Renin Inhibitors. Dipeptide Analogues of Angiotensinogen Incorporating Transition-State, Nonpeptidic Replacements at the Scissile Bond[1]

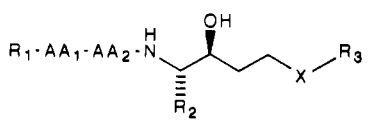
Giorgio Bolis, Anthony K. L. Fung, Jonathan Greer, Hollis D. Kleinert, Patrick A. Marcotte, Thomas J. Perun, Jacob J. Plattner,* and Herman H. Stein

Abbott Laboratories, Cardiovascular Research Division, Abbott Park, Illinois 60064. *Received February 24, 1987*

A series of dipeptide analogues of angiotensinogen have been prepared and evaluated for their ability to inhibit the aspartic proteinase renin. The compounds were derived from the renin substrate by replacing the scissile amide bond with a transition-state mimic and by incorporating bioisosteric replacements for the Val-10 amide bond. Analogue **21a** exhibited an IC_{50} of 7.6 nM against purified human renin, showed high specificity for this enzyme, and produced a hypotensive response in anesthetized, salt-depleted cynomolgus monkeys.

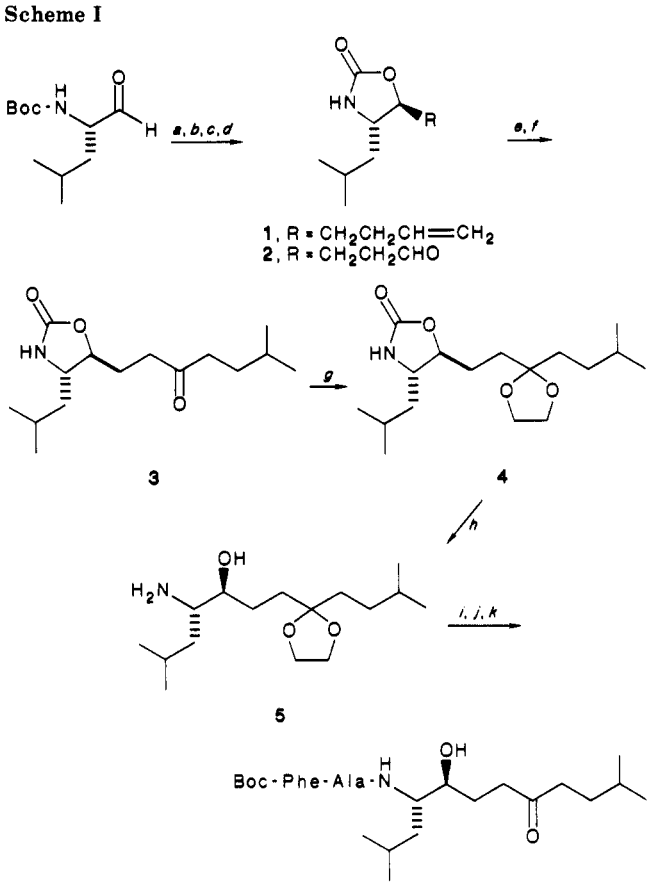
During the past several decades, the renin–angiotensin system (RAS) has represented a highly attractive biological target for attempted pharmacologic intervention. Teprotide[2] provided the first indication that modulation of the RAS would be therapeutically useful, and captopril and enalapril have since emerged as medically and commercially important cardiovascular drugs.[3] The success of the angiotensin converting enzyme (ACE) inhibitors in the marketplace has fueled the search to develop alternative antihypertensive therapy, and consequently the search for an orally active renin inhibitor has intensified in recent years.[4]

Recently, we reported a study in which a size reduction strategy was used to identify small peptide analogues of angiotensinogen that are potent inhibitors of human renin.[5] Subsequently, we described in two papers one series of renin inhibitors containing a statine residue retroinverted at the C-terminal position[6] and another series with novel Leu-Val replacements at the scissile bond.[7] We now report a continuation of this work and present the synthesis and biological activity for a series of dipeptide analogues represented generically by structure I. Formally, these compounds are derived from the renin substrate angiotensinogen by (1) modification of the scissile amide bond with a hydroxyethylene isostere,[8] (2) replacement of the valine amide bond with a bioisosteric[9] substituent (X), and (3) elimination of the remainder of the protein and incorporation of a small hydrophobic substituent (R_3).



