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SHORT CHAIN PEPTIDE INHIBITORS OF HUMAN RENIN[1)]

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The phenylalanine residue in the C-terminal sequence of angiotensin II was found to be important for the design of renin inhibitors. 1-Naphthylalanine(Nal(1))-containing tripeptide analogues such as benzyloxycarbonyl-Nal(1)-His-Leucinal (ES-188) and benzyloxycarbonyl-Nal(1)-His-(3S,4S)-4-amino-3-hydroxy-6-methylheptanoic acid (Statine)[5)] 2(S)-methylbuthylamide (ES-254) showed high potency and specificity to human renin.

Renin cleaves the substrate angiotensinogen to yield the hemodynamically inactive decapeptide, angiotensin I. Angiotensin I is transformed by the angiotensin converting enzyme into the pressor substance, angiotensin II. Important progress in the control of hypertension has been achieved by the development of the orally active inhibitors of the converting enzyme.[2)] This has evoked an interest in the blockade of the preceding step in the cascade, the renin reaction and the importance of the development of renin inhibitors. Potent inhibitors of human and canine renins have been reported by Szelke et al.[3)] and by Boger et al.,[4)] whose modifications around the cleavage site in the minimum substrate, octapeptide of the angiotensinogen sequence (6-13), have given competitive inhibitors. However, these inhibitors were not orally active.

We wish to report here short chain inhibitors of renin which are highly potent (Ki = 10^{-7}—— 10^{-8} M) and specific to human renin (Fig. 1).

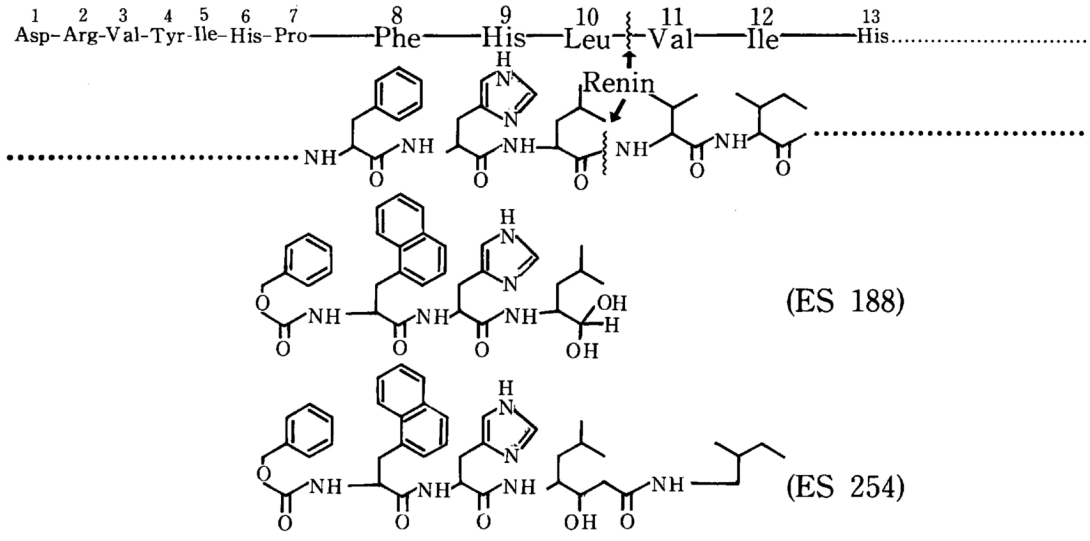


Fig. 1. Structures of human renin angiotensinogen and synthetic inhibitors.

Umezawa *et al.*[5] discovered several naturally occurring proteinase inhibitors such as leupeptin, chymostatin, and pepstatin, whose peptidic structures were characteristic in the C-terminal aldehyde groups or in the unusual amino acid statine[5] residue. We postulated that peptides in the angiotensin I sequence with leucinal at the C-terminus or with a statine residue would inhibit renin, if the peptide analogues could potentially interact with subsites in the catalytic center of renin.

