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Communications to the Editor

New Human Renin Inhibitors Containing an Unnatural Amino Acid, Norstatine

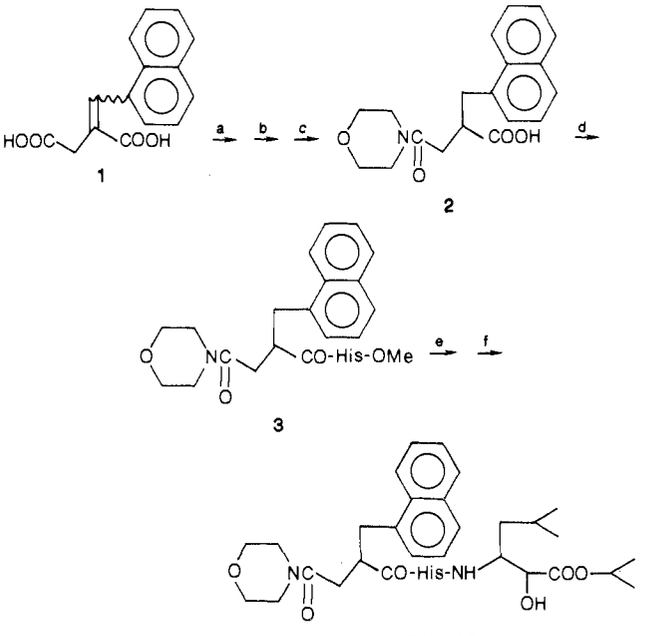
Sir:

A large number of renin inhibitors have been investigated as targets of antihypertensive drugs.[1-9] However, most of them were peptide compounds and, hence, thought to be unsuitable for oral administration because of their proteolytic lability and poor absorption in the digestive canal. We report here an orally active renin inhibitor KRI-1230 (compound **9** in Table I) containing norstatine [norSta: (2*R*,3*S*)-3-amino-2-hydroxy-5-methylhexanoic acid], an unnatural amino acid.

We initiated a study on renin inhibitors in order to analyze the mode of interaction between human renin and its substrate. Because the X-ray structure of human renin has not been determined, we deduced[10] a tertiary structure of the enzyme based upon the assumption[11] that the overall folding of human renin is very similar to that of penicillopepsin.[12] The modeling of human renin was carried out according to the method described in the ref 10a, except for the use of a computer system BIOCES.[13] The optimal conformation of the inhibitor was determined in such a way that both the maximum hydrogen bond and the minimum close contact between the inhibitor and the enzyme were attained by manual change of the dihedral angles and translation of the inhibitor.

The strategies used for designing a new renin inhibitor were as follows. The first approach was based upon the hydrophobicity of the inhibitor. On the basis of the deduced renin model,[10] it was proposed that the presence of a large hydrophobic residue in the P1 and P3 positions of the inhibitor was favorable for the inhibition. Presumably this is because binding sites S1 and S3 located under the "flap" are wide and hydrophobic. The second approach was to maintain as many hydrogen-bonding capabilities as possible. The pepstatin analogue, Iva-Val-Val-Sta-OEt [Iva = isovaleryl; Sta-OEt = statine ethyl ester (ethyl

Scheme I[a]



[a] Reagents: a, Ac_2O; b, morpholine, CH_2Cl_2; c, H_2, 10% Pd/C, MeOH; d, His-OMe·2HCl, DPPA, Et_3N, DMF; e, aqueous NaOH; f, 4·HCl, DPPA, Et_3N, DMF.

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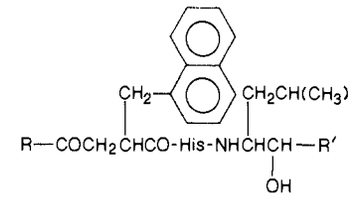
Table I. Structures and Renin Inhibitory Activities of the Norstatine and the Statine Derivatives



