Orally Potent Human Renin Inhibitors Derived from Angiotensinogen Transition State: Design, Synthesis, and Mode of Interaction

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A three-dimensional structure of the complex of human renin and the scissile site P_4 Pro to P_1' Val of angiotensinogen was deduced in order to design potent human renin inhibitors rationally. On the basis of this structure, an orally potent human renin inhibitor (**1a**) was designed from the angiotensinogen transition state and synthesized. The inhibitor **1a** contains a (2*R*)-3-(morpholinocarbonyl)-2-(1-naphthylmethyl)propionyl residue (P_4-P_3) with a retro–inverso amide bond, L-histidine, and a novel amino acid, (2*R*,3*S*)-3-amino-4-cyclohexyl-2-hydroxybutyric acid, named cyclohexylnorstatine (**2a**). The optically pure cyclohexylnorstatine was efficiently prepared from Boc-L-cyclohexylalaninol (**3**), and the stereochemistry of **1a** was established by X-ray crystal analysis. The analyses of interaction between **1a** and human renin using modeling techniques indicated that (1) the cyclohexyl group of P_1 and the naphthyl group of P_3 were accommodated in large hydrophobic subsites S_1 and S_3, respectively; (2) the imidazole of P_2 His was hydrogen bonded to the side chain OH of Ser-233 to contribute to the selectivity of renin inhibition; (3) cyclohexylnorstatine isopropyl ester residue was accommodated in S_1–S_1'. The importance of the stereochemistry in the potent and specific inhibitor was clearly shown. Oral administration to monkeys of this inhibitor resulted in a drop of 10–20 mmHg in mean blood pressure and a reduction of plasma renin activity for a 5-h period.

Renin–angiotensin system (RAS) plays a central role in the regulation of blood pressure and electrolyte balance. Renin (EC 3.4.23.15) is a highly specific aspartic protease that generates angiotensin I (Ang I) from angiotensinogen. Ang I is then cleaved by angiotensin converting enzyme, a nonspecific dipeptidyl carboxypeptidase, to yield the biologically active octapeptide angiotensin II (Ang II). Ang II acts as a potent pressor agent directly by virtue of its vasoconstrictor activity. Renin is responsible for the first and rate-limiting step in RAS. Thus, a large number of renin inhibitors have been investigated as targets of antihypertensive drugs.[1] Wolfenden[2] reported the design of enzyme inhibitors which resembled the transition state in the enzyme-catalyzed reaction. Using this transition-state inhibitor approach, Szelke et al.[3] reported potent renin inhibitors in which the scissile bond (Leu-Val, P_1-P_1')[4] of substrate analogues was replaced by a reduced peptide isostere ($-CH_2NH-$) or a hydroxy isostere ($-CH(OH)CH_2-$). Boger et al.[5] incorporated a statine residue [(3*S*,4*S*)-4-amino-3-hydroxy-6-methylheptanoic acid] as a dipeptide mimic (P_1-P_1') into substrate analogues to obtain the potent inhibitors.

However, these peptide inhibitors are unsuitable for oral administration due to proteolytic degradation and poor intestinal absorption. Consequently, considerable modifications would be needed to lead to orally active renin inhibitors suitable for clinical use. Orally active inhibitors should be of low molecular weight to achieve good intestinal absorption. In addition, they should have high potency, specificity, and metabolic stability.

Recently,[6,7] we have reported a novel class of low molecular weight human renin inhibitors that contain 1-(naphthylmethyl)succinylamide (P_4-P_3), L-histidine (P_2), and norstatine[8] [(2*R*,3*S*)-3-amino-2-hydroxy-5-methylhexanoic acid (**9**)] (P_1). There are four asymmetric carbons in these renin inhibitors, and the relation between fitness to the enzyme and the inhibitory potency suggested the importance of the stereochemistry. However, it was not easy to prepare optically pure norstatine and most inhibitors contained a mixture of diastereoisomers.[7]

In this article, we describe the details of our studies on the design and synthesis of an orally potent human renin inhibitor (**1a**) containing a novel amino acid, (2*R*,3*S*)-3-amino-4-cyclohexyl-2-hydroxybutyric acid (**2a**), named cyclohexylnorstatine.[9,10] Optically pure cyclohexylnorstatine and **1a** were efficiently synthesized, and the stereochemistry of **1a** was established by X-ray crystal analysis. The importance of the stereochemistry of **1a** and its mode of interaction with a model of human renin is also presented.

Results and Discussion

Analysis of Renin–Substrate Interaction. Analyses

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† Kissei Pharmaceutical Co.
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‖ Osaka University of Pharmaceutical Science.

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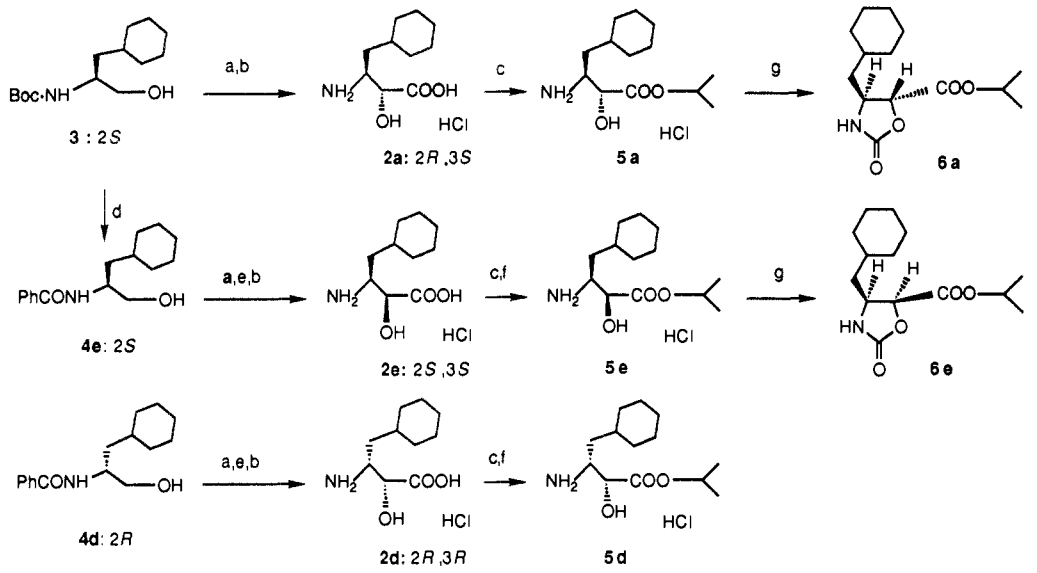
Scheme I[a]



[a] (a) Py·SO_3/DMSO, 22–25 °C, 20 min, then NaCN–HCl, 0 °C, 3 h; (b) 23% HCl, 80 °C, 11 h; (c) 2-propanolic HCl, 80 °C, 1 h; (d) 2 N HCl/MeOH, 60 °C, 2 h, then PhCOCl–Et_3N/dioxane–water, 0 °C, 1 h; (e) $SOCl_2$/benzene, 0 °C, 5 h; (f) silica gel chromatography; (g) *N,N'*-carbonyldiimidazole/CH_2Cl_2, room temperature, 4 h.

of interactions between renin and substrate provide useful information for designing specific inhibitors. In the previous study,[11] we established a three-dimensional structure of human renin[12] by using comparative modeling based on the similarity to penicillopepsin.[13] In this study, we constructed a three-dimensional structure of human renin based on porcine pepsinogen, which was recently registered in the Protein Data Bank.[14] The sequence homology of human renin to porcine pepsin[15] (41%) is higher than that to penicillopepsin (25%),[11] and the number and position of cysteine residues in porcine pepsin are equal to those in renin. It implies that the folding of renin is more similar to that of pepsin than penicillopepsin. As the result, there was not a great difference between the active site of the present model and that of the previous model, while there were differences on the outer surfaces. The region under the flap in the present model was slightly wider than in the previous model.

Then we used a model of the structure of the complex of human renin and the scissile site Pro-Phe-His-Leu-Val (P_4-P_1') of human angiotensinogen[16] (Figure 1) to design potent human renin inhibitors. Figure 2 shows the proposed mode of interaction between the proposed intermediate[17] of substrate and human renin. The hydrogen bonds between human renin and human angiotensinogen were as follows: NH of P_1 Leu and CO of Gly-228, imidazole of P_2 His and OH of Ser-233, CO of P_3 Phe and NH of Ser-230, NH of P_3 Phe and OH of Ser-230. The P_1 Leu and P_3 Phe side chains were located in the large hydrophobic subsites S_1 and S_3, respectively. We recognized[11] that in these sites there was some additional space which would permit the introduction of bulky side chains. P_4 Pro was located in the relatively polar subsite S_4.

