Synthesis and Structure–Activity Relationships of Human Renin Inhibitors Designed from Angiotensinogen Transition State

Kinji IIZUKA,*[a] Tetsuhide KAMIJO,[a] Hiromu HARADA,[a,b] Kenji AKAHANE,[a] Tetsuhiro KUBOTA,[a] Yasuo ETOH,[a] Iwao SHIMAOKA,[a] Atsushi TSUBAKI,[a] Makoto MURAKAMI,[a] Toshiaki YAMAGUCHI,[a] Akira IYOBE,[a] Hideaki UMEYAMA,[c] and Yoshiaki KISO*[,b]

Central Research Laboratories, Kissei Pharmaceutical Co., Ltd.,[a] Yoshino, Matsumoto, Nagano 399, Japan, Department of Medicinal Chemistry, Kyoto Pharmaceutical University,[b] Yamashina-ku, Kyoto 607, Japan, and School of Pharmaceutical Sciences, Kitasato University,[c] Shirokane, Minato-ku, Tokyo 108, Japan. *Received March 29, 1990*

The synthesis and the structure–activity relationships of renin inhibitors designed from the angiotensinogen transition state are described. These inhibitors contained residues modified at P_1–$P_{1'}$, P_2, and P_4–P_3. Decrease in the size of side chain alkyl group in norstatine analog at P_1 diminished the inhibitory activities of the compounds. Compound 5j, which contained valine residue instead of histidine residue at P_2, inhibited potently cathepsin D ($IC_{50} = 6.0 \times 10^{-9}$ M) and pepsin ($IC_{50} = 3.5 \times 10^{-7}$ M) to the same extent as renin ($IC_{50} = 8.5 \times 10^{-10}$ M), and thus was not specific for renin. The reduction of the β-carbonyl group to methylene group in β-carbonylpropionyl residue at P_4–P_3 decreased the potency about 2 orders against human renin (5i: $IC_{50} = 1.1 \times 10^{-7}$ M *vs.* 1: $IC_{50} = 2.4 \times 10^{-9}$ M). These results confirmed the rationality of our analysis of the interaction between an orally potent human renin inhibitor 1 and the active site of human renin using modeling techniques, showing that 1 fits the active site of renin favorably. The experimental details of the synthesis are presented.

Keywords renin inhibitor; antihypertensive drug; cyclohexylnorstatine; *retro-inverso* amide bond; angiotensinogen transition state; structure–activity relationship

Renin (EC 3.4.23.15, formerly EC 3.4.99.19) is an aspartic protease that selectively cleaves angiotensinogen to effect the formation of the decapeptide, angiotensin I (Ang I) as shown in Fig. 1. Ang I is then cleaved by angiotensin converting enzyme (ACE), which is a non-specific 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, and indirectly, by the volume expansion resulting from stimulation of aldosterone release from the adrenal cortex, leading to sodium and water retention. Inhibition of ACE is an effective therapy for the majority of hypertensive patients and is a major advance in the treatment of hypertension and congestive heart failure.[1,2] Nevertheless, ACE is not specific for Ang I and cleaves kinins and other endogenous peptides such as substance P and enkephalins. For this reason ACE inhibitors may interfere with hormonal systems other than the renin–angiotensin system (RAS). This might contribute to some of the side effects of ACE inhibitors such as cough or angioneurotic edema.[3]

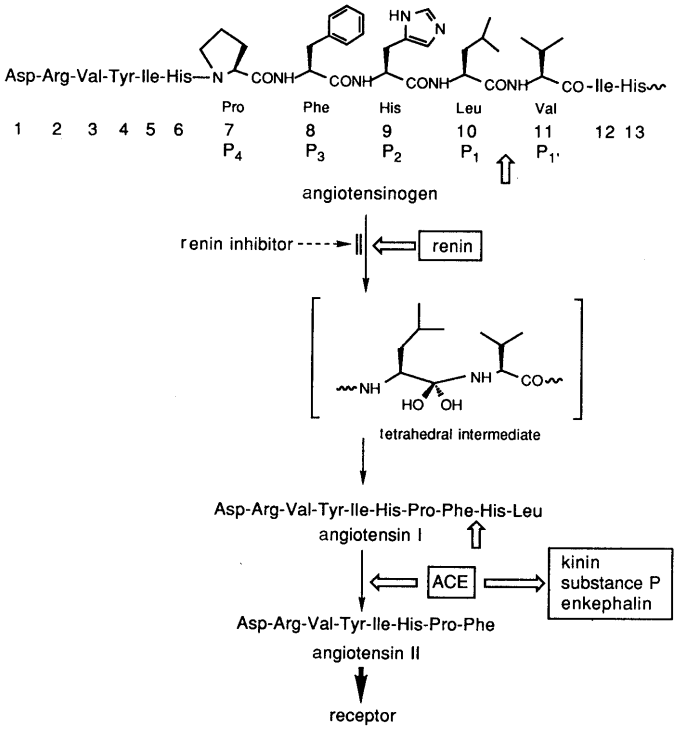
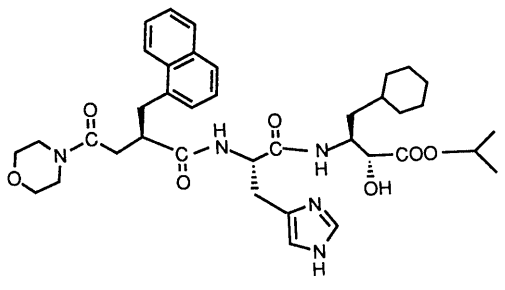


Fig. 1. Renin–Angiotensin System

Renin catalyzes the first and rate-limiting step in RAS, and the action of renin on angiotensinogen is highly specific. A large number of renin inhibitory peptides derived from substrate transition-state analogs have been investigated as targets of antihypertensive drugs.[4] The peptide compounds[5,6] are thought to be unsuitable for oral administration owing to the problems of proteolytic degradation and poor intestinal absorption. The orally active renin inhibitors suitable for clinical use should be compact to increase intestinal absorption, and, in addition, should have high potency, high specificity, and metabolic stability.

We recently reported[7–9] an orally potent human renin inhibitor **1** (Fig. 2), which was designed from the angiotensinogen transition state based on a three-



1

Fig. 2

dimensional structure of the complex of human renin and the scissile site Pro to Val (P_4 to $P_{1'}$) of angiotensinogen deduced using modeling techniques.[9,10] The inhibitor **1** contains (2*R*)-3-morpholinocarbonyl-2-(1-naphthylmethyl)propionyl residue having *retro-inverso* amide bond,[11] L-histidine, and (2*R*,3*S*)-3-amino-4-cyclohexyl-2-hydroxybutyric acid named cyclohexylnorstatine[7,9,12] as a transition-state mimic. We analyzed the mode of interaction of **1** and human renin and showed that **1** fits the active site of renin favorably.[9] In order to study the structure–activity relationships of renin inhibitors derived from the substrate transition state, we synthesized compounds **5a**—**j** related to **1** and evaluated the renin inhibitory activities. Herein we describe the experimental details of synthesis and the structure–activity relationships of these renin inhibitors.

Results and Discussion

Synthesis The compounds prepared for this study are shown in Table I and their syntheses are outlined in Charts 1—4. The inhibitors **5a**—**j** except **5i** depicted in Table I were prepared by the procedure shown in Chart 1. (2*R*)-3-Morpholinocarbonyl-2-(1-naphthylmethyl)propionic acid **2**, which was prepared according to the previous method,[13] was coupled with L-histidine methyl ester or L-valine methyl ester using DCC (dicyclohexylcarbodiimide) and HONB (*N*-hydroxy-5-norbornene-2,3-dicarboximide) to give histidine intermediate **3a** or valine intermediate **3b**. The intermediate **3a** was readily purified by recrystallization from benzene[13] and **3b** was purified by column chromatography. Coupling of the hydrolyzed product of **3a** or **3b** and transition-state analogs aminoalcohols **4a**—**g** with DCC–HONB gave the inhibitors **5a**—**h** and **5j**, which were purified by column chromatography.

