Discovery and Identification of Late Stage, Selective Nonpeptide ACTH Antagonists for the Treatment of Cushing's Disease, Ectopic ACTH Secreting Tumors, and Congenital Adrenal Hyperplasia

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MON-176

Adrenocorticotropic hormone (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 (MC2R) dictates the synthesis and secretion of cortisol (corticosterone in rats). Excess ACTH action contributes to the pathophysiology of Cushing's disease (CD), ectopic ACTH syndrome (EAS), and Congenital Adrenal Hyperplasia (CAH). Cushing's disease results from a microadenoma derived from pituitary corticotrophic cells that secretes excess ACTH, whereas EAS arises from nonpituitary ACTH secreting tumors. Excess ACTH action at the adrenal gland and resulting hypercortisolemia presents in a myriad of symptoms that result in high morbidity. CAH results from inactivating mutations in steroid synthesis pathways, the most common being a defect in 21-hydroxylase resulting in lack of cortisol and aldosterone production. Lack of negative feedback by cortisol at the level of the pituitary causes the over-secretion of ACTH, and overproduction of adrenal androgens, causing significant virilization and reduction in quality of life. We hypothesize that blocking ACTH action directly via a selective MC2R antagonist may provide an important new therapeutic mechanism to help better manage these patients.

To test this hypothesis, Crinetics launched an iterative medicinal chemistry program to identify potent and selective nonpeptide ACTH 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. Using CHO-K cells stably expressing this MC2R-MRAP complex, iterative optimization led 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 identified multiple compounds that exhibit high potency for human and rat MC2R (hMC2R K_B < 1 nM), while having no activity at the MC1R, MC3R, MC4R, or MC5R subtypes. Leading ACTH antagonists were also evaluated for drug like characteristics, including good stability in liver microsomes, lack of inhibition of cytochromes P450 and the hERG ion channel, and were shown to exhibit good exposure upon oral dosing in both rats and dogs. These ACTH antagonists acutely suppress corticosterone secretion in an ACTH-challenge model in rats. In a 7-day hypercortisolemia model in which rats are implanted with a minipump that continually secretes ACTH, corticosterone levels were decreased, and body weight loss and adrenal hypertrophy were prevented with ACTH antagonist treatment. The culmination of these studies has led to a subset of candidate molecules that are being evaluated in genotoxicity, safety pharmacology, and general toxicology studies to enable evaluation in human clinical trials.

This work was supported in part by an SBIR grant from the NIH awarded to Dr. Struthers (R43-DK115245)

Healthy and Diseased HPA Axis

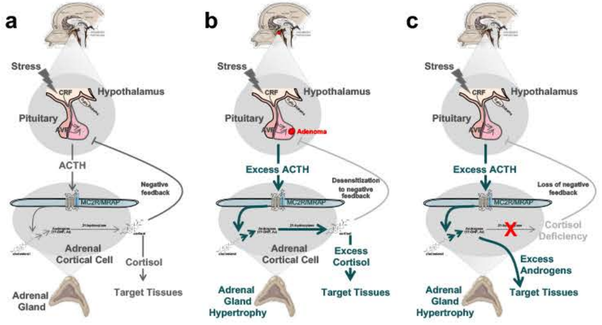

Figure 1. a) Normal HPA axis compared to (b) the axis perturbed by excess ACTH secretion from a pituitary adenoma (Cushing's disease) or non-pituitary adenoma (ectopic ACTH syndrome), and (c) the axis in CAH also characterized by excess ACTH resulting from hypocortisolemia.