I

Scheme I



[a] $MgBrCH_2CH_2CH=CH_2$. [b] Ac_2O. [c] NaOMe. [d] $NaIO_4/OsO_4$. [e] $MgBrCH_2CH_2CH(CH_3)_2$. [f] H_2CrO_4/acetone. [g] $HOCH_2CH_2OH$, *p*-TsOH. [h] $Ba(OH)_2$. [i] $ClCO_2$-*i*-Bu/Et_3N. [j] Boc-Phe-Ala-OH. [k] HOAc/H_2O/THF.

Chemistry. The compounds prepared for this study are shown in Table I, and their syntheses are outlined in Schemes I–III. Analogue **6** was prepared by the route outlined in Scheme I. Grignard reaction of 4-butenylmagnesium bromide with *N*-Boc-L-leucinal[10] was followed by base treatment of the resulting adduct to give oxazolidinone **1**. Elaboration of the butenyl side chain in **1** to the desired 3-ketoheptyl substituent was effected by $NaIO_4/OsO_4$ cleavage[11] to give aldehyde **2**, which afforded ketone **3** by successive Grignard reaction and Jones oxidation. Protection of the ketone substituent in **3** as the

(1) Portions of this work were presented in April 1986 at the 191st National Meeting of the American Chemical Society. See: *Abstracts of Papers*, 191st National Meeting of the American Chemical Society, New York, NY; American Chemical Society: Washington, DC, 1986.
(2) (a) Gavras, H.; Brunner, H. R.; Laragh, J. H.; Gavras, I.; Vukovich, R. A. *Clin. Sci. Mol. Med.* **1975**, *48*, 57s. (b) Gavras, H.; Brunner, H. R.; Laragh, J. H.; Sealey, J. E.; Gavras, I.; Vukovich, R. A. *N. Engl. J. Med.* **1974**, *219*, 817.
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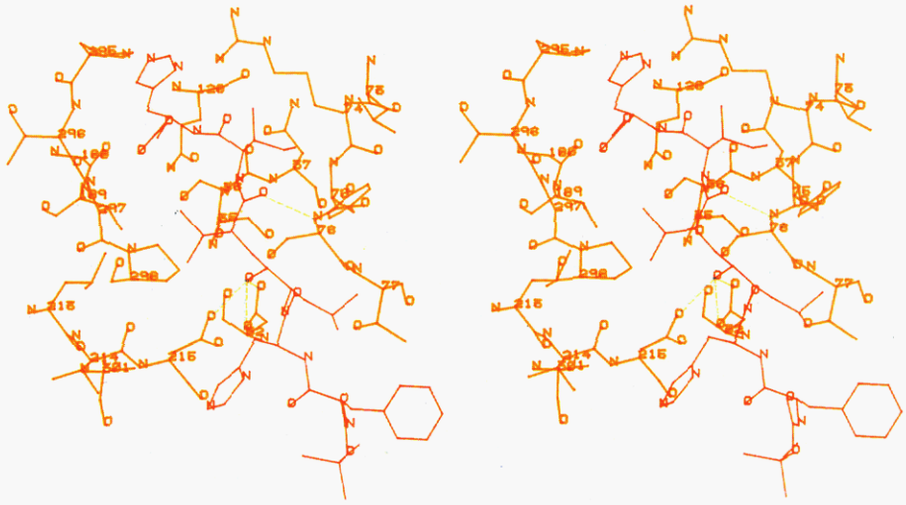
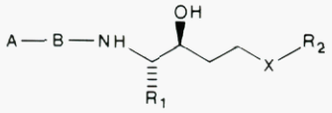


Figure 1. Stereoview of compound II (red) in the model active site of renin (orange). Note the relevant hydrogen bonds (dashed green lines) between the P_1 hydroxyl and the active site aspartates and between the Val P_1' carbonyl oxygen and the α-NH of Ser-76 as discussed in the text.