Synthesis of the norstatine analogs **10** and statine analog, ethyl *N*-Boc-(3*S*,4*S*)-4-amino-5-cyclohexyl-3-hydroxypentanoate (**11**),[14] are shown in Chart 2. Boc- or Z-L-amino acid methyl esters **7** were prepared in high yield from Boc- or Z-L-amino acids **6** by esterification using methyl iodide and $KHCO_3$ in *N*,*N*-dimethylformamide (DMF) at room temperature.[15] The esters **7** were easily reduced with LiCl– $NaBH_4$ in ethanol–tetrahydrofuran (THF) to give β-amino alcohols **8**. The amino alcohols **8** were converted

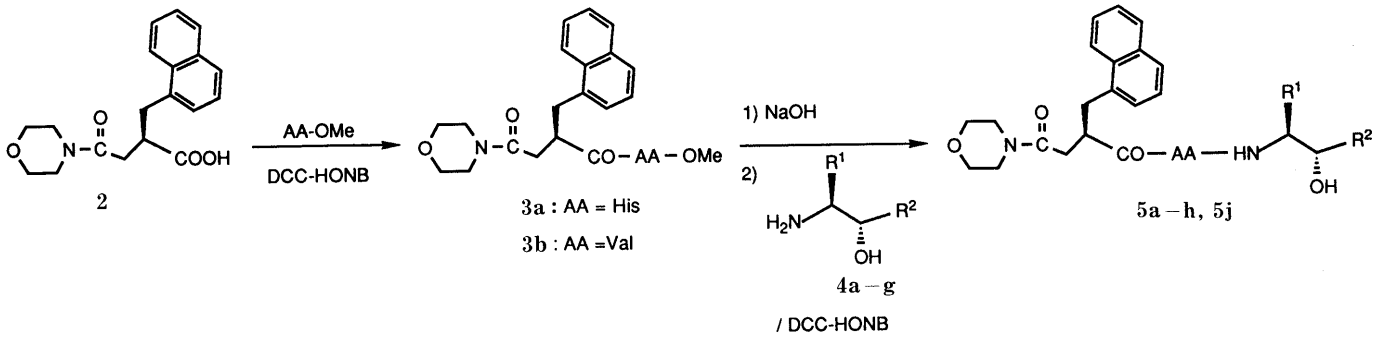


Chart 1

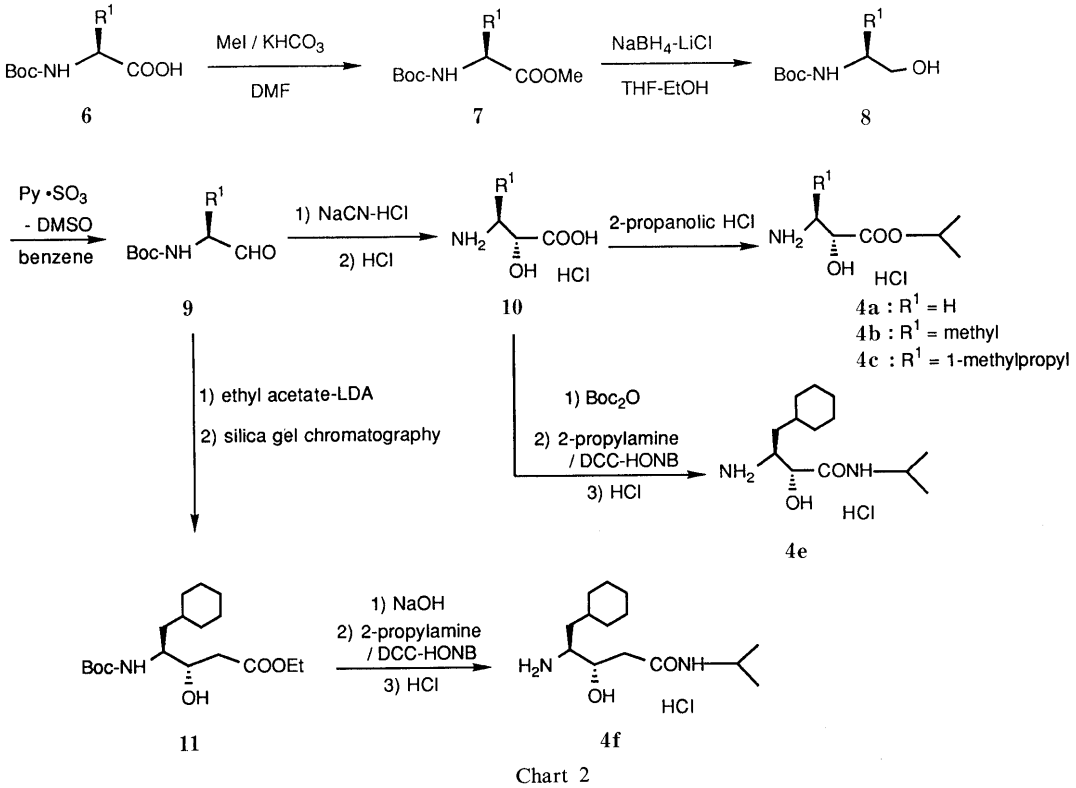


Chart 2

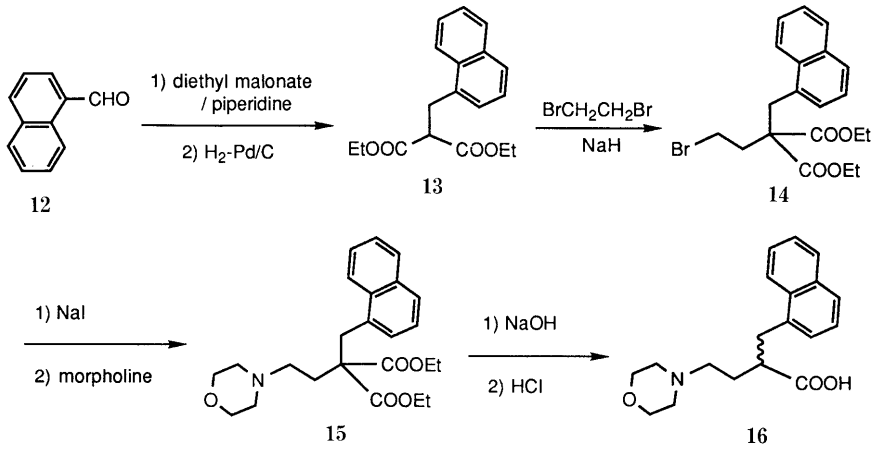
to the corresponding aldehydes **9** using $Py \cdot SO_3$–DMSO and triethylamine in benzene at 20—25 °C for 20 min. The aldehydes **9** were hydrocyanated with NaCN–HCl in ethyl acetate–H_2O at 0 °C and hydrolyzed with 23% HCl at 80°C to give the corresponding diastereomeric mixtures (2*R*,3*S*: 2*S*,3*S* ≃ 7 : 3, except the product from Boc–Gly–OH) of norstatine analogs **10**. Then the norstatine analogs **10** were converted to the corresponding isopropyl esters. Among them, optically pure **4c** (2*R*,3*S* form) was separated from the diastereomeric mixture by column chromatography. Optically pure isopropyl (2*R*,3*S*)-3-amino-2-hydroxybutyrate was not separated from the diastereomers **4b** (2*RS*,3*S* form) by silica gel chromatography. *N*-Isopropyl (2*R*,3*S*)-3-amino-4-cyclohexyl-2-hydroxybutyramide **4e** was prepared from optically pure cyclohexylnorstatine (**10**, R^1 = cyclohexylmethyl),[7,9,12] by protection with di-*tert*-butyl dicarbonate (Boc_2O) and coupling with isopropylamine using DCC–HONB followed by deprotection with alcoholic HCl. The synthesis of ethyl Boc-(3*S*,4*S*)-4-amino-5-cyclohexyl-3-hydroxypentanoate (**11**) was carried out according to the method reported by Boger *et al.*[14] Then, the corresponding ester, isopropyl (3*S*,4*S*)-4-amino-5-cyclohexyl-3-hydroxypentanoate **4d** and amide **4f** were prepared by the same procedure as norstatine analogs.

