Renin Inhibitors. II. Synthesis and Structure–Activity Relationships of N-Terminus Modified Inhibitors Containing a Homostatine Analogue[1)]

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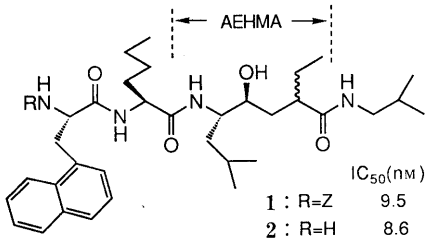
The synthesis and structure–activity relationships of N-terminus modified renin inhibitors containing the homostatine analogue, (2*RS*,4*S*,5*S*)-5-amino-2-ethyl-4-hydroxy-7-methyloctanoic acid, are described. The compounds having a 3-alkyl (or aryl)sulfonylpropionyl residue at the N-terminus are found to be potent inhibitors which contain two amino acids. (2*RS*,4*S*,5*S*)-*N*-Isobutyl-5-[*N*-[(2*S*)-3-ethylsulfonyl-2-(1-naphthylmethyl)propionyl]-L-norleucyl]-amino-2-ethyl-4-hydroxy-7-methyloctanamide (20) has an IC_{50} of 0.5 nM against human plasma renin and the oral bioavailability of 20 is 0.73% in rats. Interaction between renin and the N-terminus of 1 and 20 is discussed in molecular modeling studies.

Keywords renin inhibitor; antihypertensive agent; homostatine analogue; sulfonemethylene isostere; structure–activity relationship

The renin-angiotensin system plays a central role in the regulation of blood pressure. Renin, an aspartic protease, is the rate limiting enzyme in this cascade and is highly specific for its substrate. Inhibitors of angiotensin converting enzyme, another enzyme of this cascade, are now widely used for hypertensive patients; however adverse effects are observed because of its lower substrate specificity. Therefore, a large number of human renin inhibitors have been investigated as higher quality antihypertensive agents.[2)] In our preceding paper, compounds **1** and **2** (Fig. 1), which contained the homostatine analogue (2*RS*,4*S*,5*S*)-5-amino-2-ethyl-4-hydroxy-7-methyloctanoic acid (AEHMA), were shown to be potent renin inhibitors *in vitro*.[1)] However, they had low aqueous solubility and low oral bioavailability probably due to their peptidic character (they contained three amino acids). Therefore, in order to overcome these defects, we tried to modify the N-terminal segment of **1**.[3)] In this paper, we report the syntheses and structure–activity relationships of N-terminus modified renin inhibitors which have the homostatine analogue AEHMA. In addition, the computer modeling studies of the interaction between these inhibitors and renin are also described.



AEHMA : 5-amino-2-ethyl-4-hydroxy-7-methyloctanoic acid

Fig. 1

Synthesis The compounds prepared for this study are shown in Table I. Syntheses of compounds **5**—**8** are outlined in Chart 1. Z–Nle–AEHMA isobutylamide (**4**), synthesized by coupling of Z–Nle–OH with AEHMA isobutylamide (**3**),[4)] was hydrogenolized and the resulting amine was condensed with 3-(1-naphthyl)propionic acid using diphenyl phosphorazidate (DPPA) to yield **5** in 81% yield. The reductive alkylation of **2** with formaldehyde or glutaraldehyde using sodium cyanoborohydride ($NaBH_3CN$) in methanol gave **6** or **8** in 86%, 45% yield, respectively. Compound **7** was prepared in a similar manner using benzaldehyde and formaldehyde successively in 88% yield.

Compounds **11**, **12**, **14***R*,*S* and **17** were prepared from 2-ethoxycarbonyl-3-(1-naphthyl)propionic acid (**9**) as shown in Chart 2. The syntheses of intermediates **10** and **13***R*,*S* were described previously.[4a,b)] Deprotection of **10** with trifluoroacetic acid (TFA) followed by coupling with **3** using DPPA gave **11** in 93% yield. Sodium borohydride ($NaBH_4$) reduction of **11** afforded **12** in 92% yield. After acetylation of **13***R*, deprotection and coupling of the resulting acid with **3** gave **14***R* in 86% yield. Compound **14***S* was prepared from **13***S* by the same manner in 92% yield. Compound **17** was obtained from **15** and **16** with DPPA method. Compound **15** was prepared from **9** as

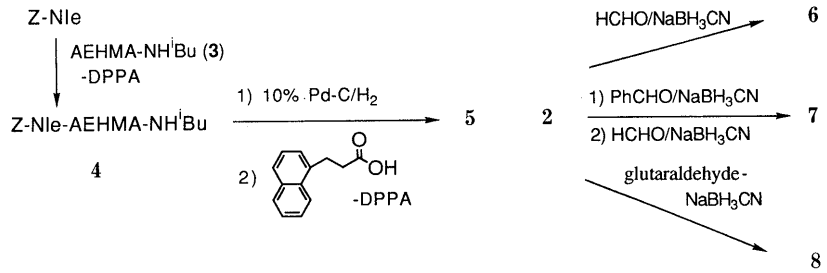


Chart 1

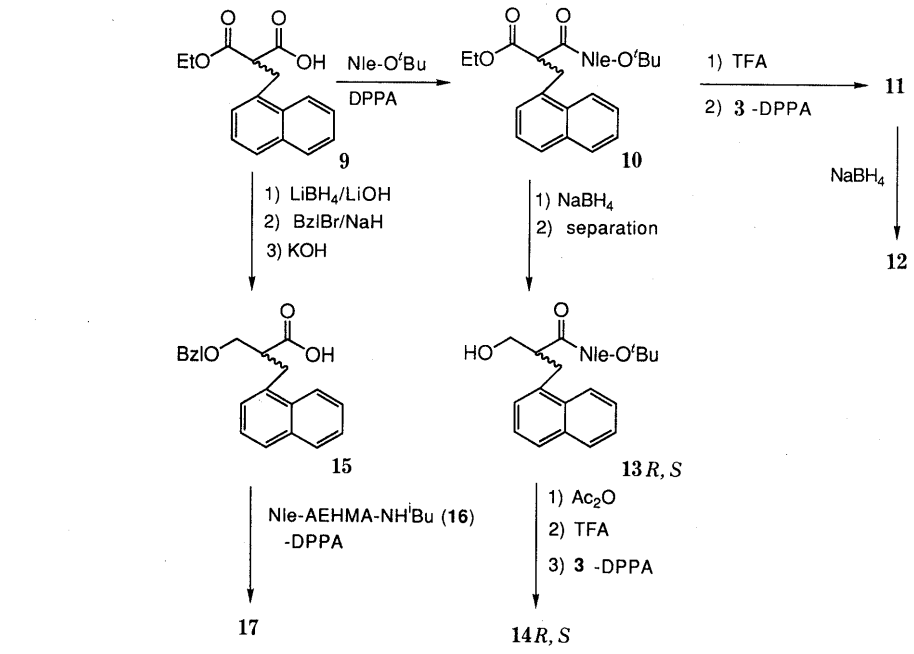
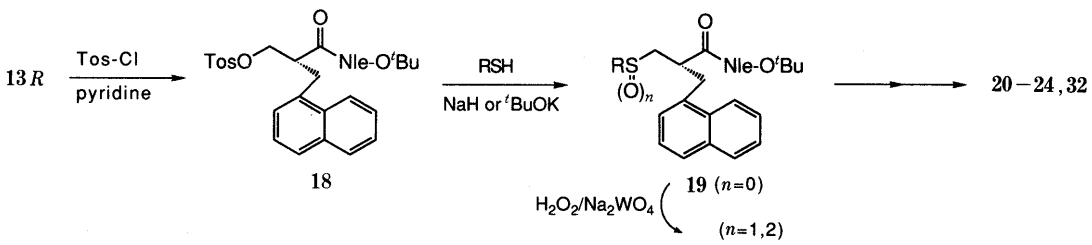


