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STRUCTURE-CONFORMATION-ACTIVITY RELATIONSHIPS OF RENIN INHIBITORY PEPTIDES HAVING P_1-P_1' Xaaψ[CH_2NH]Yaa SUBSTITUTIONS: MOLECULAR MODELING AND CRYSTALLOGRAPHY STUDIES*[1]

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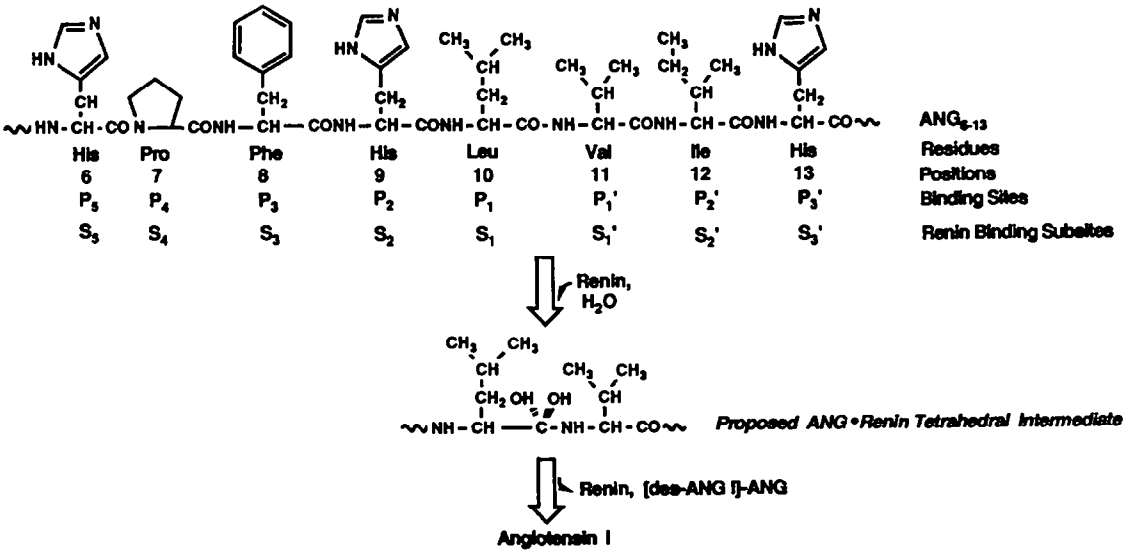
Summary: Structure-activity studies of angiotensinogen (ANG)-based renin inhibitors having carboxy-terminal pseudodipeptidyl P_1-P_1' Xaaψ[CH_2NH]Yaa-NH_2 and related transition state synthetic mimetics were performed and integrated with molecular modeling using a computer simulated human renin active site model, and compared to a known crystallographic structure of a P_1-P_1' Pheψ[CH_2NH]Phe substituted renin inhibitor bound to rhizopuspepsin.

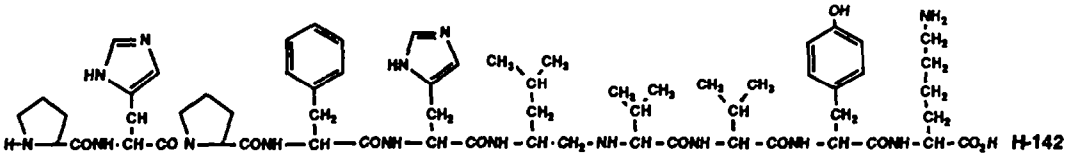
Renin is an aspartic acid protease (EC 3.4.99.19) which catalyzes the first and rate-limiting step of the enzyme cascade that exists for the biosynthesis of angiotensin II (ANG II)[2-4]. Renin inhibition may be therapeutically important in the development of useful antihypertensive agents[5-9] based on the role of the renin-angiotensin converting enzyme cascade in the physiological and, perhaps, pathophysiological control of blood pressure and electrolyte homeostasis.

In retrospect, the development of ANG-based inhibitors of human renin has essentially focused on systematic structure-activity studies[10-20] related to P_1-P_1' pseudodipeptidyl substitutions of the Leu-Val cleavage site (see below) of various substrate fragments in which the scissile amide (CONH) has been replaced by $CH(OH)CH_2$ or CH_2NH type transition state mimics of the proposed aminol ($C(OH)_2NH$) that is believed to be generated during enzyme-catalyzed hydrolysis of ANG at the renin active site.

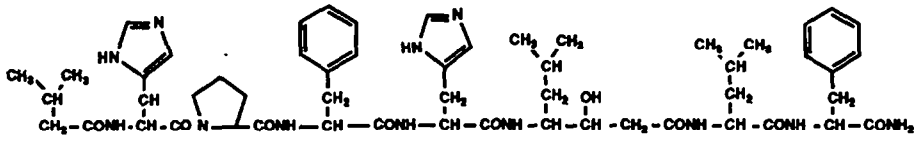


*Dedicated to The Upjohn Company in honor of its 100th anniversary, 1886-1986

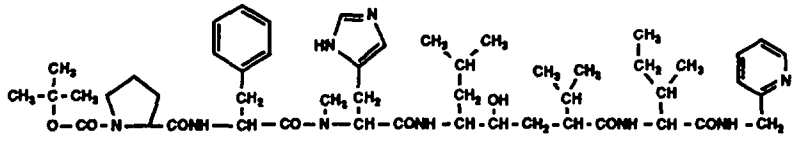
The first reported[10] renin inhibitor which was active *in vivo* was H-Pro-His-Pro-Phe-His-Phe-Phe-Val-Tyr-Lys-OH (RIP), a moderately potent (K_I = 2.0 x 10-6 M) decapeptide having P_1-P_1' Phe-Phe modification. Subsequently, noteworthy achievements in providing ANG-based congeners effecting markedly enchanced potency relative to RIP have been recently made, as exemplified by several reported renin inhibitors (shown below) which include: (1) H-Pro-His-Pro-Phe-His-Leuψ[CH_2NH]Val-Ile-His-Lys-OH (**H-142**, IC_{50} = 1.0x10-8 M)[11]; (2) Iva-His-Pro-Phe-His-Sta-Leu-Phe-NH_2 (**SCRIP**, IC_{50} = 1.6 x 10-8 M)[12]; and (3) Boc-Pro-Phe-MeHis-Leuψ[CH(OH)CH_2]Val-Ile-Amp (**U-71038**, IC_{50} = 4.2 x 10-10 M)[19].



