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

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Overview: Cushing’s disease is most commonly the result of a microadenoma derived from pituitary corticotrope cells that secretes excess adrenocorticotropic hormone (ACTH). ACTH is an important modulator of steroid hormone synthesis and secretion from the adrenal gland, and its selective activity at the melanocortin-2 receptor (MC2R) stimulates the synthesis and secretion of corticosterone in the adrenal cortex.

In the current study, the authors aimed to identify nonpeptide MC2R antagonists that could be used to suppress ACTH-induced corticosterone secretion and adrenal hypertrophy in rats.

In vitro and in vivo studies were conducted to identify MC2R antagonists with desirable biochemical and pharmacologic properties. The selected MC2R antagonists were then tested in vivo to determine their ability to suppress ACTH-induced corticosterone secretion and adrenal hypertrophy.

**Table 1. Drug-like Characteristics.** Compounds are potent at human MC2R and non-selective against MC4R.

**Results:** The selected MC2R antagonists were able to suppress ACTH-induced corticosterone secretion and adrenal hypertrophy in rats.

**Conclusions:** The nonpeptide, orally bioavailable ACTH antagonists identified in this study have potential for use in the treatment of Cushing’s disease.

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**Figure 2. Two-phase Schild Assay.**
(a) Single point pEC50 shift followed by full Schild was used to identify potent MC2R antagonists. (b) Plot of the molecular weight of nonpeptide compounds versus the potency (pEC50) or log (of the binding constant Kd) against human MC2R. Antagonist 1 is shown as a test example.

**Figure 3. Binding Assay.** (a) Receptor-competition binding assay using 1,000 nM ACTH (1-24) or the probe-receptor KREND-shadow (or the inhibition constant Kd) assay using human MC2R.

**Figure 4. Selectivity of Antagonist 1.** Versus other melanocortin receptor families. Radioligand-competition binding assay using 1,000 nM ACTH (1-24) or the probe-receptor KREND-shadow (or the inhibition constant Kd) assay using human MC2R, MC3R, MC4R, and MC5R.

**Figure 5. Oral and Oral Pharmacokinetics of Antagonist 1 in Rats and Dogs.** Antagonist 1 was tested for oral bioavailability in the rat and dog. The compound line shows acceptable half-life (4 h) and bioavailability in both species.

**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.

**Figure 7. Cushing’s Disease.** Rats were implanted in the left flank with a 200 μg/24 hminipump containing ACTH (1-24) or saline (vehicle). ACTH-induced adrenal hypertrophy on day 8 was dose-dependently reversed by daily administration of Antagonist 1 (25 or 50 mg/kg, i.p.). Antagonist 1 (500 μg/24 h) induced a 10-15% weight loss by day 8 but was dose-dependently reversed by daily administration of Antagonist 2 (100 and 200 μg/24 h).

**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