Chart 2



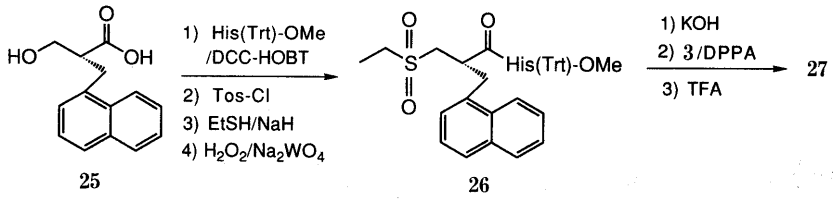


Chart 3

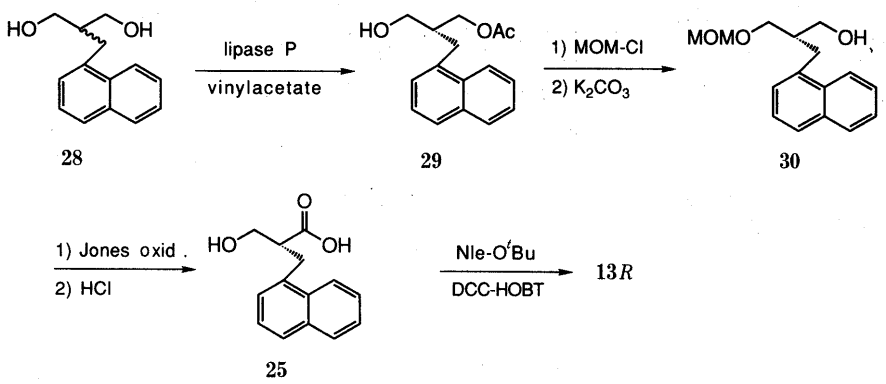
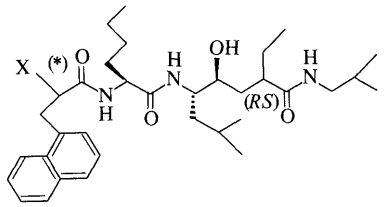


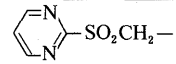
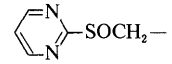
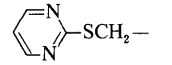
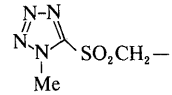
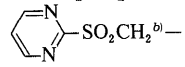
Chart 4

follows: 1) selective reduction with lithium borohydride,[5] 2) dibenzylation of the hydroxyl and carboxyl groups, and 3) hydrolysis of the ester.

The syntheses of compounds **20**—**24** and **32** followed the methods described by us previously,[4a,b] and compound **27** was prepared in a similar manner as shown in Chart 3. After (2*R*)-2-hydroxymethyl-3-(1-naphthyl)propionic acid (**25**) prepared by the route shown in Chart 4 was condensed with His(Trt)–OMe using the dicyclohexylcarbodiimide (DCC)–hydroxybenzotriazole (HOBT) method,

TABLE I. Structures and Renin Inhibitory Activities



No.	X	(*)	IC_{50} (nM)	No.	X	(*)	IC_{50} (nM)
1	ZNH-	(*S*)	9.5	**21**		(*S*)	0.17
2	H_2N-	(*S*)	8.6				
5	H		1300	**22**		(*S*)	0.8
6	Me_2N-	(*S*)	20				
7	Me(Bzl)N-	(*S*)	230				
8		(*S*)	58	**23**		(*S*)	1.6
11	EtO_2C-	(*RS*)	30				
12	$HOCH_2$-	(*RS*)	38	**24**		(*S*)	0.96
14*R*	$AcOCH_2$-	(*R*)	3.3				
14*S*	$AcOCH_2$-	(*S*)	2200				
17	$BnOCH_2$-	(*RS*)	75	**27**	$EtSO_2CH_2$-[a]	(*S*)	0.5
20	$EtSO_2CH_2$-	(*S*)	0.5	**32**		(*S*)	2.5

a) P_2 is His instead of Nle. *b*) P_3 is benzyl instead of 1-naphthylmethyl.

tosylation followed by substitution with ethyl mercaptan and oxidation by hydrogen peroxide in the presence of sodium tungstate gave **26** in 55% yield from **25**. Hydrolysis of ester **26** followed by coupling of the resulting acid with **3** and deprotection by TFA afforded **27** in 83% yield from **26**.

The stereochemistry of the asymmetric carbon in the propionyl group of **14** is important for potency against renin, and *R*-configuration is required (compound **14*R***; 3.3 nM *vs.* compound **14*S***; 2200 nM). The *R*-configuration at this position corresponds to the configuration of the L-amino acid. We already reported the enantioselective syntheses of key intermediate **25** by Evans's method[4a,b] and commercially available lipase.[6] In this paper, we described the chemoenzymatic synthesis using lipase P (Chart 4). Mono-acetylation of 2-(1-naphthylmethyl)-1,3-propanediol (**28**), which was prepared from 1-naphthaldehyde and diethyl malonate *via* 1-naphthylmethylene malonate, catalyzed by lipase P using vinyl acetate[7] as the acyl donor proceeded enantioselectively to afford (2*R*)-2-(1-naphthylmethyl)-1,3-propanediol-1-acetate (**29**) in a 86% ee and a 93% chemical yield.[8] Treatment of **29** with chloromethyl methyl ether followed by hydrolysis with potassium carbonate gave **30** in 91% yield. After Jones oxidation of **30**, deprotection with hydrochloric acid afforded crude **25**. A single recrystallization yielded **25** in a 96% ee and a 50% chemical yield.[9]

Structure–Activity Relationships The renin inhibitory potencies of compounds synthesized in this study were measured with human plasma renin by the method described previously,[1] and IC_{50} values are summarized in Table I.

Elimination of the amino group at position 2 of the 3-(1-naphthylmethyl)propionic acid residue decreased potency dramatically (compound **2** *vs.* compound **5**). A substituent at this position may be essential for showing inhibitory activity against renin.[10] *N*-Alkylation of 3-(1-naphthyl)-alanine in **2** also caused the loss of potency (compounds **6**—**8**), especially in compound **7** which was 26-fold less potent than **2**. Compound **6** was stable in rat liver homogenate and had improved aqueous solubility (43.7 μg/ml in saline, Table IV). However, in our *in vivo* study on the metabolic fate of **6** using ^{13}C-labeled compound, the *N*-methyl group was oxidized and excreted in expired air as carbon dioxide.[11] Therefore, further investigation at N-terminus was carried out. Replacement of the amino group in **2** with an ethoxycarbonyl group or a hydroxymethyl group slightly decreased potency (compounds **11** and **12**). Although acetylation of **12** gave