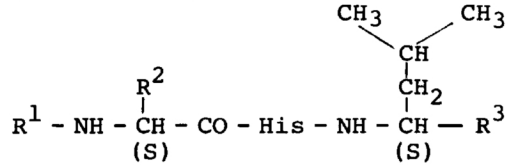
In our preliminary report,[6] we showed that Z-Phe-His-Leucinal (IC_{50} 7.5×10^{-7}M) and Z-His-Leucinal (IC_{50} 5×10^{-4}M) were inhibitors of human renin, whereas longer chain analogues such as Z-Tyr-Ile-His-Pro-Phe-His-Leucinal and Z-Ile-His-Pro-Phe-His-Leucinal, which inhibited hog renin,[7] showed no potency to human renin. These short chain analogues do not have the minimum sequence (10-13) of angiotensinogen which we reported previously to be capable as an inhibitor.[8] It was assumed that some parts of these inhibitors were acting as the key center for interacting with the catalytic center of renin and that the hydroxyl groups of the hemiacetals derived from the aldehyde residues were analogues of the tetrahedral intermediate of the enzyme reaction.[9]

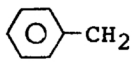
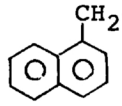
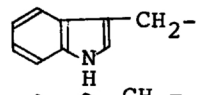
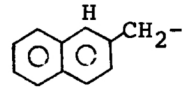
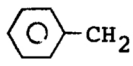
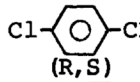
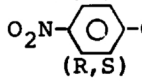
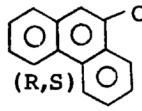
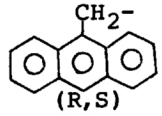
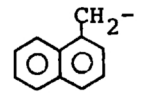
Thus, various kind of tripeptide analogues which have either aldehyde group or hydroxyl group were prepared. Some of these analogues and their inhibitory potencies against human renin are listed in Table 1.

All the peptide aldehydes were conveniently prepared by the coupling of N-protected dipeptide azides and the aminoaldehyde semicarbazones. In a typical experiment, 0.82 ml of a solution of 4.1 M hydrogen chloride in dioxane was added to 10 ml of DMF solution of 500 mg (1 mmol) of benzyloxycarbonyl-L-[3-(1-naphthyl)]-alanyl-L-histidine hydrazide (mp 157-159 °C, $[\alpha]_D^{22}$-58.7° (*c*1, DMF)) which was prepared by the coupling of benzyloxycarbonyl-L-[3-(1-naphthyl)]alanine (Nal(1))[10] with L-histidine methyl ester utilizing dicyclohexylcarbodiimide + 1-hydroxybenzotriazole and subsequent hydrazinolysis in MeOH. The mixture was cooled to -60 °C, 0.2 ml of isoamyl nitrite was added and the result was warmed to -20 °C. After cooling again to -60 °C and neutralization with 340 mg of N-methylmorpholine, 5 ml of DMF solution containing 172 mg (1.1 mmol) of L-leucinal semicarbazone[11] was added and the mixture stirred overnight at 4 °C. The solvent was removed *in vacuo* and 5% aqueous solution of sodium bicarbonate was added to the residue to form a precipitate.The precipitate,was collected by filtration and washed thoroughly with water and ethyl acetate to give Z-Nal(1)-His-Leucinal semicarbazone, 564 mg (88% yield), mp 192-195 °C, $[\alpha]_D^{22}$-21.4° (*c*0.5, MeOH). Deprotection of the semicarbazide group and purification of the products (350 mg) by a partition chromatography on Sephadex G-25 using a solvent system of n-butanol : acetic acid : carbon tetrachloride and water (4 : 1 : 1 : 1.5)[7] gave pure Z-Nal(1)-His-leucinal, 270 mg (85% yield based on semicarbazone), mp 106-108 °C, $[\alpha]_D^{22}$-49.0° (*c*0.5, MeOH).

Considering the susceptivility of peptide aldehydes[7] to racemization, we further investigated tripeptide analogues having alcoholic hydroxyl group for enhancing stability and potency. We postulated that amino acids with a hydroxyl group could replace leucinal if the Schiff's base formation was not involved in the inhibition by aldehyde derivatives. Analogues with alcoholic hydroxyl groups were prepared by the azide method similar to that described above or by the coupling of N-protected amino acids with the amino terminus of suitably protected dipeptides utilizing active esters such as succinimide or 1-benzotriazole esters.