Table I. Dipeptide Analogues of Angiotensinogen



compd	A	B	R_1	CO	R_2	mp, °C	formula[a]
6	Boc-Phe	Ala	isobutyl	CO	i-C_5H_{11}	137–138	$C_{31}H_{51}N_3O_6$
7	Boc-Phe	Ala	isobutyl	CHOH[b]	i-C_5H_{11}	129–133	$C_{31}H_{53}N_3O_6$
12a	Boc-Phe	Ala	isobutyl	S	i-C_5H_{11}	137–138	$C_{30}H_{51}N_3O_5S$
12b	Boc-Phe	Ala	isobutyl	S	CH_2CH_2Ph	glass	$C_{33}H_{49}N_3O_5S$
12c	Boc-Phe	Ala	isobutyl	S	i-C_4H_9	137–139	$C_{29}H_{49}N_3O_5S$
12d	Boc-Phe	Ala	isobutyl	S	i-C_3H_7	126–128	$C_{28}H_{47}N_3O_5S$
13	Boc-Phe	Ala	isobutyl	SO[b]	i-C_5H_{11}	150–153	$C_{30}H_{51}N_3O_6S$
14a	Boc-Phe	Ala	isobutyl	SO_2	i-C_5H_{11}	165–166	$C_{30}H_{51}N_3O_7S$
14b	Boc-Phe	Ala	isobutyl	SO_2	CH_2CH_2Ph	186–187	$C_{33}H_{49}N_3O_7S$
14c	Boc-Phe	Ala	isobutyl	SO_2	i-C_4H_9	147–149	$C_{29}H_{49}N_3O_7S$
14d	Boc-Phe	Ala	isobutyl	SO_2	i-C_3H_7	156–158	$C_{28}H_{47}N_3O_7S$
21a	Boc-Phe	His	cyclohexylmethyl	SO_2	i-C_3H_7	145–147	$C_{34}H_{53}N_5O_7S$
21b	Boc-Phe	His	cyclohexylmethyl	SO_2	C_2H_5	158–160	$C_{33}H_{51}N_5O_7S$
22a	Boc-Phe	Ala	cyclohexylmethyl	SO_2	i-C_3H_7	92–94	$C_{31}H_{51}N_3O_7S$
22b	Boc-Phe	Ala	cyclohexylmethyl	SO_2	C_2H_5	150–151	$C_{30}H_{49}N_3O_7S$
23	Boc-Phe	Leu	cyclohexylmethyl	SO_2	i-C_3H_7	152–154	$C_{34}H_{57}N_3O_7S$
24	Boc-Phe	Phe	cyclohexylmethyl	SO_2	i-C_3H_7	139–141	$C_{37}H_{55}N_3O_7S$[c]
25	Boc-(Me)Tyr[d]	His	cyclohexylmethyl	SO_2	i-C_3H_7	160–162	$C_{35}H_{55}N_5O_8S$
26	Tba-Phe[e]	His	cyclohexylmethyl	SO_2	i-C_3H_7	146–148	$C_{35}H_{55}N_5O_6S$
27	Etoc-Phe[f]	His	cyclohexylmethyl	SO_2	i-C_3H_7	168–170	$C_{32}H_{49}N_5O_7S$

[a] All compounds gave satisfactory C, H, and N analyses. [b] *R, S* mixture of diastereomers at this center. [c] C: calcd, 64.79; found, 64.36. [d] (*tert*-Butyloxycarbonyl)tyrosine methyl ether. [e] (*tert*-Butylacetyl)phenylalanine. [f] (Ethoxycarbonyl)phenylalanine.

ethylene ketal was followed by hydrolysis with $Ba(OH)_2$ to give amino alcohol **5**. Mixed anhydride coupling of **5** with Boc-Phe-Ala-OH then gave the desired product **6**. The corresponding diol derivative **7** was prepared by $NaBH_4$ reduction of **6**.

Analogues containing a sulfur atom in the side chain and an isobutyl substituent at the P_1 subsite were prepared as outlined in Scheme II. Oxazolidinone **8** was synthesized analogously to **1** described above. Hydroboration of **8** with 9-BBN[12] afforded alcohol **9**, which gave thioethers **10a–d** by successive mesylation and mercaptide displacement. Oxazolidinone hydrolysis was followed by peptide coupling to complete the synthesis. The sulfoxide analogue **13** and sulfones **14a–d** were prepared by oxidation of the corresponding sulfides with MCPBA.

The syntheses of analogues containing a cyclohexylmethyl side chain at the P_1 subsite[13] were carried out as depicted in Scheme III. Dibal reduction of *N*-Boc-L-cyclohexylalanine methyl ester[14] was followed by in situ reaction of the resulting aldehyde with vinylmagnesium bromide to give oxazolidinone **15** in 54% yield. Hydroboration and mesylation as described above provided mesylate **17**. Displacement of **17** with isopropyl mercaptide and ethyl mercaptide produced **18a** and **18b**, respectively.