TABLE II. Characterization of Renin Inhibitors

No.	t_R (min)[a]	Purity (%)	Formula	FAB-MS[d]	
				Calcd	Found
5	5.60, 5.81	98	$C_{34}H_{53}N_3O_4$	568.4114	568.4137
6	5.91, 6.28[b]	93	$C_{36}H_{59}N_4O_4$	611.4536	611.4534
7	9.90, 11.08[b]	76	$C_{42}H_{62}N_4O_4$	687.4849	687.4857
8	8.52, 9.40[b]	71	$C_{39}H_{62}N_4O_4$	651.4849	651.4847
11	6.12, 6.45, 6.74	97	$C_{37}H_{57}N_3O_6$	640.4326	640.4314
12	5.16, 5.45, 5.66, 6.02	94	$C_{35}H_{55}N_3O_5$	598.4220	598.4194
14*R*	5.67, 5.99	88	$C_{37}H_{57}N_3O_6$	640.4326	640.4305
14*S*	6.06, 6.32	87	$C_{37}H_{57}N_3O_6$	640.4326	640.4314
17	8.89, 9.56	86	$C_{42}H_{61}N_3O_5$	688.4690	688.4714
20	4.76, 5.00	95	$C_{37}H_{59}N_3O_6S$	674.4203	674.4217
21	6.42, 6.77	88	$C_{39}H_{57}N_5O_4S$	692.4210	692.4214
22	4.88, 5.18	93	$C_{39}H_{57}N_5O_5S$	708.4159	708.4151
23	4.56, 4.78	94	$C_{39}H_{57}N_5O_6S$	724.4108	724.4100
24	5.06, 5.34	77	$C_{37}H_{57}N_7O_6S$	728.4169	728.4171
27	4.95, 5.00[c]	88	$C_{37}H_{55}N_5O_6S$	698.3951	698.3962
32	4.08	97	$C_{35}H_{55}N_5O_6S$	674.3951	674.3959

a) See the experimental section for conditions. *b*) Solvent, MeOH : 10 mM $AcONH_4$ = 7 : 1. *c*) Solvent, MeOH : 10 mM $AcONH_4$ = 5 : 1. *d*) For $[M+H]^+$.

a more potent inhibitor (compound **14***R*), benzylation afforded a 2-fold decrease in potency (compound **17**). The result suggested that the carbonyl of the acetyl group in **14** formed a hydrogen bond with the enzyme. Replacement of the 2(*R*)-3-acetoxypropionyl group with 2(*S*)-isomer abolished activity (compound **14***S*). Substitution of the acetoxy group in **14***R* with the ethylsulfonyl group, which was thought to form a hydrogen bond with the enzyme like the acetoxy group, afforded a 6-fold increase in potency (compound **20**).[12] Furthermore, replacement of the ethyl group in **20** with the 2-pyrimidinyl group gave the most potent inhibitor in this study (compound **21**, IC_{50}: 0.17 nM). The corresponding sulfoxide (**22**) and sulfide (**23**) were less potent than the sulfone. The compound containing histidine at P_2 instead of norleucine was equipotent (compound **20** *vs.* compound **27**). Substitution of the naphthylmethyl group at P_3 with the benzyl group gave a 15-fold decrease in potency (compound **21** *vs.* compound **32**). This result was consistent with that reported previously.[1]

Modeling Studies Although the three dimensional structure of renin[13] or renin and its inhibitors[14] were investigated by X-ray analysis, the coordinate data have not yet been shown. To investigate the interaction between renin and its inhibitors, we constructed a model of renin referring to the X-ray structure of pepsin[15] which has better sequence homology to renin than any other aspartic proteinases, by virtue of BIOCES[E],[16] a computer aided

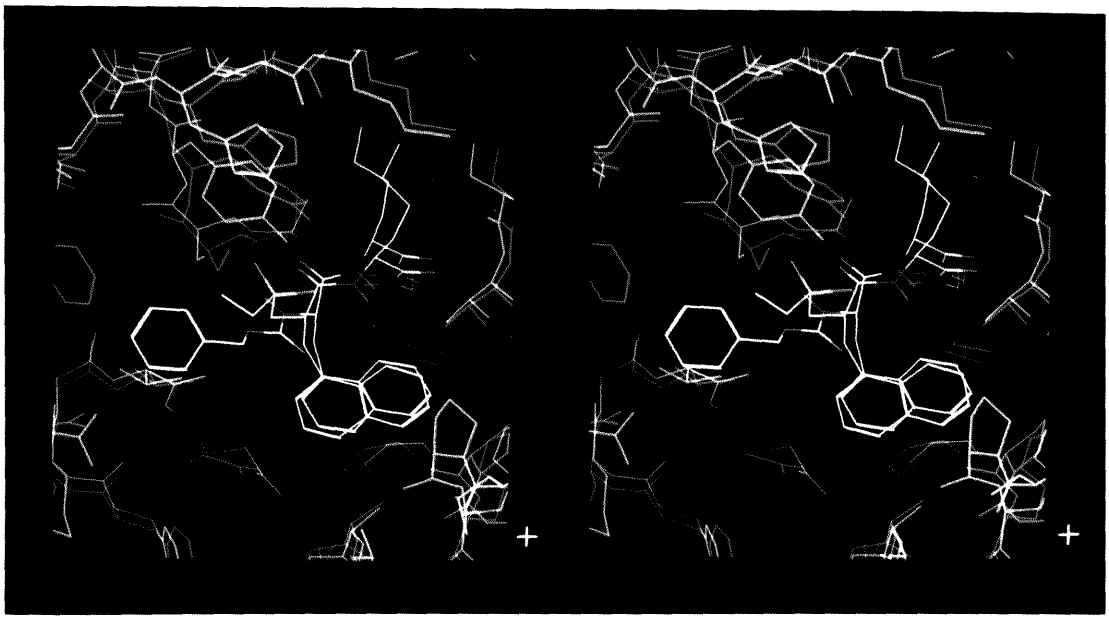


Fig. 2. Stereo View of **1** with Renin (Yellow) and **20** with Renin (Cyan)
Inhibitors are colored as follows; carbon (white), oxygen (red), nitrogen (blue), sulfur (yellow).

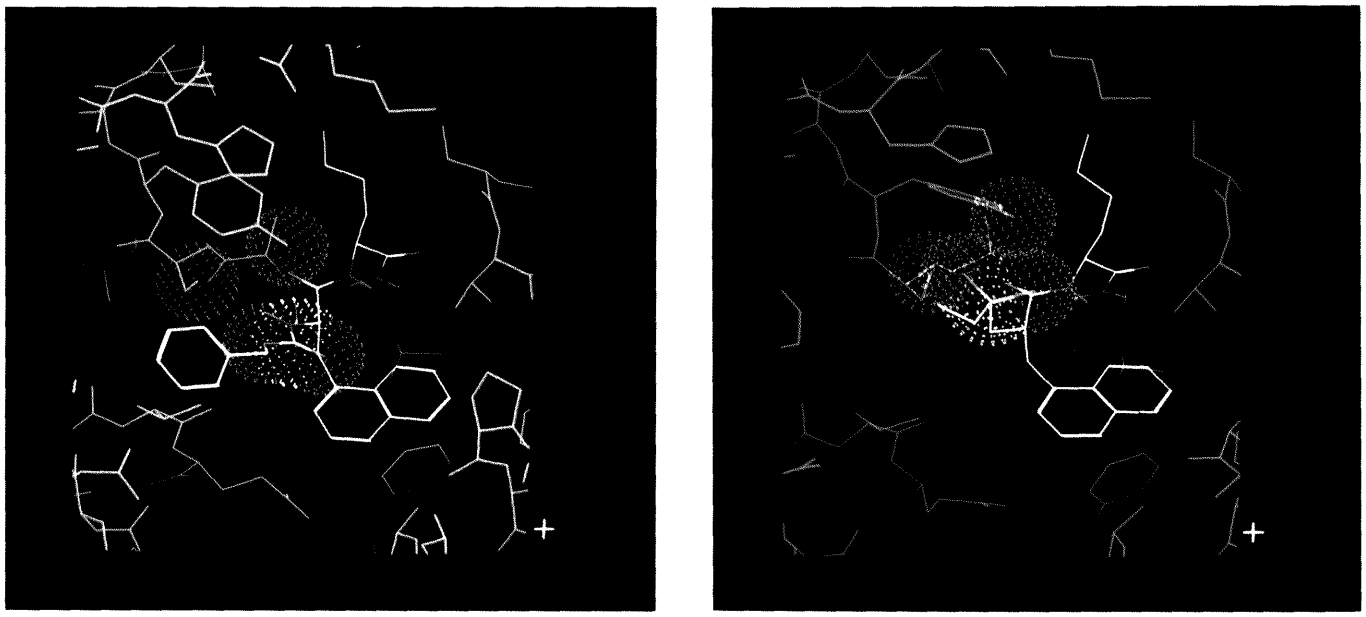


Fig. 3. Dot-Surface Display of Van Der Waals Radii of Atoms around N-Terminus of **1** (Left) or **20** (Right)
Atoms are colored as follows; oxygen (red), carbon (white), sulfur (yellow).

