

Communications to the Editor

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NEW HUMAN RENIN INHIBITORY PEPTIDES: ANGIOTENSINOGEN TRANSITION-STATE ANALOGUES
CONTAINING NOVEL LEU-VAL REPLACEMENT AND A RETRO-INVERSO AMIDE BOND¹⁾

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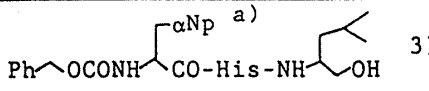
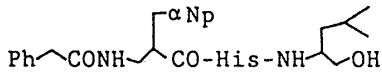
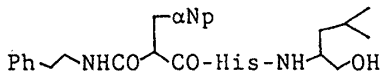
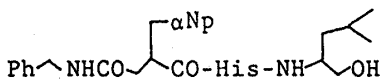
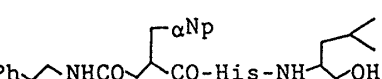
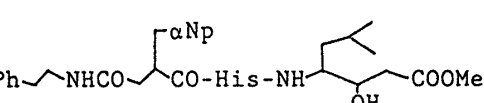
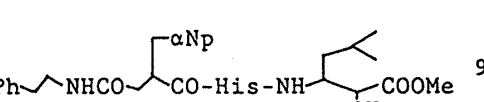
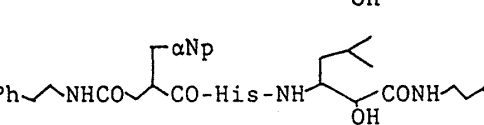
New human renin inhibitors were designed from transition-state analogues of angiotensinogen, synthesized and evaluated. The peptide derivative, which contained 1-naphthylmethylsuccinylamide residue (P3) with a retro-inverso amide bond and a norstatine isoamylamide residue (P1-P1'), was stable to proteases and had potent human renin inhibitory activity. This compact inhibitor exhibited hypotension when administered orally to a monkey.

KEYWORDS — renin inhibitor; antihypertensive drug; angiotensinogen; retro-inverso amide; transition-state analogue; norstatine; bioisostere

Renin is a highly specific aspartic protease which generates angiotensin I from angiotensinogen, and a large number of renin inhibitors have been investigated as targets of antihypertensive drugs.²⁻⁵⁾ Kokubu et al.³⁾ reported that peptides with aldehyde or alcohol at the C-terminal position of angiotensin I were potent inhibitors of human renin. However, these inhibitors would not be expected to be potential inhibitors of renin in vivo due to their chemical or metabolic instability. Considering the similarities between the structure of pepstatin and the transition-state tetrahedral intermediate in the hydrolysis by aspartic proteases, Boger et al.⁴⁾ introduced statine into the angiotensinogen sequence to enhance the inhibitory potency for human renin. The importance of the configuration of the hydroxyl group of statine in the inhibitors was demonstrated by the fact that the 3S isomer was 500-fold more potent than the 3R isomer. An enzyme-bound water molecule has been reported to be displaced by the hydroxyl group of statine upon binding of the inhibitor.^{4,5)}

In this paper, we describe the design, synthesis and inhibitory potencies of new human renin inhibitors derived from angiotensinogen transition-state analogues containing a hydroxyl function at the Leu¹⁰-Val¹¹ (P1-P1') scissile site. The strategies to obtain a stable and potent inhibitor were as follows. (i) The natural peptide bonds, which are metabolized easily by proteases, should be modified to avoid metabolic degradation. As shown in Fig. 1, compound I³⁾ was hydrolyzed by a serine endopeptidase chymotrypsin, which was specific to aromatic L-amino acids, e.g.,

Table I. Structures and Renin Inhibitory Activities

No.	Structure	IC ₅₀ (M)	
		against human renin	against human plasma renin
I		4.3x10 ⁻⁶	3.6x10 ⁻⁶
II		> 10 ⁻⁴	N.D. ^{b)}
III		> 10 ⁻⁴	N.D.
IV		1.8x10 ⁻⁵	N.D.
V		7.4x10 ⁻⁶	8.3x10 ⁻⁶
VI		3.1x10 ⁻⁷	1.7x10 ⁻⁶
VII		3.1x10 ⁻⁸	7.7x10 ⁻⁸
VIII		2.5x10 ⁻⁷	7.9x10 ⁻⁷

a) αNp = 1-naphthyl. b) N.D. = not determined.