H-142



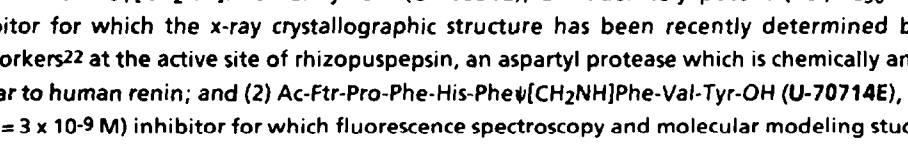
SCRIP



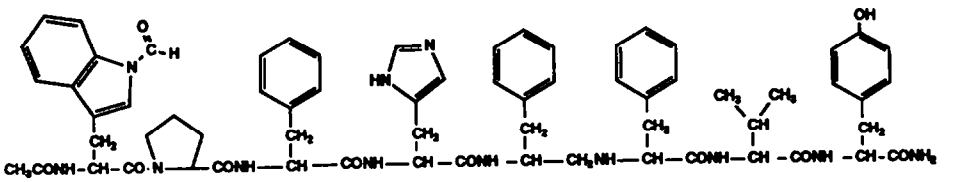
U-71038

X-ray crystallographic structures have been recently determined[21,22] for several ANG-based renin inhibitors bound to the active sites of two different aspartyl proteases, endothiapepsin and rhizopuspepsin. These 3-dimensional structures provide insight to interpret structure-activity relationships and to predict the likely conformational properties and, perhaps, potential binding interactions (*e.g.*, H-bonding and hydrophobic) between such inhibitors and the human renin active site. In fact, significant research efforts[23-25] have already been directed towards the examination of renin-inhibitor interactions by computer-assisted molecular modeling using 3-D models of human renin constructed from its known primary sequence[26] and x-ray crystallographic structures of homologous aspartyl proteases.

In this report we describe primarily the structure-conformation-activity relationships of several linear and cyclic, semi-rigid ANG-based inhibitors of human renin modified by pseudodipeptidyl P_1-P_1' Xaaψ[CH_2NH]Yaa in which the P_1 Xaa and P_1' Yaa residues were systematically varied. Two previously documented compounds (shown below) have provided leads for this investigation: (1) H-D-His-Pro-Phe-His-Pheψ[CH_2NH]Phe-Val-Tyr-OH (**U-70531E**), a moderately potent (*i.e.*, IC_{50} = 4.2 x 10-7 M) inhibitor for which the x-ray crystallographic structure has been recently determined by Davies and co-workers[22] at the active site of rhizopuspepsin, an aspartyl protease which is chemically and functionally similar to human renin; and (2) Ac-Ftr-Pro-Phe-His-Pheψ[CH_2NH]Phe-Val-Tyr-OH (**U-70714E**), a more potent (IC_{50} = 3 x 10-9 M) inhibitor for which fluorescence spectroscopy and molecular modeling studies have been performed by Epps and co-workers[27] to determine an average conformation of the inhibitor in solution and at the active site of a computer simulated molecular model of human renin (CKH-RENIN) originally developed by Carlson and co-workers[23].



U-70531E



U-70714E

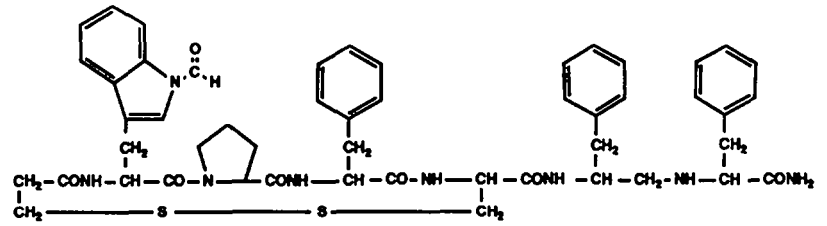
Structure-Activity Relationships of P_1-P_1' Pheψ[CH_2NH]Phe Modified ANG Congeners. As summarized in Table I, several congeners of U-70714E (1) were synthesized and tested for their comparative human plasma renin inhibitory activities. Highlights of this structure-activity study are summarized as follows: (1) C-Terminal truncation of 1 provided a superpotent hexapeptide derivative, Ac-Ftr-Pro-Phe-His-Pheψ[CH_2NH]Phe-NH_2 (6, $IC_{50} = 5.6 \times 10^{-10}$ M); (2) Stereoinversion of the Cα-carbons of the Pheψ[CH_2NH]Phe of 6 effected greatly diminished (>1000-fold) renin inhibitory potency; (3) Cyclization of the Ac-Ftr-Pro-Phe-His sequence of 6 by replacement of P_2 His by Cys or Hcy and disulfide linkage to the N-terminal Mpr or Mbu which substituted for the Ac group yielded conformationally-constrained hexapeptide derivatives 9 and 10, respectively (shown below), that demonstrated about a 100,000-fold potency reduction; and (5) modification of the P_2 His in compound 6 by Pro was detrimental to potency. However, Nα-methylation of His provided Ac-Ftr-Pro-Phe-MeHis-Pheψ[CH_2NH]Phe-NH_2 (12), a superpotent ($IC_{50} = 4.4 \times 10^{-10}$ M) inhibitor that was very selective for human versus porcine renin ($IC_{50} = 1.0 \times 10^{-6}$ M). In addition, the N-terminal Ftr residue contributed to the high affinity binding of 12 as demonstrated by the marked decrease (*i.e.*, about 300-fold) in potency of the pentapeptide congener, Ac-Pro-Phe-MeHis-Pheψ[CH_2NH]Phe-NH_2 (13). Relative to previously reported ANG-based inhibitors of renin incorporating P_1-P_1' pseudodipeptidyl transition state mimetics, hexapeptide 12 was noteworthy in terms of both its chemical simplicity, biological potency and stability to selected proteolytic enzymes (*e.g.*, chymotrypsin and carboxypeptidase-A, unpublished results). The *in vivo* hypotensive properties of 12 were also investigated and will be described in detail elsewhere.

Table I. Renin inhibitory activities of RIP derivatives.