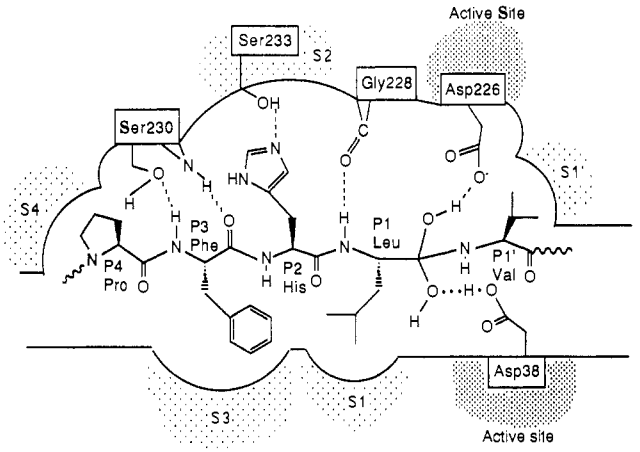


Figure 2. Schematic representation of a binding mode of the proposed intermediate of substrate to the proposed binding sites (S_1'-S_4).

Design. Based on the analysis of the renin–substrate interaction, we designed an orally active human renin inhibitor from the angiotensinogen transition-state analogues[3,5] as follows. (i) A succinic acid residue having a retro–inverso amide bond (P_3-P_4) was introduced to reduce the likelihood of enzymatic degradation while the hydrogen bond to Ser-233 was maintained. (ii) A cyclohexylnorstatine residue (P_1) in the place of the more common statine-type residue[18] was incorporated as a transition-state

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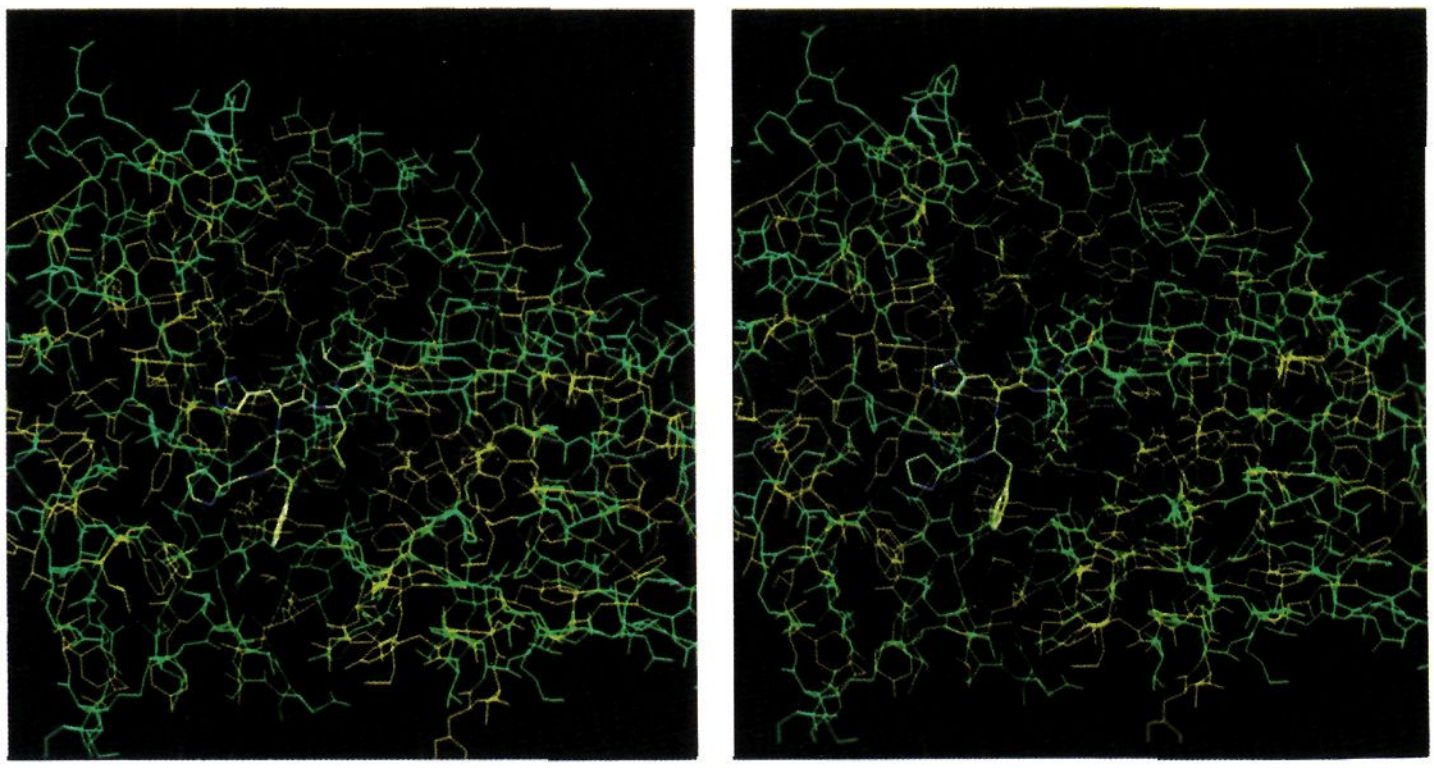


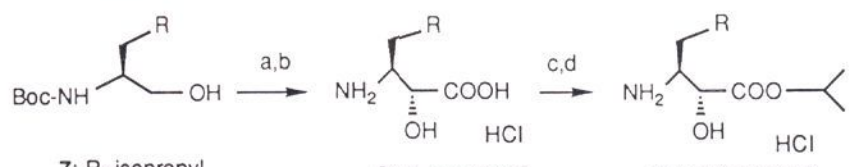
Figure 1. Stereoview of the active site of human renin (blue = hydrophilic residue, yellow = hydrophobic residue) with the scissile site P_4 Pro to P_1' Val of human angiotensinogen (white = carbon, purple = nitrogen, red = oxygen).

mimic, because it has an important hydroxy group for hydrogen bonding to Asp-38 and a large hydrophobic cyclohexyl group for filling the large S_1 site. Thus, the Leu-Val (P_1-P_1') scissile site was replaced by the cyclohexylnorstatine isopropyl ester residue. The isopropyl ester group fits favorably in the S_1' site. (iii) Histidine was placed at P_2, because the side chain imidazole forms a hydrogen bond with side chain OH of Ser-233.

Synthesis. On the basis of the above specifications, we synthesized the inhibitors listed in Table I. Cyclohexylnorstatine isopropyl ester (**5a**) was prepared as shown in Scheme I. Boc-L-cyclohexylalaninal, which was prepared from Boc-L-cyclohexylalaninol (**3**) with Py·SO_3–DMSO[19] at 20–25 °C for 20 min, was hydrocyanated with NaCN–HCl in $CHCl_3$–H_2O at 0 °C and hydrolyzed with 23% HCl at 80 °C. When the reaction mixture[20] was kept overnight, optically pure cyclohexylnorstatine (**2a**) exclusively crystallized out of the mixture in the form of HCl salt in 60% yield from alcohol **3**. Thus, we efficiently prepared optically pure **2a**, which was then converted to the isopropyl ester **5a** (95% yield).

The isomer **5e** (2*S*,3*S* form) was prepared from *N*-benzoyl-L-cyclohexylalaninol (**4e**), which was prepared by reprotection with benzoyl chloride. The diastereomeric cyanohydrin (2*R*:2*S* ~ 4:1), which was prepared from **4e** by oxidation and hydrocyanation in a similar way for the synthesis of **2a**, was treated with $SOCl_2$ in benzene to give the oxazoline inverted from the 2*R* to 2*S* configuration.[10] The resulting oxazoline was hydrolyzed to give a mixture (2*S*,3*S*:2*R*,3*S* = 3:1) of **2e**. Pure **5e** was prepared by esterification followed by separation of diastereomers using column chromatography (40% yield from **4e**). The stereochemistry of **5a** and **5e** was determined by conversion to the corresponding oxazolidinones **6a** and **6e** using *N*,*N*′-carbonyldiimidazole in CH_2Cl_2. Coupling constants of 4.9 and 8.8 Hz and NOEs of 2.7 and 23.4% for the ring protons of **6a** and **6e**, respectively, are consistent with trans and cis stereochemistry. These results show that the configurations at the C2-carbon are *R* for **5a** and *S* for **5e**. The synthesis of **5d** was carried out with **4d** derived from Boc-D-cyclohexylalaninol, as described above for **5e**.

