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Renin inhibitors

Improvements in the stability and biological activity of small peptides containing novel Leu-Val replacements

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We have designed a novel class of potent (0.3–7 nM) renin inhibitors which contain a dihydroxyethylene replacement for what is formally the Leu[10]-Val[11] amide bond. Good potency (0.6 nM), water solubility (> 10 mg/ml at 37°C), stability toward degradation by chymotrypsin ($t_{1/2}$ = 820 min), and in vivo activity in a primate model (15% drop in mean arterial pressure in association with complete inhibition of plasma renin activity) are properties which have been incorporated into compound **10**, an interesting new agent to be used in the study of hypertension.

Enzyme inhibitor; Renin; Dihydroxyethylene replacement; Antihypertension

1. INTRODUCTION

Research in the area of renin inhibition has occupied a significant portion of the efforts devoted to the search for new drugs useful in the treatment of hypertension and congestive heart failure. Recently, we reported a novel series of renin inhibitors [1] composed of dipeptides attached to a non-peptide replacement for the scissile Leu-Val amide bond (exemplified by **1**, table 1). The new Leu-Val surrogate was composed of a hydroxyethylene isostere to replace the amide bond, and it was truncated at the C-terminus in order to remove all functionality beyond the Val side chain. When compared to Boc-Phe-His-L-Leucinol (IC_{50} = 1.3×10^{-5} M), it is clear that the additional isopentyl appendage in **1** leads to enhanced binding to the enzyme. In an effort to improve the biological properties for this series of small inhibitors, we felt it important to optimize a number of characteristics, the first of which is potency. We intended to do this by improving existing, or creating new inhibitor-enzyme interactions rather than by incorporating additional amino acid residues. By taking this approach to potency enhancement, we simplify our second problem, that of stabilizing the inhibitor to potential degradative enzymes such as chymotrypsin, by having fewer amide bonds to stabilize. The third goal was to find a position in the inhibitor molecule which would tolerate a wide variety of structural modifications without sacrificing inhibitory potency. Such a site would allow the variation of lipophilicity and water solubility of the inhibitor, two important considerations in the design of an orally active agent.

2. MATERIALS AND METHODS

2.1. *In vitro renin assay*

Purified human renal renin [2] was assayed utilizing pure human angiotensinogen [3] at pH 6.0 in maleate buffer. Test

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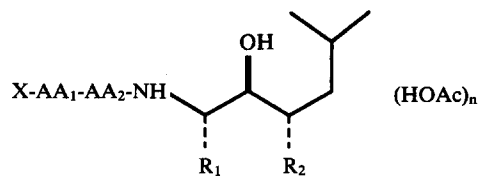
Table 1



Compound	X	AA_1	AA_2	R_1	R_2	n	IC_{50} (nM)[a,b]	Chymotrypsin stability $t_{1/2}$ (min)[b]	Water solubility (mg/ml at 37°C)
1	Boc	Phe	His	isobutyl	H	0	1500		
2	Boc	Phe	His	(cyclohexyl)methyl	H	0	10		
3	Boc	Phe	His	isobutyl	OH	0	11		
4a	Boc	Phe	His	(cyclohexyl)methyl	OH	0	1.5		
5	Etoc	Phe	His	(cyclohexyl)methyl	OH	0	0.5–0.7	3.2	0.026
6	Etoc	Phe	Leu	(cyclohexyl)methyl	OH	0	0.3		0.0003
7	β-Ala	Phe	His	(cyclohexyl)methyl	OH	2	2.5	9.4	>20
8	β-Ala	Phe	Leu	(cyclohexyl)methyl	OH	1	3	48	
9	β-Val	Phe	His	(cyclohexyl)methyl	OH	2	0.5	3.4	>10
10	β-Val	(4-OCH_3)Phe	His	(cyclohexyl)methyl	OH	2	0.6	820	>10
11	β-Ala	(4-OCH_3)Phe	His	(cyclohexyl)methyl	OH	2	7		

[a] IC_{50} values using purified human renin at pH = 6.0
[b] Assay conditions detailed in [5]

compounds were dissolved in DMSO and diluted so that prior to addition to the assay system the solutions were 10% in DMSO and 0.5% in BSA. The final incubation mixture (100 μl) contained 0.135 M maleate buffer (pH 6.0), 3 mM EDTA, 1.4 mM PMSF, 0.21 μM angiotensinogen, 0.24 mGU renin, 0.44% BSA and 1% DMSO. The inhibitory activity was determined by radioimmunoassay for angiotensin I as described [4].