	Compound*	IC_{50} (M)
RIP	H-Pro-His-Pro-Phe-His-Phe-Phe-Val-Tyr-Lys-OH	8.0×10^{-6}
1	Ac-Ftr-Pro-Phe-His-Pheψ[CH_2NH]Phe-Val-Tyr-NH_2	3.0×10^{-9}
2	H-D-His-Pro-Phe-His-Pheψ[CH_2NH]Phe-Val-Tyr-OH	4.2×10^{-7}
3	Tba-Phe-His-Pheψ[CH_2NH]Phe-Val-Tyr-NH_2	1.9×10^{-7}
4	Ac-Pro-Phe-His-Pheψ[CH_2NH]Phe-Val-Tyr-NH_2	7.9×10^{-8}
5	Ac-Ftr-Pro-Phe-His-Pheψ[CH_2NH]Phe-Val-NH_2	6.0×10^{-8}
6	Ac-Ftr-Pro-Phe-His-Pheψ[CH_2NH]Phe-NH_2	5.6×10^{-10}
7	Ac-Ftr-Pro-Phe-His-Pheψ[CH_2NH]D-Phe-NH_2	5.9×10^{-7}
8	Ac-Ftr-Pro-Phe-His-D-Pheψ[CH_2NH]Phe-NH_2	1.1×10^{-6}
9	Mpr-Ftr-Pro-Phe-Cys-Pheψ[CH_2NH]Phe-NH_2	28% @ 10^{-5}
10	Mbu-Ftr-Pro-Phe-Hcy-Pheψ[CH_2NH]Phe-NH_2	40% @ 10^{-5}
11	Ac-Ftr-Pro-Phe-Pro-Pheψ[CH_2NH]Phe-NH_2	25% @ 10^{-5}
12	Ac-Ftr-Pro-Phe-MeHis-Pheψ[CH_2NH]Phe-NH_2	4.4×10^{-10}
13	Ac-Pro-Phe-MeHis-Pheψ[CH_2NH]Phe-NH_2	1.1×10^{-7}

*See Reference 1 for abbreviations.

Molecular Modeling Analysis of P_1-P_1' Pheψ[CH_2NH]Phe Modified ANG Congeners. Molecular modeling studies of 1 and 6 at the CKH-RENIN active site were performed using molecular dynamics simulation and energy minimization subroutines of the CHARMM macromolecular refinement program (version 19) developed by Karplus and co-workers[28] The dynamics-averaged and co-minimized structures



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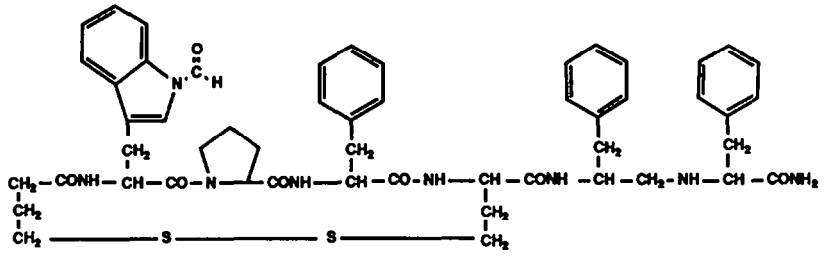

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of both CKH-RENIN•1 and CKH-RENIN•6 provided 3-D molecular models for each of the two inhibitors as shown in Fig. 1. For comparative analysis, the x-ray crystallographic structure of 2 derived from the rhizopuspepsin•2 complex[22] is also shown. Noteworthy observations made from these studies are summarized as follows: (1) The P_5 Ftr of both compounds 1 and 6 interacts with a hydrophobic pocket derived from five aromatic residues (*i.e.*, Tyr-14, Tyr-230, Tyr-254, Phe-123 and Tyr-20); (2) The spatial orientation of the P_5 Ftr, P_3 Phe and P_1 Phe residues in both the CKH-RENIN•1 and CKH-RENIN•6 models were capable of providing significant shielding (>50%) of the enzyme subsites (*i.e.*, S_5, S_3 and S_1) from accessible solvent; (3) The backbone torsion angles (*i.e.*, ϕ and ψ) of the inhibitors from each the CKH-RENIN•1 model and rhizopuspepsin•2 complex were determined to be similar at their common P_4-P_2' sequence, Pro-Phe-His-Pheψ[CH_2NH]Phe-Val. For the dynamics-averaged structure of 1 at the CKH-RENIN active site the ϕ and ψ torsion angles were: P_5 Ftr, ϕ, ψ (-127°, 94°); P_4 Pro, ϕ, ψ (-83°, 108°); P_3 Phe, ϕ, ψ (-96°, 116°); P_2 His, ϕ, ψ (-150°, 78°); P_1 Phe, ϕ, ψ (-110°, 70°); P_1' Phe, ϕ, ψ (-86°, 171°); P_2' Val, ϕ, ψ (-80°, 90°); and P_3' Tyr, ϕ (-81°).; and (4) several potential H-bond interactions between the enzyme CKH-RENIN active site and compound 1 were identified. These intermolecular H-bonding contacts include: (i) P_5 Ftr Cα-N-H····O = C-Cα Glu-287; (ii) P_4 Pro Cα-C = O····H-N-Cα Thr-84; (iii) P_3 Phe C = O····H-N-Cα Ser-229; (iv) P_3 Phe Cα-N-H····OH-Cβ Ser-229; (v) P_1 Phe Cα-N-H····O = C-Cα Gly-227; and (vi) P_2' Val Cα-NH····O = C-Cα Gly-39. The intermolecular H-bond distances and angles for these specified interactions ranged from 2.7 Å to 3.3 Å (where H-bond distance is defined between donor and acceptor atoms) and from 3° to 31° (where 0° defines colinearity of the H-bond donor, H-bond acceptor and H atom), respectively. The P_1-P_1' Pheψ[CH_2NH]Phe aminomethylene of 1 was found to be 4.9 Å from the Asp-225 side-chain carbonyl of the CKH-RENIN active site. Similarly, a 2.8 Å distance between the aminomethylene of 2 and Asp-218 side-chain carbonyl of the rhizopuspepsin complex has been reported[22].

Overall, the above 3-D molecular models for the CKH-RENIN•inhibitor complexes may explain, in part, the enhanced binding affinities of compounds 1 and 6 versus structurally-related compounds (refer to Tables I and II) and account for the observed regioselective and/or stereoselective intermolecular hydrophobic, π-π bonding and/or H-bonding interactions between the N-terminal P_5 Ftr and C-terminal P_1-P_1' Pheψ[CH_2NH]Phe residues and their complementary S_5 and S_1-S_1' binding subsites. In our 3-D inhibitor models of 1 and 6 the P_5 Ftr and P_2 His side-chains are not spatially proximate to each other, and the secondary structure of the P_5-P_2 sequence more closely approximates an extended conformation versus the postulated β-turn or reverse-turn conformation as previously reported[29,30] for cyclic analogues (*i.e.* P_5 to P_2 disulfide-bridged) of P_1-P_1' Leu-Leu or Sta modified, ANG-based inhibitors of renin. In addition, the extremely low potencies of the cyclic, semi-rigid compounds 9 and 10 did not support the existence of a reverse-turn secondary structure for the N-terminal P_5-P_2 tetrapeptide sequence.