Scheme II[a]



7: R=isopropyl
8: R=phenyl

9: R=isopropyl
10: R=phenyl

11: R=isopropyl
12: R=phenyl

[a] (a) Py·SO_3/DMSO, 22–25 °C, 20 min, then NaCN–HCl, 0 °C, 3 h; (b) 23% HCl, 80 °C, 11 h; (c) 2-propanolic HCl, 80 °C, 1 h; (d) silica gel chromatography.

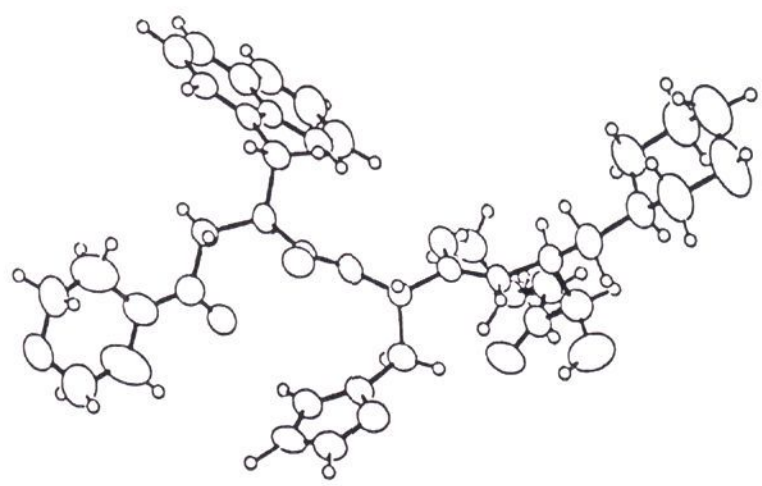


Figure 3. The X-ray crystal structure of **1a**.

Norstatine isopropyl ester (**11**) and phenylnorstatine[21] isopropyl ester (**12**) were synthesized in a similar way to the synthesis of **2a** as shown in Scheme II. Diastereomerically pure **9** and **10** could not be obtained, but diastereomerically pure **11** and **12** were prepared by esterification followed by separation using column chromatography in 55% and 50% yields from **7** and **8**, respectively.

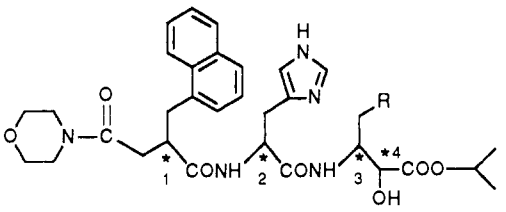
Scheme III shows the synthetic pathway of **1a**–**e**. Propionic acid **14a** prepared from 1-naphthaldehyde was coupled with L-histidine methyl ester and provided **15a**

(19) Hamada, Y.; Shibata, M.; Sugiura, T.; Kato, S.; Shioiri, T. *J. Org. Chem.* **1987**, *52*, 1252.

(20) A 4:1 mixture of **2a** (2*R*,3*S* form) and **2e** (2*S*,3*S* form). Diastereomeric ratio was determined by the proton NMR spectroscopic analysis.

(21) Nishizawa, R.; Saino, T.; Takita, T.; Suda, H.; Aoyagi, T.; Umezawa, H. *J. Med. Chem.* **1977**, *20*, 510.

Table I. Structures and Activities of Renin Inhibitors



no.	*1	*2	*3	*4	R	IC_{50}, M, against human renin	IC_{50}, M, against human plasma renin
1a	*R*	*S*	*S*	*R*	cyclohexyl	2.4×10^{-9}	4.7×10^{-9}
1b	***S***	*S*	*S*	*R*	cyclohexyl	4.2×10^{-6}	1.5×10^{-6}
1c	*R*	***R***	*S*	*R*	cyclohexyl	2.5×10^{-6}	2.3×10^{-6}
1d	*R*	*S*	***R***	*R*	cyclohexyl	3.5×10^{-6}	2.1×10^{-6}
1e	*R*	*S*	*S*	***S***	cyclohexyl	2.7×10^{-6}	4.2×10^{-6}
16	*R*	*S*	*S*	*R*	isopropyl	6.7×10^{-9}	6.2×10^{-9}
17	*R*	*S*	*S*	*R*	phenyl	1.1×10^{-7}	2.7×10^{-7}

Table II. Enzyme Inhibition Selectivity[a]

	IC_{50}, M renin[b] (human)	cathepsin D[c] (bovine)	pepsin[d] (porcine)	chymotrypsin[e] (bovine)	ACE[f] (rabbit)
1a	2.4×10^{-9}	8.0×10^{-5}	$>10^{-4}$	$>10^{-4}$	$>10^{-4}$
pepstatin A	9.2×10^{-5}	1.0×10^{-8}	1.8×10^{-8}	$>10^{-4}$	$>10^{-4}$

[a] The inhibitory effects were assayed at 37 °C. [b] Sheep angiotensinogen as the substrate at pH 7.4. [c] Hemoglobin as the substrate at pH 3.2. [d] Casein as the substrate at pH 2.0. [e] Casein as the substrate at pH 8.0. [f] Hip-His-Leu as the substrate at pH 8.3.

as described.[22] Condensation of the hydrolyzed product of **15a** and **5a** with dicyclohexylcarbodiimide (DCC) and *N*-hydroxy-5-norbornene-2,3-dicarboximide (HONB) gave **1a**, which was readily purified by recrystallization of the *p*-toluenesulfonic acid salt from ethyl acetate without using column chromatography (65% yield). After the salts of **1a** with several acids were examined, we obtained suitable crystals of **1a** with cinnamic acid and analyzed the X-ray crystal structure (Figure 3), which verified the configuration of the residues in **1a** deduced from NMR analysis.[10,22] The inhibitors listed in Table I were synthesized by essentially the same method. Column chromatography was needed for purification of these inhibitors.

Renin Inhibitory Activity. The human renin inhibitory potencies of the compounds were measured with both human renin–sheep substrate system and human plasma renin (Table I). The side chain isopropyl group of norstatine residue in **16**[7] was replaced with the larger and more hydrophobic phenyl in **17** or a cyclohexyl group in **1a**. By such replacements, the potency of **1a** was enhanced, while that of **17** was decreased against our expectation. Boger et al.[23] reported that replacing the isopropyl group of statine with either a phenyl group [(3*S*,4*S*)-4-amino-3-hydroxy-5-phenylpentanoic acid (AHPPA)] or a cyclohexyl group [(3*S*,4*S*)-4-amino-5-cyclohexyl-3-hydroxypentanoic acid (ACHPA)] enhanced the inhibitory potency. The detailed reason for the discrepancy between the norstatine analogue and the statine analogues by replacement with a phenyl group is unclear. A structural difference between the norstatine and the statine is the number of carbon atom in the main chain. In the case of statine, the rigidity of phenyl group affecting the conformation would be relaxed by the additional carbon atom in the main chain.

