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CP-71,362, AN UNUSUALLY POTENT INHIBITOR OF RAT AND DOG RENIN[1]

Edward F. Kleinman,*† Andrew H. Fray,† William F. Holt,‡ M. A. Ravi Kiron,‡ William R. Murphy,‡ Irene M. Purcell‡ and Robert L. Rosati†

Pfizer Central Research

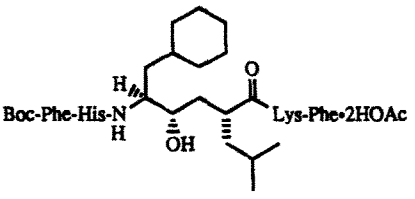
Groton, CT 06340

†Department of Medicinal Chemistry, ‡Department of Metabolic Diseases and General Pharmacology

Abstract: CP-71,362 (Boc-Phe-His-hexahydroPhe[OH]Leu-Lys-Phe) has been identified as the most potent inhibitor of rat plasma renin (IC_{50} = 3 nM) and dog plasma renin (IC_{50} = 3 pM) to date and thus can be used as an important experimental tool in the study of the renin angiotensin system in these established hypertensive models.

In this communication we report the identification of CP-71,362 as the most potent inhibitor of dog and rat renin described to date. This is significant because it is now possible to study the acute and chronic effects of RAS blockade mediated by renin inhibition in these animal models.[2] Previously this had been difficult or impossible because species specificity among renins has meant that agents that are potent inhibitors of human renin have had little activity against the renin of dogs and rats. These animal models are most often used in cardiovascular studies as most investigators lack accessibility to primates.

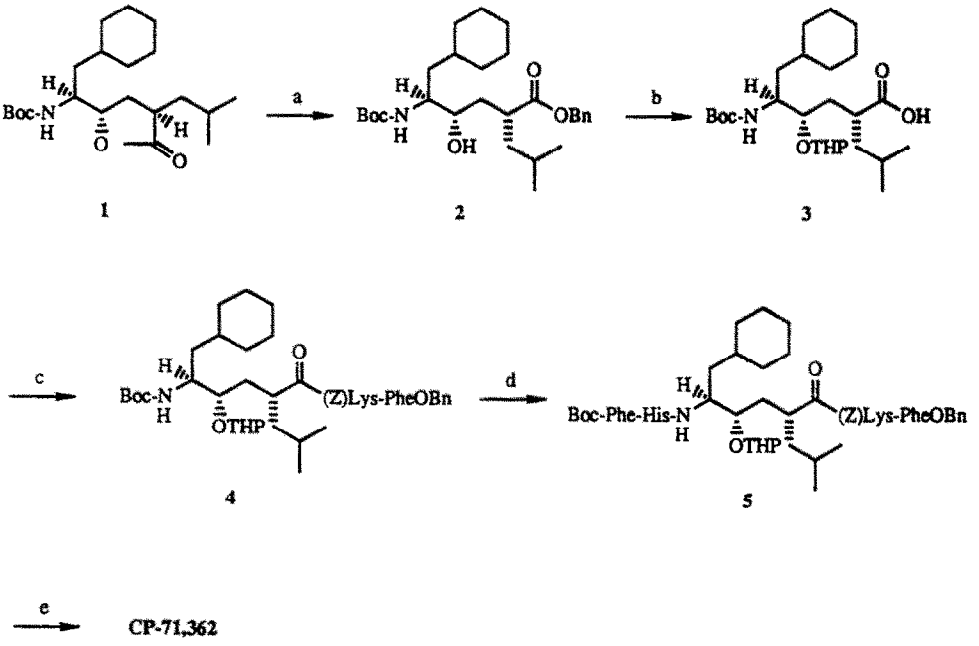
CP-71,362 is a substrate analog of renin spanning residues P_4-P_3' and contains a hydroxyethylene dipeptide isostere[3] transition state mimic in place of the P_1-P_1' residues surrounding the cleavage (scissile bond) site. The P_1 side chain contains a cyclohexylmethyl group which has been shown to have an *in vitro* potency advantage over the natural leucyl side chain at this position.[4,5] Also, a lysine residue is inserted at P_2' based on earlier observations in our laboratory that this modification in the related series of peptide inhibitors containing statine at P_1-P_1'[6] leads to improved duration of action *in vivo*.



CP-71,362

Chemistry. Lactone 1 (oil, $[\alpha]_D^{25}$ -28.2° (c1 5, $CHCl_3$)) is prepared in analogy to the previously described synthesis of the corresponding lactone containing a leucyl group at P_1,[7] and is incorporated into the peptide framework as shown in Scheme 1. Saponification of **1** followed by alkylation of the lyophilized sodium salt with benzyl bromide gives the oily benzyl ester **2**. The hydroxyl group is protected as its THP ether and the benzyl ester is cleaved using catalytic hydrogenation. The resulting acid, **3**, is coupled to N^{ε}-Z-Lys-PheOBn•HCl using DCC/HOBT which affords the protected P_1-P_3' segment, **4** (m.p. 153-175°C), following hydrolysis of the THP group. Cleavage of the Boc group of **4** using TFA and coupling to N^{α}-Boc-Phe-(N^{im}-Boc)His^8 using DCC/HOBT gives the protected peptide **5** (m.p. 150-155°C) after removal of the N^{im}-Boc group of the histidine. Deprotection of **5** by catalytic hydrogenation then furnishes CP-71,362 as a diacetic acid salt (m.p. >200°C (dec.), reverse phase HPLC retention time = 3.09 min, [Zobax C_8, 4.66 mm diameter x 250 mm height, 50% pH 2.1 0.1M phosphate buffer/50% acetonitrile, flow rate = 1.5 mL/min]).