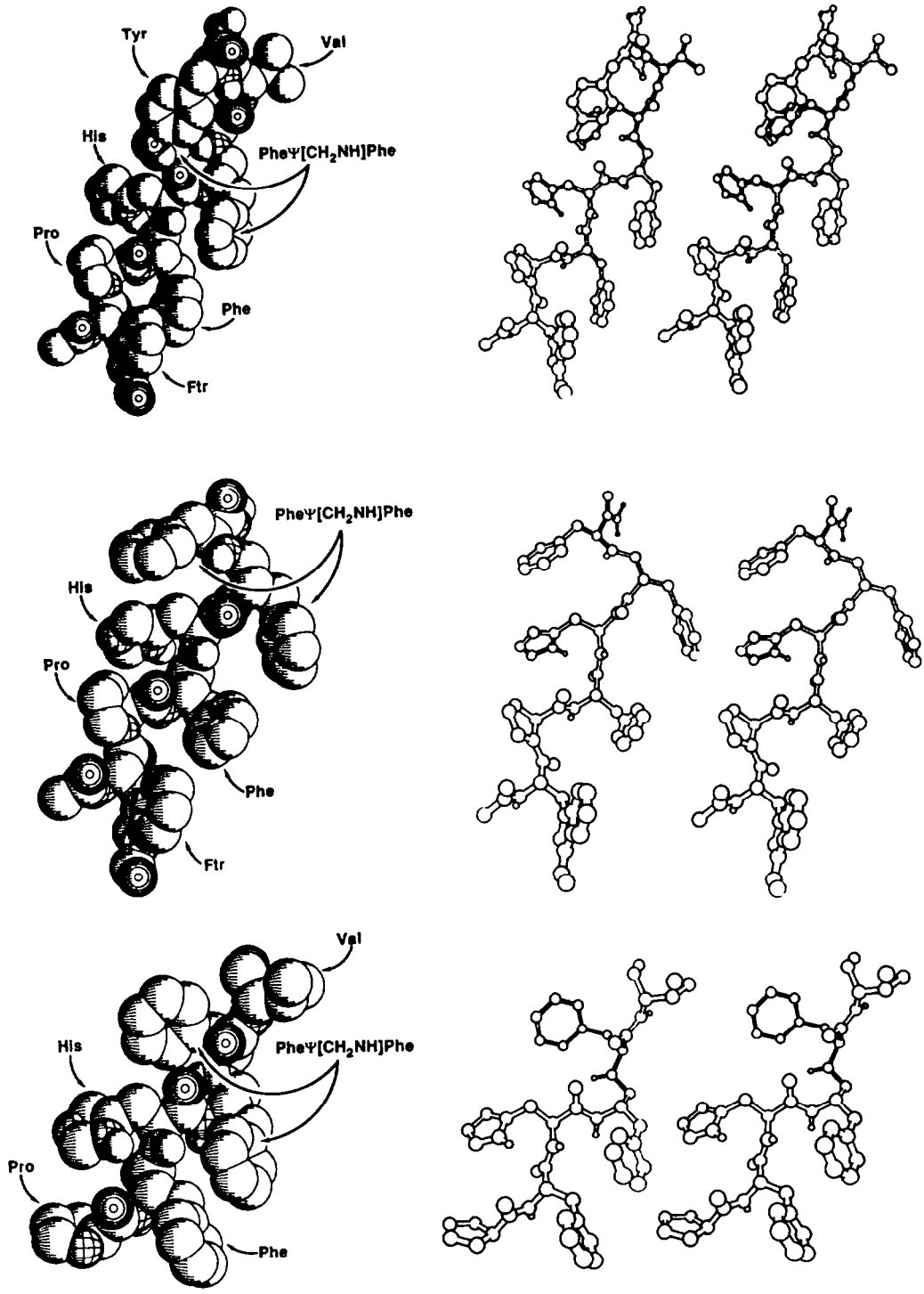


Fig. 1. Computer graphics representations of the molecular dynamics-averaged 3-D structures of compounds 1 (top left) and 6 (center left) derived from modeling at the CKH-RENIN active site and the x-ray crystallographic structure of compound 2 (bottom left) derived from its rhizopuspepsin active site complex. It is noted that only the P_4-P_2' sequence, Pro-Phe-His-Pheψ[CH_2NH]Phe-Val, of 2 in the rhizopuspepsin•2 complex were determined. In addition, ball-and-stick stereopairs for these compounds are shown.

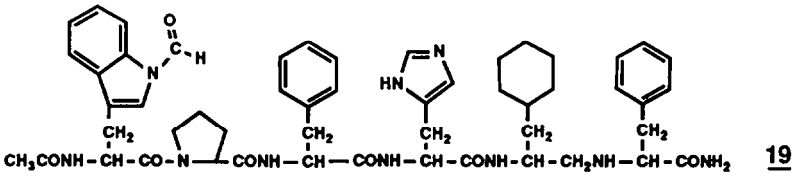
Recent fluorescence quenching studies by Epps and co-workers[31] on recombinant human renin, and anti-human renin antibody experiments by Carlson and co-workers[32] have provided data which have been in accord with the predicted 3-D structural properties of the CKH-RENIN model. In addition, an independent molecular modeling study of a renin•inhibitor complex which may be compared to our data has been previously reported by Plattner and co-workers[18] for the hexapeptide, Boc-Phe-His-Leuψ[CH_2NH]Val-Ile-His-OMe. In general, the predicted enzyme•ligand intermolecular interactions (*e.g.*, hydrophobic and H-bond) and conformational properties of the peptide backbone of their inhibitor appear similar to our molecular modeling studies on compounds **1** and **6** at the CKH-RENIN active site. Such molecular mapping of the renin-derived binding subsites to ANG_{6-13}-based analogues may provide insight to the design of future renin inhibitory peptides and/or peptidemimetics. This general strategy of integrating protein crystallography, comparative model building and computer graphics to provide insight into rational drug design for various therapeutic objectives has been recently reviewed[33] by Hol.