tryptophan, tyrosine and phenylalanine. The hydrolysis apparently occurs at the 1-naphthylalanyl-His amide bond. (ii) The inhibitors should have as many hydrogen bonds to renin as possible. From the information regarding a tertiary structural model of renin,⁶⁾ we deduced that there were relevant hydrogen bonds between the P3 Phe carbonyl oxygen and the backbone NH of Ser-230 and between the imidazole group of P2 His and the hydroxyl group of Ser-233 characteristic of renin. So, we modified the P4-P3 site with a residue having a retro-inverso amide bond in order to maintain these hydrogen bonds and to resist proteases. (iii) It is preferred to substitute statine⁷⁾ at the P1 position with other bioisostere, since the mass production of statine is rather troublesome. Therefore, we focused on norstatine^{8,9)} [(2R,3S)-3-amino-2-hydroxy-5-methylhexanoic acid] which could be mass-produced and had a hydroxyl group in the same direction as that of statine. And we replaced the Leu-Val (P1-P1') scissile site with the norstatine isoamylamide residue. (iv) The molecular weight of the inhibitor should be as low as possible to increase intestinal absorption after oral administration, because high molecular weight compounds were poorly absorbed through the digestive organ in general.²⁾

On the basis of the above specifications, we synthesized the inhibitors listed in Table I. Chart 1 shows the synthetic pathway of VIII. The diacid prepared by condensation (step a) of 1-naphthaldehyde and diethyl succinate was dehydrated (step b) with acetic anhydride, treated (step c) with phenethylamine, and reduced (step d)

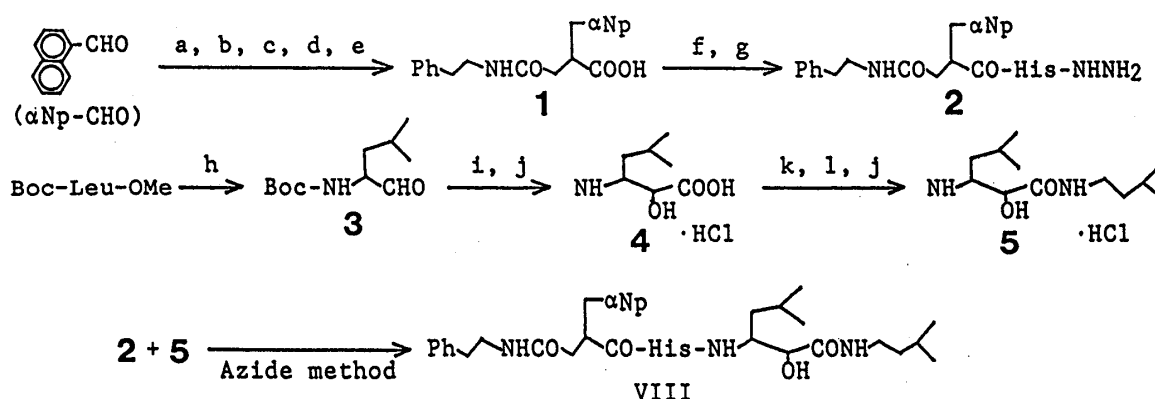


Chart 1. Reagents: a, diethyl succinate / NaOEt; b, Ac_2O ; c, $\text{PhCH}_2\text{CH}_2\text{NH}_2$; d, $\text{H}_2\text{-Pd/C}$; e, (+)- α -methylbenzylamine; f, His-OMe / DPPA; g, NH_2NH_2 ; h, DIBAL; i, NaHSO_3 / KCN; j, HCl; k, Boc-ON; l, $\text{NH}_2\text{CH}_2\text{CH}_2\text{CH}(\text{CH}_3)_2$ / DCC-HOBt.