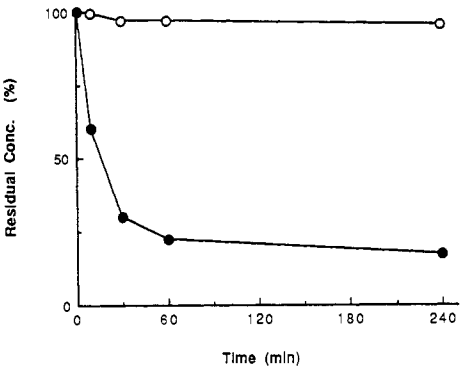
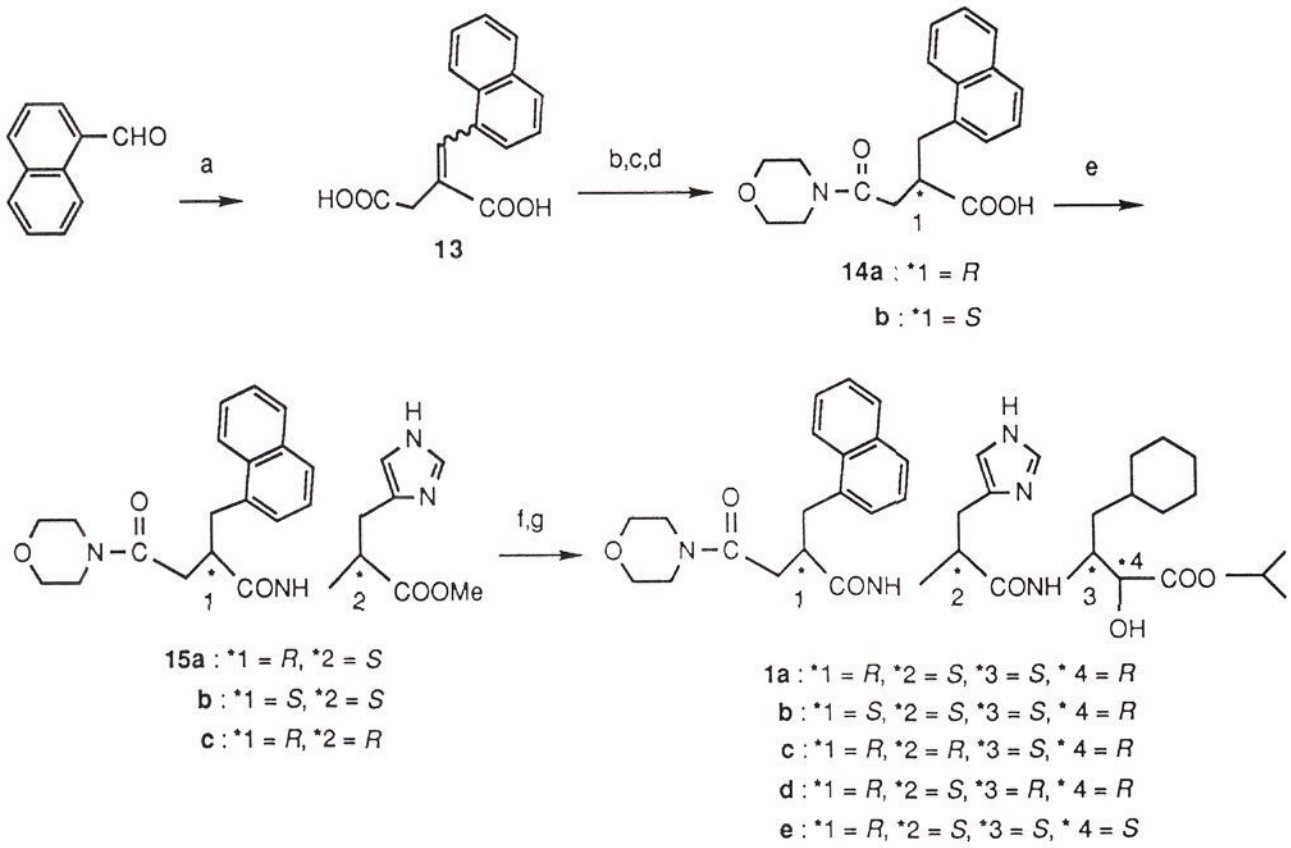


Figure 4. Stabilities of **1a** and MCA–substrate in monkey liver homogenates: ○, **1a** (0.1 mM); ●, MCA–substrate (0.1 mM).

Of the compounds listed in Table I, compound **1a** proved to be the most potent inhibitor against both the isolated human renin (2.4×10^{-9} M) and human plasma renin (4.7×10^{-9} M). The stereoisomers (**1b–e**) of **1a** showed very weak renin inhibitory activities ($>10^{-6}$ M). These results showed clearly that the stereochemistry of four asymmetric carbons in **1a** was very important for the potency. Also, **1a** showed little inhibition of other proteases such as cathepsin D ($>10^{-5}$ M), pepsin ($>10^{-4}$ M), and chymotrypsin ($>10^{-4}$ M), as shown in Table II. Compound **1a** was stable against monkey liver homogenates (Figure 4), human plasma, and chymotrypsin.[24] Oral administration of 10 mg/kg of **1a** to salt-depleted Japanese monkeys[25] resulted in a fall of 10–20 mmHg of mean blood pressure and reduced plasma renin activity for a 5-h period.

(22) Harada, H.; Yamaguchi, T.; Iyobe, A.; Tsubaki, A.; Kamijo, T.; Iizuka, K.; Ogura, K.; Kiso, Y. *J. Org. Chem.* **1990**, *55*, 1679.
(23) Boger, J.; Payne, L. S.; Perlow, D. S.; Lohr, N. S.; Poe, M.; Blaine, E. H.; Ulm, E. H.; Schorn, T. W.; LaMont, B. I.; Lin, T.-Y.; Kawai, M.; Rich, D. H.; Veber, D. F. *J. Med. Chem.* **1985**, *28*, 1779.
(24) Iizuka, K.; Kamijo, T.; Harada, H.; Akahane, K.; Kubota, T.; Umeyama, H.; Kiso, Y. In Abstracts of the 9th Symposium on Medicinal Chemistry in Tokyo, Nov 29–30, 1988, pp 9–13.
(25) Miyazaki, M.; Etoh, Y.; Iizuka, K.; Toda, N. *J. Hypertens.* **1989**, *7* (*suppl. 2*), S25. The detailed results of intravenous and oral administrations will be published elsewhere.

Scheme III[a]



[a] (a) diethyl malonate–NaOMe/MeOH, reflux, 2 h, then 2 N NaOH, reflux, 6 h; (b) $SOCl_2/CH_2Cl_2$, reflux, 2 h, then morpholine/EtOAc, room temperature, 20 h; (c) H_2–Pd/C/MeOH, room temperature, 20 h, then (*S*)- or (*R*)-methyl mandelate–DCC/EtOAc, room temperature, 20 h; (d) crystallized from methanol, then 2 N NaOH, room temperature, 20 h; (e) L- or D-His-OMe·HCl, DCC–HONB, 0 °C, 2 h and then room temperature, 20 h; (f) 1 N NaOH/MeOH, 0 °C, 2 h and then room temperature, 20 h; (g) **5a**, **5d**, or **5e**, DCC–HONB/CH_3CN, 0 °C, 2 h and then room temperature, 18 h.

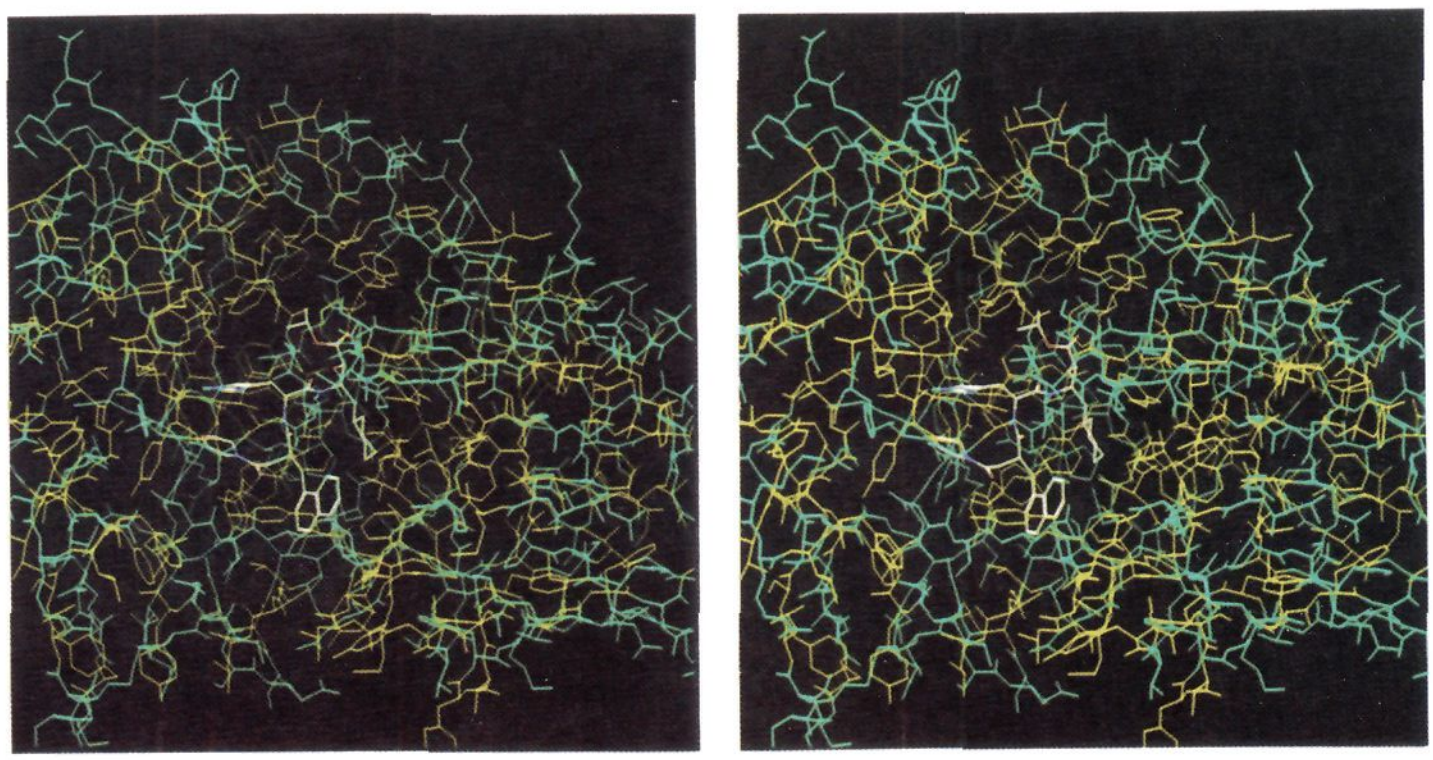


Figure 5. Stereoview of the active site of human renin with the inhibitor **1a** (white = carbon, purple = nitrogen, red = oxygen).