Comparative Structure-Activity Relationships of P_1-P_1' Xaaψ[CH_2NH]Yaa, Leuψ[$CH(OH)CH_2$]Val, Sta or Ast Modified ANG Congeners. As summarized in Table II, the hexapeptide template of **6** was further explored by the comparative biological analysis of several **RIP** derivatives having related synthetic mimetics of the proposed P_1-P_1' transition state. In addition, the reported decapeptide **H-142** (**14**) was prepared to evaluate its potency relative to this series of renin inhibitors. Highlights of the above structure-activity study are summarized as follows: (1) The P_4-P_1' pentapeptide core, Pro-Phe-His-Leuψ[CH_2NH]Val, of **H-142** was about 1000-fold less potent than the parent decapeptide as indicated by compound **15** (IC_{50} = 1 x 10^{-5}M). However, N-terminal modification of pentapeptide **15** by Ftr to yield **16** (IC_{50} = 2.1 x 10^{-8} M) provided 500-fold enchanced potency. (2) Substitution of cyclohexylmethyl and benzyl side-chain groups at the P_1 and P_1' positions, respectively, of **16** each provided more potent derivatives (*i.e.*, **18**-**20**). Surprisingly, compound **19** was about 10-fold less potent than it was predicted to be based on the structure-activity relationships of compounds **16**, **18** and **20**. Interestingly, Boger and co-workers[14a] have reported data for a highly lipophilic **SCRIP** congener, POA-His-ACHPA-Leu-Phe-NH_2, in which its IC_{50} (human plasma renin) value was 100-fold higher than its K_I (human kidney renin). This potency discrepency was attributed to adsorption of the **SCRIP** derivative to plasma components and subsequent diminished bioavailability for renin inhibition. (3) Conversion of Pheψ[CH_2NH]Phe in hexapeptide **6** to Phe-Phe yielded **21**, a compound which was inactive as an inhibitor. (4) Comparison of Leuψ[$CH(OH)CH_2$]Val, Sta, Ast and Leuψ[CH_2NH]Val modified congeners of **16** showed the superiority of the hydroxyethylene moiety as a CONH surrogate relative to the aminoethylene or aminomethylene substituents based on the higher potencies of Ac-Ftr-Pro-Phe-His-Leuψ[$CH(OH)CH_2$]Val-NH_2 (**22**, IC_{50} = 3.1 x 10^{-10} M) and Ac-Ftr-Pro-Phe-His-Sta-NH_2 (**23**, IC_{50} = 3.8 x 10^{-9} M) relative to **16** and Ac-Ftr-Pro-Phe-His-Ast-NH_2 (**24**, IC_{50} = 1.1 x 10^{-6}M), respectively.

Table II. Renin inhibitory activities of RIP derivatives.

	Compound*	IC_{50} (M)
RIP	H-Pro-His-Pro-Phe-His-Phe-Phe-Val-Tyr-Lys-OH	8.0 x 10^{-6}
14	H-Pro-His-Pro-Phe-His-Leuψ[CH_2NH]Val-Ile-His-Lys-OH	9.1 x 10^{-9}
15	Ac-Pro-Phe-His-Leuψ[CH_2NH]Val-NH_2	1.0 x 10^{-5}
16	Ac-Ftr-Pro-Phe-His-Leuψ[CH_2NH]Val-NH_2	2.1 x 10^{-8}
17	Ac-Ftr-Pro-Phe-His-Pheψ[CH_2NH]Val-NH_2	1.0 x 10^{-8}
18	Ac-Ftr-Pro-Phe-His-Chaψ[CH_2NH]Val-NH_2	2.0 x 10^{-9}
19	Ac-Ftr-Pro-Phe-His-Chaψ[CH_2NH]Phe-NH_2	3.0 x 10^{-9}
20	Ac-Ftr-Pro-Phe-His-Leuψ[CH_2NH]Phe-NH_2	1.3 x 10^{-9}
6	Ac-Ftr-Pro-Phe-His-Pheψ[CH_2NH]Phe-NH_2	5.6 x 10^{-10}
21	Ac-Ftr-Pro-Phe-His-Phe-Phe-NH_2	20% @ 10^{-5}
22	Ac-Ftr-Pro-Phe-His-Leuψ[$CH(OH)CH_2$]Val-NH_2	3.1 x 10^{-10}
23	Ac-Ftr-Pro-Phe-His-Sta-NH_2	3.8 x 10^{-9}
24	Ac-Ftr-Pro-Phe-His-Ast-NH_2	1.1 x 10^{-6}

*See Reference 1 for abbreviations.



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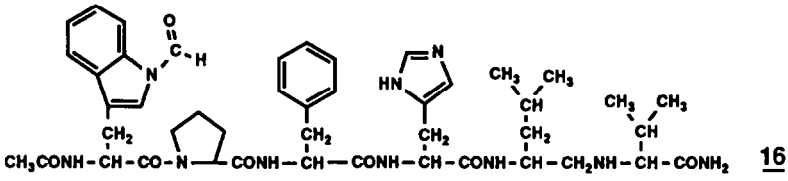

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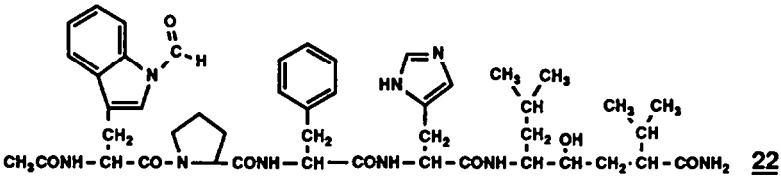

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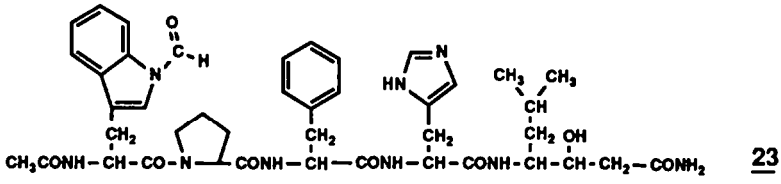

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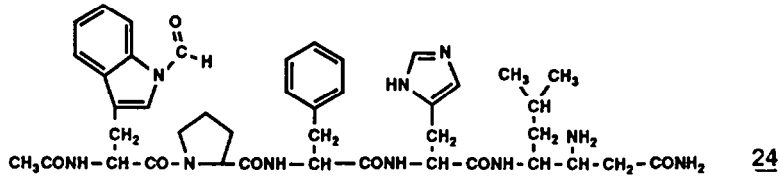

