Design and Synthesis of an Orally Potent Human Renin Inhibitor containing a Novel Amino Acid, Cyclohexylnorstatine

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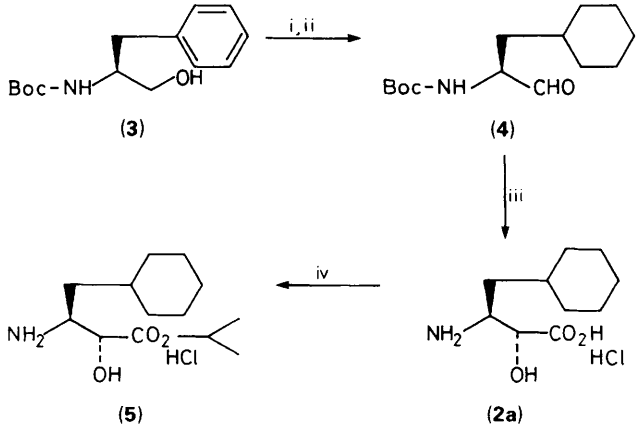
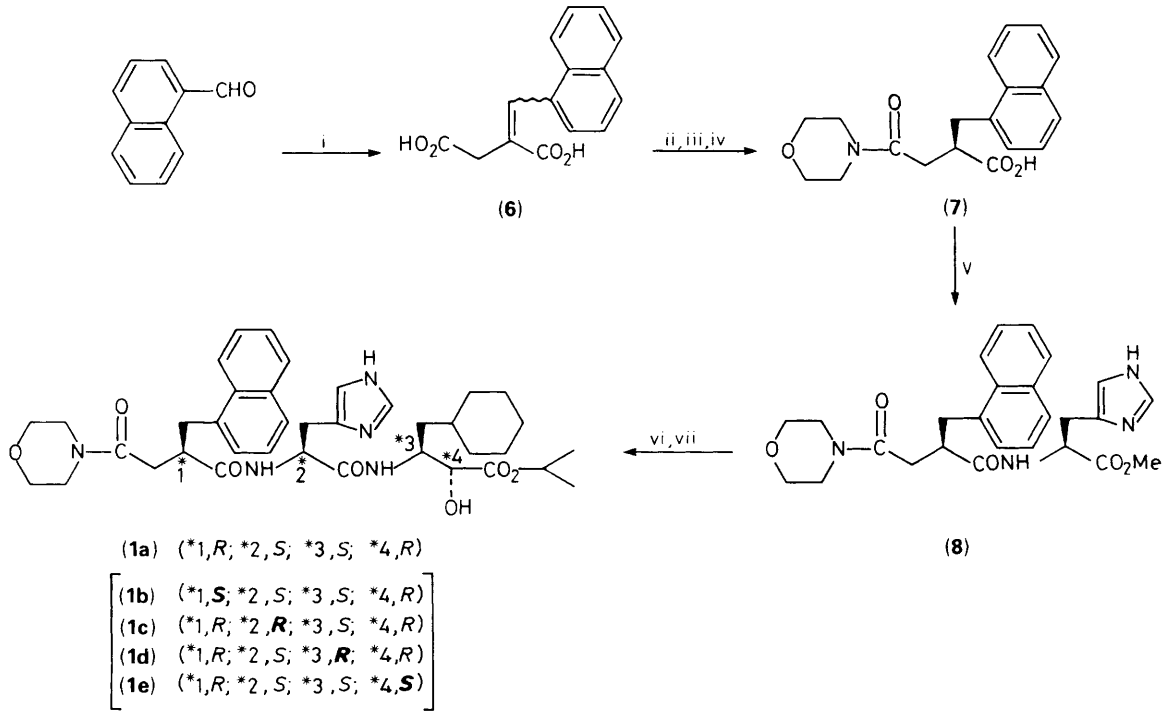
An orally potent human renin inhibitor (**1a**) containing a novel amino acid, (2*R*,3*S*)-3-amino-4-cyclohexyl-2-hydroxybutyric acid named cyclohexylnorstatine, has been designed from the angiotensinogen transition-state and synthesized.