no.	R	R'	IC_{50},[a] M, against human renin	IC_{50},[b] M, against plasma renin from human	dog	rat
5[c] (KRI-1177)	$Ph(CH_2)_2NH$-	-$COOCH_3$	7.8×10^{-8}	9.0×10^{-8}	2.1×10^{-6}	$>10^{-4}$
6[d]	$(CH_3)_2N$-	-$COOCH_3$	7.8×10^{-8}	2.5×10^{-8}	6.4×10^{-7}	$>10^{-4}$
7[e]		-$COOCH_3$	4.1×10^{-8}	2.4×10^{-8}	4.2×10^{-7}	$>10^{-4}$
8[f]		-$COOCH_3$	5.2×10^{-8}	1.1×10^{-8}	1.0×10^{-6}	$>10^{-4}$
9[g] (KRI-1230)		-$COOCH(CH_3)_2$	2.5×10^{-8}	7.8×10^{-9}	1.5×10^{-7}	1.7×10^{-5}
10[h]		-$CONHCH(CH_3)_2$	1.3×10^{-7}	1.3×10^{-7}	1.1×10^{-6}	4.4×10^{-6}
11[i]		-$CH_2COOCH(CH_3)_2$	1.8×10^{-7}	6.6×10^{-8}	3.5×10^{-7}	$>10^{-4}$

[a] A 25-μL aqueous solution of human renin (20–30 ng of AngI/mL per hour) was incubated at 37 °C with a mixture of sheep angiotensinogen (2000 ng of AngI/mL, 50 μL), Phe-Ala-Pro (25 μL of 20 mM aqueous solution), a DMSO solution of the inhibitor (50 μL), water (150 μL), and 125 mM of pyrophosphate buffer (pH 7.4, 200 μL). Angiotensin I that formed after 15 min of incubation was measured by radioimmunoassay. [b] A human plasma (500 μL) containing EDTA-2Na (14 mM) and neomycin sulfate (0.3%) was added to a mixture of 0.5 M phosphate buffer (pH 7.0, 350 μL), Phe-Ala-Pro (50 μL of 20 mM aqueous solution) and a DMSO solution of the inhibitor (100 μL). After incubation (37 °C, 60 min), angiotensin I that was produced was measured by radioimmunoasay. Anal.: [c] ($C_{37}H_{45}N_5O_6$) C, H, N. [d] ($C_{31}H_{41}N_5O_6 \cdot {}^1/_6CHCl_3$) C, H, N. [e] ($C_{34}H_{45}N_5O_6$) C, H, N. [f] ($C_{33}H_{43}N_5O_7 \cdot {}^1/_5CHCl_3$) C, H, N. [g] ($C_{35}H_{47}N_5O_7$) C, H, N. [h] ($C_{35}H_{48}N_6O_6$) C, H, N. [i] ($C_{36}H_{49}N_5O_7$) C, H, N.

(3*S*,4*S*)-4-amino-3-hydroxy-6-methylheptanoate)],[14] was found to form hydrogen bonds with penicillopepsin in the enzyme–inhibitor complex crystals. A similar hydrogen-bonding network can be expected for renin–inhibitor interaction.[10] These hydrogen bonds contribute not only to the control of inhibitor orientation in the enzyme but also to stabilization of the complex. Thirdly, it is necessary to minimize the number of natural peptide bonds for a long-lived inhibitor, since a naturally occurring peptide bond is subject to enzyme-catalyzed hydrolytic cleavage. Lastly, we focused on not widely known norstatine in the place of the popular statine residue. One of the advantages of norstatine over statine is its ease of preparation. The hydroxyl group of the statine residue is very important for the inhibition and thought to contribute to binding energy through substituting with a water molecule bound to the active site.[15] The same effect would be expected in the inhibition of renin by the norstatine derivatives. On the basis of the requisites mentioned above for a good inhibitor, the inhibitors listed in Table I were designed and synthesized.

Scheme I outlines a synthetic pathway of **9**. The acid anhydride of 1 was treated with morpholine (step b), and the product was reduced (step c) under hydrogen to give **2**. Condensation (step d) of **2** and histidine methyl ester using diphenyl phosphorazidate (DPPA) yielded **3** as a mixture of diastereomers. The diastereomers were separated, hydrolyzed (step e), and condensed (step f) with norstatine isopropyl ester (4) to obtain **9**. Most of the inhibitors listed in Table I were synthesized according to Scheme I. Details on these syntheses will be reported in the near future.