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Comparative Structure-Activity Relationships to Previously Cited P_1-P_1' Xaa-Yaa Modified ANG Congeners and Concluding Remarks. The above studies on P_1-P_1' Xaaψ[CH_2NH]Yaa modified hexapeptide congeners of Ac-Ftr-Pro-Phe-His-Leuψ[CH_2NH]Val-NH_2 (**16**) are similar to previous studies reported by Burton and co-workers[34] on a series of ANG analogues that led to the discovery of **RIP**. In summary, their investigation of the template H-Pro-His-Pro-Phe-His-Xaa-Yaa-Val-Tyr-OH by systematic replacement of the P_1 Xaa and P_1' Yaa sites showed that P_1' Phe was a key substitution to provide increased inhibitor potency (*i.e.*, Phe-Phe ≈ Leu-Phe > Leu-Leu as Xaa-Yaa insertions). The superiority of P_1' Phe versus Val in our series of P_1-P_1' Xaaψ[CH_2NH]Yaa substituted **RIP** fragment analogues suggest that the S_1' binding pocket for the P_1' Phe side-chain might be identical for both the P_1-P_1' Xaa-Yaa or Xaaψ[CH_2NH]Yaa modified series of inhibitors. However, the aminomethylene surrogate of the P_1-P_1' amide bond is apparently responsible for improved intermolecular interactions between the inhibitor and the renin active site as based on several observations: (1) the comparative renin inhibitory potencies of Ac-Ftr-Pro-Phe-His-Phe-Phe-NH_2 (**21**, IC_{50} $>10^{-5}$ M) and Ac-Ftr-Pro-Phe-His-Pheψ[CH_2NH]Phe-NH_2 (**6**, $IC_{50} = 5.6 \times 10^{-10}$ M); (2) the x-ray crystallographic structures of **U-70531E** at the active site of rhizopuspepsin[22] and **H-142** at the active site of endothiapepsin[21] which have shown the spatial proximity of the CH_2NH group to the two catalytically important Asp residues of the enzyme; and (3) the identification of similar H-bonding interactions in the dynamics-averaged molecular model of CKH-RENIN•**1** and the rhizopuspepsin•**2** crystallographic structure. Interestingly, in our own investigation of P_1-P_1' Phe-Phe modified ANG congeners we have previously shown[20] that C-terminal extension of **21** by Val transformed it from being inactive against renin to eliciting moderate inhibitory activity (*cf.*, Ac-Ftr-Pro-Phe-His-Phe-Phe-Val-NH_2, IC_{50} *ca.* 10^{-6} M). In contrast, C-terminal extension of **6** by Val resulted in 100-fold decreased potency (*cf.* compound **5**, Table I)

In conclusion, we have described the structure-activity relationships of C-terminal P_1-P_1' Xaaψ[CH_2NH]Yaa-NH_2 modified renin inhibitors and identified several congeners of the sequence Ac-Ftr-Pro-Phe-His-Xaaψ[CH_2NH]Phe-NH_2 (where Xaa = Leu, Cha or Phe) which effected IC_{50} values in the 10^{-10}-10^{-9} range. One hexapeptide, Ac-Ftr-Pro-Phe-MeHis-Pheψ[CH_2NH]Phe-NH_2, was found to be superpotent and very selective against human renin (versus porcine renin), stable to selected proteolytic enzymes, and suitable for *in vivo* evaluation. Further studies are in progress in these laboratories using human recombinant renin[35] and the CKH-RENIN active site computer model to determine the comparative kinetic properties of such C-terminal pseudodipeptidylcarboxamide modified peptides and their predicted intermolecular interactions (*e.g.*, hydrophobic, electrostatic and H-bonding) with the target enzyme active site.

EXPERIMENTAL SECTION

Materials. p-Methylbenzhydrylamine (p-MBHA) and benzhydrylamine (BHA) resins (each *ca.* 0.5 mequiv amine g-1) were purchased from U.S. Biochemical Corporation and Peptides International. p-Chloromethyl-poly(styrene-co-divinylbenzene) (Merrifield) resin (*ca.* 0.7 mequiv chloride g-1) was purchased from Lab Systems Incorporated. Nα-tert-butyloxycarbonyl (Boc) protected amino acid derivatives were purchased from Pennisula Laboratories, Advanced ChemTech and Peptides International. Nα-Boc-Pheψ[CH_2NH]Phe-OH, Nα-Boc-Pheψ[CH_2NH]D-Phe-OH, Nα-Boc-D-Pheψ[CH_2NH]Phe-OH, Nα-Boc-Leuψ[CH_2NH]Val-OH, Nα-Boc-Leuψ[CH(OTBDMS)CH_2]Val-OH, Nα-Boc-Sta-OH, Nα-Boc-Ast-OH and Nα-Boc-MeHis(N^{im}-Tos)-OH were prepared by reported[14a,14c,19a,20] procedures. The reactive side-chains of the amino acids were protected as follows His, N^{im}-Tos or N^{im}-Bom; Lys, N^{ϵ}-2-Cl-Z, Tyr, 0-2,6-Cl_2-Bzl, Trp, N^{in}-For; Cys, S-4-Me-Bzl; and Hcy, S-4-Me-Bzl. In addition, both Mpr and Mbu were protected by S-4-Me-Bzl. Before use all amino acid derivatives were tested for authenticity and purity by melting point determination and ninhydrin assay [36] ACS grade (or higher) methylene chloride (CH_2Cl_2), N,N'dimethylformamide (DMF) and absolute ethanol (EtOH) were purchased from Burdick and Jackson. Trifluoroacetic acid (TFA) was purchased from Columbia Organic Chemical Diisopropylethylamine (DIEA), anisole, acetic anhydride (Ac_2O), and N-acetylimidazole were purchased from Aldrich. All reagents and solvents were used without further purification, except the DMF, which was stored over 4 Å molecular sieves, and the TFA which was redistilled prior to use.

Peptide Synthesis. The general methodology used for the preparation of **RIP** and compounds **1**-**24** is described here. The synthesis of compounds **17** - **20** utilized the solid-phase reductive amination procedures previously described by Coy and co-workers[37] to incorporate their Xaaψ[CH_2NH]Yaa pseudodipeptides. The peptides were each prepared on either automated (Beckman 990B or Peptides International PS-2000) or manually-operated instruments by a previously reported[20] solid-phase synthetic procedure. The coupling reactions were effected by the use of N,N'-dicylohexylcarbodiimide (DCC) and 1-hydroxybenzotriazole (HOBT), except for the case of Boc-His(N^{im}-Tos) · dicyclohexylamine (Dcha) salt which was coupled with benzotriazol-1-yloxytris(dimethylamino)-phosphonium hexafluorophosphate (BOP). N-Acetylation was achieved by Ac_2O for **RIP** and compound **21** and by N-acetylimidizole for all other compounds. This procedure eliminated possible O- or N-acetylations of the P_1-P_1' Xaaψ[CH_2NH]Yaa, Leuψ[CH(OH)CH_2]Val, Sta or Ast functionalities. Following solid-phase synthesis, the title peptides were cleaved (60 min at 0-4°C) from their resins by 16% anisole-84% hydrogen fluoride (*ca.* 15 mL g-1 peptide-resin intermediate) which simultaneously removed all side-chain protecting groups except for the N^{in}-For of Ftr substituted compounds. After evaporation *in vacuo* of the hydrogen fluoride-anisole the peptide-resin products were washed with ethyl ether and the crude peptides extracted with aqueous acetic acid (HOAc) and lyophilized to yield amorphous powders. Cyclization of the disulfhydryl precursors of compounds **19** and **20** were achieved using diiodoethane to effect monomeric disulfide product formation under dilute DMF solvent conditions at about pH 7.