ACTH Antagonist Assay Cascade

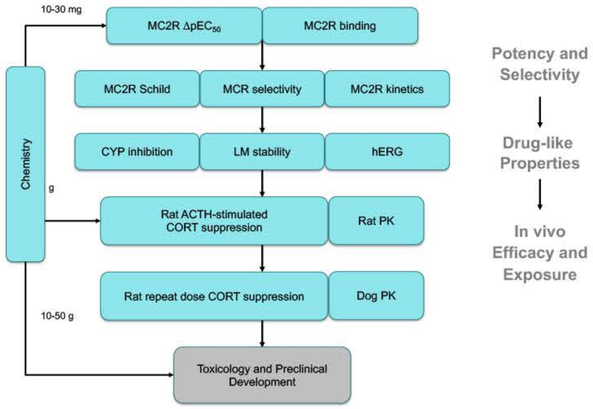

Figure 2. Assay cascade used to identify potent, selective, and drug-like orally bioavailable ACTH antagonists. Molecules were screened by a combination of functional cAMP and binding assays. Potent molecules were counter-screened against other MCR family members. Properties such as liver microsomal stability and hERG channel activity were screened to move suitable compounds forward. A subset of molecules with acceptable properties were evaluated for in vivo efficacy in rats and exposure in rats and dogs. A further subset of these compounds were advanced to dose range finding toxicology and preclinical development.

ACTH Antagonists Are Potent and Selective

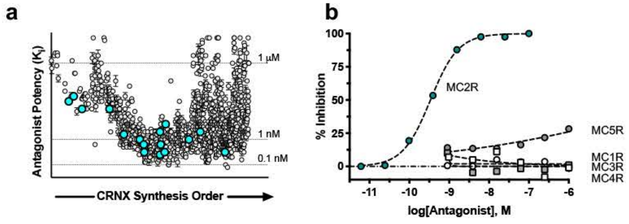

Figure 3. Potency history and selectivity of nonpeptide ACTH antagonists. (a) Crinetics has generated a library of ~ 2600 compounds to target MC2R. Of these, ~ 1300 were run in a competition binding assay using ^{125}I-ACTH(1-39) as the probe ligand shown above, and ~ 500 compounds were run in a full Schild. Compounds detailed in Table 1 are shown in teal **(b)** Radioactive competition binding assays of ANT-8 for human MCR family members.

ACTH Antagonists Have Good Drug-Like Properties and Are Orally Bioavailable

	MC2R Avg Potency (K_B nM)		Selectivity (K_i, nM)				CYP450 Inhibition (µM)				hERG Inhibition (µM)	LM Stability $t_{1/2}$ (min)			PK (F,%)	
	human	rat	MC1R	MC3R	MC4R	MC5R	2C9	2C19	2D6	3A4		human	rat	dog	rat	dog
ANT-1	26	252					> 10	> 10	8.9	> 10	0.64	231	231			
ANT-2	15	30					3.6	1.8	4.4	1.9	3.7			≥ 693		
ANT-3	11	25					> 10	6.7	> 10	> 10	3.3	≥ 693	139			
ANT-4	3.8	5.3		>1000*	>1000*				>10	1.8	4.4	> 462	> 363			
ANT-5	1.7	19							> 10	4.6	2.0	11	3	26	12	19
ANT-6	0.90	18							> 10	7.7	2.7	14	20	5	19	4
ANT-7	1.1	32	>1000	>1000	>1000	>1000	3.1	3	> 10	7.8	2.7	58	173	87	38	41
ANT-8	0.20	1.0	>1000	>1000	>1000	>1000	> 10	9	> 10	5.6	2.0	35	43	87	17	34
ANT-9	0.50	0.50	>1000	>1000	>1000	>1000			> 10	> 10	6.5	> 375	> 375	77	27	68
ANT-10	0.20	0.45					> 10	7.5	> 10	> 10	4.0	39	28	77		
ANT-11	0.90	2.9				>1000*	6.5	5.2	> 10	> 10	> 10	77	≥ 693	≥ 693	5	34
ANT-12	0.10	0.90	>1000	>1000	>1000	>1000	> 10	> 10	> 10	> 10	3.0	58	37	58	26	43
ANT-13	1.4	0.40	>1000	>1000	>1000	>1000	> 10	> 10	> 10		12	77	≥ 693	139	45	58
ANT-14	0.30	0.20	>1000	>1000	>1000	>1000	> 10	> 10			19	231	231	173	47	107
ANT-15	0.80	1.2	>1000	>1000	>1000	>1000	> 10	> 10	> 10	> 10	16	116	≥ 693	231	91	36
ANT-16	0.10	1.7	>1000	>1000	>1000	>1000	4.9	> 10	> 10	> 10	1.6	99	77	43	46	100