The inhibitors in Table I, except for 11, contain norstatine instead of statine in the P1 position. It was proposed[16] for the statine derivative that the P1′ side chain interacted not with an S1′ site but an S2′ site of the enzyme because of an additional carbon atom in its main chain. The amino acid residue of the P1′ position is Val in human angiotensinogen.[17] The conversion of the methoxy group into an isopropoxy group, which resembles the Val side chain, enhanced the inhibitory potency (**8** vs **9**, Table I), while the conversion into an isopropylamino group (compound **10**) decreased the potency. The statine-containing peptide 11 was less active than the corresponding norstatine derivative **9**. The valine-mimicked isopropoxy group of compound 11 would not be able to interact any longer with the S1′ site because of the additional carbon atom in the main chain. The conversion of the primary phenethylamino group of the P4 position of **5** into a secondary dimethylamino group did not improve the inhibitory activity, while cyclization on this position slightly enhanced the activity (compounds **6** and **7**). The replacement of the P4 residue of **5** with relatively polar morpholine (**8**) slightly improved the activity against isolated human renin; however, nearly a 1 order increment was found in the inhibition of human plasma renin (**5** vs **8**, Table I). This effect seems to be related in part to the binding to unspecified plasma components[18] because of

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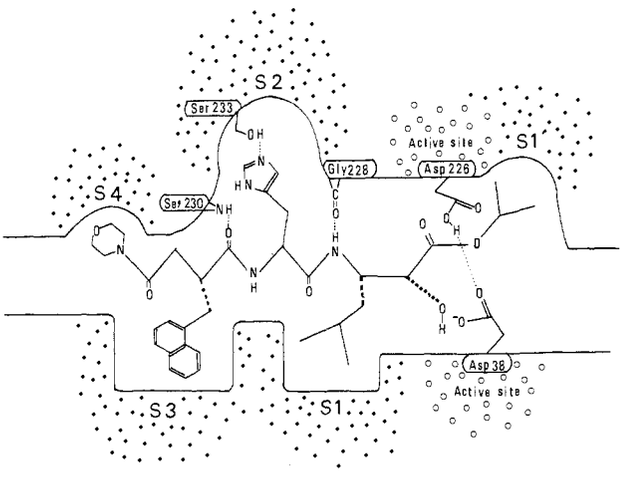

Figure 1. Schematic representation of a binding mode of **9** to the proposed binding sites (S1'–S4).

high lipophilicity of **5** compared with **8**. Of the compounds listed in Table I, **9** (KRI-1230) was the most potent inhibitor not only against isolated human renin but also against human plasma renin. These compounds selectively inhibited human renin (Table I).

There are four asymmetric carbons in these inhibitors. Both the α-carbon of P2 His and the C3 carbon having an isobutyl group of P1 norstatine are of the *S* configuration. Table II confirms that **13** with a 2(*R*)-hydroxy group is more active than the 2*S* isomer **14**, while the 3(*S*)-hydroxy group is favored over the 3*R* one in the case of the statine derivatives.[6] This discrepancy between norstatine and statine arises only from the difference in priority for designating the absolute configuration because of the presence of additional C2 carbon atom in statine. The hydroxy groups of the more active stereoisomers of norstatine and statine (2*R* of norstatine and 3*S* of statine) appear to fit into the active site in the same manner. Previously, (*Z*)-[3-(1-naphthyl)Ala]-His-AHMH-OMe [AHMH-OMe = methyl (2*S*,3*S*)-3-amino-2-hydroxy-5-methylhexanoate, a stereoisomer of norstatine] was reported as a renin inhibitor.[19] However, the inhibition was weak (53% inhibition at 10 μM), because the C2 carbon atom of the norstatine residue was of the *S* configuration. Johnson also reported[20] inhibitors containing the stereoisomers of norstatine with inhibitory constants in the range of 10^{-3}–10^{-4} M. However, the advantages of the 2*R* isomer in the activity was not clarified.

The absolute configuration of the carbon atom having 1-naphthylmethyl moiety has not yet been determined. Compound **5** derived from (+)-2-(-1-naphthylmethyl)-3-(phenylethylcarbamoyl)propionic acid was more potent than **12**, which was synthesized from the corresponding acid with opposite optical character. In the case of **9**, the (−)-isomer produced more active compound than the (+)-isomer of 2-(1-naphthylmethyl)-3-(morpholinocarbonyl)propionic acid (**2**) as a starting material (see **9** and **15**, Table II). In view of the fitness to the enzyme, we speculate that the favorable configuration around the carbon atom having the naphthylmethyl group is *R* type (the same configuration as a naturally occurring amino acid).

The proposed mode of interaction between human renin and **9** is illustrated schematically in Figure 1. The P1 and the P3 side chains are accommodated in the large and hydrophobic subsites S1 and S3, respectively. The morpholine moiety of the P4 position interacts with relatively polar subsite S4.