Chart 3 shows the synthetic pathway of **16** in which β carbonyl group of **2** is reduced to methylene group. The diethyl malonate (**13**) was prepared by condensation of 1-naphthaldehyde and diethyl malonate in the presence of piperidine under benzene reflux followed by hydrogenation over Pd/C in ethyl acetate in 90% yield. Alkylation of **13** with NaH and excess 1,2-dibromoethane under ethylene glycol dimethylether reflux gave the bromide **14**, which was purified by silica gel chromatography in 59% yield. After the bromide **14** was converted to the iodide with NaI in 2-butanone, *N*-alkylation of morpholine with the iodide under acetonitrile reflux followed by column chromatography gave the morpholino compound **15** in 59% yield. Decarboxylation of **15** with aqueous NaOH and then hydrochloric acid under reflux followed by column chromatography gave **16** in 85% yield.

Chart 4 shows the synthetic pathway of **5i** in which β-carbonyl group of **1** is reduced to methylene group. (4-Methoxybenzyloxycarbonyl)-L-histidine hydrazide (PMZ–His–$NHNH_2$) **17** was coupled with cyclohexylnorstatine isopropyl ester (**4g**)[7c,9,12] by the azide method using isoamyl nitrite to afford **18** in 64% yield. After the deprotection of amino group in **18** by hydrogenation over Pd/C, coupling of the product and **16** using diphenylphosphoryl azide (DPPA)[16] followed by preparative chromatography provided **5i**.

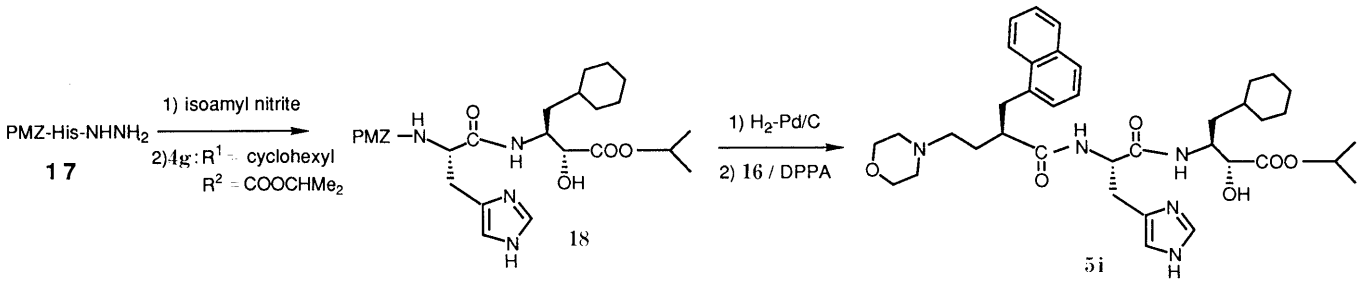
Structure–Activity Relationships The renin inhibitory potencies of the compounds were measured with both human renin–sheep substrate system and human plasma renin (Table I).

1. Modification of the Cyclohexylnorstatine Residue at P_1–$P_{1'}$ We have reported[9] that replacement of the side chain isopropyl group (P_1) of norstatine [(2*R*,3*S*)-3-amino-2-hydroxy-5-methylhexanoic acid] in **5a** with more hydrophobic cyclohexyl group enhanced the inhibitory potency (**5a**: $IC_{50} = 7.6 \times 10^{-9}$ M *vs.* **1**: $IC_{50} = 2.4 \times 10^{-9}$ M), while phenyl group substitution resulted in reduced inhibitory potency (**1** *vs.* **5b**: $IC_{50} = 1.1 \times 10^{-7}$ M). The reduction implies decrease of the fitness to renin due to the rigidity of phenyl group. To further test the effect of side chain (P_1) in the norstatine analogs on inhibitory potency, we prepared inhibitors **5c**—**e** which contained norstatine



12 13 14 15 16

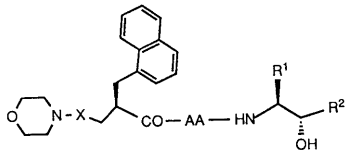
Chart 3



17 18 5i

Chart 4

TABLE I. *In Vitro* Renin Inhibitory Activity for Inhibitors



No.	X	AA	R^1	R^2	IC_{50} (M) Human renin	IC_{50} (M) Human plasma renin
1[9]	CO	His	Cyclohexylmethyl	$-COOCHMe_2$	2.4×10^{-9}	4.7×10^{-9}
5a[9]	CO	His	Isobutyl	$-COOCHMe_2$	6.7×10^{-9}	6.2×10^{-9}
5b[9]	CO	His	$PhCH_2-$	$-COOCHMe_2$	1.1×10^{-7}	2.7×10^{-7}
5c	CO	His	H	$-COOCHMe_2$[a]	7.3×10^{-7}	N.D.[c]
5d	CO	His	CH_3-	$-COOCHMe_2$[b]	5.7×10^{-6}	N.D.[c]
5e	CO	His	Et(Me)CH–	$-COOCHMe_2$	1.5×10^{-6}	N.D.[c]
5f	CO	His	Cyclohexylmethyl	$-CONHCHMe_2$	3.9×10^{-8}	2.1×10^{-8}
5g	CO	His	Cyclohexylmethyl	$-CH_2COOCHMe_2$	1.3×10^{-8}	3.5×10^{-8}
5h	CO	His	Cyclohexylmethyl	$-CH_2CONHCHMe_2$	1.5×10^{-8}	6.6×10^{-9}
5i	CH_2	His	Cyclohexylmethyl	$-COOCHMe_2$	1.1×10^{-7}	N.D.[c]
5j	CO	Val	Cyclohexylmethyl	$-COOCHMe_2$	8.5×10^{-10}	7.7×10^{-10}

a) Mixture (2*R*:2*S*=1:1) of stereoisomers. *b*) Mixture (2*R*,3*S*:2*S*,3*S*=13:7) of diastereoisomers. *c*) N.D.=not determined.

analogs **4a**—**c**. Decrease in the size of alkyl group of side chain diminished the inhibitory activity of compounds (**1** *vs.* **5c**: $IC_{50}=7.3\times10^{-7}$ M, **5d**: $IC_{50}=5.7\times10^{-6}$ M). On the other hand, substitution with more bulky 1-methylpropyl group [(2*S*,3*S*,4*S*)-3-amino-2-hydroxy-4-methylheptanoic acid named isonorstatine **4c**] caused a dramatic reduction in renin inhibitory potency (**1** *vs.* **5e**: $IC_{50}=1.5\times10^{-6}$ M). Isonorstatine **4c** has a methyl group at the β-position. This methyl group affects the conformation due to steric hindrance to the vicinal hydroxy group, which is essential for the inhibitory activity, and histidine residue (P_2). The stable conformation of **5e** would be different from the active conformation.