Molecular weight range for all antagonists is 540- 650 * functional cAMP assay

Table 1. Drug-like characteristics of selected ACTH antagonists. The primary screen at human MC2R led to hits in the 10-50 nM potency range. Some initial hits were poorly suited for efficacy in animal models due to low potency against the rat MC2R. Later iterations improved both rat selectivity and drug-like properties. Promising compounds were tested for oral bioavailability in the rat and dog.

ACTH Antagonists Suppresses ACTH-induced Corticosterone Secretion in Rats

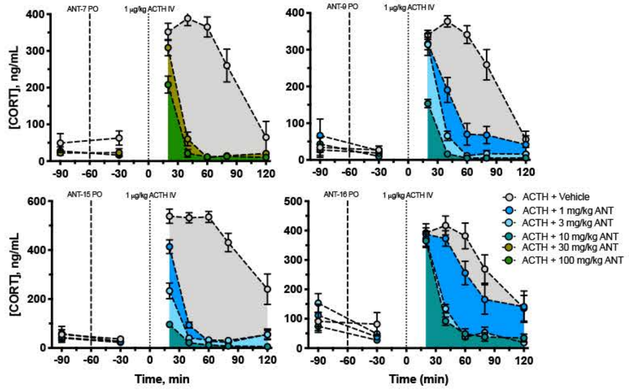

Figure 4. Suppression of ACTH-stimulated corticosterone (CORT) secretion in rats. Intravenous administration of an acute ACTH bolus in male Sprague Dawley rats results in a predictable increase in CORT secretion that is suppressed by ACTH antagonists in a dose-dependent manner.

ACTH Antagonists Prevent Weight Loss and Adrenal Hypertrophy from Sustained ACTH Exposure in Rats

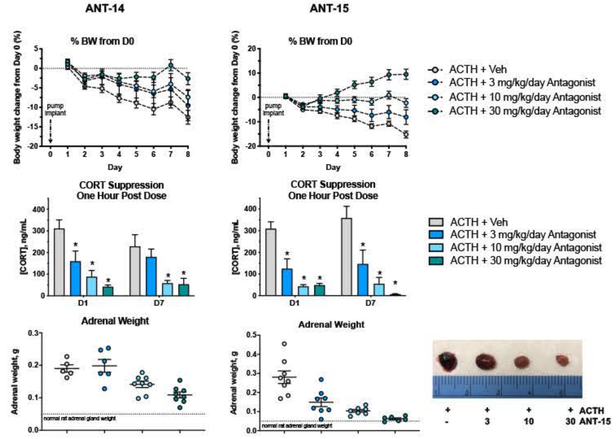

Figure 5. Osmotic pumps delivering 100 µg/kg/day ACTH were subcutaneously implanted in male Sprague-Dawley rats 24 h before daily oral administration of antagonist (or vehicle) for seven days. Administration of ANT-14 and ANT-15 dose-dependently reversed ACTH-induced weight loss, CORT secretion, and adrenal hypertrophy. Representative adrenal glands after 7-day treatment with ACTH or ACTH plus 3, 10 or 30 mg/kg/day of ANT-15 are shown.

Conclusions

Crinetics has discovered potent, selective, and drug-like ACTH antagonists. We describe several antagonists that:

- **are potent antagonists and are selective over other MCR subtypes**
- **have desirable drug-like characteristics and good oral bioavailability in rats and dogs**
- **suppress acute ACTH-stimulated corticosterone secretion in rats**
- **reverse the effects of sustained ACTH exposure in repeat-dose studies in rats**

We have nominated one antagonist for further development and are conducting first in human trial enabling GLP toxicity studies

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