Mode of Interaction between 1a and Renin. The proposed conformation of **1a** in the model of the active site of human renin is depicted in Figure 5 and a schematic representation of the mode of interaction of **1a** with human renin is shown in Figure 6. The cyclohexyl group of P_1, the naphthyl group[26] of P_3, and morpholine moiety of P_4 are accommodated in S_1, S_3, and S_4, respectively. The valine-mimicked isopropoxy group[27] of P_1' is accommodated in S_1', and the hydroxy group of P_1 is interacting with both Asp-38 and Asp-226. It is proposed that the cyclohexylnorstatine residue is an analogue of the P_1 leucine unit in the renin substrate, while Boger et al. proposed[28] that the statine residue was an analogue of a dipeptide unit. The difference between the interaction of **1a** and that of human angiotensinogen (Pro-Val, P_4-P_1') with human renin is that the β-carbonyl group[29] of the P_3

(26) Replacement of the naphthyl group with a phenyl group decreased the potency (isopropyl (2*R*,3*S*)-3-[[*N*-[(2*RS*)-3-(morpholinocarbonyl)-2-(phenylmethyl)propionyl]-L-histidyl]-amino]-4-cyclohexyl-2-hydroxybutyrate: $IC_{50} = 9.0 \times 10^{-7}$ M).

(27) Conversion of the isopropyl group into a methyl group decreased the inhibitory potency.[7]

(28) Boger, J. In *Peptides Structure and Function. Proceedings of the Eighth American Peptide Symposium*; Hruby, V. J., Rich, D. H., Eds.; Pierce Chemical Co.: Rockford, IL, 1983; pp 569–578.

(29) Reduction of the carbonyl group to a methylene group decreased the potency: Iizuka, K.; Kamijo, T.; Harada, H.; Akahane, K.; Kubota, T.; Etoh, Y.; Shimaoka, I.; Tsubaki, A.; Murakami, M.; Yamaguchi, T.; Iyobe, A.; Umeyama, H.; Kiso Y. *Chem. Pharm. Bull.* **1990**, *38*, 2487.

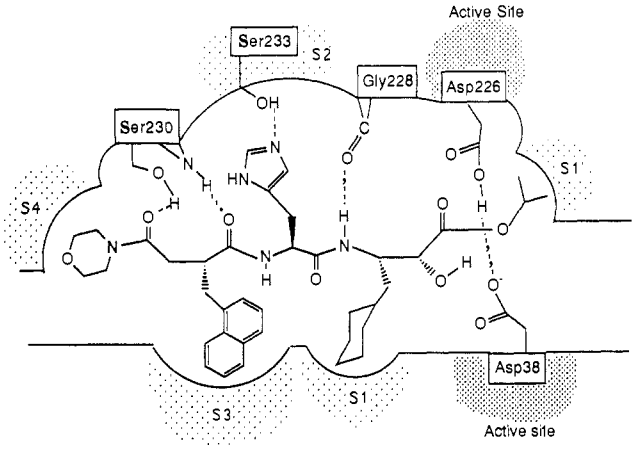


Figure 6. Schematic representation of a binding mode of **1a** to the proposed binding sites (S_1'-S_4).

succinic acid residue of **1a** rather than the NH of P_3 Phe in angiotensinogen may be hydrogen bonded to the side chain OH of Ser-230 of renin. The imidazole of P_2 His is hydrogen bonded to the side chain OH of Ser-233. This hydrogen bond seems to contribute to the specific inhibition against renin vs other aspartic proteinases.[30] Maibaum et al.[31] suggested that the protonated state of the imidazole group at P_2 contributes to the enzyme specificity. The weak inhibition ($>10^{-6}$ M) of the stereoisomers (**1b–e**) of **1a** may be attributable to poor interaction causing a decrease in affinity for renin. Recently, Sielecki et al.[32] reported the X-ray crystal structure of recombinant human renin. We cannot compare our three-dimensional structure with their X-ray structure because the coordinates are not available, but the general structure of the active site model seems to be similar to that of X-ray structure which appeared in the literature. Sielecki et al. described in the literature that the residues likely involved in binding the substrate P_4 Pro to P_3' His were similar to those proposed in our renin model.[11]

Conclusion

From an analysis of a model of the interaction between the substrate transition state and human renin, we designed and synthesized a specific human renin inhibitor, **1a**, which contained (2*R*)-3-(morpholinocarbonyl)-2-(1-naphthylmethyl)propionyl residue with a retro–inverso amide bond, L-histidine, and novel cyclohexylnorstatine. Optically pure cyclohexylnorstatine and **1a** were efficiently prepared, and the crystal structure of **1a** was determined by X-ray analysis. The stereochemistry of the four asymmetric carbons in **1a** was very important for potency. Compound **1a** is one of the most compact human renin inhibitors[33] and exhibits high and long-lasting activities in vitro and after oral administration. This methodology should be generally applicable for the rational discovery of inhibitors of other proteases, especially aspartic proteases such as the HIV protease.

Experimental Section

Proton magnetic resonance spectra were measured on a JEOL JMX-GX270 (270-MHz) instrument. Chemical shifts are reported as δ values (parts per million) relative to Me_4Si or $(CH_3)_3Si(CH_2)_3SO_3Na$ as an internal standard. Mass spectra were obtained with a JEOL JMX-DX300 (FAB) spectrometer having a JMA-DA5000 data processor. Infrared spectra (IR) were measured on JASCO IR-810 infrared spectrophotometer. HPLC analyses were performed on a Shimadzu LC-6A liquid chromatograph instrument, with YMC-packed column R-ODS-5 or Cosmosil $5C_{18}$ and 0.05 M aqueous NH_4OAc–CH_3CN elutions, with UV detection at 223 nm (JASCO UVIDEC-100-V). Optical rotations were measured with Horiba SEPA-200 high sensitivity polarimeter. Melting points were measured on a Yamato micro melting point apparatus and are uncorrected. Preparative thin-layer chromatography was carried out with Merck precoated silica gel 60 F-254 plates (thickness 0.5 mm). Flash column chromatography was carried out with Merck silica gel 60 (Art. 9385, 230–400 mesh). Elemental analyses were performed by the Analytical Research Department, Central Research Laboratories, Kissei Pharmaceutical Co., Ltd.

***N*-Benzoyl-L-cyclohexylalaninol (4e).** A solution of Boc-L-cyclohexylalaninol (**3**; 7.64 g, 0.03 mol) and 2 N HCl (74 mL, 0.15 mol) in methanol (90 mL) was stirred at 60 °C for 2 h, and the solvent was removed in vacuo. To a stirred 0 °C solution of the residue in dioxane (20 mL) and water (20 mL) were added dropwise triethylamine (8.7 mL, 0.063 mol) and benzoyl chloride (3.8 mL, 0.033 mol), and the mixture was stirred at 0 °C for 1 h. The solvent was removed and the residue was portioned between 1 N HCl (50 mL) and ethyl acetate (50 mL). The organic layer was washed with 5% $NaHCO_3$ (50 mL) and brine (30 mL), dried, and evaporated to give **4e** (3.9 g, quant.) as a white solid: mp 91–93 °C; $[\alpha]^{22}_D$ −38.4° (*c* 0.99, $CHCl_3$); IR (KBr) 1635 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.85–1.9 (m, 13 H), 3.66 (dd, 1 H, *J* = 11.0, 5.5 Hz), 3.78 (dd, 1 H, *J* = 11.0, 3.8 Hz), 4.2–4.4 (m, 1 H), 6.25 (bs, 1 H), 7.4–7.6 (m, 3 H), 7.76 (dd, 2 H, *J* = 6.6, 1.6 Hz). Anal. ($C_{16}H_{23}NO_2$) C, H, N.

