Discovery of Potent and Orally Bioavailable Nonpeptide Parathyroid Hormone Receptor Type-1 (PTHR1) Antagonists for the Treatment of Primary Hyperparathyroidism (PHPT)

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Parathyroid hormone (PTH) regulates calcium and phosphate homeostasis in bone and kidney through activation of its receptor, PTHR1. Primary hyperparathyroidism (PHPT) is caused by the enlargement of one or more of the parathyroid glands resulting in over-secretion of PTH, which causes increased blood calcium levels. PHPT affects approximately 100,000 patients per year and is often asymptomatic. However, approximately 20% of patients exhibit symptomatic PHPT which is characterized by skeletal, renal, gastrointestinal, and neurological manifestations with increased mortality. Partial to total parathyroidectomy is first line therapy, however, a subset of patients (10-15%) require continuous monitoring and medical therapy due to recurrent PHPT symptoms, or the inability or refusal to have surgery. The goals for medical therapy are to normalize blood calcium levels and urinary calcium excretion, increase bone mineral density (BMD), lower the risk of bone fracture, and reduce the risk of kidney stones. However, calcimimetics decrease circulating calcium levels but have no effect on BMD, while bisphosphonates can improve BMD but have no effect on circulating calcium levels, resulting in a large unmet need for recurrent and non-surgical patients. We hypothesize that blocking PTH action via a PTH receptor antagonist may provide an improved therapeutic mechanism to treat PHPT patients.

Using an iterative medicinal chemistry approach, Crinetics has identified potent and orally available nonpeptide PTH antagonists using a combination of binding and functional in vitro assays. These compounds have low nanomolar affinity for both human and rat PTHR1 and are potent functional antagonists in vitro (IC_{50} <100 nM). The antagonists also exhibit good oral exposure in preclinical species and appropriate drug-like properties, including stability in liver microsomes and lack of inhibition of cytochromes P450 and the hERG ion channel. In a rat model of PTH-induced hypercalcemia, the antagonists dose-dependently suppress ionized blood calcium, providing evidence that a nonpeptide PTH antagonist has potential use as an effective therapeutic for PHPT and possibly other diseases of PTH excess. Currently, a subset of potential candidate molecules is being evaluated in a battery of safety studies to select the optimal molecule(s) suitable for evaluation in human clinical trials.

Targeting PTHR1 in the bone and kidney should decrease hypercalcemia associated with primary hyperparathyroidism

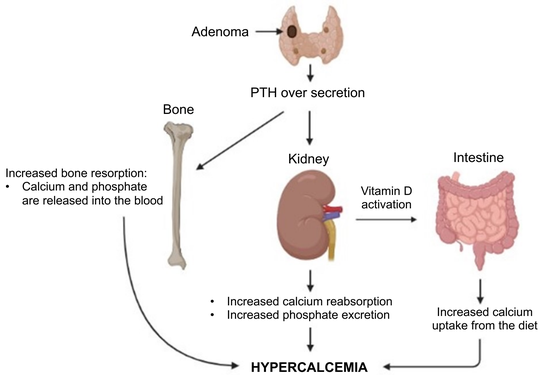

Figure 1. Hypercalcemia associated with primary hyperparathyroidism. Over secretion of PTH would hyperactivate PTH1R expressed in osteoblasts (bone) and renal proximal and distal tubule cells resulting in gene expression and protein changes of important modulators of calcium homeostasis, ultimately resulting in hypercalcemia.

PTHR1 antagonist assay cascade

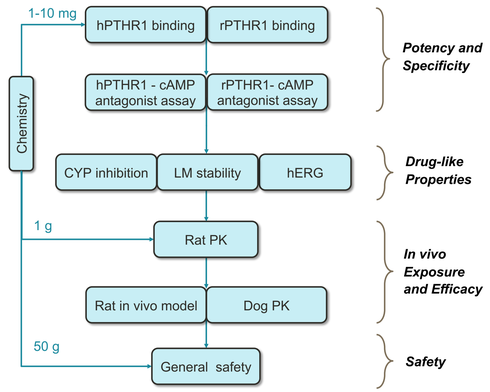

Figure 2. Assay cascade used to identify potent and drug-like orally bioavailable PTHR1 antagonists. Molecules with desirable characteristics proceed to preclinical development.

Discovery of potent PTHR1 antagonists

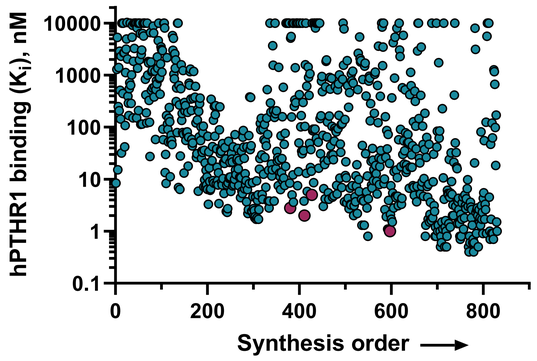

Figure 3. Identification of PTHR1 Antagonists. A binding assay using membranes from cells overexpressing the human PTHR1 was used to identify new compound series. Affinity for the human receptor is expressed as the mean K_i (nM) of at least 2 replicates. ANT-1, ANT-2, ANT-3 and ANT-4 are shown in magenta.