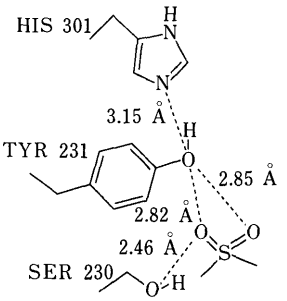


Fig. 4. Schematic Representation of Distances between the Sulfonyl Group of **20** and Surrounding Residues of Renin

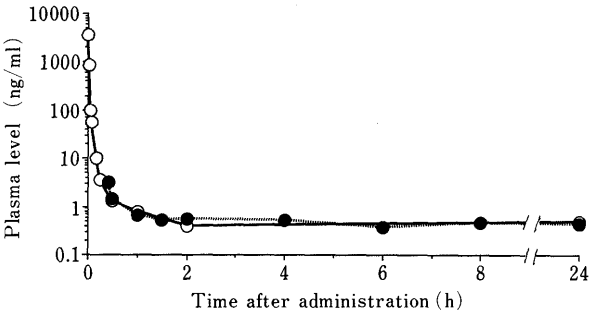


Fig. 5. Plasma Levels of Compound **20** in Rats after Administrations of 0.35 mg/kg i.v. (—○—) and 8.4 mg/kg *p.o.* (---●---)

Blood samples were collected at 20 s, 1, 3, 5, 10, 15, 30 min, 1, 2, and 24 h (i.v.), and 15, 30 min, 1, 1.5, 2, 4, 6, 8, and 24 h (*p.o.*).

modeling system. Docking of an inhibitor to the model enzyme was proceeded by referring to the structure of the rhizopuspepsin-inhibitor complex.[17] The modeled enzyme was superimposed on rhizopuspepsin and then inhibitor **20** was fitted onto the inhibitor of rhizopuspepsin. Inhibitor **1** was treated as in the same way. The enzyme–inhibitor complexes were energetically minimized and subjected to analysis on graphic display.

Comparison of the complex structures of **1** and renin or **20** and renin shows a fairly good overlap of the two inhibitors except at the N-termini (Fig. 2). In the case of **20**, sulfonyl group gains good van der Waals contact with side chains of Ser^{230} and Tyr^{231}, while the oxycarbonyl group of **1** cannot make favorable interactions with those residues (Fig. 3). One of the sulfonyl oxygens of **20** may form a hydrogen bond with the hydroxyl group of Ser^{230} as the distance between the two oxygen atoms is suitable for hydrogen bonding and the hydrogen is in a favorable direction (Fig. 4). However, the hydroxyl group of Tyr^{231} may not form a hydrogen bond with the sulfonyl oxygen of **20** because the hydrogen atom of the hydroxyl group of Try^{231} points to $N^{\varepsilon 2}$ of His^{301}, although the hydroxyl oxygen of Tyr^{231} and the oxygen atom of the sulfonyl group are in favorable distance for hydrogen bonding.

The result of the modeling studies is consistent with the experimental data of IC_{50} values (Table I). Modification of N-terminus of **1** with the ethylsulfonyl group could bring about better van der Waals contact and hydrogen bonding interactions, so that **20** could show 20-fold more potent inhibitory activity than **1**.

Enzyme Specificity and Aqueous Solubility High enzyme specificity is a factor for more useful renin inhibitors. Compounds **20** and **21** inhibited cathepsin D and pepsin as well as renin, however compound **27** containing histidine at P_2 had good selectivity for renin (Table III). This result was consistent with that reported by the Kissei group[18] and Abbott group.[19]

Table III. Enzyme Specificity

No.	Renin (Human)	IC_{50} (nM) Cathepsin D (Bovine)	Pepsin (Porcine)
20	0.50	15	260
21	0.17	16	230
27	0.50	8500	(25% at 100 μM)

Table IV. Aqueous Solubility

No.	Solubility (μg/ml in saline)[a]
1	<0.07
6	43.7[b]
20	0.37
21	0.09

a) Determined by enzyme inhibition assay. *b*) HCl salt.

Aqueous solubilities were determined for inhibitors **6**, **20** and **21** (Table IV). Compound **6** was considerably, and compounds **20** and **21** were slightly, more soluble than the lead compound **1**.

Oral Bioavailability of Inhibitor 20 in Rats In order to determine the oral bioavailability of **20** in rats, preliminary experiments ($n=2$) were carried out (Fig. 5). We selected compound **20** because it was superior in terms of ease of synthesis and His of compound **27** was thought to augment biliary excretion and adversely affect oral absorption.[19] The compound was dissolved in dimethyl sulfoxide (DMSO)/saline or DMSO/water and was administered to conscious, fed rats either intravenously (i.v.) or orally (*p.o.*). Plasma levels of **20** were determined by enzyme inhibition assay after extraction with methanol. After an i.v. dose of 0.35 mg/kg of **20**, the plasma level was 3506.7 ± 195.8 ng/ml at 20 s, followed by 101.3 ± 6.5 ng/ml at 3 min and 3.7 ± 0.1 ng/ml at 15 min. About 99% of the initial dose was eliminated from circulation within the first 15 min ($t_{1/2}$: 1.5 min). When given orally, a maximum plasma level of 3.20 ± 1.40 ng/ml was observed at 15 min at a dose of 8.4 mg/kg. Plasma levels fell rapidly by 1 h (0.66 ± 0.22 ng/ml) ($t_{1/2}$: 21 min). Values of AUC_{0-24} at 0.35 mg/kg i.v. and 8.4 mg/kg *p.o.* were 67.9 ± 0.3 and 11.9 ± 0.5 ng·h/ml, respectively, and the oral bioavailability was calculated as 0.73%.

Conclusion

Structure–activity studies directed toward improving the aqueous solubility and the oral bioavailability of starting compound **1** led to compound **20**. Although compound **20** was a potent renin inhibitor *in vitro*, its oral bioavailability was still poor. Modification of the physicochemical properties of **20** were required to achieve the desired profile. Further investigation is in progress.

Experimental

Melting points were determined with a Yanagimoto melting point apparatus and uncorrected. Infrared (IR) spectra were measured with a

Hitachi 270-30 infrared spectrophotometer. Proton nuclear magnetic resonance (^{1}H-NMR, 300 MHz) spectra were recorded with a Varian VXR-300 spectrometer in deuteriochloroform ($CDCl_3$). Chemical shifts are reported relative to residual protons of deuterated NMR solvents. Fast atom bombardment mass spectra (FAB-MS) were obtained with a JEOL JMS-DX 300 mass spectrometer. Optical rotations were determined with a Horiba SEPA-200 high-sensitivity polarimeter. Analytical high-performance liquid chromatography (HPLC) was carried out on Hitachi L-6200 system, using packed column Inertsil ODS (5 μm, 4.6 × 250 mm), and MeOH–water (90 : 10) elutions unless otherwise stated (flow rate, 1 ml/min), with ultraviolet (UV) detection at 254 nm (Hitachi L-4000 UV detector). Column chromatography was done on Kieselgel 60 (E. Merck, 70—230 mesh). The organic solutions were dried over $MgSO_4$ before vacuum evaporation.