***N*-Benzoyl-D-cyclohexylalaninol (4d)** was synthesized as described above for **4e** with Boc-D-cyclohexylalaninol. **4d** (87% yield) as a white solid: mp 106–108 °C; $[\alpha]^{24}_D$ +42.3° (*c* 0.60, $CHCl_3$); other physical and spectral characteristics were identical with those of **4e**. Anal. ($C_{16}H_{23}NO_2$) C, H, N.

(2*R*,3*S*)-3-Amino-4-cyclohexyl-2-hydroxybutyric Acid Hydrochloride (2a). To a stirred solution of **3** (0.49 g, 1.9 mmol), DMSO (1.4 mL, 19 mmol), and triethylamine (0.81 mL, 5.7 mmol) in benzene (0.7 mL) at 20–25 °C was added portionwise sulfur trioxide–pyridine complex (0.92 g, 5.7 mmol). After 20 min, the mixture was poured into ice water and extracted with $CHCl_3$ (20 mL). To the organic layer was added water (5 mL), followed, upon cooling to 0 °C, by NaCN (0.28 g, 5.7 mmol) and 1 N HCl (5.7 mL, 5.7 mmol). After 18 h, the solvent was removed in vacuo and concentrated HCl (13 mL) was added to the residue. The mixture was heated at 80 °C for 11 h and concentrated to about 15 mL. After standing overnight, **2a** (0.28 g, 60% yield) was collected by filtration as white crystals: mp 172–175 °C; $[\alpha]^{23}_D$ −11.2° (*c* 2.0, H_2O); IR (KBr) 1720 cm^{-1}; ^1H NMR (D_2O) δ 0.85–1.8 (m, 13 H), 3.65–3.8 (m, 1 H), 4.36 (d, 1 H, *J* = 3.3 Hz). Anal. ($C_{10}H_{19}NO_3$·HCl) C, H, N.

Isopropyl (2*R*,3*S*)-3-Amino-4-cyclohexyl-2-hydroxybutyrate Hydrochloride (5a). A solution of **2a** (0.28 g, 1.2 mmol) in 2-propanolic HCl (5 mL) was heated at 80 °C for 1 h. The solution was concentrated and crystallized from ethyl acetate (1 mL) to give **5a** (0.31 g, 95% yield) as white crystals: mp 118–119 °C; $[\alpha]^{23}_D$ −7.4° (*c* 2.4, H_2O); IR (KBr) 1720 cm^{-1}; ^1H NMR (D_2O) δ 0.85–1.25 (m, 5 H), 1.30 (d, 6 H, *J* = 6.6 Hz), 1.35–1.8 (m, 8 H), 3.6–3.75 (m, 1 H), 4.37 (d, 1 H, *J* = 5.0 Hz), 5.10 (quint, 1 H, *J* = 6.6 Hz). Anal. ($C_{13}H_{25}NO_3$·HCl) C, H, N.

(4*S*,5*R*)-4-(Cyclohexylmethyl)-5-(isopropoxycarbonyl)-2-oxazolidinone (6a). To a stirred solution of **5a** (279 mg, 1 mmol) in $CHCl_3$ (30 mL) at room temperature were added triethylamine (0.14 mL, 1 mmol) and *N*,*N*'-carbonyldiimidazole (190 mg, 1.2 mmol). After 4 h, the mixture was washed with 1 N HCl, 5% $NaHCO_3$, and brine. The solution was dried over $MgSO_4$ and

(30) Replacement of the L-histidine with L-valine inhibited cathepsin D and pepsin as well as renin.[29]

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concentrated in vacuo to give **6a** (260 mg, 97%) as a white solid: mp 100–102 °C; $[\alpha]^{23}_D$ −39.0° (*c* 0.616, $CHCl_3$); IR (KBr) 1760, 1735, 1720 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.9–1.25 (m, 5 H), 1.28 (d, 3 H, *J* = 6.0 Hz), 1.31 (d, 3 H, *J* = 6.0 Hz), 1.35–1.45 (m, 1 H), 1.58 (t, 2 H, *J* = 7.1 Hz), 1.6–1.8 (m, 6 H), 3.92 (dd, 1 H, *J* = 11.5, 4.9 Hz), 4.52 (d, 1 H, *J* = 4.9 Hz), 5.13 (quint, 1 H, *J* = 6.0 Hz), 5.64 (s, 1 H). Anal. ($C_{14}H_{23}NO_4$) C, H, N.

Isopropyl (2*S*,3*S*)-3-Amino-4-cyclohexyl-2-hydroxybutyrate Hydrochloride (5e). To a stirred solution of **4e** (0.392 g, 1.5 mmol), DMSO (1 mL, 15 mmol), and triethylamine (0.63 mL, 4.5 mmol) in benzene (0.5 mL) at 20–25 °C was added portionwise sulfur trioxide–pyridine complex (0.72 g, 4.5 mmol). After 20 min, the mixture was poured into ice water and extracted with $CHCl_3$ (10 mL). To the organic layer was added water (4.5 mL), followed, upon cooling to 0 °C, by NaCN (0.22 g, 4.5 mmol) and 1 N HCl (4.5 mL, 4.5 mmol). After 18 h, the organic layer was washed with brine, dried over $MgSO_4$, and evaporated. To the stirred, 0 °C solution of the residue in benzene (10 mL) was added $SOCl_2$ (0.11 mL, 1.5 mmol). The mixture was stirred overnight and concentrated in vacuo, followed by addition of 23% HCl and heating at 80 °C for 11 h. The aqueous solution was washed with ether and evaporated to give a mixture of **2e** and **2a** (3:1) (0.42 g). After the mixture in 2-propanolic HCl (5 mL) was heated at 80 °C for 1 h, the solvent was removed in vacuo. The residue was chromatographed on silica gel with $CHCl_3$–MeOH (15:1) for eluent. To the combined fraction was added 2-propanolic HCl and the solution was evaporated to give **5e** (0.156 g, 40% total yield) as a white solid: mp 127–133 °C; $[\alpha]^{22}_D$ −15.4° (*c* 1.00, H_2O); IR (KBr) 1730 cm^{-1}; ^1H NMR (D_2O) δ 0.8–1.2 (m, 5 H), 1.31 (d, 6 H, *J* = 6.0 Hz), 1.35–1.85 (m, 8 H), 3.8–3.9 (m, 1 H), 4.59 (d, 1 H, *J* = 2.7 Hz), 5.10 (quint, 1 H, *J* = 6.0 Hz). Anal. ($C_{13}H_{25}NO_3$·HCl) C, H, N.

(4*S*,5*S*)-4-(Cyclohexylmethyl)-5-(isopropoxycarbonyl)-2-oxazolidinone (6e) was synthesized as described above for **6a** using **5e**. **6e** (quant.) as a white solid: mp 113–114 °C; $[\alpha]^{23}_D$ −30.0° (*c* 0.644, $CHCl_3$); IR (KBr) 1760, 1725 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.8–1.25 (m, 6 H), 1.30 (d, 6 H, *J* = 6.0 Hz), 1.39 (t, 2 H, *J* = 6.6 Hz), 1.55–1.8 (m, 5 H), 4.2 (dd, 1 H, *J* = 14.3, 8.8 Hz), 4.98 (d, 1 H, *J* = 8.8 Hz), 5.16 (quint, 1 H, *J* = 6.0 Hz), 5.35–5.55 (m, 1 H). Anal. ($C_{14}H_{23}NO_4$) C, H, N.

Isopropyl (2*R*,3*R*)-3-amino-4-cyclohexyl-2-hydroxybutyrate hydrochloride (5d) was synthesized from **4d** as described above for **5e**. **5d** (35% yield) as a white solid: mp 110–118 °C; $[\alpha]^{22}_D$ +13.1° (*c* 0.52, H_2O); other physical and spectral characteristics were identical with those of **5e**. Anal. ($C_{13}H_{25}NO_3$·HCl) C, H, N.

