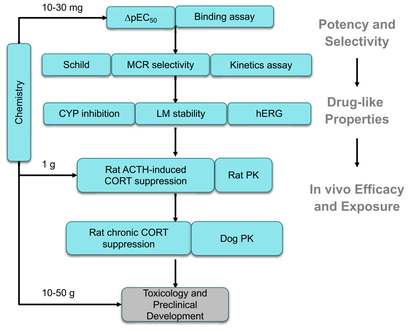

Figure 1. Assay cascade used to identify potent, selective, and drug-like orally bioavailable MC2R antagonists. Molecules with desirable characteristics proceed to preclinical development.

In vitro Identification of Potent MC2R Antagonists

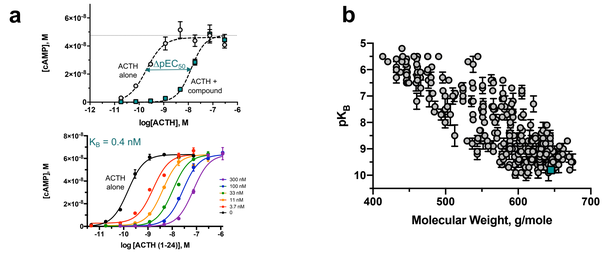

Figure 2. Two-phase Schild Assay. (a) Single point pEC_{50} shift followed by full Schild was used to identify potent MC2R antagonists. **(b)** Plot of the molecular weight of Crinetics compounds versus the potency (pK_B or -log of the binding constant K_B) against human MC2R. Antagonist 1 is shown as a teal square.

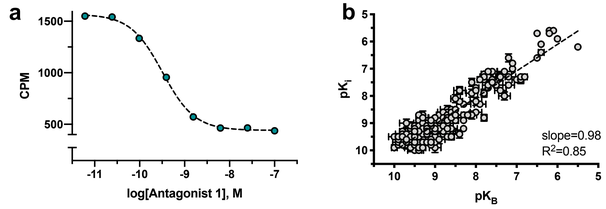

Figure 3. Binding Assay. (a) Radioactive competition binding assay using ^{125}I-ACTH(1-39) as the probe ligand for human MC2R. **(b)**There is good correlation between the functional (pK_B) and binding (pK_i or -log of the inhibition constant K_i) assays for human MC2R.

MC2R Antagonists Are Selective

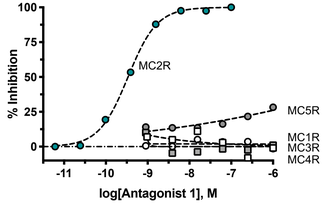

Figure 4. Selectivity of Antagonist 1 Versus other Melanocortin Receptor Family Members. Radioactive competition binding assay using ^{125}I-ACTH(1-39) as the probe ligand for human MC2R and ^{125}I-NDP-α-MSH for human MC1R, MC3R, MC4R, and MC5R.

MC2R Antagonists Have Drug-Like Characteristics and Are Orally Bioavailable

	Potency (K_B, nM)		Cytochrome P450 inhibition (IC_{50}, μM)				Microsomal Stability: $t_{1/2}$ (min)			hERG inhibition
	Human	Rat	CYP3A4	CYP2D6	CYP2C9	CYP2C19	Human	Rat	Dog	IC_{50} (μM)
Antagonist 1	0.2	7.1	5.6	> 10	> 10	9	35	43	87	2.8
Antagonist 2	1.4	2.2	ND	> 10	> 10	> 10	77	>690	ND	> 10
Antagonist 3	0.2	0.5	10	> 10	> 10	7.5	38	28	ND	4.0
Antagonist 4	0.1	1.8	> 10	> 10	> 10	> 10	58	37	58	7.3

Table 1. Drug-like Characteristics. Compounds are potent at human MC2R and not selective against rat MC2R. Additionally, the lack of CYP enzyme activity, adequate liver microsomal stability, and lack of hERG channel activity are consistent with favorable drug-like properties. ND, not determined.

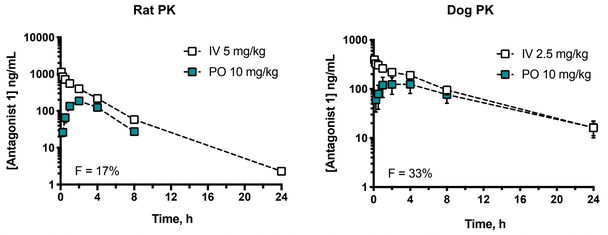

Figure 5. Oral and IV Pharmacokinetics of Antagonist 1 in Rats and Dogs. Antagonist 1 was tested for oral bioavailability in the rat and dog. The compound has shown acceptable half-life (2-6 hours) and bioavailability in both species.

MC2R Antagonist Suppresses ACTH-induced Corticosterone Secretion

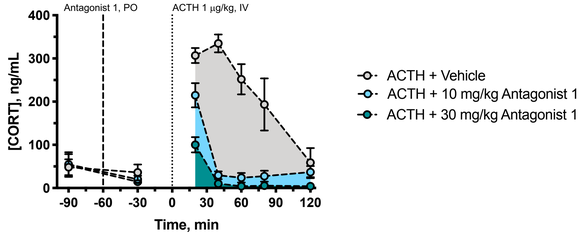

Figure 6. Suppression of ACTH-induced Corticosterone (CORT) Secretion in Rats. An ACTH challenge results in a predictable increase in CORT levels that can be suppressed by Antagonist 1.

Daily Dosing of an MC2R Antagonist Prevents Weight Loss and Adrenal Hypertrophy from Chronic ACTH Exposure

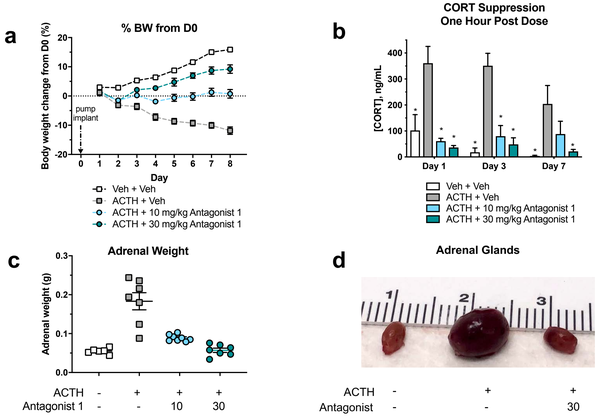

Figure 7. Osmotic pumps with 100 μg/kg/d ACTH (or vehicle) were subcutaneously implanted in male Sprague-Dawley rats 24 h before administration of Antagonist 1 (or vehicle) via oral gavage. **(a)** ACTH induced a 10-15% weight loss by day 8 that was dose-dependently reversed by daily administration of Antagonist 1. **(b)** CORT secretion was suppressed by Antagonist 1. **(c)** ACTH-induced adrenal hypertrophy on day 8 was dose-dependently reversed by the Antagonist 1. **(d)** Representative rat adrenal glands after 7-day treatment with vehicle, ACTH, or ACTH plus the antagonist.

Conclusions

We have discovered potent, selective, and drug-like MC2R antagonists. We describe a representative antagonist that:

- **is a potent MC2R antagonist and selective over other melanocortin receptor subtypes**
- **has desirable drug-like characteristics and achieves good exposure following oral administration in rats and dogs**
- **suppresses acute ACTH-induced corticosterone secretion in rats**
- **reverses the effects of chronic ACTH infusion in repeat-dose studies**

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