to give a mixture of stereoisomers. The isomers were separated by optical resolution (step e) with (+)- α -methylbenzylamine to give the isomer (1) with the R configuration.¹⁰ Coupling (step f) the acid (1) with histidine methyl ester using diphenyl phosphorazidate (DPPA) followed by hydrazinolysis (step g) provided the hydrazide (2). The aldehyde (3) prepared by reduction (step h) of Boc-Leu-OMe with diisobutylaluminum hydride (DIBAL)⁸ was converted to a mixture (2R,3S/2S,3S = 7/3) of diastereoisomers of norstatine (4) with aqueous NaHSO_3 and KCN (step i) followed by hydrolysis (step j). A mixture (2R,3S/2S,3S = 7/3) of diastereoisomers of the norstatine isoamylamide (5) was prepared by protection (step k) of the amino group of the diastereoisomers (4) with 2-t-butoxycarbonyloximino-2-phenylacetonitrile (Boc-ON), condensation (step l) with isoamylamine, and deprotection (step j).¹¹ Coupling of the hydrazide (2) with the norstatine isoamylamide (5) by the azide method, using isoamyl nitrite, provided VIII. Most of the inhibitors listed in Table I were synthesized by essentially the same method.

The renin inhibitory potencies of the compounds were measured with both human

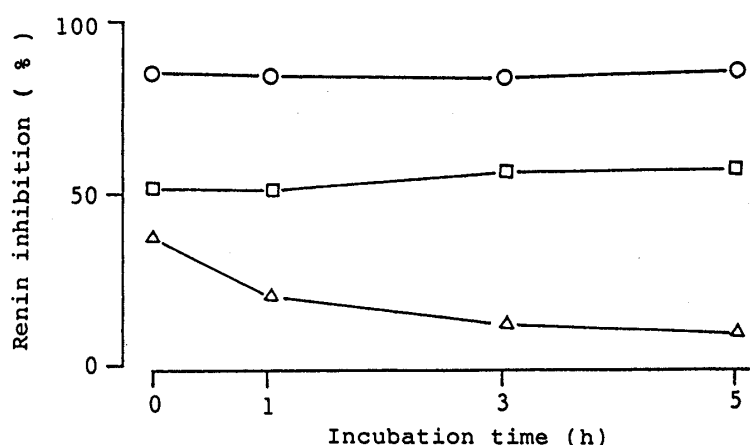


Fig. 1. Hydrolysis of Renin Inhibitors by Chymotrypsin
 The degradation of the inhibitors with 0.1 mg/mL of bovine chymotrypsin (Sigma) in 0.1 M borate buffer (pH 8.0) at 37°C was assayed by measuring the decrease of renin inhibition on human renin-sheep substrate:
 5×10^{-6} M VII (O); 1×10^{-5} M VIII (□); 1×10^{-4} M I (Δ).

renin-sheep substrate and human high renin plasma.¹²⁾ The IC_{50} values are shown in Table I. The β -amino acid derivative (II) and the malonylamide derivative (III) showed very weak inhibition. However, the 1-naphthylmethylsuccinylamide derivatives (IV and V) with a carbonyl group at the same position as that of P4 Pro showed inhibitory activities comparable to compound I. The phenethylamino group-containing compound (V) was more active than the corresponding benzylamino derivative (IV). We deduced that the phenethyl group fitted more favorably to renin than the benzyl group because the former was more flexible than the latter. The replacement of the leucinol residue with the statine residue (VI) enhanced the potency 10-fold and with norstatine (VII) enhanced it 100-fold. The isoamylamide derivative (VIII) was less active than the ester derivative (VII). This is attributed to the decreased fitness to renin because of the rigidity of VIII. These compounds were also very specific for human renin over other related aspartic proteases, e.g. cathepsin D and pepsin.^{12,13)}

As shown in Fig. 1, compound I was decomposed by chymotrypsin, while compounds VII and VIII were stable. The intravenous administration of 5 mg/kg of VII to a Japanese monkey led to a rapid drop in the mean blood pressure.¹²⁾ The oral administration of 30 mg/kg of VIII, containing the norstatine isoamylamide residue, resulted in a lowering of approximately 20 mmHg of mean blood pressure for about 7 hr in the common marmoset,¹³⁾ but VII showed no significant oral activity. One cause may be attributed to the hydrolysis of methyl ester in compound VII in vivo.

In conclusion, the P3 modification with the 1-naphthylmethylsuccinylamide residue with the retro-inverso amide bond stabilized the P3-P2 bond to chymotrypsin. Replacement of the Leu-Val (P1-P1') scissile site with norstatine isoamylamide residue enhanced the renin inhibitory potency and stability to proteases. Thus, the hydroxymethylcarboxamide bioisostere was effective in both potency and stability. Compound VIII containing these residues is a new class of orally potent renin inhibitor which has possibilities as a clinically useful antihypertensive drug.

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