(12) Brown, H. C.; Krishnamurthy, S.; Yoon, N. M. *J. Org. Chem.* **1976**, *41*, 1778.

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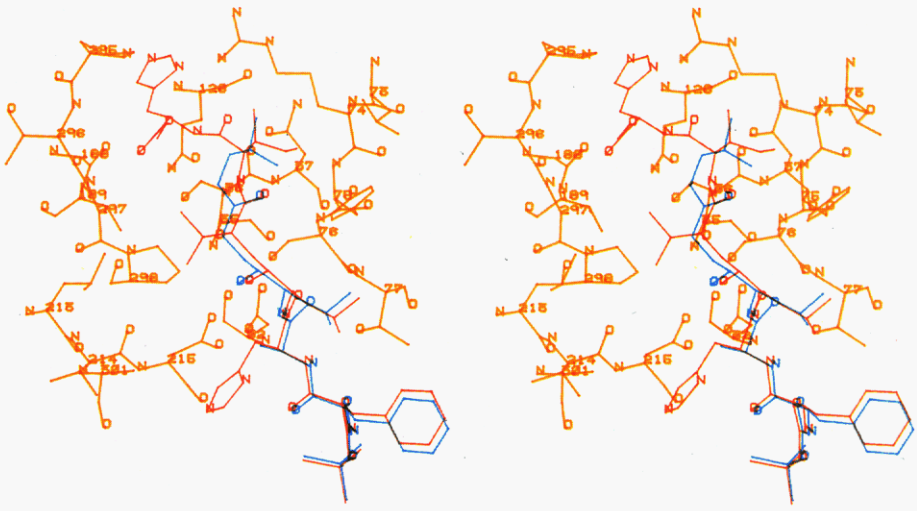


Figure 2. Stereoview of inhibitor **6** (blue) superimposed on compound II (red) in the active site of renin (orange). The renin active site was minimized along with the inhibitor **6**, but since the structure of the former did not change significantly, for purpose of comparison the enzyme structure in this figure is the same as in Figure 1.

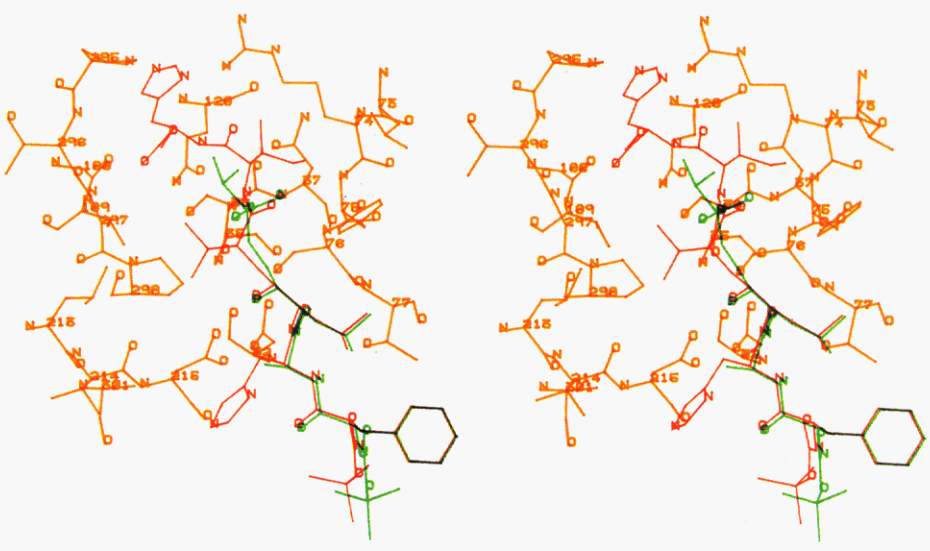
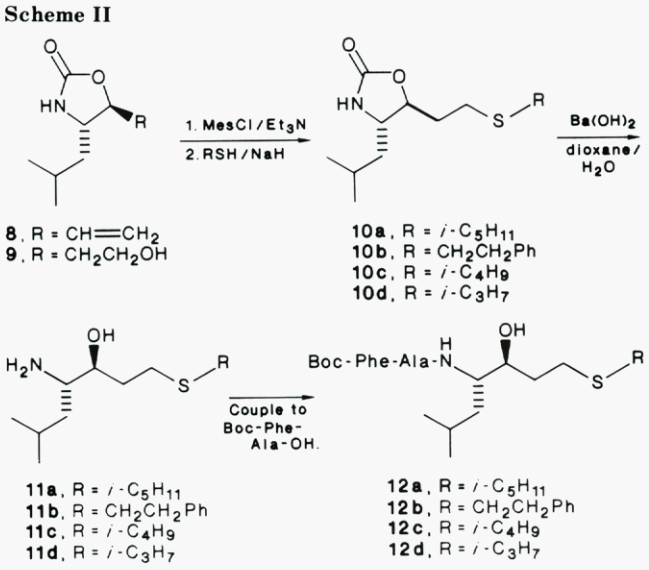