(2*RS*,4*S*,5*S*)-*N*-Isobutyl-5-[*N*-[3-(1-naphthyl)propionyl]-L-norleucyl]-amino-2-ethyl-4-hydroxy-7-methyloctanamide (5) To a solution of *N*-benzyloxycarbonyl-L-norleucine (100 mg, 0.38 mmol), AEHMA isobutylamide (**3**) hydrochloride (105 mg, 0.34 mmol) and DPPA (54 mg, 0.41 mmol) in dimethylformamide (DMF, 1 ml) was added triethylamine (41 mg, 0.85 mmol) at −10 °C. The mixture was stirred for 2 h at −10 °C, and further stirred at room temperature overnight. The reaction mixture was diluted with AcOEt and washed with 1 N HCl, saturated $NaHCO_3$, water and brine. Drying followed by evaporation and purification by silica gel chromatography ($CHCl_3$: MeOH = 100 : 1) afforded **4** as a colorless powder in 69.2% yield, mp 160.0—163.0 °C, $[\alpha]_D^{20}$ −18.6° (c = 1.02, $CHCl_3$). IR (KBr): 3316, 1713, 1647 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.78—1.00 (18H, m), 1.15—1.92 (14H, m), 2.13—2.35 (1H, m), 2.93—3.20 (5/2H, m), 3.55—3.70 (1H, m), 3.78—4.04 (3/2H, m), 4.04—4.17 (1H, m), 5.10 (2H, s), 5.15—5.32 (1H, br), 5.72—5.85 (1H, m), 6.05—6.20 (1H, br), 7.22—7.42 (5H, m). FAB-MS m/z: $[M+H]^+$ Calcd for $C_{29}H_{50}N_3O_5$: 520.3751. Found: 520.3746.

A suspension of **4** (40 mg, 0.077 mmol) and 10% Pd–C (20 mg) in EtOH (2 ml) was stirred under hydrogen atmosphere for 2 h at room temperature. The catalyst was filtered off and the filtrate was concentrated. The residue was dissolved in DMF (1 ml), and then 3-(1-naphthyl)propionic acid (17 mg, 0.111 mmol) were added at −10 °C. Compound **5** was prepared by a procedure similar to that described for **4**, and was chromatographed on silica gel with $CHCl_3$–MeOH (100 : 1); 81.2% yield as a colorless solid, mp 188.0—191.0 °C. ^{1}H-NMR ($CDCl_3$) δ: 0.78—1.04 (18H, m), 1.04—1.90 (14H, m), 2.14—2.26 (1/2H, m), 2.26—2.39 (1/2H, m), 2.55—2.70 (2H, m), 2.95—3.20 (2H, m), 3.30 (1/2H, br d, J = 3.1 Hz), 3.33—3.49 (2H, m), 3.52—3.68 (1H, m), 3.78—3.97 (1H, m), 4.24 (1/2H, m), 4.32—4.44 (1H, m), 5.68 (1/2H, t, J = 5.4 Hz), 5.98 (1/2H, d, J = 9.5 Hz), 7.28—7.42 (2H, m), 7.42—7.56 (2H, m), 7.72 (1H, d, J = 7.8 Hz), 7.85 (1H, m), 8.02 (1H, t, J = 8.0 Hz).

(2*RS*,4*S*,5*S*)-*N*-Isobutyl-5-[*N*-[*N*,*N*-dimethyl-3-(1-naphthyl)-L-alanyl]-L-norleucyl]amino-2-ethyl-4-hydroxy-7-methyloctanamide (6) A solution of **2** (6.6 mg, 0.011 mmol), and formaldehyde (33% solution in water, 4.0 μl, 0.053 mmol) in MeOH (0.3 ml) was acidified with 0.1 N HCl to pH 4 and a solution of sodium cyanoborohydride (3.4 mg, 0.055 mmol) in MeOH (0.2 ml) was added. The mixture was stirred for 3 h at room temperature and acidified to pH 2 with 50% acetic acid. The resulting mixture was adjusted to pH 9 with saturated $NaHCO_3$ and extracted with AcOEt. The organic layer was washed with saturated $NaHCO_3$, water and brine. Drying and evaporation gave **6** (5.6 mg, 86.0%). Compound **6** was used as a hydrochloride salt prepared by treatment with 1 N HCl (1 eq) in MeOH and evaporation, mp 125.0—127.0 °C. ^{1}H-NMR ($CDCl_3$) δ: 0.68—0.92 (15H, m), 1.07—1.90 (17H, m), 2.05—2.38 (7H, m), 2.93 (2H, m), 3.05 (1H, m), 3.35 (1H, m), 3.45—3.60 (2H, m), 3.80 (1H, m), 4.24 (1H, m), 7.21—7.52 (4H, m), 7.60—7.85 (2H, m), 8.05 (1H, m).

(2*RS*,4*S*,5*S*)-*N*-Isobutyl-5-[*N*-[*N*-benzyl-*N*-methyl-3-(1-naphthyl)-L-alanyl]-L-norleucyl]amino-2-ethyl-4-hydroxy-7-methyloctanamide (7) A solution of **2** (17.7 mg, 0.030 mmol) and benzaldehyde (16.1 mg, 0.15 mmol) in MeOH (0.5 ml) was acidified with 0.1 N HCl to pH 4, and then a solution of $NaBH_3CN$ (9.5 mg, 0.15 mmol) in MeOH (0.2 ml) was added. The same manner described for **6** gave *N*-benzyl derivative (16.8 mg, 82.1%) of **2**. The title compound was prepared from *N*-benzyl compound (7.4 mg, 0.01 mmol) and formaldehyde (4 ml, 0.05 mmol) with $NaBH_3CN$ (3.6 mg, 0.06 mmol) by a procedure similar to that described for **6**; yield 7.6 mg (quant.) as a hydrochloride salt, mp 109.0—114.0 °C. ^{1}H-NMR (CD_3OD) δ: 0.65—1.10 (18H, m), 1.10—1.95 (14H, m), 2.19 (1/2H, m), 2.37 (1/2H, m), 2.65—3.12 (5H, m), 3.35—4.65 (8H, m), 7.28—7.65 (9H, m), 7.78—7.98 (2H, m), 8.02 (1/2H, m), 8.12 (1/2H, m).

(2*RS*,4*S*,5*S*)-*N*-Isobutyl-5-[*N*-[(2*S*)-3-(1-naphthyl)-2-piperidinopropionyl]-L-norleucyl]amino-2-ethyl-4-hydroxy-7-methyloctanamide (8) The title compound was prepared from **2** (100 mg, 0.172 mmol), glutaraldehyde (25% solution in water, 68 μl, 0.172 mmol), and $NaBH_3CN$ (76 mg, 1.20 mmol) by a procedure similar to that described for **6**, and was chromatographed on silica gel with $CHCl_3$–MeOH (40 : 1); yield 75.2 mg (70.2%). It was used as a hydrochloride salt, mp 114.0—118.0 °C. ^{1}H-NMR ($CDCl_3$) δ: 0.78—1.02 (18H, m), 1.10—2.10 (20H, m), 2.23 (1H, m), 2.47—2.75 (3H, m), 2.80—3.50 (4H, m), 3.50—4.00 (5H, m), 4.30 (1H, m), 5.76 (1/2H, t, J = 7.2 Hz), 5.97 (1/2H, br), 6.38 (1/2H, d, J = 8.3 Hz), 6.75 (1/2H, d, J = 9.0 Hz), 7.10—7.60 (4H, m), 7.70 (1H, m), 7.85 (1H, m), 8.08 (1/2H, m), 8.16 (1/2H, m).