PTHR1 antagonists have high affinity for the human and rat PTHR1

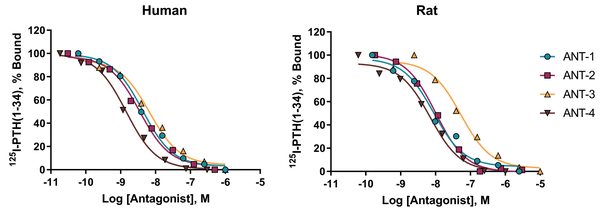

Figure 4. Radioactive competition binding assay using ^{125}I-PTH(1-34) as the probe ligand for human and rat PTHR1. Dose response curves from binding assays using membranes overexpressing human and rat PTHR1 are expressed as % bound ^{125}I-PTH(1-34). K_i values are reported as a measure of compound affinity for each receptor (see Table 1).

PTHR1 antagonists are negative allosteric modulators

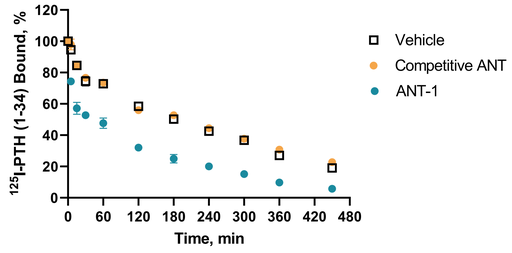

Figure 5. Dissociation kinetics of ^{125}I-PTH(1-34). Dissociation rate of ^{125}I-PTH(1-34) was measured in the presence of 10 µM cold PTH(1-34) ± 10 µM Antagonists. Vehicle represents cold PTH(1-34) only, Competitive ANT is a known competitive peptide PTHR1 antagonist and ANT-1 is a representative of the newly identified small molecule PTHR1 antagonists. Dissociation rate of ^{125}I-PTH(1-34) is not affected by a competitive antagonist but it is significantly increased by ANT-1. Data is expressed as % bound of ^{125}I-PTH(1-34) and it is representative of n=4 experiments.

PTHR1 antagonists have good drug-like properties

	Binding, K_i (nM)		IC_{50} (nM)		CYP450 Inhibition (µM)		hERG (µM)	LM stability $t_{1/2}$ (min)	
	Human	Rat	Human	Rat	2D6	3A4		Human	Rat
ANT-1	2.8	9.1	47	100	>10	>10	4.3	115	20
ANT-2	2.0	9.0	120	140	>10	>10	3.7	87	24
ANT-3	5.0	46	190	370	>10	>10	>10	139	77
ANT-4	1.0	25	4.5	150	>10	>10	8.6	>600	>600

Table 1. Drug-like characteristics of selected PTH1R antagonists. Compounds were screened for PTHR1 binding and antagonist activity at the human and rat receptor, CYP and hERG inhibition, and liver microsomal (LM) stability.

PTHR1 antagonists are orally bioavailable

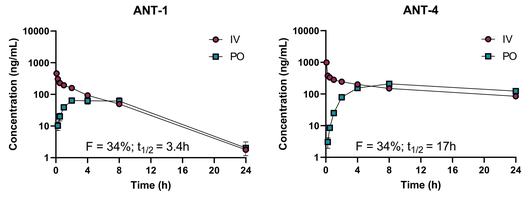

Figure 6. PTHR1 antagonists' pharmacokinetic plasma profile in rats. Sprague Dawley rats were administered PTHR1 antagonists (ANT-1 or ANT-4) at 5 mg/kg (IV-intravenous) and 10 mg/kg (PO-oral). Antagonists' concentration was measured in plasma at specific time points. Points represent mean ± SEM (n=3).

ANT-4 suppresses ionized calcium in rat model of PTH-induced hypercalcemia

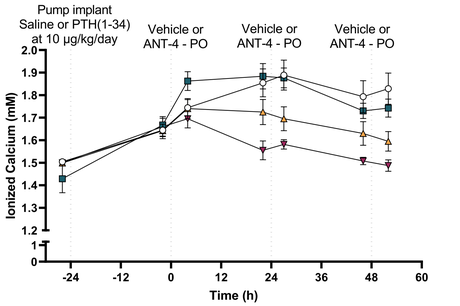

Figure 7. Effect of oral administration of PTHR1 antagonist on ionized calcium levels in a rat model of hypercalcemia. Continuous subcutaneous administration of PTH(1-34) via osmotic pump increased blood ionized calcium by 24h post pump implant in male Sprague Dawley rats (n=8/group). Once daily oral administration of ≥30 mg/kg ANT-4 over 3 days decreased blood ionized calcium measured at -2h and 4h post dose.

Conclusion

Crinetics has developed several examples of potent, drug-like PTHR1 antagonists that inhibit hypercalcemia in a rat model and could be a viable treatment for primary hyperparathyroidism in humans.

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