Peptide Purification and Physiochemical Characterization. The crude peptides (*ca.* 100 mg/sample load) were purified by semi-preparative reverse-phase HPLC on a Vydac C18 (15-20 μ particle size) column (2.2 cm i.d x 29.0 cm length) using a binary solvent system consisting of solvent A (10% acetonitrile-

0.2% TFA in H_2O) and solvent B (70% acetonitrile-0.2% TFA in H_2O) An exception to this solvent system was made for the purification of compound **22** in which the TFA was not added to solvents A and B in order to minimize lactonization of the C-terminal Leuψ[CH(OH)CH_2]Val-NH_2 which otherwise was essentially complete within 24 h in aqueous acid solutions. A flow rate of 2.0-2.5 mL min^{-1} (*ca.* 100 p.s.i. back pressure) was used and a typical chromatographic mobile-phase program was as follows: 17% solvent B isocratic (15 min); 17-67% solvent B linear gradient (210 min); and 67% solvent B isocratic (75 min). Elution of the peptide was monitored at 280 (or 254) nm, and 4.0-5.0 mL post-column fractions were collected. The title peptides were obtained by pooling of the desired fractions, removal of the acetonitrile by rotary evaporation *in vacuo* and lyophilization. AAA of compounds 1-20 (Table III) were performed using a Dionex D-500 following hydrolysis (0.1% phenol in 6M hydrochloric acid in a vacuum sealed tube at 100°C for 24 h). No corrections were made for the destruction of amino acids during hydrolysis. Fast atom bombardment-mass spectrometry (FAB-MS) of **RIP** and compounds **1-24** (Table III) were performed using a Varian-MAT-CH5-DF or a VG-ZAB-2F mass spectrophotometer. Analytical HPLC on a Beckman-344 gradient liquid chromatography system was used to determine the k' values and % purities of **RIP** and compounds **1-24** (Table III) on a Synchropak C18 (6.5 μ particle size) column (4.1 mm i.d. by 250 mm length) and other chromatographic protocol included a flow rate of 1.5 mL min^{-1} (*ca.* 2000 p.s.i. back pressure), a standard binary solvent mobile phase program (17% solvent B isocratic for 2 min; 17-100% solvent B linear gradient over 20 min), and detection at 220 nm and 280 (or 254) nm. Optical rotations of selected compounds were performed using a Perkin-Elmer-141 polarimeter.

Molecular Modeling Methods. The 3-D human renin model (CKH-RENIN) used in this study has been previously described[23], and was displayed using an Evans and Sutherland PS340 high performance graphics system and MOSAIC[38] software. The 3-D structures of compounds **1** and **6** at the active site of CKH-RENIN model were generated as previously described[20] for Ac-Ftr-Pro-Phe-His-Sta-Ile-NH_2. For example, the P_3-P_1' residues of pepstatin (*i.e.*, Val-Val-Sta sequence) were structurally mutated to provide the corresponding Phe-His-Pheψ[CH_2NH]Phe sequence of **1** and **6**, and the X_1 and X_2 angles of pepstatin were conserved accordingly. Further structural mutation was required to provide the N-terminal dipeptide sequence, Ac-Ftr-Pro of both **1** and **6**, and this required computer graphics-based modeling at the CKH-RENIN active site. A similar modeling strategy was required to provide the C-terminal Val-Tyr-NH_2 and amide of compounds **1** and **6**, respectively. This modeling was performed in order to maximize H-bonds from the polyamide backbone of each inhibitor to the CKH-RENIN active site as well as hydrophobic interactions from the amino acid side-chains of the ligand to the enzyme. In addition, unfavorable steric interactions were minimized, and the resultant CKH-RENIN•inhibitor complexes were energy refined using the program CHARMM.[28] Constraints were placed on the spatial disposition of ligand atoms from their initial coordinates and reduced every 25 cycles for the first 100 cycles. An additional 100 cycles were completed without constraints on either the enzyme or ligand.

Torsion angles at the N-terminus of the polyamide backbone of compound **1** were varied in order to explore the P_5 Ftr side-chain, in particular, with respect to CKH-RENIN binding subsites which might provide significant hydrophobic interactions. Specifically, a conformation of **1** was obtained in which the P_5 Ftr side-chain attained close contact with several aromatic amino acid residues (*e.g.*, Tyr-230 at 6.0 Å and Tyr-254 at 4.5 Å) derived from the C-terminal lobe of the CKH-RENIN model. To explore the conformation space availabe to compound **1** in this putative binding geometry, molecular dynamics simulation of the CKH-RENIN•**1** complex was performed in which constraints were placed on residues outside of an approximate 10 Å core of the CKH-RENIN about the docked compound **1**. The energy refined structure of this enzyme inhibitor complex was first thermally perturbed to and equilibrated at about 300°K for 20 ps, and then another 20 ps of simulation was conducted to provide the experimental analysis. The average 3-D structure of compound **1** during the second 20 ps simulation showed that, although the intermolecular distances between the P_5 Ftr side chain of **1** and specific CKH-RENIN active site residues increased slightly (*e.g.*, from 6.0 to 7.0 Å for Tyr-230, and from 4.5 to 8.0 Å for Tyr-254), close contacts were conserved between those side chain functionalities which contributed to the P_5•S_5 interaction. This putative P_5•S_5 interaction provided solvent shielding for both the Ftr side-chain and aromatic side-chains of residues Tyr-14, Tyr-230, Phe-123, Tyr-254 and Tyr-20 of the CKH-RENIN active site Furthermore, this spatial

disposition allowed the P_5 Ftr to exist in an orientation similar to previously described[39] aromatic clusters of other proteins determined by x-ray diffraction studies.

Table III. AAA, FAB-MS and HPLC data for RIP analogues.