Inhibitor **1** was noticeably more active than the corresponding statine derivative **5g** ($IC_{50}=1.3\times10^{-8}$ M), showing that norstatine-type analogs fitted to renin more favorably than statine-type analogs. However, in the case of norstatine analog, conversion of the ester bond in the C-terminal to amide bond attenuated the potency (**1** *vs.* **5f**: $IC_{50}=3.9\times10^{-8}$ M). In contrast, in the case of statine analog, the conversion did not greatly influence the potency (**5g** *vs.* **5h**: $IC_{50}=1.5\times10^{-8}$ M). Decrease of the fitness to renin seems to cause the attenuation of the inhibitory potency because the norstatine amide derivative might be more rigid than the statine amide having an additional carbon atom in its main chain.

2. Modification of the β-Carbonylpropionyl Residue at P_4–P_3 In our model, β-carbonyl group of (2*R*)-3-morpholinocarbonyl-2-(1-naphthylmethyl)propionyl residue (P_4–P_3) hydrogen-bonded to the side chain OH of Ser-230 in renin. To confirm the hydrogen bond, we synthesized **5i** in which the β-carbonyl group in **1** was reduced to methylene group. The reduction of the carbonyl group dramatically decreased the potency (**1** *vs.* **5i**: $IC_{50}=1.1\times10^{-7}$ M). In addition, the inhibitors, which contained the related residues with a carbonyl group at a different position from that of **2**, showed very weak inhibition.[11b] These results supported importance of the hydrogen bond between the β-carbonyl group and the side chain OH of Ser-230, which contributed to the control of inhibitor orientation in the renin and stabilization of the complex.

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)
1[9]	2.4×10^{-9}	8.0×10^{-5}	$>10^{-4}$	$>10^{-4}$	$>10^{-4}$
5j	8.5×10^{-10}	6.0×10^{-9}	3.5×10^{-7}	N.D.[g]	N.D.[g]

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. *g*) N.D.=not determined.

3. Modification of the Histidine Residue at P_2 Replacement of L-histidine residue with L-valine residue enhanced the potency (**1** *vs.* **5j**: $IC_{50}=8.5\times10^{-10}$ M) to a certain extent. However, **5j** inhibited cathepsin D ($IC_{50}=6.0\times10^{-9}$ M) and pepsin ($IC_{50}=3.5\times10^{-7}$ M) as well as renin (Table II). **1** did not practically inhibit other proteases such as cathepsin D, pepsin, and chymotrypsin, that is, it was specific for renin.[9] In our model, the imidazole of P_2 His in **1** hydrogen-bonded to the side chain OH of Ser-233. In the model constructed by Hui *et al.*,[17] there was a hydrogen bond between the ring nitrogen of the P_2 His of renin substrate analog inhibitory peptides and the hydrogen of the backbone nitrogen of Tyr-230. We do not have direct evidence that a hydrogen bond actually exists between the compound and renin. However, our data suggest that the bond between His and Ser-233 contributes to selectivity, that is, the imidazole group of P_2 His is indispensable to selective inhibition against renin.

Conclusion

We synthesized compounds **5a**—**j** related to **1** and evaluated their renin inhibitory activities. The modification of residues at P_1–$P_{1'}$, P_2, and P_4–P_3 decreased the inhibitory potency or the selectivity against renin. The decrease in size of the side chain alkyl group in norstatine analog at

P_1 reduced the inhibitory activities. The replacement of histidine residue with valine residue at P_2 potently inhibited cathepsin D and pepsin to the same extent as renin, and was not specific for renin. The reduction of the β carbonyl group in (2*R*)-3-morpholinocarbonyl-2-(1-naphthylmethyl)propionyl residue to methylene group at P_4–P_3 decreased the potency. The results show that **1** fits favorably the active site of renin and confirm our analysis of the interaction between **1** and the active site of renin as follows: 1) The presence of a large hydrophobic residue in the P_1 position of the inhibitor is favorable for the inhibition; 2) The histidine imidazole of **1** is hydrogen-bonded to the side chain OH of Ser-233 and indispensable to selective inhibitory potency against renin; 3) The β-carbonyl group of propionyl residue (P_4–P_3) is hydrogen-bonded to the side chain OH of Ser-230. The hydrogen bond may be one of the bonds which contributes to the control of inhibitor orientation in the renin and stabilization of the complex. Thus, our methodology for designing the orally potent inhibitor **1** would be generally applicable for the rational discovery of inhibitors of other proteases.

Experimental

Proton nuclear magnetic resonance (^{1}H-NMR) 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 (MS) were obtained with a JEOL JMX-DX300 (FAB) spectrometer having JMA-DA5000 data processor. Infrared (IR) spectra were measured on a JASCO IR-810 IR-spectrophotometer. High performance liquid chromatography (HPLC) analyses were performed on a Shimadzu LC-6A liquid chromatograph instrument, YMC-Packed Column R-ODS-5, and 0.05 M aq. NH_4OAc–CH_3CN elutions, with ultraviolet (UV) detection at 223 nm (JASCO UVIDEC-100-V). Optical rotations were measured with a Horiba SEPA-200 high sensitive polarimeter. Melting points were measured on a Yamato micro melting point apparatus and are uncorrected. Preparative thin-layer chromatography (TLC) was carried out using Merck precoated Silica gel 60 F-254 plates (thickness 0.5 mm). Flash column chromatography was carried out using 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*-[(2*R*)-3-Morpholinocarbonyl-2-(1-naphthylmethyl)propionyl]-L-valine Methyl Ester (3b)** To a stirred solution of **2**[13] (103 mg, 0.31 mmol) and L-valine methyl ester (hydrochloride, 53 mg, 0.31 mmol) in acetonitrile (5 ml) were sequentially added triethylamine (0.04 ml, 0.31 mmol), HONB (60 mg, 0.31 mmol) and DCC (64 mg, 0.31 mmol) at 0 °C. 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 and the solution was washed with aqueous citric acid, 5% $NaHCO_3$ and brine, and dried over $MgSO_4$. The solution was evaporated *in vacuo* and the residue was purified by preparative TLC with $CHCl_3$ and methanol (25 : 1) for development to afford **3b** (98 mg, 72%) as a white solid: mp 110—114 °C. $[\alpha]_D^{22}$ +20.0° (c=0.22, MeOH). IR (KBr): 1740, 1640 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.84 (t, 6H, J=7.1 Hz), 1.9—2.1 (m, 1H), 2.43 (dd, 1H, J=16.2, 3.6 Hz), 2.86 (dd, 1H, J=16.2, 9.1 Hz), 3.2—3.65 (m, 14H), 4.38 (dd, 1H, J=8.8, 5.2 Hz), 6.23 (d, 1H, J=8.8 Hz), 7.3—7.6 (m, 4H), 7.74 (d, 1H, J=7.7 Hz), 7.85 (d, 1H, J=7.7 Hz), 8.11 (d, 1H, J=8.2 Hz). FABMS m/z: 441 (M+1). *Anal.* Calcd for $C_{25}H_{32}N_2O_5$; C, 68.16; H, 7.32; N, 6.36. Found: C, 67.79; H, 7.15; N, 6.14.