(2*RS*,4*S*,5*S*)-*N*-Isobutyl-5-[*N*-[(2*RS*)-2-ethoxycarbonyl-3-(1-naphthyl)-propionyl]-L-norleucyl]amino-2-ethyl-4-hydroxy-7-methyloctanamide (11) To a solution of **10** (51.6 mg, 0.117 mmol) in CH_2Cl_2 (0.4 ml) was added TFA (0.4 ml). The solution was stirred for 2 h at room temperature and evaporated. The residue was dissolved in DMF (0.5 ml), and then triethylamine (33 μl, 0.24 mmol), DPPA (33 μl, 0.15 mmol) and a solution of AEHMA isobutylamide hydrochloride (54 mg, 0.18 mmol) in DMF (0.8 ml) were added at −10 °C. The mixture was stirred for 1 h at −10 °C and further stirred at 5 °C overnight. The mixture was diluted with AcOEt and washed with 5% $KHSO_4$, 4% $NaHCO_3$ and brine. Drying followed by evaporation and purification by flash chromatography ($CHCl_3$: MeOH = 100 : 1) afforded **11** (69.7 mg, 93.3%) as a colorless solid, mp 143.0—147.0 °C. ^{1}H-NMR ($CDCl_3$) δ: 0.70—1.95 (35H, m), 2.20 (1/2H, m), 2.29 (1/2H, m), 2.88—3.20 (3H, m), 3.55—3.99 (5H, m), 4.10 (2H, m), 4.29 (1H, m), 7.30—7.42 (2H, m), 7.45—7.60 (2H, m), 7.74 (1H, m), 7.85 (1H, d, J = 8.2 Hz), 8.03 (1H, d, J = 8.2 Hz).

(2*RS*,4*S*,5*S*)-*N*-Isobutyl-5-[*N*-[(2*RS*)-2-(hydroxymethyl)-3-(1-naphthyl)-propionyl]-L-norleucyl]amino-2-ethyl-4-hydroxy-7-methyloctanamide (12) To a solution of **11** (43 mg, 0.067 mmol) in EtOH (1.6 ml) was added $NaBH_4$ (50 mg, 1.32 mmol), and the mixture was stirred for 2 h at room temperature. After neutralization with 3.5 N acetic acid (0.2 ml), the mixture was concentrated. The residue was partitioned between AcOEt and water, and then the organic layer was washed with water and brine. Drying followed by evaporation afforded **12** (37.3 mg, 92.8%) as a colorless solid, mp 115.0—123.0 °C. ^{1}H-NMR ($CDCl_3$) δ: 0.60—1.02 (18H, m), 1.05—1.95 (14H, m), 2.30 (1/2H, m), 2.31 (1/2H, m), 2.88 (1H, m), 2.95—3.38 (4H, m), 3.40—4.10 (6H, m), 4.16 (1H, m), 5.64 (1/2H, d, J = 7.2 Hz), 5.85—6.20 (1H, m), 5.22—5.35 (1/2H, m), 7.85 (1H, m), 7.90—8.10 (1H, m).

(2*RS*,4*S*,5*S*)-*N*-Isobutyl-5-[*N*-[(2*R*)-2-(acetoxymethyl)-3-(1-naphthyl)-propionyl]-L-norleucyl]amino-2-ethyl-4-hydroxy-7-methyloctanamide (14*R*) To a solution of *N*-[(2*R*)-2-(hydroxymethyl)-3-(1-naphthyl)propionyl]-L-norleucine *tert*-butyl ester (**13*R***, 23.0 mg, 0.058 mmol) in pyridine (0.5 ml) was added acetic anhydride (0.1 ml) at room temperature. After 3 h, water (0.1 ml) was added and the solution was concentrated. The residue was dissolved in AcOEt, and washed with water and brine. After evaporation, the residue was dissolved in CH_2Cl_2 (0.5 ml) and TFA (0.5 ml) was added. The mixture was stirred for 2 h at room temperature and concentrated. The residue was dissolved in DMF (0.5 ml), and then triethylamine (16 μl, 0.12 mmol), DPPA (16 μl, 0.075 mmol) and a solution of AEHMA isobutylamide hydrochloride (27 mg, 0.086 mmol) and triethylamine (16 μl, 0.12 mmol) in DMF (0.8 ml) were added at −10 °C. The mixture was stirred at −10 °C for 30 min and further stirred at 5 °C overnight. The reaction mixture was diluted with AcOEt and washed with 5% $KHSO_4$, 4% $NaHCO_3$ and brine. Drying followed by evaporation and purification by flash silica gel chromatography ($CHCl_3$: MeOH = 50 : 1) gave **14*R*** (31.8 mg, 86.3%) as a colorless solid, mp 186.0—189.0 °C. ^{1}H-NMR ($CDCl_3$) δ: 0.75—1.00 (18H, m), 1.16—1.92 (14H, m), 2.03 (3H, s), 2.19 (1/2H, m), 2.29 (1/2H, m), 2.90—3.30 (5H, m), 3.30—3.48 (1H, m), 3.60 (1H, m), 3.72—3.93 (1H, m), 4.15—4.40 (3H, m), 5.77 (1/2H, m), 5.87 (1/2H, m), 5.98 (1/2H, d, J = 9.8 Hz), 6.06 (1H, m), 6.12 (1/2H, d, J = 7.7 Hz), 7.30—7.40 (2H, m), 7.42—7.60 (2H, d, J = 8.8 Hz), 7.85 (1H, d, J = 8.8 Hz), 7.95—8.05 (1H, m).

(2*RS*,4*S*,5*S*)-*N*-Isobutyl-5-[*N*-[(2*S*)-2-(acetoxymethyl)-3-(1-naphthyl)-propionyl]-L-norleucyl]amino-2-ethyl-4-hydroxy-7-methyloctanamide (14*S*) The title compound was prepared from **13*S*** (33 mg, 0.084 mmol) by a procedure similar to that described for **14*R***, and was purified by flash silica gel chromatography ($CHCl_3$: MeOH = 50 : 1); yield 49.3 mg (92.2%) as a colorless solid, mp 186.0—189.0 °C. ^{1}H-NMR ($CDCl_3$) δ: 0.62—1.00 (18H, m), 1.00—1.90 (14H, m), 2.05 (3H, m), 2.10—2.22 (1/2H, m), 2.22—2.33 (1/2H, m), 2.86—3.08 (2H, m), 2.08—3.20 (2H, m), 3.30—3.38 (1H, m), 3.68 (1H, m), 3.81 (1H, m), 4.14 (1H, m), 4.25—4.42 (2H, m), 7.25—7.40 (2H, m), 7.40—7.58 (2H, m), 7.72 (1H, d, J = 9.0 Hz), 7.85

(1H, d, J=9.0 Hz), 8.00 (1H, m).