Compound	Amino Acid Analysis*	FAB-MS†	HPLC‡	
RIP	P (2.08), H (2.00), F (2.94), V (0.99), Y (0.97), K (1.02)	1318	7.7	(>98%)
1	W (0.95), P (0.98), H (0.96), F (1.05), V (0.64), Y (1.03)	1197	10.8	(>98%)
2	P (0.95), H (2.00), F (1.04), V (0.97), Y (1.02)	1079	8.2	(>98%)
3	H (0.97), F (1.05), V (0.71), Y (0.99)	942	10.2	(>96%)
4	P (1.00), H (0.99), F (1.02), V (0.77), Y (0.98)	983	8.9	(>98%)
5	W (1.04), P (0.95), H (1.04), F (0.97)	1034	11.0	(>95%)
6	W (0.99), P (1.01), H (0.98), F (1.01)	935	10.5	(>96%)
7	P (0.98), H (1.02), F (1.05)	935	10.6	(>96%)
8	P (1.03), H (0.91), F (1.02)	935	10.4	(>96%)
9	P (1.01), F (0.99)	945	11.5	(>95%)
10	P (0.99), F (1.01)	973	12.1	(>95%)
11	W (1.00), P (2.00), F (0.98)	895	12.0	(>98%)
12	W (1.07), P (0.95), F (0.89)	949	10.1	(>95%)
13	P (1.05), F (0.95)	735	9.4	(>95%)
14	P (1.90), H (2.98), F (0.99), K (1.01), I (0.97)	1210	6.1	(>95%)
15	P (1.00), H (0.97), F (1.01)	638	8.4	(>97%)
16	W (1.03), P (0.86), H (0.98), F (1.03)	853	8.5	(>97%)
17	P (0.93), H (1.04), F (1.03)	887	9.6	(>96%)
18	P (1.02), H (0.96), F (1.02)	893	10.8	(>96%)
19	P (0.90), H (1.08), F (1.02)	942	11.8	(>96%)
20	P (0.96), H (0.96), F (1.07)	901	10.4	(>96%)
21	W (0.94), P (1.03), H (1.02), F (3.01)	949	11.6	(>97%)
22	W (0.90), P (1.02), H (1.00), F (1.00)	868	9.8	(>96%)
23	W (1.04), P (0.98), H (0.95), F (1.01)	812	8.2	(>95%)
24	P (0.98), H (0.98), F (1.04)	811	8.3	(>95%)

* AAA values of common amino acids, except for Cys, were determined. Trp and Val values were frequently observed to be low due to oxidative decomposition and incomplete hydrolysis, respectively.
† FAB-MS values refer to $(M + 1)^+$.
‡ HPLC values refer to k' and % purity, respectively.

Renin Inhibition *In Vitro* Assays. All of the compounds were assayed for their human plasma renin inhibitory activity (*i.e.*, IC_{50} determinations) using reconstituted, lyophilized human plasma with 0.1% ethylenediaminetetraacetic acid (EDTA) which was commercially obtained (New England Nuclear) The

angiotensin I generation step utilized 250 μL of plasma, 2.5 μL phenylmethylsulfonyl fluoride, 25 μL of maleate buffer (pH 6.0), and 10 μL of an appropriate concentration of inhibitor in a 1.0% Tween 80 in water vehicle. Incubation was for 90 min at 37°C. Radioimmunoassay for angiotensin I was carried out with a commercial kit (Clinical Assays). Plasma renin activity values for inhibitor tubes were compared to control tubes to estimate the % inhibiton. The IC_{50} values were obtained by plotting three to four inhibitor concentrations on semilog graph paper and estimating the concentration producing 50% inhibition.

In addition, compound **12** was also assayed for its ability to inhibit porcine kidney renin. One unit of partially purified porcine kidney renin (Sigma; 8.4 unit mg^{-1} protein) was reconstituted and diluted 8100-fold with tris-acetate buffer. The substrate for porcine renin was the tetradecapeptide (TDP, H-Asp-Arg-Val-Tyr-Ile-His-Pro-Phe-His-Leu-Leu-Val-Tyr-Ser-OH; from Peninsula). The TDP was prepared as an 0.16 mg ml^{-1} solution in Na_2HPO_4 buffer at pH 6.0. Compound **12** was dissolved in 100 μL dimethyl sulfoxide (DMSO) and diluted with Na_2HPO_4 buffer to the desired concentration. Each incubation mixture of 340 μL contained 200 μL Na_2HPO_4 buffer, 10 μL phenylmethylsulfonylfluoride, 20 μL porcine renin, 50 μL TDP, and 60 μL of the inhibitor solution. Incubation was for 30 min at 37°C. Following incubation, the mixture was analyzed in duplicate via radioimmunoassay for angiotensin I and the IC_{50} value determined as described above for human plasma renin inhibition.

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REFERENCES AND NOTES

(1) Symbols and abbreviations are in accord with the recommendations of the IUPAC-IUB Commission on Biochemical Nomenclature; Eur. J. Biochem., 1984, **158**, 9. All optically active amino acids are of the L variety unless otherwise specified. Additional abbreviations used are: Ftr, Trp(Nin-For); Sta, (3S, 4S)-4-amino-3-hydroxy-6-methylhepatanoic acid; Ast, (3S, 4S)-3,4-diamino-6-methylheptanoic acid; Leuψ[CH(OH)CH_2]Val, (2S, 4S, 5S)-5-amino-4-hydroxy-2-isopropyl-7-methyloctanoic acid; Leuψ[CH_2NH]Val, (S)-2-amino-4-methylpentyl-L-valine; Pheψ[CH_2NH]Phe, (S)-2-amino-3-phenylpropyl-L-phenylalanine; MeHis, Nα-methyl-L-histidine; Mpr, 3-mercaptopropionic acid; Mbu, 4-mercaptobutyric acid; Tba, t-butylacetic acid; and Hcy, L-homocysteine; POA, phenoxyacetyl; and ACHPA, (3S, 4S)-4-amino-5-cyclohexyl-3-hydroxypentanoic acid. In accord with nomenclature described by Schechter and Berger (Biochem. Biophys. Res. Commun. 1967, **27**, 157), P_n-P_n' refer to the side chain positions of the peptide substrate, whereas S_n-S_n' refer to the subsites on the enzyme that bind the corresponding side chain of the substrate. Other abbreviations are referenced as used in text. The term "transition state" has been used to implicate the P_1-P_1' scissile amide bond isosteres of CH_2NH, $CH(OH)CH_2$ and $CH(OH)CH_2$-NH as possible tetrahedral mimics of the substrate P_1-P_1' $C(OH)_2NH$ transition-state which is believed to be produced during the renin-ANG reaction. Also refer to: Rich, D.H. J. Med. Chem. 1985, **28**, 263.

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