Renin, an aspartic protease, generates angiotensin I from angiotensinogen, and a large number of inhibitory peptides of human renin have been investigated as potential agents of antihypertensive therapy.[1] Using the transition-state inhibitor approach, Szelke *et al.*[2] reported potent renin inhibitors in which the scissile bond of substrate analogues was replaced by a reduced peptide isostere or a hydroxy isostere. Boger *et al.*[3] incorporated a statine residue as a dipeptide mimic into substrate analogues to obtain the potent renin inhibitors. However, the peptide compounds are thought to be unsuitable for oral administration owing to the problems of proteolytic degradation and poor intestinal absorption. Herein, we report 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.

Analysis of renin–substrate interactions is essential in the design of specific inhibitors. Firstly, we constructed[4] a three-dimensional structure of human renin using modelling techniques. Then, we deduced the structure of a complex of



Scheme 1. *Reagents*: i, H_2–Rh/Al_2O_3, 18 h; ii, Py·SO_3/Me_2SO, 25 °C, 15 min; iii, HCN, 0 °C, 3 h, then 23% HCl, reflux, 11 h; iv, isopropanol/HCl, 80 °C, 1 h.



(1a) (*1,*R*; *2,*S*; *3,*S*; *4,*R*)

(1b) (*1,***S**; *2,*S*; *3,*S*; *4,*R*)
(1c) (*1,*R*; *2,***R**; *3,*S*; *4,*R*)
(1d) (*1,*R*; *2,*S*; *3,***R**; *4,*R*)
(1e) (*1,*R*; *2,*S*; *3,*S*; *4,***S**)

Scheme 2. *Reagents*: i, diethyl succinate/NaOMe, reflux, 2 h, then aq. NaOH, reflux, 2 h; ii, Ac_2O, 60 °C, 1 h, then morpholine, 2 h; iii, H_2–Pd/C, 14 h, then (*S*)-methyl mandelate/DCC, 18 h; iv, aq. NaOH, 14 h; v, His-OMe/DCC–HONB, 18 h; vi, aq. NaOH, 18 h; vii, (**5**) DCC–HONB, 18 h.

human renin and the scissile site Pro-Phe-His-Leu-Val (P4-P1′) of human angiotensinogen according to our previous method.[5]

Subsequently, we designed (**1a**) from the angiotensinogen transition-state analogues[2,3] by the following strategies. (i) The molecular weight should be as low as possible to increase intestinal absorption.[6] Also, the natural peptide bonds, metabolized easily by proteases, should be modified. Thus, the succinic acid residue having a retro-inverso amide bond was introduced to maintain the hydrogen bond to renin and to avoid enzymatic degradation. (ii) Cyclohexyl group (P1) and naphthyl group (P3) were introduced as large hydrophobic residues, fitting more favourably to renin. (iii) We focused on cyclohexylnorstatine instead of the popular statine-type residue [statine: (3*S*,4*S*)-4-amino-3-hydroxy-6-methylheptanoic acid][7] as a transition-state mimic, because cyclohexylnorstatine could be mass-produced in the optically pure form. Thus, we replaced the Leu-Val (P1-P1′) scissile site with the cyclohexylnorstatine isopropyl ester residue.

Cyclohexylnorstatine isopropyl ester (**5**) was prepared as shown in Scheme 1. Boc-L-cyclohexylalaninal (**4**) (Boc = t-butoxycarbonyl) was prepared by hydrogenation of (**3**) followed by oxidation. The aldehyde (**4**) was hydrocyanated in AcOEt–H_2O and hydrolysed using 23% HCl. When the reaction mixture† was kept overnight, (**2a**)‡ exclusively crystallized out of the mixture in the form of the HCl salt. Thus, optically pure (**2a**) {$[\alpha]_D^{23}$ −11.2° (*c* 2.0, H_2O)} was prepared efficiently (60%, 4 steps) and then converted to the isopropyl ester (**5**) (95%).