Isopropyl (2*RS*)-3-Amino-2-hydroxypropionate·HCl (4a) To a solution of Boc–Gly–OH (1.0 g, 5.7 mmol) in dimethylformamide (5 ml) was added $KHCO_3$ (1.14 g, 11.4 mmol), followed by methyl iodide (0.57 ml, 9.1 mmol). The mixture was stirred at room temperature for 4 h. Ethyl acetate was added and the solution was washed with aqueous citric acid, 5% $NaHCO_3$, and brine. After the solution was dried over $MgSO_4$, removal of the solvent gave *N*-Boc–Gly–OMe **7** (1.0 g, 93%) as a colorless oil. To a solution of **7** (0.99 g, 5.2 mmol) in THF (5 ml) were added anhydrous lithium chloride (0.67 g, 15.6 mmol), sodium borohydride (0.59 g, 15.6 mmol), and ethanol (10 ml). After the mixture was stirred at room temperature overnight, aqueous citric acid was added and the mixture was extracted with CH_2Cl_2. The organic extracts were washed with 5% $NaHCO_3$ and brine, and dried over $MgSO_4$. Removal of the solvent gave Boc-2-amino-ethanol **8** (0.59 g, 70%) as a colorless oil. To a stirred solution of **8** (0.16 g, 1.0 mmol), DMSO (0.8 ml, 10 mmol), and triethylamine (0.42 ml, 3.0 mmol) in benzene (0.4 ml) at 20—25 °C was added portionwise sulfur trioxide pyridine complex (0.748 g, 3.0 mmol). After 20 min, the mixture was poured into ice water and extracted with ethyl acetate (10 ml). To the organic layer was added water (2.5 ml) followed, upon cooling to 0 °C, by NaCN (0.15 g, 3.0 mmol) and 1 N HCl (3 ml, 3.0 mmol). After 18 h, the organic layer was washed with brine and evaporated *in vacuo*. To the residue was added 23% HCl, and heating followed at 80°C for 11 h. The aqueous solution was washed with ether and evaporated to give a racemate **10** (0.18 g, 128%). The mixture in 2-propanolic HCl (5 ml) was heated 80 °C for 1 h and then the solvent was removed *in vacuo* to give **4a** (0.15 g, 84% total yield) as a colorless oil: IR (neat): 1730 cm^{-1}, ^{1}H-NMR (D_2O) δ: 1.28 (d, 6H, J=6.0 Hz), 3.20 (dd, 1H, J=13.2, 8.8 Hz), 3.45 (dd, 1H, J=13.2, 3.9 Hz), 3.20 (dd, 1H, J=13.2, 8.8 Hz), 3.45 (dd, 1H, J=13.2, 3.9 Hz), 4.52 (dd, 1H, J=8.8, 3.9 Hz), 5.08 (quint, 1H, J=6.0 Hz). *Anal.* Calcd for $C_6H_{13}NO_3 \cdot HCl \cdot 0.3H_2O$: C, 38.12; H, 7.79; N, 7.41. Found: C, 37.91; H. 7.63; N, 7.25.

Isopropyl (2*RS*,3*S*)-3-Amino-2-hydroxybutyrate·HCl (4b) The synthesis of **4b** (2*R*,3*S* : 2*S*,3*S* ≃ 13 : 7) was carried out as described above for **4a** with Boc–Ala–OH as a starting material. Pure stereoisomer (2*R*,3*S* form) was not separated from the diastereomers by silica gel chromatography. **4b** (70% total yield) as a colorless oil: IR (neat): 1735 cm^{-1}, ^{1}H-NMR (D_2O) δ: 1.22 (d, 1H, J=6.6 Hz), 1.25—1.35 (m, 6H), 1.36 (d, 2H, J=6.6 Hz), 3.6—3.9 (m, 1H), 4.29 (d, 0.65H, J=6.0 Hz), 4.54 (d, 0.35H, J=3.3 Hz), 5.10 (quint, 1H, J=6.0 Hz). *Anal.* Calcd for $C_7H_{15}NO_3 \cdot HCl \cdot 0.2H_2O$: C, 41.77; H, 8.21; N, 6.96. Found: C, 41.53; H, 8.15; N, 6.79.

Isopropyl (2*S*,3*S*,4*S*)-3-Amino-2-hydroxy-4-methylhexanoate·HCl (4c) The synthesis of **4c** (2*R*,3*S*,4*S*) was carried out as described above for **4a** with Boc–Ile–OH as a starting material. The diastereoisomers of **4c** were separated by silica gel chromatography with $CHCl_3$ and methanol (15 : 1) for eluent followed by recrystallization from benzene and hexane to afford pure **4c** (20% total yield) as a white solid: mp 108—114 °C; $[\alpha]_D^{22}$ −51.5° (c=0.56, H_2O). IR (KBr): 1730 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.96 (t, 3H, J=7.2 Hz), 1.13 (d, 3H, J=6.6 Hz), 1.28 (d, 3H, J=6.6 Hz), 1.32 (d, 3H, J=6.6 Hz), 1.35—2.0 (m, 3H), 3.54 (br s, 1H), 4.43 (d, 1H, J=4.4 Hz), 5.16 (quint, 1H, J=6.6 Hz). *Anal.* Calcd for $C_{10}H_{21}NO_3 \cdot HCl$: C, 50.10; H, 9.25; N, 5.84. Found: C, 49.81; H, 8.87; N, 5.81.

Isopropyl (3*S*,4*S*)-4-Amino-5-cyclohexyl-3-hydroxypentanoate·HCl (4d) Ethyl *N*-Boc-(3*S*,4*S*)-4-amino-5-cyclohexyl-3-hydroxypentanoate (**11**) was prepared by the method described in the literature.[14] A solution of **11** in 2-propanolic HCl was heated at 80°C for 2 h. The solvent was removed *in vacuo* to give **4d** as a white solid (32% total yield): mp 90—94 °C. $[\alpha]_D^{22}$ −18.8° (c=0.52, H_2O). IR (KBr): 1730 cm^{-1}. ^{1}H-NMR (D_2O) δ: 0.85—1.2 (m, 5H), 1.26 (d, 6H, J=6.0 Hz), 1.35—1.8 (m, 8H), 2.58 (dd, 1H, J=15.9, 8.8 Hz), 2.73 (dd, 1H, J=15.9, 3.9 Hz), 3.3—3.4 (m, 1H), 4.05—4.15 (m, 1H), 5.03 (quint, 1H, J=6.0 Hz). *Anal.* Calcd for $C_{14}H_{28}NO_3 \cdot HCl \cdot 0.1H_2O$: C, 56.69; H, 9.92; N, 4.72. Found: C, 56.41; H, 9.86; N, 4.59.

***N*-Isopropyl (2*R*,3*S*)-3-Amino-4-cyclohexyl-2-hydroxybutyramide·HCl (4e)** To a stirred 0 °C solution of cyclohexylnorstatine·HCl[7,9,12] (2.0 g, 8.4 mmol) in dioxane (6 ml) and water (6 ml) were added triethylamine (2.3 ml, 16.8 mmol) and Boc_2O (2.0 g, 9.24 mmol). After 2 h the mixture was warmed to ambient temperature gradually, and stirred overnight. The solution was washed with ether and the aqueous solution was acidified with aqueous citric acid. This solution was extracted with ethyl acetate and the ethyl acetate layer was washed with brine, dried over $MgSO_4$, and evaporated to give *N*-Boc-cyclohexylnorstatine (2.01 g, 79%). To a solution of *N*-Boc-cyclohexylnorstatine (200 mg, 0.66 mmol) and isopropylamine (0.085 ml, 1.0 mmol) in ethyl acetate (6 ml) were added HONB (120 mg, 0.66 mmol) and DCC (140 mg, 0.66 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 and the solution was washed with aqueous citric acid, 5% $NaHCO_3$ and brine, dried over $MgSO_4$, and evaporated *in vacuo* to give Boc-cyclohexylnorstatine isopropylamide (232 mg, quant.). To a solution of Boc-cyclohexylnorstatine isopropylamide (232 mg, 0.66 mmol) in methanol (5 ml) was added 2 N HCl (2 ml) and the mixture was heated at 60 °C for 3 h. The solution was washed with ether and evaporated *in vacuo* to afford **4e** (172 mg, 95%) as a white solid: mp 106—112 °C. $[\alpha]_D^{22}$ −29.9° (c=0.67,

H_2O). IR (KBr): 1655 cm^{-1}. ^{1}H-NMR (D_2O) δ: 0.8—1.1 (m, 2H), 1.17 (dd, 6H, J=6.6, 3.7 Hz), 1.2—1.8 (m, 9H), 3.55—3.7 (m, 1H), 3.97 (quint, 1H, J=6.6 Hz), 4.17 (d, 1H, J=5.0 Hz). *Anal.* Calcd for $C_{13}H_{26}N_2O_2 \cdot$ HCl: C, 56.00; H, 9.76; N, 10.05. Found: C, 55.75; H, 9.83; N, 9.89.