(2*RS*,4*S*,5*S*)-*N*-Isobutyl-5-[*N*-[(2*RS*)-3-benzyloxymethyl-3-(1-naphthyl)-propionyl]-L-norleucyl]amino-2-ethyl-4-hydroxy-7-methyloctanamide (17) To a suspension of **9** (900 mg, 3.33 mmol) and LiOH (86 mg, 3.50 mmol) in THF (30 ml) was added $LiBH_4$ (291 mg, 13.3 mmol) at 0 °C. After being stirred at 0 °C for 3 h and further stirred at room temperature overnight, the mixture was concentrated. The residue was partitioned between AcOEt and water, and the organic layer was washed with water and brine. Drying followed by evaporation and crystallization from Et_2O–hexane gave 2(*RS*)-hydroxymethyl-3-(1-naphthyl)propionic acid (655 mg, 85.4%) as a colorless powder. A solution of this acid (30 mg, 0.13 mmol) in DMF (1 ml) and benzyl bromide (133 mg, 0.78 mmol) were added to a suspension of NaH (60% in oil, 13 mg, 0.32 mmol) in DMF (0.5 ml) at room temperature under argon. After being stirred overnight, the mixture was poured into ice-water. The resulting mixture was extracted with AcOEt and the organic layer was washed with brine. Drying followed by evaporation and purified by preparative TLC with AcOEt–hexane (1 : 5) afforded 2(*RS*)-benzyloxymethyl-3-(1-naphthyl)-propionic acid benzyl ester (14.8 mg, 27.7%) as a colorless oil. To a solution of the benzyl ester (27.5 mg, 0.067 mmol) in EtOH–water (9 : 1, 1 ml) was added 2 N KOH (0.17 ml). The mixture was stirred at room temperature overnight and acidified to pH 2 with 1 N HCl. The mixture was extracted with AcOEt and the organic layer was washed with water and brine. Drying and evaporation gave **15** (25.5 mg, quant.) as a pale yellow oil. ^{1}H-NMR ($CDCl_3$) δ: 3.17 (1H, m), 3.34 (1H, dd, J=8.0, 15.0 Hz), 3.58 (1H, dd, J=7.1, 15.0 Hz), 3.67 (2H, d, J=8.0 Hz), 4.53 (2H, s), 7.24—7.42 (7H, m), 7.50 (2H, m), 7.74 (1H, d, J=8.0 Hz), 7.85 (1H, m), 8.05 (1H, d, J=8.0 Hz). The title compound was prepared from **4** (54 mg, 0.104 mmol) and **15** (25 mg, 0.078 mmol) using DPPA (25.6 mg, 0.93 mmol) by a procedure similar to that described for **5**, and was purified by preparative TLC ($CHCl_3$: MeOH = 20 : 1); yield 38.0 mg (70.8%) as a colorless solid, mp 129.0—131.0 °C. ^{1}H-NMR ($CDCl_3$) δ: 0.75—1.00 (18H, m), 1.02—1.95 (14H, m), 2.18 (1/2H, m), 2.32 (1/2H, m), 2.75—4.65 (12H, m), 7.20—7.40 (7H, m), 7.50 (2H, m), 7.72 (1H, d, J=8.0 Hz), 7.85 (1H, m), 8.05 (1H, m).

***N*-[2(*S*)-Ethylsulfonyl-3-(1-naphthyl)propionyl]-(N^{im}-trityl)-L-histidine Methyl Ester (26)** To a solution of 2(*R*)-hydroxymethyl-3-(1-naphthyl)-propionic acid (**25**) (1.26 g, 5.47 mmol), N^{im}-trityl-L-histidine methyl ester (2.7 g, 6.56 mmol) and HOBT (1.09 g, 7.11 mmol) in DMF (15 ml) was added DCC (1.47 g, 7.11 mmol) at −10 °C. The reaction mixture was stirred at −5 °C for 2 h and further stirred at 5 °C overnight. The precipitate was filtered off and the filtrate was concentrated. The residue was dissolved in AcOEt and washed with 5% $KHSO_4$, 4% $NaHCO_3$ and brine. Drying followed by evaporation and purification by silica gel chromatography (AcOEt : hexane = 4 : 1) afforded *N*-acyl derivative (3.20 g, 93.8%) as a colorless solid, mp 194.0—201.0 °C, $[\alpha]_D^{20}$ +42.3° (c=1.02, $CHCl_3$). ^{1}H-NMR ($CDCl_3$) δ: 2.87 (2H, m), 3.12 (1H, dd, J=7.4, 14.6 Hz), 3.29 (1H, dd, J=3.7, 14.6 Hz), 3.53 (3H, s), 3.74 (2H, m), 3.85 (1H, dd, J=8.7, 11.7 Hz), 4.85 (1H, m), 6.49 (1H, d, J=1.5 Hz), 6.72 (1H, d, J=7.8 Hz), 7.04—7.12 (6H, m), 7.30—7.42 (12H, m), 7.47 (2H, m), 7.70 (1H, m), 7.83 (1H, m), 8.03 (1H, m). To a solution of *N*-acylhistidine derivative (1.87 g, 3.00 mmol) in pyridine (15 ml) was added *p*-toluenesulfonyl chloride (1.16 g, 6.00 mmol). The solution was stirred at room temperature overnight and concentrated. The residue was dissolved in AcOEt and washed with 5% $KHSO_4$, 4% $NaHCO_3$, water and brine. Drying followed by evaporation and purification by silica gel chromatography (AcOEt : hexane = 3 : 1) afforded the tosylate (1.94 g, 83.1%) as a pale yellow amorphous. To a suspension of ethyl mercaptan (285 μl, 3.86 mmol) and NaH (60% in oil, 62 mg, 1.54 mmol) was added a solution of the tosylate (66 mg, 0.77 mmol) in DMF (4 ml) at −10 °C. After being stirred at room temperature for 1 h, the mixture was diluted with AcOEt and washed with water and brine. Drying and evaporation gave a yellow residue. The residue was dissolved in MeOH (20 ml) and 30% H_2O_2 (2 ml) and Na_2WO_4 (100 mg) were added. The mixture was stirred at room temperature overnight and concentrated. The residue was dissolved in AcOEt and washed with water and brine. Drying followed by evaporation and purification by silica gel chromatography ($CHCl_3$: MeOH = 100 : 1) afforded **26** (378 mg, 70.1%) as a colorless oil, $[\alpha]_D^{20}$ +32.3° (c=1.07, $CHCl_3$). IR (neat): 3352, 1749, 1677 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.10 (3H, t, J=7.4 Hz), 2.62—3.00 (5H, m), 3.10—3.25 (2H, m), 3.35—3.55 (1H, m), 3.44 (3H, s), 3.60—3.72 (1H, m), 4.62 (1H, m), 6.43 (1H, s), 6.90—7.04 (6H, m), 7.14—7.44 (11H, m), 7.51 (1H, d, J=7.8 Hz), 7.61 (1H, dd, J=1.2, 7.8 Hz), 7.66—7.72 (1H, m), 7.94—8.02 (1H, m). FAB-MS m/z: $[M+H]^+$ Calcd for $C_{42}H_{42}N_3O_5S$: 700.2845. Found: 700.2853.

(2*RS*,4*S*,5*S*)-*N*-Isobutyl-5-[*N*-[(2*S*)-2-ethylsulfonylmethyl-3-(1-naphthyl)propionyl]-L-histidyl]amino-2-ethyl-4-hydroxy-7-methyloctanamide (27) To a solution of **26** (53 mg, 0.076 mmol) in MeOH–water (10 : 1, 1.5 ml) was added 1 N KOH (EtOH : water = 10 : 1, 0.38 ml) and the mixture was stirred at room temperature for 1 h. After acidification with 1 N HCl, the mixture was diluted with AcOEt and washed with water and brine. Drying and evaporation gave the corresponding acid (49.4 mg, 95.1%) as a pale yellow oil. This acid (33 mg, 0.05 mmol) was condensed with AEHMA isobutylamide (**3**) (21 mg, 0.06 mmol) using DPPA by the usual method to afford N^{im}-trityl **27** (37.5 mg, 81.7%) as a colorless solid. N^{im}-trityl **27** (28.6 mg, 0.037 mmol) was dissolved in CH_2Cl_2 (0.5 ml) and TFA (0.1 ml) was added. The mixture was stirred at room temperature for 3 h and concentrated. The residue was purified by silica gel chromatography ($CHCl_3$: MeOH = 20 : 1) to afford **27** (16.8 mg, 87.3%) as a colorless solid, mp 108.0—114.0 °C. ^{1}H-NMR ($CDCl_3$) δ: 0.56—1.00 (14H, m), 1.10—1.81 (12H, m), 2.24 (1/2H, m), 2.70—3.28 (9H, m), 3.40—3.88 (5H, m), 4.68 (1/2H, m), 4.78 (1/2H, m), 6.44 (1H, br), 6.56 (1H, br), 6.94 (1H, m), 7.22—7.40 (2H, m), 7.40—7.55 (3H, m), 7.72 (1H, d, J=7.9 Hz), 7.82 (1H, m), 8.02 (1H, m).