2.2. *In vitro stability assay*

The degradation of the inhibitor (0.05 mg/ml) with 0.1 mg/ml bovine chymotrypsin in phosphate buffer was monitored by high-pressure liquid chromatographic analysis of the incubations (Waters μBondapak C18 analytical column; CH_3CN/H_2O/TFA, 35:65:0.1–80:20:0.1, 20 min gradient, 214 nm detection).

2.3. *In vivo model*

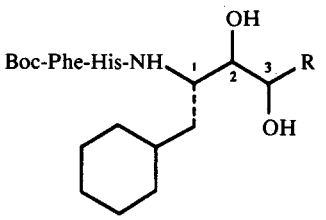
Compounds were administered by bolus injection to salt-depleted, anesthetized cynomolgus monkeys, and their effect on plasma renin activity (PRA), mean arterial blood pressure and heart rate was measured as described [5].

3. RESULTS AND DISCUSSION

3.1. *Inhibitory potency*

Addressing the potency issue first, we found that the inhibitors were improved by changing the P_1 side chain from isobutyl to cyclohexylmethyl, a side chain which after extensive investigation proved to be optimal in a series of closely related sulfidoethanol analogues [6]. Thus, compound **2** is more than 100-times more potent than **1**. Introducing a second hydroxyl group into the Leu-Val replacement [7–11] also increased inhibitory potency. Comparison of compounds **1** and **3** reveals a greater than 100-fold increase in inhibitory potency. Indeed, others have also incorporated a dihydroxyethylene isostere into various renin inhibitors [12] since Matsueda et al. [12a] first showed in a simple system that a 1,2-diol possessed inhibitory activity. We reasoned that these dihydroxyethylene isostere-containing inhibitors were profiting by interaction with an additional binding site in renin such as an active site carboxyl group (Asp-32 or Asp-215) or with some other hydrogen bond donating or accepting functionality. One would expect this type of interaction to be stereospecific, and inhibitory potency should be sensitive to variation of the hydroxyl group geometry. For this reason we explored all four

Table 2



Compound	R	Configuration		IC_{50} (nM)
		C2	C3	
4a	isobutyl	*R*	*S*	1.5
4b	isobutyl	*R*	*R*	35
4c	isobutyl	*S*	*S*	70
4d	isobutyl	*S*	*R*	95
4e	H	*R*	–	50

possible configurations of the 1-amino-2,3-dihydroxy nucleus shown in table 2. A stepwise drop in activity can be seen in going from the configuration of **4a** to that of **4d**. It is interesting to note that, like most renin inhibitors, the two most active compounds in this series, **4a** and **4b**, are more potent than their counterparts, **4c** and **4d**, respectively, which do not have the statine-like absolute configuration at C2 [13]. Like the monohydroxyethylene compounds, removal of the aliphatic appendage, R, from **4a** to give **4e** results in a significant loss in inhibitory potency underscoring the importance of each interaction in the Leu-Val surrogate.

3.2. *Inhibitor solubility*

Next, we exchanged the acid-labile *t*-butyloxycarbonyl (Boc) group with the smaller and more stable ethoxycarbonyl (Etoc). This substitution resulted in a slight improvement in activity in both the Phe-His and Phe-Leu series of compounds, and resulted in inhibitors with subnanomolar potency in both cases (**5** and **6**, respectively). While inhibitor **6** could not be examined reliably in vivo due to extremely low water solubility (table 1), compound **5** consistently lowered blood pressure in a primate model (vide infra) after i.v. administration. After oral dosing, however, hypotensive activity for both was low and variable. We felt that this might be due to a combination Phe-His amide bond lability and low water solubility. Water solubility could be improved dramatically by using the isosteric replacement, β-Ala for Etoc. Implementing this change in **5** allowed formation of diacetic acid salt **7** which had three orders of magnitude greater water solubility, but both **7** and **8** were somewhat less potent than their Etoc analogues. We found that potency could be preserved while still maintaining high water solubility by introducing lipophilicity into the basic N-terminus. This was done conveniently with β,β-dimethyl-β-Ala (or β-Val); compare **9** to **5**.