***N*-Isopropyl (3*S*,4*S*)-3-Amino-5-cyclohexyl-3-hydroxypentanamide·HCl (4f)** The synthesis of **4f** was carried out as described above for **4e** with *N*-Boc-(3*S*,4*S*)-4-amino-5-cyclohexyl-3-hydroxypentanoic acid (the hydrolyzed product of **11**). **4f** (84% yield) as white solid: mp 180—186 °C, $[\alpha]_D^{22}$ −62.0° (c=0.61, H_2O). IR (KBr): 1650 cm^{-1}. ^{1}H-NMR (D_2O) δ: 0.85—1.2 (m, 2H), 1.13 (d, 6H, J=6.6 Hz), 1.2—1.8 (m, 9H), 2.42 (dd, 1H, J=14.8, 8.2 Hz), 2.56 (dd, 1H, J=14.8, 5.0 Hz), 3.25—3.4 (m, 1H), 3.92 (quint, 1H, J=6.6 Hz), 4.0—4.1 (m, 1H). *Anal.* Calcd for $C_{14}H_{28}N_2O_2 \cdot$HCl: C, 57.42; H, 9.98; N, 9.57. Found: C, 57.11; H, 10.05; N, 9.26.

Diethyl 1-Naphthylmethylmalonate (13) A solution of 1-naphthaldehyde **12** (9.75 g, 62.5 mmol), diethyl malonate (10.0 g, 62.5 mmol), piperidine (3 ml, 30 mol) in benzene (250 ml) was refluxed for 18 h. The reaction mixture was washed with 1 N HCl, 5% $NaHCO_3$, and brine, dried over $MgSO_4$, and evaporated *in vacuo*. The residue was purified by silica gel chromatography with benzene for eluent to give diethyl 1-naphthylmethylidene malonate (17.4 g, 93%). Then, the suspension of the malonate (17.4 g, 58.4 mmol) and 10% Pd on activated carbon (2 g) in ethyl acetate (300 ml) was hydrogenated at atmospheric pressure overnight. After Pd on activated carbon was filtered out, the filtrate was concentrated *in vacuo* to afford **13** (17.0 g, 97%) as a colorless oil: IR (neat): 1730 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.19 (t, 6H, J=7.1 Hz), 3.71 (d, 2H, J=7.1 Hz), 3.84 (t, 1H, J=7.1 Hz), 4.1—4.25 (m, 4H), 7.37 (d, 1H, J=6.5 Hz), 7.45—7.6 (m, 2H), 7.74 (t, 1H, J=4.8 Hz), 7.86 (d, 1H, J=8.2 Hz), 8.05 (d, 1H, J=7.7 Hz). *Anal.* Calcd for $C_{18}H_{20}O_4$: C, 71.98; H, 6.71. Found: C, 71.71; H, 6.63.

Ethyl 4-Bromo-2-ethoxycarbonyl-2-(1-naphthylmethyl)butyrate (14) To a solution of **13** (5.0 g, 16.7 mmol) in ethylene glycol dimethylether (50 ml) under argon were added NaH (0.96 g, 20 mmol) and then 1,2-dibromoethane (7.5 ml, 87 mmol). After reflux for 16 h, water and ethyl acetate were added to the reaction mixture and the organic layer was washed with 1 N HCl, 5% $NaHCO_3$, and brine. The solution was dried over $MgSO_4$ and evaporated *in vacuo*. The residue was purified by silica gel chromatography with hexane and benzene (1 : 2) for eluent to afford **14** (4.0 g, 59%) as a dark brown oil: IR (neat): 1775, 1720 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.15 (t, 6H, J=7.1 Hz), 2.43 (t, 2H, J=8.2 Hz), 3.40 (t, 1H, J=8.2 Hz), 3.77 (s, 2H), 4.0—4.15 (m, 4H), 7.3—7.55 (m, 4H), 7.76 (d, 1H, J=8.2 Hz), 7.8—8.1 (m, 2H). *Anal.* Calcd for $C_{20}H_{23}BrO_4$: C, 58.98; H, 5.69. Found: C, 58.69; H, 5.57.

Ethyl 2-Ethoxycarbonyl-4-morpholino-2-(1-naphthylmethyl)butyrate (15) A solution of **14** (1.0 g, 2.45 mmol) and NaI (1.1 g, 7.35 mmol) in 2-butanone (15 ml) was refluxed for 3 h. To the reaction mixture was added ether and the ether solution was washed with water. The solution was evaporated *in vacuo* and the residue was purified by silica gel chromatography with $CHCl_3$ for eluent to give ethyl 2-ethoxycarbonyl-4-iodo-2-(1-naphthylmethyl)butyrate (1.06 g, 95%). Morpholine (5 ml, 57.7 mmol) was added to the iodide (1.06 g, 2.3 mmol) in CH_3CN (5 ml). After reflux for 7 h, the mixture was evaporated and the residue was dissolved in ether. The ether solution was washed with 5% $NaHCO_3$ and brine, dried over $MgSO_4$, and evaporated. The residue was purified by silica gel chromatography with $CHCl_3$ for eluent to afford **15** (0.60 g, 62%) as a pale yellow oil: IR (neat): 1730 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.13 (t, 6H, J=7.1 Hz), 2.09 (t, 2H, J=7.7 Hz), 2.3—2.5 (m, 6H), 3.65 (t, 4H, J=4.9 Hz), 3.77 (s, 2H), 3.95—4.1 (m, 4H), 7.35—7.8 (m, 5H), 7.83 (d, 1H, J=7.1 Hz), 8.06 (d, 1H, J=6.6 Hz), *Anal.* Calcd for $C_{24}H_{31}NO_5$: C, 69.71; H, 7.56; N, 3.39. Found. C, 69.35; H, 7.31; N, 3.13.

4-Morpholino-2-(1-naphthylmethyl)butyric Acid (16) A solution of **15** (500 mg, 1.2 mmol) and 2 N NaOH (12 ml, 24 mmol) in ethanol (15 ml) was refluxed for 17 h followed by the addition of 2 N HCl (15 ml, 26.4 mmol). After reflux for 5 h, 2 N NaOH (3 ml, 2.4 mmol) was added to the mixture and the solvent was evaporated. The residue was purified by silica gel chromatography with $CHCl_3$ and methanol (15 : 1) for eluent to afford **16** (320 mg, 85%) as a white solid: mp 67—70°C. IR (KBr): 1720 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.8—2.3 (m, 2H), 2.8—3.15 (m, 8H), 3.7—4.0 (m, 5H), 7.33 (d, 1H, J=8.2 Hz), 7.39 (t, 1H, J=8.2 Hz), 7.45–7.6 (m, 2H), 7.76 (d, 1H, J=8.2 Hz), 7.86 (d, 1H, J=7.1 Hz), 8.07 (d, 1H, J=6.6 Hz). *Anal.* Calcd for $C_{19}H_{23}NO_3$: C, 72.82; H, 7.40; N, 4.47. Found. C, 72.50; H, 7.21; N, 4.25.