Figure 3. Stereoview of inhibitor **14d** (green) superimposed on compound II (red) in the active site of renin (orange). The renin active site was minimized along with inhibitor **14d**, but since the structure of the former did not change significantly, for purpose of comparison the enzyme structure in this figure is the same as in Figure 1.

Scheme II



8, R = $CH{=}CH_2$
9, R = CH_2CH_2OH

10a, R = *i*-C_5H_{11}
10b, R = CH_2CH_2Ph
10c, R = *i*-C_4H_9
10d, R = *i*-C_3H_7

11a, R = *i*-C_5H_{11}
11b, R = CH_2CH_2Ph
11c, R = *i*-C_4H_9
11d, R = *i*-C_3H_7

12a, R = *i*-C_5H_{11}
12b, R = CH_2CH_2Ph
12c, R = *i*-C_4H_9
12d, R = *i*-C_3H_7

Oxidation to the corresponding sulfones and oxazolidinone cleavage with $Ba(OH)_2$ led to amino alcohols **20a** and **20b**. Coupling of these products, either stepwise or with the appropriate dipeptides, then gave the desired final products.

The hydroxyl stereochemistry for the above products was established by examining the ^1H NMR spectra for the corresponding 2-oxazolidinones. The chemical shifts and coupling constants of ring hydrogens for the desired trans oxazolidinones are consistent with ample literature precedent.[15]

Biological Results and Discussion

Structure–Activity Relationships. Previously[5] we reported the effect of peptide chain length on renin in-

(15) (a) Rich, D. J.; Sun, E. T. O. *J. Med. Chem.* **1980**, *23*, 27. (b) Futagawa, S.; Inui, T.; Shiba, T. *Bull. Chem. Soc. Jpn.* **1973**, *46*, 3308. (c) Foglia, T. A.; Swern, D. *J. Org. Chem.* **1969**, *34*, 1680. (d) Cardillo, G.; Orena, M.; Sandri, S.; Tomasini, C. *Tetrahedron* **1985**, *41*, 163.

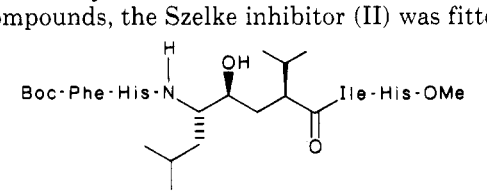
Table II. Enzyme Inhibition by Renin Substrate Analogues

compd	IC_{50},[a] μM: renin, purified human	renin, human plasma	renin, rat plasma	cathepsin D
6	2.4	NT[b]	NT	NT
7	3.8	NT	NT	NT
12a	5.5	NT	NT	NT
12b	4.2	NT	NT	NT
12c	4.1	NT	NT	NT
12d	4.8	NT	NT	NT
13	5.2	NT	NT	NT
14a	2.4	NT	NT	NT
14b	1.8	NT	NT	NT
14c	3.2	NT	NT	NT
14d	1.6	NT	NT	NT
21a	0.0076	0.027	>10 (11)	>10 (0)
21b	0.010	0.028	>10 (0)	>10 (0)
22a	0.076	0.85	>10 (12)	>10 (22)
22b	0.14	1.0	>10 (2)	>10 (18)
23	0.014	0.077	>10 (18)	8.0
24	0.020	0.34	>10 (26)	>10 (0)
25	0.0091	0.041	>10 (0)	>10 (0)
26	0.0060	0.019	>10 (30)	>10 (0)
27	0.010	0.023	>10 (12)	>10 (0)
H-261[c]		0.0007		