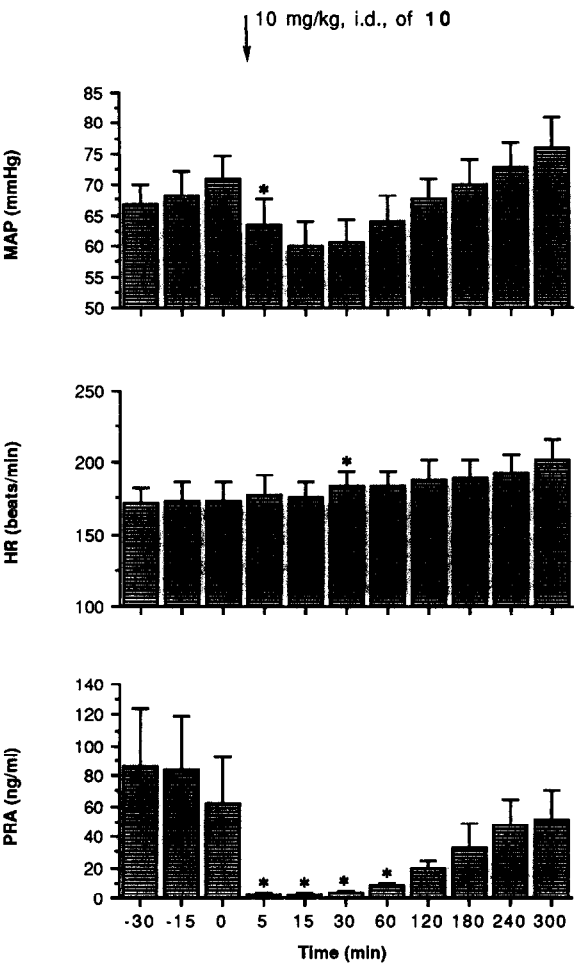


Fig.1. The effects of a single intraduodenal administration of **10** given at a dose of 10 mg/kg are shown in salt-depleted, anesthetized cynomolgus monkeys ($n = 3$). Values are expressed as mean ± SE and were considered significantly different from time = 0, based on the one-sample *t*-test, if * $P \leq 0.05$. MAP, mean arterial blood pressure; HR, heart rate; PRA, plasma renin activity.

3.3. *Stability to enzymatic degradation*

Compound **9**, although ideal in many respects, still shows an extreme susceptibility toward degradation by chymotrypsin (table 1). We have determined that the cause of this instability is hydrolysis of the Phe-AA_2 amide bond. To stabilize this bond, we employed the replacement of Phe with (4-OCH_3)Phe [5,10] (cf. **10** to **9**). Compound **10** seemed to have a desirable combination of potency, water solubility and acid/enzymatic stability, and was therefore examined in detail in vivo.

3.4. *In vivo activity*

The in vivo determination of hypotensive and renin inhibitory activity in monkeys has been previously described [5]. A bolus intravenous injection of 0.1 mg/kg of **10** to salt-depleted, anesthetized cynomolgus monkeys produced a 15% drop in mean arterial blood pressure, in association with complete inhibition of plasma renin activity within 5 min of injection. The duration of both responses persisted for 1–2 h, during which time both parameters gradually returned to control values [11]. Compound **10** also demonstrated intraduodenal activity at 10 mg/kg, as it induced a fall in both blood pressure and PRA (fig.1). Within 5 min post-dosing, mean arterial pressure fell approx. 15% below baseline, accompanied by 95% suppression of plasma renin activity. The average duration of action of **10** on both parameters was between 2 and 3 h. A slight yet significant reflex tachycardia was noted at the 30 min time point, following dosing. The duration of the hypotensive effect of **10** was variable; the maximum effect lasted for 5 h.

3.5. *Summary*

In summary, the monohydroxyethylene inhibitors have been improved by fulfilling our design objectives. Inhibitory potency was increased both by finding new, as well as by improving existing, binding groups through introduction of a second hydroxyl group of specific stereoconfiguration and through modification of the P_1 lipophilic side chain, respectively. Solubility was improved by the introduction of a basic group in the N-terminus. Stability toward chymotrypsin was imparted, without a drop in potency, by the replacement of Phe with (4-OCH_3)Phe. The combination of high inhibitory potency and optimized physicochemical properties for **10** leads to significant hypotensive effects after intravenous and intraduodenal administration to cynomolgus monkeys, and has prompted us to evaluate it in human clinical trials. Details of the methods of synthesis, stereochemical proof, structure activity relationships, and biological activity of this new class of inhibitors will be provided in future reports.

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[13] 'Statine-like' refers to compounds which have an absolute stereochemistry of the carbon bearing the hydroxyl group like that of statine (4*S*-amino-3*S*-hydroxy-6-methyl-heptanoic acid). This will avoid possible confusion brought on by the '*S/R*' stereochemical convention [14]. For example, the stereochemistry of C2 of inhibitor **4a** is statine-like, but of the *R* configuration.

[14] The '*S*' and '*R*' configurations are as defined by the IUPAC 1974 Recommendations for Section E, Fundamental Stereochemistry (1976) Pure Appl. Chem. 45, 13–30.