Isopropyl (2*R*,3*S*)-3-Amino-2-hydroxy-5-methylhexanoate Hydrochloride (11). The synthesis of **9** (2*R*:2*S* ∼ 7:3) was carried out as described above for **2a** with Boc-L-leucinol (**7**) as a starting material. Then, compound **9** (2*R*:2*S* ∼ 7:3) was esterified and separated by chromatography to give **11** (55% yield from **7**) as a viscous oil: $[\alpha]^{24}_D$ −9.2° (*c* 0.26, H_2O); IR 1740 cm^{-1}; ^1H NMR (D_2O) δ 0.93 (d, 3 H, *J* = 3.3 Hz), 0.95 (d, 3 H, *J* = 2.8 Hz), 1.28 (d, 3 H, *J* = 1.7 Hz), 1.31 (d, 3 H, *J* = 1.1 Hz), 1.5–1.8 (m, 3 H), 3.6–3.7 (m, 1 H), 4.38 (d, 1 H, *J* = 4.4 Hz), 5.0–5.15 (m, 1 H). Anal. ($C_{10}H_{21}NO_3$·HCl) C, H, N.

Isopropyl (2*R*,3*S*)-3-Amino-2-hydroxy-4-phenylbutyrate Hydrochloride (12). The synthesis of **10** (2*R*:2*S* ∼ 7:3) was carried out as described above for **2a** with Boc-L-phenylalaninol (**8**) as a starting material. Then, compound **10** (2*R*:2*S* ∼ 7:3) was esterified and separated by chromatography to give **12** (50% yield from **8**) as a white solid: mp 138–142 °C; $[\alpha]^{24}_D$ −10.9° (*c* 1.4, H_2O); IR (KBr) 1730 cm^{-1}; ^1H NMR (D_2O) δ 1.26 (dd, 6 H, *J* = 6.0, 4.4 Hz), 3.04 (dd, 1 H, *J* = 14.3, 7.7 Hz), 3.12 (dd, 1 H, *J* = 14.3, 7.1 Hz), 3.9–4.0 (m, 1 H), 4.32 (d, 1 H, *J* = 3.8 Hz), 5.03 (quint, 1 H, *J* = 6.0 Hz), 7.3–7.5 (m, 5 H). Anal. ($C_{13}H_{19}NO_3$·HCl) C, H, N.

***N*-[(2*S*)-3-(Morpholinocarbonyl)-2-(1-naphthylmethyl)propionyl]-L-histidine methyl ester (15b)** was synthesized as described for **15a** with **14b**.[22] **15b** (75% yield) as a white solid: mp 90–95 °C; $[\alpha]^{21}_D$ −52.9° (*c* 0.72, MeOH); IR (KBr) 1740, 1630 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.56 (dd, 1 H, *J* = 15.5, 4.9 Hz), 2.7–2.85 (m, 2 H), 3.04 (dd, 1 H, *J* = 15.5, 5.5 Hz), 3.2–3.65 (m, 9 H), 3.70 (s, 1 H), 4.45–4.55 (m, 1 H), 6.44 (s, 1 H), 6.66 (d, 1 H, *J* = 7.2 Hz), 7.3–7.8 (m, 5 H), 7.87 (d, 1 H, *J* = 7.7 Hz), 8.15 (d, 1 H, *J* = 8.2 Hz); HPLC 98% (elution time 5.36 min). Anal. ($C_{26}H_{30}N_4O_5$) C, H, N.

***N*-[(2*R*)-3-(Morpholinocarbonyl)-2-(1-naphthylmethyl)propionyl]-D-histidine methyl ester (15c)** was synthesized as described for **15a**[22] with D-histidine methyl ester. **15c** (73% yield) as a white solid: mp 90–94 °C; $[\alpha]^{25}_D$ +58.76° (*c* 0.65, MeOH); other physical and spectral characteristics were identical with those of **15b**. Anal. ($C_{26}H_{30}N_4O_5$) C, H, N.

Isopropyl (2*R*,3*S*)-3-[[*N*-[(2*R*)-3-(Morpholinocarbonyl)-2-(1-naphthylmethyl)propionyl]-L-histidyl]amino]-4-cyclohexyl-2-hydroxybutyrate (1a). To a stirred, 0 °C solution of **15a**[22] (2.2 g, 3.9 mmol) in MeOH (10 mL) was added 1 N NaOH (4.7 mL, 4.7 mmol). After 2 h the solution was warmed to ambient temperature gradually and stirred overnight. Addition of 1 N HCl (4.7 mL, 4.7 mmol) was followed by evaporation in vacuo. To the stirred, 0 °C mixture of the residue and **5a** (1.1 g, 3.9 mmol) in CH_3CN (10 mL) were added triethylamine (0.54 mL, 3.9 mmol), HONB (0.71 g, 3.9 mmol), and DCC (0.81 g, 3.9 mmol). After 2 h the mixture was warmed to ambient temperature gradually, stirred overnight, filtered, and evaporated in vacuo. The residue was dissolved in ethyl acetate, washed with 5% $NaHCO_3$ and brine, dried over $MgSO_4$, and concentrated in vacuo. The mixture of the residue and *p*-toluenesulfonic acid monohydrate (0.75 g, 3.9 mmol) was crystallized from ethyl acetate to give pure **1a**·*p*-toluenesulfonic acid salt as white crystals. These crystals were poured into 5% $NaHCO_3$ (10 mL) and extracted with $CHCl_3$ (5 mL × 3). The organic phase was washed with brine, dried over $MgSO_4$, and concentrated in vacuo to give **1a** (1.76 g, 65%) as a white solid: mp 99–104 °C; $[\alpha]^{21.5}_D$ −28.6° (*c* 1.04, MeOH); IR (KBr) 1735, 1635 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.7–1.2 (m, 5 H), 1.23 (t, 6 H, *J* = 6.6 Hz), 1.43 (t, 2 H, *J* = 7.1 Hz), 1.55–1.85 (m, 5 H), 2.3–2.8 (m, 2 H), 3.0–3.7 (m, 13 H), 4.08 (d, 1 H, *J* = 2.2 Hz), 4.3–4.6 (m, 2 H), 5.02 (quint, 1 H, *J* = 6.6 Hz), 6.83 (s, 1 H), 7.01 (d, 1 H, *J* = 9.3 Hz), 7.28 (d, 1 H, *J* = 8.8 Hz), 7.25–7.55 (m, 4 H), 7.74 (d, 1 H, *J* = 8.2 Hz), 7.85 (d, 1 H, *J* = 7.1 Hz), 8.04 (d, 1 H, *J* = 7.7 Hz); HPLC 98% (column, YMC-packed column R-ODS-5, 4.6 × 250 mm; eluent, acetonitrile–0.05 M NH_4OAc (aqueous) (11:9); flow rate, 1 mL/min; elution time 9.7 min); FABMS *m/z* 690 (M + 1). Anal. ($C_{38}H_{51}N_5O_7 \cdot {}^1/_2H_2O$) C, H, N.

X-ray Crystal Structure of 1a. The crystal of **1a** with cinnamic acid suitable for X-ray analysis was obtained by recrystallization from aqueous methanol and analyzed by the X-ray diffraction method. The crystal data are as follows: $C_{38}H_{51}N_5O_7 \cdot C_9H_8O_2 \cdot H_2O$, Mr = 856.03, monoclinic, space group $P2_1$, *a* = 23.101 (7) Å, *b* = 6.223 (1) Å, c = 16.628 (6) Å, β = 108.69 (4)°, *V* = 2264.5 (12) Å^3, *Z* = 2, D_m = 1.252 (1), D_x = 1.255 g·cm^{-3}, μ(Cu Kα) = 6.85 cm^{-1}, *F*(000) = 916. The intensities of independent reflections within 2θ = 130° were measured on a Rigaku AFC-5 diffractometer with graphite-monochromated Cu Kα radiation and corrected for the Lorentz and polarization factors, but not for absorption effects. Out of 4251 collected reflections, 3864 ($F_o^2 > 0.0$) were used for the structure determination and refinement. The structure was finally solved by the combination of the direct method and successive Fourier syntheses and refined by a least-squares method using the anisotropic temperature factors for non-hydrogen atoms. Ideal positions of hydrogen atoms were calculated and used only for the calculations of structure factors. The present discrepancy indexes *R* and *wR* are 0.066 and 0.048, respectively.

Isomers 1b–1e, 16, and 17 were prepared by essentially the same procedure as for **1a**, but these compounds needed the further purification by silica gel column chromatography with $CHCl_3$ and methanol (15:1) as eluent.