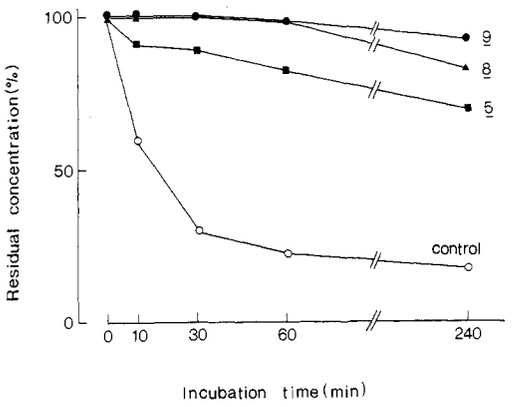

Figure 2. Stabilities **5**, **8**, and **9** in monkey liver homogenates. One gram of liver from monkey was homogenized with aqueous KCl solution (1.15%, 4 mL). An aqueous solution of the inhibitor (0.1 mL) was added to the mixture of the liver homogenates (0.5 mL) and 0.1 M phosphate buffer (pH 7.4, 0.4 mL), and the mixture was incubated at 37 °C. Final concentration of the inhibitor was 10^{-4} M. Residual concentration of each compound was measured by HPLC (control; Suc-Arg-Pro-Phe-His-Leu-Leu-Val-Tyr-MCA).

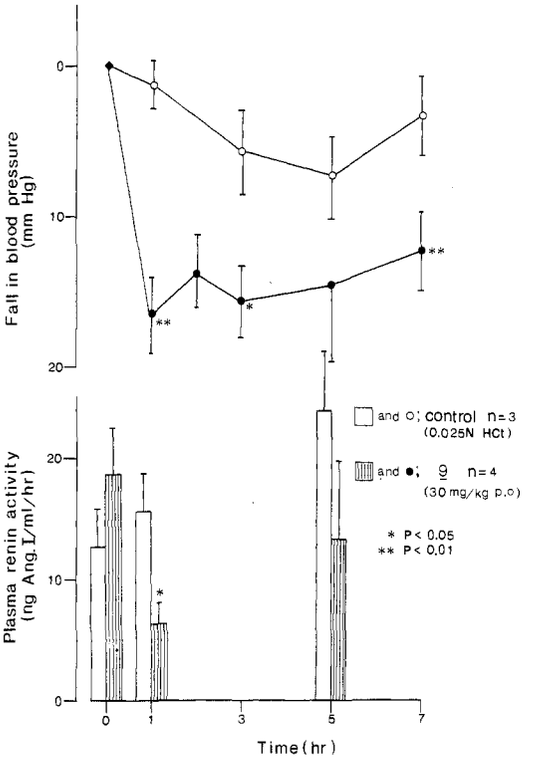

Figure 3. Effect of oral administration of **9** on blood pressure and plasma renin activity of marmosets. Male marmosets were fed a low-sodium diet. Frosemide (15 mg/kg) was orally applied every other day. After 1 week, 30 mg/kg of **9** as 0.025 N HCl solution was administered orally (2 mL/kg). Blood pressure was measured by the tail-cuff method. This method gave a value near the mean blood pressure measured by the direct method. The plasma renin activity was measured by radioimmunoassay.

A long-lasting antihypertensive drug should be resistant to proteolytic enzymes. As shown in Figure 2, compound **5** was decomposed about 30% by incubation in monkey liver homogenates for 4 h, while **9** was decomposed about 10%. This stability of **9** is thought to arise not only from a steric effect of isopropyl ester but also from total structural effects, because **8** having the methyl ester res-

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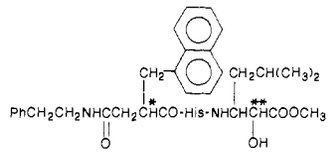
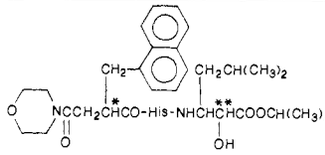
Table II. Renin Inhibitory Potencies of the Stereoisomers[a]



no.	*	**	IC_{50}, M, against human renin	IC_{50}, M, against human plasma renin
5 (KRI-1177)	+	*R,S* (7:3)	7.8×10^{-8}	9.0×10^{-8}
12[b]	-	*R,S* (7:3)	$>10^{-4}$	
13[c]	+	*R*	3.1×10^{-8}	7.7×10^{-8}
14[d]	+	*S*	1.3×10^{-6}	



no.	*	**	IC_{50}, M, against human renin	IC_{50}, M, against human plasma renin
9 (KRI-1230)	-	*R,S* (7:3)	2.5×10^{-8}	7.8×10^{-9}
15[e]	+	*R,S* (7:3)	$>10^{-4}$	