Scheme 2 shows the synthetic pathway to (**1a**). The diacid (**6**), prepared by condensation of 1-naphthaldehyde and diethylsuccinate followed by hydrolysis, was dehydrated, treated with morpholine, and reduced to give a racemate of (**7**) (89%, 5 steps). The isomers were coupled with (*S*)-methyl mandelate and the diastereoisomer, which crystallized from methanol, was hydrolysed to give the isomer (**7**) {$[\alpha]_D^{22}$ −11.8° (*c* 2.0, MeOH)} with the *R* configuration (44%, 2 steps).§ Coupling of the acid (**7**) and L-histidine methyl ester using dicyclohexylcarbodiimide (DCC) and *N*-hydroxy-5-norbornene-2,3-dicarboximide (HONB)[9] provided (**8**). Condensation of the hydrolysed product of (**8**) and (**5**) using DCC–HONB gave (**1a**) {$[\alpha]_D^{21.5}$ −28.6° (*c* 1.0, MeOH)} without racemization. The stereoisomers (**1b—e**) of (**1a**) were synthesized by essentially the same method. In addition, we synthesized several related compounds such as statine-type derivatives.

The renin inhibitory potencies of the compounds were measured with both a human renin–sheep substrate system and human plasma renin.[10] Of the compounds synthesized, (**1a**) was the most potent inhibitor against both the isolated human renin and human plasma renin: $IC_{50} = 2.4 \times 10^{-9}$ and 4.7×10^{-9} M, respectively. On the other hand, (**1a**) did not practically inhibit other proteases, such as cathepsin D

† A 7:3 mixture of (**2a**) (2*R*,3*S* form) and (**2b**) (2*S*,3*S* form). Diastereoisomeric ratio was determined by ^{1}H n.m.r. spectroscopic analysis.

‡ The stereochemistry of (**2a**) was estimated by conversion to the corresponding oxazolidinone. A coupling constant of 4.94 Hz and nuclear Overhauser enhancement (n.O.e.) of 2.7% for the ring protons (H of C-4 and H of C-5) are consistent with *trans* stereochemistry, showing that the configuration at C-2 is *R* for the oxazolidinone.

§ The stereochemistry of (**7**) was estimated by conversion to the corresponding (+)- and (−)-α-methoxy-α-(trifluoromethyl)phenylacetic acid (MTPA) esters. Examination of the ^{1}H n.m.r. spectra of the resulting diastereoisomers with tris(1,1,1,2,2,3,3-heptafluoro-7,7-dimethyloctane-4,6-dionato)europium(III) [Eu(fod)$_3$] as shift reagent indicated that the configuration was (*R*).[8]

($>10^{-5}$ M), pepsin ($>10^{-4}$ M), and chymotrypsin ($>10^{-4}$ M). The stereoisomers (**1b**—**e**) of (**1a**) showed very weak renin inhibitory activities ($>10^{-6}$ M).

The deduced active conformation of (**1a**) in the active site of renin was constructed,¶ and (**1a**) was shown to fit to renin favourably. The difference between the interaction of (**1a**) and that of the angiotensinogen (P4 Pro to P1′ Val) with renin was that the side chain OH of Ser-230 was hydrogen-bonded to the β-CO of the P3 succinic acid residue of (**1a**) instead of the NH of the P3 Phe of angiotensinogen. The weak inhibition of the stereoisomers (**1b**—**e**) may be attributable to poor interaction, causing a decrease in affinity for renin.

Compound (**1a**) was stable against monkey liver homogenates, human plasma, and chymotrypsin. The oral administration of 10 mg kg^{-1} of (**1a**) to salt-depleted Japanese monkeys resulted in a fall of 10—20 mmHg in blood pressure and reduced plasma renin activity for a 5 h period.

It has been reported previously that some substrate-derived peptides exhibit potent renin inhibitory activities.[13] Compound (**1a**) is superior to such derivatives since it exhibits high and long-lasting activities *in vitro* and after oral administration and is the most compact compound among such human renin inhibitors.

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¶ The orientation of (**1a**) in the active site of renin was deduced by a Monte Carlo Simulation with Metropolis algorithm.[11] In this simulation, the enzyme was fixed in its 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.[12]