Scheme 1



(a) i. NaOH, H_2O, DME, 25°C, ii. $PhCH_2Br$, DMF, 25°C (63%); (b) ii. DHP, PPTS, CH_2Cl_2, 25°C, ii. H_2, Pd/C, EtOAc (100%); (c) i. N^{ε}-Z-LysPheOBn•HCl, DCC, HOBT, TEA, CH_2Cl_2, 0°C, ii. 70% HOAc-H_2O, 25°C (72%); (d) i. TFA, 0°C, ii. N^{α}-Boc-His(N^{im}-Boc)Phe, DCC, HOBT, TEA, CH_2Cl_2, 0°C, iii. Et_2NH, $CHCl_3$, 25°C (55%); (e) H_2, $Pd(OH)_2$, 4:1-MeOH:HOAc (90%).

In Vitro Activity. Plasma renin inhibitory activity of CP-71,362 measured against canine, rat, and human renin is summarized in Table 1.[9] CP-71,362 demonstrates picomolar potency and a high level of specificity towards canine plasma renin, while maintaining nanomolar potency against human plasma renin (IC_{50} = 20 nM). Compared to published values for other renin inhibitors against the canine plasma enzyme, CP-71,362 (IC_{50} = 3.3 x 10^{-12}M), is 10,000x more potent than SCRIP (IC_{50} = 1 x 10^{-8}M)[10] or H-77 (IC_{50} = 2.4 x 10^{-8}M).[11]

The inhibitory potency of CP-71,362 against rat renin is also remarkable (IC_{50} = 3.3 x 10^{-9}M) being 100x that of SCRIP (IC_{50} = 1 x 10^{-7}M)[10] or H-77 (IC_{50} = 6 x 10^{-7}M).[11] The origin of the specificity of CP-71,362 to rat and dog renin is unknown; we speculate that there may be an anionic group in S_2' specificity pocket of these enzymes which interacts with the positively charged P_2' lysine side chain.

Table 1
***IN VITRO* ACTIVITY OF CP-71,362**

IC_{50} (nM)		
Rat Plasma	Dog Plasma	Human Plasma
3	003	20

In Vivo Activity. In anesthetized, sodium depleted dogs, a continuous infusion of CP-71,362 reduces mean arterial pressure (MAP) in a dose dependent manner, as shown in Figure 1. A 35 mmHg depression of MAP (from 119 mmHg to 84 mmHg, n = 5) is observed at the highest infusion rate (3.7 ± 0.4 µg/kg/min). The infusion rate producing half of the maximal decrease in renin dependent blood pressure is calculated to be 1.1 µg/kg/minute. A maximally effective dose of the ACE inhibitor captopril (10 mg/kg, i.v.) produces an equivalent drop in MAP. An infusion rate of 4.5 ± 0.7 µg/kg/min produces near maximal effects on MAP (~4x the ED_{50} dose), which returns to pre-infusion levels within 30-60 minutes following cessation of the infusion, suggesting rapid metabolism and/or clearance of the compound.

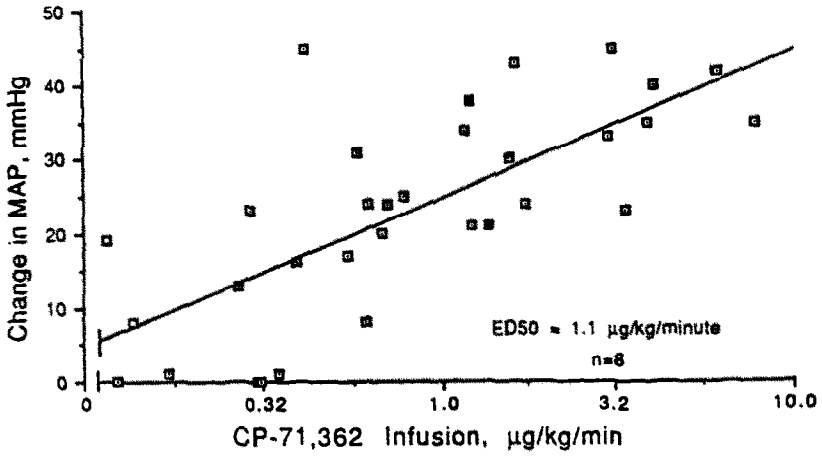


Figure 1
EFFECT OF CP-71,362 INFUSION ON MEAN ARTERIAL PRESSURE IN THE ANESTHETIZED, SODIUM DEPLETED DOG

CP-71,362 also lowers blood pressure in the conscious, sodium depleted Sprague-Dawley rat. An infusion rate of approximately 30 μg/kg/min produces a decrease in MAP of between 30 and 40 mmHg in the rat. Based on a comparison with a maximal captopril dose (captopril's maximal effect is 43 ± 4.2 mmHg), the infusion rate of CP-71,362 producing half of the maximal fall in renin dependent blood pressure is calculated to be 9.7 μg/kg/minute.

These studies clearly demonstrate that, unlike previously published renin inhibitors which lack *in vitro* and *in vivo* potency against the canine and rat enzyme, CP-71,362 can be used as a very important tool to investigate the renin angiotensin system of dogs and other laboratory animals (e.g., rats), particularly in chronic studies.

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