Isopropyl (2*R*,3*S*)-3-[[*N*-[(2*S*)-3-(morpholinocarbonyl)-2-(1-naphthylmethyl)propionyl]-L-histidyl]amino]-4-cyclohexyl-2-hydroxybutyrate (1b) (55% yield) as a white solid: mp 105–108 °C; $[\alpha]^{21}_D$ −70.6° (*c* 0.66, MeOH); IR (KBr) 1740, 1635 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.7–2.0 (m, 19 H), 2.4–3.8 (m, 15 H), 4.05 (d, 1 H, *J* = 3.3 Hz), 4.2–4.3 (m, 1 H), 4.4–4.5 (m, 1 H), 4.96 (quint, 1 H, *J* = 6.0 Hz), 6.68 (s, 1 H), 7.2–7.8 (m, 6 H), 7.87 (d, 1 H, *J* = 7.7 Hz), 8.1–8.2 (m, 1 H); HPLC 98.5% (elution time 9.4 min). Anal. ($C_{38}H_{51}N_5O_7 \cdot {}^1/_5CHCl_3$) C, H, N.

Isopropyl (2*R*,3*S*)-3-[[*N*-[(2*R*)-3-(morpholinocarbonyl)-2-(1-naphthylmethyl)propionyl]-D-histidyl]amino]-4-cyclohexyl-2-hydroxybutyrate (1c) (35% yield) as a white solid: mp 105–107 °C; $[\alpha]^{21}_D$ +12.9° (*c* 0.62, MeOH); IR (KBr) 1730, 1635 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.7–2.1 (m, 19 H),

2.4–3.7 (m, 15 H), 4.00 (d, 1 H, J = 2.8 Hz), 4.2–4.3 (m, 1 H), 4.7–4.8 (m, 1 H), 4.93 (quint, 1 H, J = 6.0 Hz), 6.13 (s, 1 H), 7.3–7.65 (m, 5 H), 7.75–7.95 (m, 2 H), 8.1–8.25 (m, 2 H); HPLC 97% (elution time 8.0 min). Anal. ($C_{38}H_{51}N_5O_7 \cdot {}^1/_4CHCl_3$) C, H, N.

Isopropyl (2*R*,3*R*)-3-[[*N*-[(2*R*)-3-(morpholinocarbonyl)-2-(1-naphthylmethyl)propionyl]-L-histidyl]amino]-4-cyclohexyl-2-hydroxybutyrate (1d) (56% yield) as a white solid: mp 106–108 °C; $[\alpha]^{24}_D$ +14.0° (c 0.53, MeOH); IR (KBr) 1735, 1640 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.6–2.6 (m, 20 H), 2.8–3.7 (m, 14 H), 4.24 (d, 1 H, J = 2.8 Hz), 4.3–4.5 (m, 1 H), 4.8–4.9 (m, 1 H), 5.06 (quint, 1 H, J = 6.0 Hz), 6.6–6.8 (m, 1 H), 7.00 (s, 1 H), 7.25–7.6 (m, 5 H), 7.73 (d, 1 H, J = 7.1 Hz), 7.84 (d, 1 H, J = 8.2 Hz), 8.09 (s, 1 H), 8.18 (d, 1 H, J = 8.2 Hz); HPLC 99% (elution time 7.7 min). Anal. ($C_{38}H_{51}N_5O_7 \cdot {}^1/_4CHCl_3$) C, H, N.

Isopropyl (2*S*,3*S*)-3-[[*N*-[(2*R*)-3-(morpholinocarbonyl)-2-(1-naphthylmethyl)propionyl]-L-histidyl]amino]-4-cyclohexyl-2-hydroxybutyrate (1e) (68% yield) as a white solid: mp 110–114 °C; $[\alpha]^{21}_D$ +6.85° (c 0.70, MeOH); IR (KBr) 1735, 1640 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.6–1.8 (m, 19 H), 2.3–3.8 (m, 15 H), 4.25 (s, 1 H), 4.65–4.75 (m, 1 H), 5.09 (quint, 1 H, J = 6.3 Hz), 6.8–6.9 (m, 1 H), 7.3–7.8 (m, 5 H), 7.84 (d, 1 H, J = 7.9 Hz), 8.05–8.15 (m, 1 H), 8.4–8.5 (m, 1 H); HPLC 95% (elution time 9.9 min). Anal. ($C_{38}H_{51}N_5O_7 \cdot {}^1/_5CHCl_3$) C, H, N.

Isopropyl (2*R*,3*S*)-3-[[*N*-[(2*R*)-3-(morpholinocarbonyl)-2-(1-naphthylmethyl)propionyl]-L-histidyl]amino]-2-hydroxy-5-methylhexanoate (16) (37% yield) as a white solid: mp 94–98 °C; $[\alpha]^{24}_D$ −20.0° (c 0.73, MeOH); IR (KBr) 1740, 1640 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.81 (d, 3 H, J = 1.7 Hz), 0.84 (d, 3 H, J = 2.2 Hz), 1.15 (t, 6 H, J = 6.6 Hz), 2.1–3.6 (m, 15 H), 3.96 (s, 1 H), 4.1–4.2 (m, 1 H), 4.3–4.45 (m, 1 H), 4.83 (quint, 1 H, J = 6.6 Hz), 5.5 (bs, 1 H), 6.8 (bs, 1 H), 7.25–7.6 (m, 4 H), 7.77 (d, 1 H, J = 8.2 Hz), 7.91 (d, 1 H, J = 7.7 Hz), 8.26 (d, 1 H, J = 7.7 Hz), 8.27 (d, 1 H, J = 8.2 Hz); HPLC 95% (elution time 5.7 min); FABMS m/z 650 (M + 1). Anal. ($C_{35}H_{47}N_5O_7 \cdot {}^1/_4CHCl_3$) C, H, N.

Isopropyl (2*R*,3*S*)-3-[[*N*-[(2*R*)-3-(morpholinocarbonyl)-2-(1-naphthylmethyl)propionyl]-L-histidyl]amino]-2-hydroxy-4-phenylbutyrate (17) (54% yield) as a white solid: mp 108–111 °C; $[\alpha]^{24}_D$ −33.4° (c 0.79, MeOH); IR (KBr) 1735, 1640 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.17 (t, 6 H, J = 6.6 Hz), 2.4–3.7 (m, 17 H), 4.03 (d, 1 H, J = 2.2 Hz), 4.4–4.6 (m, 2 H), 5.00 (quint, 1 H, J = 6.6 Hz), 6.83 (s, 1 H), 6.95–7.6 (m, 11 H), 7.68 (s, 1 H), 7.76 (d, 1 H, J = 8.2 Hz), 7.86 (d, 1 H, J = 7.7 Hz), 8.05 (d, 1 H, J = 8.2 Hz); HPLC 97% (elution time 6.0 min); FABMS m/z 684 (M + 1). Anal. ($C_{38}H_{45}N_5O_7 \cdot {}^1/_4CHCl_3$) C, H, N.

In Vitro Renin Inhibition Assay. The renin inhibitory activity was measured as described.[34] A 25-μL aqueous solution of human renin (20–30 ng of Ang I/mL per h) was incubated at 37 °C with a mixture of sheep angiotensinogen (2000 ng of Ang I/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 formed after 15 min of incubation was measured by radioimmunoassay.

Alternatively, 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 produced was measured by radioimmunoassay.

In Vitro Stability Assay. One gram of liver from a 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). Final concentration of the inhibitor was 10^{-4} M. The mixture was incubated at 37 °C and residual concentration of each intact compound was measured by HPLC (control; Suc-Arg-Pro-Phe-His-Leu-Leu-Val-Tyr-MCA).

Molecular Modeling. (a) Complex of Human Renin and the Substrate Fragment. The three-dimensional structure of human renin was constructed by essentially the previous method[34] based on the coordinates of porcine pepsinogen (1PSG) from the Protein Data Bank[14] by using a computer system BIOCES.[35] The substrate human angiotensin fragment positions 7–11, Pro-Phe-His-Leu-Val (P_4-P_1'), was fitted into the active site manually.

(b) **Complex of Human Renin and Inhibitor 1a.** The orientation of **1a** in the active site of renin was determined by the Monte Carlo Simulation[36] with the Metropolis algorithm.[37] In this simulation, the enzyme was fixed to the starting position and only the rotational degrees of freedom of the inhibitor were taken into account. The potential function and parameters were taken from the literature.[38] Figure 5 shows the most stable conformation of the inhibitor in the ensemble obtained by the Metropolis method.

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