(2*R*)-2-(1-Naphthyl)methyl-1,3-propanediol 1-Acetate (29) The suspension of 2-(1-naphthyl)methyl-1,3-propanediol (20 g, 92.6 mmol) in vinyl acetate (50 ml) was added lipase P (3 g), and the mixture was stirred at room temperature for 4.5 h and further stirred at 30 °C for 2 h. Another portion of lipase P (1.2 g) was added and stirred at 8 °C for 2 d. Lipase P was filtered off and the filtrate was concentrated. The residue was purified by silica gel chromatography (AcOEt : hexane = 1 : 2) to afford **29** (22.2 g, 93.1%) as a colorless oil, $[\alpha]_D^{20}$ +35.7° (c=1.03, $CHCl_3$). ^{1}H-NMR ($CDCl_3$) δ: 2.00 (3H, t, J=5.7 Hz), 2.12 (3H, s), 2.32 (1H, m), 3.07 (1H, dd, J=7.6, 13.7 Hz), 3.18 (1H, dd, J=7.6, 13.7 Hz), 3.54—3.72 (2H, m), 4.18 (1H, dd, J=6.7, 11.4 Hz), 4.24 (1H, dd, J=6.7, 11.4 Hz), 7.30—7.55 (4H, m), 7.75 (1H, d, J=7.8 Hz), 7.86 (1H, m), 8.04 (1H, m). The enantiomer excess was determined to be 86% ee by HPLC analysis using a column packed with Chiralcel OC (iso-PrOH : hexane = 1 : 9, flow rate; 0.8 ml/min). t_R, 2*R* compound, 31.4 min; 2*S* compound, 35.4 min.

(2*S*)-2-(1-Naphthyl)methyl-1-*O*-methoxymethyl-1,3-propanediol (30) To a solution of **29** (19.1 g, 74.0 mmol) and diisopropylethylamine (28.7 g, 222.1 mmol) in CH_2Cl_2 (100 ml) was added chloromethyl methyl ether (17.9 g, 222.1 mmol), and the solution was stirred at room temperature overnight. After concentration, the residue was dissolved in AcOEt and the solution was washed with 1 N HCl, saturated $NaHCO_3$, water and brine. Drying and evaporation gave pale yellow oil (21.3 g). To a solution of the residue in MeOH–water (4 : 1, 250 ml) was added 2 M K_2CO_3 (70 ml), and the mixture was stirred at room temperature for 2 d. The mixture was concentrated and the residue was partitioned between AcOEt and water. After washing the organic layer with water and brine, drying and evaporation gave **30** (17.6 g, 91.4%), $[\alpha]_D^{20}$ −20.7° (c=0.98, $CHCl_3$). ^{1}H-NMR ($CDCl_3$) δ: 2.25 (1H, m), 2.30 (1H, m), 3.14 (2H, dd, J=2.9, 7.1 Hz), 3.40 (3H, s), 3.60—3.85 (4H, m), 4.61 and 4.67 (2H, ABq, J=6.1 Hz), 7.35 (2H, m), 7.50 (2H, m), 7.75 (1H, d, J=8.9 Hz), 7.87 (1H, d, J=8.9 Hz), 8.07 (1H, d, J=8.9 Hz). The enantiomer excess was determined to be 86% ee by HPLC analysis using a column packed with Chiralcel OC (iso-PrOH : hexane = 1 : 19; flow rate, 0.8 ml/min). t_R, 2*S*-compound, 19.8 min; 2*R*-compound, 17.5 min.

(2*R*)-2-Hydroxymethyl-3-(1-naphthyl)propionic Acid (25) To a solution of **30** (17.5 g, 67.3 mmol) in acetone (350 ml) was added Jones reagent (30 ml) at 0 °C over 30 min, and the mixture was stirred at 0 °C for 2 h. iso-PrOH was added and concentrated. The residue was dissolved in THF (200 ml) and 6 N HCl (200 ml) was added. The mixture was stirred at 50 °C for 1.5 h and concentrated. The residue was dissolved in AcOEt and the solution was extracted with saturated $NaHCO_3$. The extract was acidified with 6 N HCl and extracted with AcOEt. Drying followed by evaporation and recrystallization from benzene gave **25** (7.8 g, 50.2%) as a pale brown powder, mp 116.5—118.0 °C, $[\alpha]_D^{20}$ +33.3° (c=1.02, $CHCl_3$). ^{1}H-NMR ($CDCl_3$) δ: 3.08 (1H, m), 3.30 (1H, dd, J=9.7, 14.1 Hz), 3.65 (1H, dd, J=5.3, 14.0 Hz), 3.75 (1H, dd, J=6.1, 11.3 Hz), 3.74 (1H, dd, J=3.9, 11.3 Hz). The enantiomer excess was determined to be 96% ee by HPLC analysis using a packed column with Senshu Pak. Silica gel 1151N (hexane : AcOEt = 1 : 1) after coupling with L-norleucine. t_R, **13***R*, 4.70 min; **13***S*, 7.58 min.

***N*-[(2*R*)-2-Hydroxymethyl-3-(1-naphthyl)propionyl]-L-norleucine *tert*-Butyl Ester (13*R*)** The title compound was prepared from **25** (3.0 g, 13.0 mmol) and L-norleucine *tert*-butyl ester (2.68 g, 14.3 mmol) by DCC–HOBT method, and was recrystallization from AcOEt–hexane; yield 4.57 g (88.1%) as colorless needles, mp 92.0—93.0 °C, $[\alpha]_D^{20}$ +50.9° (c=1.04, $CHCl_3$). ^{1}H-NMR ($CDCl_3$) δ: 0.86 (3H, t, J=5.6 Hz), 1.00—

1.40 (4H, m), 1.42 (9H, s), 1.78 (1H, m), 2.70—2.85 (2H, m), 3.32 (1H, dd, $J=7.9$, 13.2 Hz), 3.50 (1H, dd, $J=7.5$, 13.2 Hz), 3.81 (2H, m), 4.37 (1H, m), 6.12 (1H, d, $J=7.6$ Hz), 7.38 (2H, d, $J=4.8$ Hz), 7.51 (2H, m), 7.73 (1H, t, $J=4.3$ Hz), 7.86 (1H, d, $J=8.9$ Hz), 8.05 (1H, d, $J=8.9$ Hz).

Bioavailability in Rats Compound **20** dissolved in DMSO–saline (1 : 19, for i.v.) or in DMSO–water (1 : 19, for *p.o.*) was injected into the tail vein at a dose of 0.35 mg/ml/kg (i.v.) or given into the stomach at 8.4 mg/ml/kg (*p.o.*) using an esophageal sound, to unrestrained conscious rats with cervical cannula. Blood samples were collected through the cervical cannula into EDTA-containing glass tubes and centrifuged. Then a methanol extract of plasma (after dryness) was subjected to the PRA inhibition assay, which was the same as described previously.[1] The percent inhibition of human PRA was converted to the plasma concentration of **20** using a standard curve constructed with the known concentrations of **20** dissolved in control rat plasma.

The areas under the plasma concentration–time curve (AUC) were calculated by using the trapezoidal rule, and the bioavailability was calculated by the following equation:

$$\text{bioavailability } (\%) = (\text{AUC}_{p.o.}/\text{AUC}_{\text{i.v.}}) \times (\text{dose}_{\text{i.v.}}/\text{dose}_{p.o.}) \times 100$$

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