Isopropyl (2*R*,3*S*)-3-{(4-Methoxybenzyloxycarbonyl)-L-histidyl}amino-4-cyclohexyl-2-hydroxybutyrate (18) To a stirred −20 °C solution of **17** (3.5 g, 10.5 mmol) in DMF (20 ml) were added dropwise 5.95 N HCl–DMF solution (5.82 ml, 34.6 mmol) and then isoamyl nitrite (1.69 ml, 12.6 mmol). After stirring at −20—−10 °C for 20 min, triethylamine (4.81 ml, 34.6 mmol) was added to the solution to give (4-methoxybenzyloxycarbonyl)-L-histidine azide solution. The azide solution was added to a stirred 0 °C solution of **4g**[7c,9,12] (2.93 g, 10.5 mmol) and triethylamine (1.46 ml, 10.5 mmol) in DMF (40 ml). After stirring overnight, the mixture was dissolved in ethyl acetate and the ethyl acetate solution was washed with 5% $NaHCO_3$ and brine, dried over $MgSO_4$, and evaporated *in vacuo*. The residue was purified by silica gel chromatography with $CHCl_3$ and methanol (15 : 1) for eluent to afford **18** (3.97 g, 64%) as a white solid: mp74—78 °C. $[\alpha]_D^{21}$ −44.4° (c=0.45, MeOH). IR (KBr): 1725, 1665 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.8—1.2 (m, 6H), 1.26 (d, 6H, J=6.0 Hz), 1.3—1.85 (m, 7H), 2.97 (dd, 1H, J=14.3, 6.5 Hz), 3.09 (dd, 1H, J=14.3, 3.3 Hz), 3.81 (s, 3H), 4.05 (s, 1H), 4.3—4.5 (m, 2H), 4.95—5.1 (m, 2H), 6.1—6.2 (m, 1H), 6.8—6.95 (m, 4H), 7.31 (d, 2H, J=7.3 Hz), 7.63 (s, 1H). FABMS m/z: 544 (M+1). *Anal.* Calcd for $C_{28}H_{40}N_4O_7 \cdot 0.15CHCl_3$: C, 60.10; H, 7.19; N, 9.96. Found: C, 61.09; H, 7.14; N, 10.25.

Isopropyl (2*RS*)-3-{*N*-[(2*R*)-3-Morpholinocarbonyl-2-(1-naphthylmethyl)propionyl]-L-histidyl}amino-2-hydroxypropionate (5c) To a stirred 0 °C solution of **3a**[13] (556 mg, 1.0 mmol) in MeOH (5 ml) was added 1 N NaOH 1.2 ml, 1.2 mmol). After 2 h, the solution was warmed to ambient temperature gradually and stirred overnight. Addition of 1 N HCl (1.2 ml, 1.2 mmol) was followed by evaporation *in vacuo*. To the stirred 0 °C mixture of the residue and **4a** (183 g, 1.0 mmol) in CH_3CN (5 ml) were added triethylamine (0.14 ml, 1.0 mmol), HONB (180 mg, 1.0 mmol) and DCC (210 mg, 1.0 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 and the solution was washed with 5% $NaHCO_3$ and brine, dried over $MgSO_4$, and concentrated *in vacuo*. The residue was purified by silica gel preparative TLC with $CHCl_3$ and methanol (5 : 1) for development to afford **5c** (178 mg, 31%) as a white solid: mp 100—105 °C. IR (KBr): 1735, 1655 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.8—1.2 (m, 3H), 1.28 (t, 6H, J=6.6 Hz), 1.3—3.9 (m, 14H), 4.1—4.7 (m, 2H), 5.03 (quint, 1H, J=6.6 Hz), 6.95 (s, 1H), 7.3—8.2 (m, 8H). HPLC 93% (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 3.5 min). FABMS m/z: 594 (M+1). *Anal.* Calcd for $C_{31}H_{39}N_5O_7 \cdot 1/3CHCl_3$: C, 59.44; H, 6.26; N, 11.06. Found: C, 59.21; H, 6.25; N, 9.81.

5d—5j (except **5i**) were prepared by essentially the same procedure as used to prepare **5c**.

Isopropyl (2*RS*,3*S*)-3-{*N*-[(2*R*)-3-Morpholinocarbonyl-2-(1-naphthylmethyl)propionyl]-L-histidyl}amino-2-hydroxybutyrate (5d) (35% yield) as a white powder: mp 104—106 °C. IR (KBr): 1735, 1645 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.9—2.6 (m, 13H), 2.7—3.7 (m, 9H), 4.0—4.6 (m, 3H), 5.0—5.2 (m, 1H), 6.35—7.2 (m, 3H), 7.31 (d, 1H, J=7.6 Hz), 7.39 (t, 1H, J=7.6 Hz), 7.45—7.65 (m, 2H), 7.75 (d, 1H, J=8.2 Hz), 7.86 (d, 1H, J=8.2 Hz), 8.05 (d, 1H, J=8.2 Hz). HPLC 94% (elution time 3.8 min). FABMS m/z: 622 (M+1). *Anal.* Calcd for $C_{32}H_{41}N_5O_7 \cdot 1/4CHCl_3$: C, 60.76; H, 6.52; N, 10.98. Found: C, 60.63; H, 6.48; N, 10.71.

Isopropyl (2*S*,3*S*,4*S*)-3-{*N*-[(2*R*)-3-Morpholinocarbonyl-2-(1-naphthylmethyl)propionyl]-L-histidyl}amino-2-hydroxy-4-methylhexanoate (5e) (49% yield) as a white powder: mp 102—105 °C. $[\alpha]_D^{24.5}$ −26.6° (c=0.60, MeOH). IR (KBr): 1735, 1650 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.75—1.1 (m, 4H), 0.96 (d, 3H, J=7.1 Hz), 1.21 (dd, 6H, J=13.2, 6.0 Hz), 1.25—1.7 (m, 2H), 2.4—2.8 (m, 2H), 3.0—3.7 (m, 13H), 3.95—4.1 (m, 1H), 4.29 (d, 1H, J=1.7 Hz), 4.4—4.55 (m, 1H), 5.04 (quint, 1H, J=6.0 Hz), 6.83 (S, 1H), 6.92 (d, 1H, J=7.3 Hz), 7.30 (d, 1H, J=6.6 Hz), 7.35—7.6 (m, 5H), 7.74 (d, 1H, J=7.7 Hz), 7.86 (d, 1H, J=7.7 Hz), 8.04 (d, 1H, J=7.7 Hz). HPLC 96% (elution time 5.7 min); FABMS m/z: 650 (M+1). *Anal.* Calcd for $C_{35}H_{47}N_5O_7 \cdot 1/5CHCl_3$: C, 62.76; H, 7.06; N, 10.40. Found: C, 62.53; H, 7.01; N, 10.15.

***N*-Isopropyl (2*R*,3*S*)-3-{*N*-[(2*R*)-3-Morpholinocarbonyl-2-(1-naphthylmethyl)propionyl]-L-histidyl}amino-4-cyclohexyl-2-hydroxybutyramide (5f)** (42% yield) as a white solid: mp 115—121 °C. $[\alpha]_D^{24.5}$ −9.8° (c=0.55, MeOH). IR (KBr): 1645 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.75—1.05 (m, 4H), 1.14 (t, 6H, J=6.6 Hz), 1.25—1.85 (m, 9H), 2.3—3.7 (m, 16H), 3.96 (d, 1H, J=3.9 Hz), 4.0—4.15 (m, 2H), 4.55—4.65 (m, 1H), 6.88 (s, 1H), 6.92 (d, 1H, J=8.2 Hz), 6.99 (d, 1H, J=9.3 Hz), 7.28 (d, 1H, J=7.7 Hz), 7.37 (t, 1H, J=7.7 Hz), 7.45—7.6 (m, 3H), 7.73 (d, 1H, J=7.7 Hz), 7.85 (d, 1H, J=7.7 Hz), 8.05 (d, 1H, J=8.8 Hz). HPLC 94% (elution time 5.8 min). FABMS m/z: 689 (M+1). *Anal.* Calcd for $C_{38}H_{52}N_6O_6 \cdot 1/4CHCl_3$: C, 63.92; H, 7.33; N, 11.69. Found: C, 63.75; H, 7.21; N, 11.51.