Table 1. Synthetic Peptide Analogues and Their Inhibitory Potencies to Human Renin



Compound No.	R^1	R^2	R^3	Mp θ_m/°C ($[\alpha]_D^{22}$/° (c 0.5, MeOH))	Inhibition % at 1×10^{-6}M[e] [IC_{50}/M]	Ki Value[e]/M
1	Z		-CHO	101-104 (-40.1 (EtOH))	65 [7.5×10^{-7}]	
2 (ES-188)	"		"	106-108 (-49.0)	88 [8.0×10^{-8}]	6.5×10^{-8} (noncompetitive)
3	"		"	109-110 (-20.2)	61	
4	"		"	110-112 (-3.2)	33	
5	"		$-CH_2OH$	181-183 (-19.4)	22	
6	"		"	153-163 (-13.8)	12	
7	"		"	151-154 (-16.6)	2	
8	Ac	 a)	"	182-3 (-14.8)	0	
9	"	 b)	"	208-211 (-28.8)	2	
10	Z		"	163-165 (-56.2)	61 [3.6×10^{-6}]	5.6×10^{-6} (competitive)
11	"	"	(S) $-CH(OH)-CH_2OH$ c)	176-178 (-57.4)	52	
12	"	"	(S) $-CH(OH)-CH_2-CH_2OH$	196-198 (-57.8)	63 [6.4×10^{-6}]	
13 (ES-226)	"	"	(S) $-CH(OH)-CH_2-COOC_2H_5$ d)	138-140 (-70.6)	85 [2.8×10^{-7}]	2.3×10^{-7} (competitive)
14	"	"	(S) $-CH(OH)-CH_2-COOH$ d)	191-192 (-59.2)	42	
15 (ES-254)	"	"	(S) (S) $-CH(OH)-CH_2-CO-NH-CH_2-CH(CH_3)-C_2H_5$	164-166 (-6.8)	94 [4.5×10^{-7}]	1.9×10^{-7} (competitive)

a) The acid: mp 220-223 °C. b) The acid: mp 220-240 °C (dec). c) Boc-amino-2-hydroxy-5-methylhexanol (mp 58-59 °C, $[\alpha]_D^{22}$ -10.2° (c1, MeOH)) was prepared by sodium borohydride reduction of the corresponding acid methyl ester (mp 84-85 °C, $[\alpha]_D^{22}$ -10.2° (c1, MeOH)). d) D.H. Rich, E.T. Sun and A. Bopari, J. Org. Chem., 43, 3624 (1978). e) Inhibitory potencies and Ki values were determined using sheep angiotensinogen as reported previously.6) Human angiotensinogen was used in the determination of Ki value of the compound 15 (ES-254).

Z-Phe-His-Leucinol (Compound 5) was found to be potent. This stimulated us to investigate structural modifications of this compound for enhancing potencies. To improve active site binding affinity, we tried to replace the benzene ring of phenylalanine with 4-nitrobenzene, 1-naphthalene, 2-naphthalene, 9-phenanthrene or 9-anthracene. 1-Naphthylalanine containing analogues such as Z-Nal(1)-His-Leucinol (Compound 10) showed a high potency but Compounds 8 and 9 showed none. This suggests that the rings of 9-phenanthrene and 9-anthracene are too bulky to fit in the pocket of the active site cleft[12] of human renin and 1-naphthylalanine residue fits more closely than phenylalanine residue. Replacement of leucinol with statine (Compound 14) or statinol(Compound 12)did not increase potency but a major increase in inhibition was established by replacement with a suitably protected statine residue. Ethyl ester (Compound 13, ES-226) and 2(S)-methylbutylamide (Compound 15, ES-254) of Z-Nal-His-Statine were found to be as highly potent as the aldehyde derivative (Compound 2, ES-188). Ki values of these inhibitors are also listed in Table 1.

ES-188, 226, and 254 markedly inhibited human and primate renins but were very weak inhibitors of renins from pigs, goats, dogs, and rats.[13] ES-188 and 226 had little discermible effect on cathepsin D, pepsin or human angiotensin converting enzyme.[13] It was found in the *in vivo* experiments in monkeys that they abolished the renin-induced blood pressure elevation. Details will be reported elsewhere.

In conclusion, the phenylalanine residue in the C-terminal sequence of angiotensin II is important for the design of renin inhibitors. 1-Naphthylalanine-containing short chain inhibitors are highly potent and specific to human renin. This family of inhibitors may provide a possibility for the development of orally active renin inhibitors in the treatment of hypertension. Further investigations are in progress.

References

1) Amino acids with no prefix are of the L-configuration. The abbreviations used are those recommended by the IUPAC-IUB: J. Biol. Chem., 247, 977 (1972). The following abbreviations are also used: Z=benzyloxycarbonyl, Boc=t-butyloxycarbonyl, DMF=dimethylformamide.
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