[a] The IC_{50} values of the inhibitors against isolated human renin and human plasma renin were measured by the method described in Table I. Anal.: [b] ($C_{37}H_{45}N_5O_6$) C, H, N. [c] ($C_{37}H_{45}N_5O_6$) C, H, N. [d] ($C_{37}H_{45}N_5O_6$) C, H, N. [e] ($C_{35}H_{47}N_5O_7 \cdot {}^1/_5CHCl_3$) C, H, N.

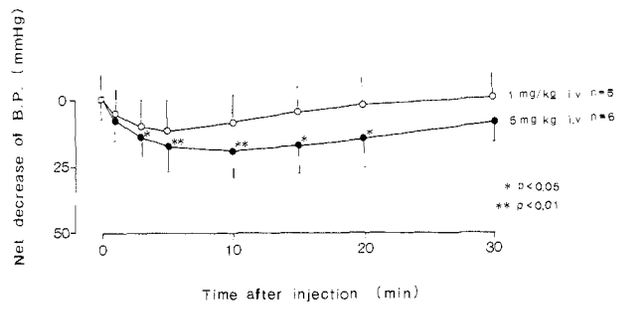


Figure 4. Effect of intravenous injection of **9** on blood pressure. Frosemide was applied by the method described in Figure 3 to sodium-depleted male marmosets. A catheter was inserted under anesthesia into the femoral artery. The catheter was connected to the pressure transducer for measurement of blood pressure. After come out from under the anesthesia, compound **9** was injected into the femoral vein as 1 mL/kg aqueous solution.

idue was moderately stable in the same condition. Compound **9** was stable also in the human plasma.

Oral administration of 30 mg/kg of **9** to common marmosets resulted in a lowering of mean blood pressure accompanying a reduction of the plasma renin activity (Figure 3). Figure 4 shows changes in blood pressure after intravenous injection of **9** in doses of 1 or 5 mg/kg. The lowering effect of a 5 mg/kg injection was comparable to that of oral administration of a 30 mg/kg dose. In the case of intravenous injection, the hypotensive response was dose dependent, and the maximum response occurred within 10 min after injection. On the other hand, long-lasting hypotensive effect was found when **9** was orally administered. The maximum response occurred 1 h after the administration and both blood pressure and plasma renin activity recovered gradually. However, recovery of the blood pressure was very slow, and even after 7 h the blood pressure was significantly depressed (Figure 3).

In conclusion, the present study shows that norstatine is a useful component of the renin inhibitors compared with statine and KRI-1230 is one of the most compact and highly potent renin inhibitors.

[†] Kissei Pharmaceutical Co., Ltd.
[‡] Kitasato University.
[§] Kyoto Pharmaceutical University.

Kinji Iizuka,*† Tetsuhide Kamijo,† Tetsuhiro Kubota† Kenji Akahane,† Hideaki Umeyama,‡ Yoshiaki Kiso§

Division of Medicinal Chemistry
Central Research Laboratories
Kissei Pharmaceutical Co., Ltd.
Nagano 399 Japan
School of Pharmaceutical Sciences
Kitasato University
Shirokane, Minato-Ku, Tokyo 108, Japan
Kyoto Pharmaceutical University
Yamashina-Ku, Kyoto 607, Japan

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β-Substituted Phenethylamines as High-Affinity Mechanism-Based Inhibitors of Dopamine β-Hydroxylase

Sir:

Dopamine β-hydroxylase (DBH; E.C. 1.14.17.1) presents an appealing target for the design of inhibitors as potential new cardiovascular agents. We have recently reported potent, reversible inhibitors of DBH that are effective antihypertensive agents[1-4] and, in an alternative approach, have described several structurally simple mechanism-based inhibitors of DBH.[5,6] Whereas a multitude of other mechanism-based inhibitors of DBH have been reported previously,[7-14] the high, millimolar K_m for dopamine substrate makes critically important the design of k_{cat} inhibitors with enhanced binding to DBH. To date, only one class of mechanism-based inhibitors, some heterocyclic allylamines, appear to fulfill this criterion.[14] In this paper we describe a simple ethynyl-substituted tyramine that is an effective mechanism-based inhibitor of DBH; it binds enzyme in the micromolar range, nearly 100-fold more

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