Isopropyl (3*S*,4*S*)-4-{*N*-[(2*R*)-3-Morpholinocarbonyl-2-(1-naphthylmethyl)propionyl]-L-histidyl}amino-5-cyclohexyl-3-hydroxypentanoate

(5g) (40% yield) as a white solid: mp 98—102 °C. $[\alpha]_D^{24} -24.8°$ ($c=0.25$, MeOH). IR (KBr): 1730, 1640 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.7—2.1 (m, 19H), 2.23 (dd, 1H, $J=13.3$, 8.2 Hz), 2.33 (d, 1H, $J=6.0$ Hz), 2.5—2.8 (m, 1H), 3.0—4.0 (m, 14H), 4.55–4.65 (m, 1H), 5.02 (quint, 1H, $J=6.0$ Hz), 5.3—5.4 (m, 1H), 6.6—6.7 (m, 1H), 6.88 (s, 1H), 7.31 (d, 1H, $J=6.6$ Hz), 7.35—7.6 (m, 3H), 7.76 (d, 1H, $J=8.2$ Hz), 7.87 (d, 1H, $J=7.6$ Hz), 8.05 (d, 1H, $J=8.2$ Hz). HPLC 97% (elution time 9.4 min). FABMS *m/z*: 704 (M+1). *Anal.* Calcd for $C_{39}H_{53}N_5O_7 \cdot 1/4CHCl_3$: C, 64.25; H, 7.32; N, 9.55. Found: C, 63.98; H, 7.21; N, 9.43.

***N*-Isopropyl (3*S*,4*S*)-4-{*N*-[(2*R*)-3-Morpholinocarbonyl-2-(1-naphthylmethyl)propionyl]-L-histidyl}amino-5-cyclohexyl-3-hydroxypentanamide (5h)** (38% yield) as a white solid: mp 109—114 °C. $[\alpha]_D^{24.5} -12.1°$ ($c=0.78$, MeOH). IR (KBr): 1640 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.7—1.1 (m, 4H), 1.15 (d, 3H, $J=2.2$ Hz), 1.18 (d, 3H, $J=2.2$ Hz), 1.2—1.8 (m, 9H), 2.1—4.1 (m, 20H),4.5—4.6 (m, 1H), 6.45—6.55 (m, 1H), 6.65—6.75 (m, 1H), 6.91 (bs, 1H), 7.31 (d, 1H, $J=6.6$ Hz), 7.40 (t, 1H, $J=7.7$ Hz), 7.45—7.6 (m, 3H), 7.76 (d, 1H, $J=7.7$ Hz), 7.87 (d, 1H, $J=7.1$ Hz) 8.06 (d, 1H, $J=7.7$ Hz). HPLC 91% (elution time 5.5 min). FABMS, *m/z*: 703 (M+1). *Anal.* Calcd for $C_{39}H_{54}N_6O_6 \cdot 1/3CHCl_3$: C, 63.64; H, 7.38; N, 11.32. Found: C, 63.41; H, 7.29; N, 11.12.

Isopropyl (2*R*,3*S*)-3-{*N*-[(2*R*)-4-Morpholino-2-(1-naphthylmethyl)butyryl]-L-histidyl}amino-4-cyclohexyl-2-hydroxybutyrate (5i) A suspension of **18** (34.6 mg, 0.064 mmol) and 10% Pd on activated carbon (5 mg) in methanol (1 ml) was hydrogenated at atmospheric pressure overnight. After Pd on activated carbon was filtered out, the filtrate was evaporated *in vacuo*. To a stirred 0 °C solution of the residue and **16** (20 mg, 0.064 mmol) in DMF (1 ml) were added triethylamine (0.028 ml, 0.2 mmol) and DPPA (0.017 ml, 0.077 mmol). After stirring overnight, the mixture was dissolved in ethyl acetate and the ethyl acetate solution was washed with 5% $NaHCO_3$ and brine, dried over $MgSO_4$, and evaporated *in vacuo*. The residue was separated by silica gel preparative chromatography with $CHCl_3$ and methanol (5:1) for development to afford **5i** ($Rf=0.42$: 8.1 mg, 19%) as a white powder: mp 89—95 °C. $[\alpha]_D^{24.5} -40.0°$ ($c=0.40$, MeOH). IR (KBr): 1735, 1640 cm^{-1}, ^{1}H-NMR ($CDCl_3$) δ: 0.8—1.2 (m, 3H), 1.27 (t, 6H, $J=6.6$ Hz), 1.3—3.8 (m, 27H), 4.05—4.7 (m, 3H), 4.95 (quint, 1H, $J=6.6$ Hz), 6.8—7.0 (m, 1H), 7.37 (d, 1H, $J=7.1$ Hz), 7.4—7.7 (m, 3H), 7.87 (d, 1H, $J=8.8$ Hz), 8.02 (d, 1H, $J=7.7$ Hz), 8.28 (d, 1H, $J=9.3$ Hz), HPLC 94% (elution time 11.5 min). FABMS *m/z*: 676 (M+1). *Anal.* Calcd for $C_{38}H_{53}N_5O_6 \cdot 1/3CHCl_3$: C, 64.37; H, 7.52; N, 9.79. Found: C, 64.06; H, 5.21; N, 9.59.

Isopropyl (2*R*,3*S*)-3-{*N*-[(2*R*)-3-Morpholinocarbonyl-2-(1-naphthylmethyl)propionyl]-L-valyl}amino-4-cyclohexyl-2-hydroxybutyrate (5j) The synthesis of **5j** was carried out as described above for **5c** with **3b**. **5j** (10% yield) as a white solid: mp 81—84 °C. $[\alpha]_D^{24} -35.0°$ ($c=0.40$, MeOH). IR (KBr): 1735, 1645 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.86 (dd, 6H, $J=11.0$, 7.1 Hz), 0.9—1.2 (m, 3H), 1.28 (dd, 6H, $J=6.0$, 4.4 Hz), 1.4—1.9 (m, 9H), 2.15—2.3 (m, 2H), 2.68 (dd, 1H, $J=16.5$, 8.2 Hz), 3.1—3.7 (m, 12H), 4.07 (s, 1H), 4.12 (dd, 1H, $J=8.2$, 5.5 Hz), 4.4—4.5 (m, 1H), 5.05 (quint, 1H, $J=6.0$ Hz), 6.19 (d, 1H, $J=9.3$ Hz), 6.66 (d, 1H, $J=8.2$ Hz), 7.33 (d, 1H, $J=6.7$ Hz), 7.35—7.6 (m, 3H), 7.75 (d, 1H, $J=8.2$ Hz), 7.86 (d, 1H, $J=7.7$ Hz), 8.08 (d, 1H, $J=7.7$ Hz). HPLC 98% (elution time 19.7 min). FABMS *m/z*: 652 (M+1). *Anal.* Calcd for $C_{37}H_{53}N_3O_7 \cdot 1/5CHCl_3$: C, 66.12; H, 7.94; N, 6.22. Found: C, 65.89; H, 7.85; N, 6.02.

Biological Methods. *In Vitro* Renin Assay A 25 μl aqueous solution of human renin (20—30 ng of Ang I/ml per hour) 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 that formed after 15 min of incubation was measured by radioimmunoassay. 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 produced was measured by radioimmunoassay.

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