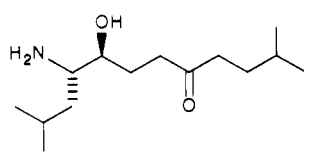
[a] Value in parentheses is the percent inhibition at 1.0×10^{-5} M. None of the compounds inhibited pepsin at 1.0×10^{-5} M. [b] Not tested. [c] H-261 = Boc-His-Pro-Phe-His-Leu$\overset{OH}{=}$Val-Ile-His-OH (ref 8).

hibition for a series of angiotensinogen fragments containing a reduced amide function at the scissile bond. In that study we found that amino acid residues beyond the P_1' portion of angiotensinogen were not required to maintain a significant renin inhibitory potency. A tetrapeptide analogue truncated beyond the P_1' position maintained inhibitor potency equivalent to that of the parent hexapeptide. These results encouraged us to design even "smaller" renin inhibitors by eliminating residues beyond the scissile bond and in their place incorporating small, stable, nonpeptidic substituents capable of binding in the active site region of renin.

Szelke[8] has previously reported that a hydroxyethylene isostere replacement for the scissile amide bond of angiotensinogen affords a 70-fold better inhibitor than the corresponding reduced amide bond. In analogy with this concept, we therefore chose to incorporate a tetrahedral hydroxyl group as a transition-state mimic to replace the leucine carbonyl function. In order to aid the design of new compounds, the Szelke inhibitor (II) was fitted into



II



III

our three-dimensional model structure for human renin and the complex minimized. Details of the modeling and energy calculations have been described.[5] After energy minimization of the active site–inhibitor complex,[5] several new interactions were observed between the enzyme and the inhibitor, which were not present in the previously reported reduced amide inhibitors. Figure 1 shows the interactions of the hydroxyl group at position P_1 forming a network of hydrogen bonds with the active site residues Asp-32 and Asp-215. The side chain of residue Val P_1' continues to lie in the vicinity of Leu-213, Asp-215, and Pro-298, and Ile P_2' is in a pocket lined by residues Tyr-75, Leu-73, Gln-128, and Asn-37. Also notable was the close proximity of the Val P_1' carbonyl oxygen to the α-NH of Ser-76, suggesting the possible formation of a hydrogen bond at this position.

In order to maintain the maximum number of interactions between the inhibitor and the enzyme while shortening the C-terminal portion of the inhibitor as much as possible, the hydroxy ketone III was designed as a stable, transition-state mimic to replace the Leu-Val-Ile-His residues of the Szelke inhibitor. In this moiety, both the interactions of the hydroxyl with the active site aspartates and the possibility of a hydrogen bond of the carbonyl should be preserved. The isopentyl group was intended to occupy the hydrophobic pocket of the Ile P_2' side chain.

The first compound designed was **6**. Figure 2 shows the structure of **6** bound to the active site after energy minimization. It is clear that the hydroxy ketone fragment III superimposes very nicely upon the backbone of Val P_1' and Ile P_2' with the isopentyl group fitting into the pocket at position P_2'. In this conformation, the pocket for the side chain of position P_1' would remain empty. The ketone function is found hydrogen bonding with the α-NH of Ser-76.

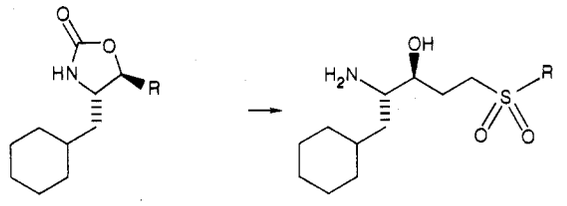
The synthesis of **6** was carried out as described in Scheme I. Use of an Ala residue at the P_2 subsite rather than a His as found in angiotensinogen facilitated both the synthesis and purification of target analogues at the early stages of this work. As seen from data in Table II, compound **6**, which incorporates the design features mentioned above, inhibited human renin at a moderate level of 2.4×10^{-6} M. Some time ago Hirschmann noted[16] that groups that are apparently required for high potency in a biologically active compound can be eliminated if additional or alternative binding can be realized. Since analogue **6** represents a substantial reduction in size of the minimal substrate octapeptide,[17] we next sought to optimize binding in the remainder of the molecule so as to increase the inhibitor potency of this compound; SAR studies were therefore undertaken.

The effect of replacing the ketone moiety with alternative functional groups is seen with compounds **7**, **12a**, **13**, and **14a** (Table II). Of the four compounds, only the sulfone analogue (**14a**) matched the inhibitor potency of the ketone **6**, and although the differences are not striking, the trend supports the possibility of favorable electrostatic or hydrogen-bonding interaction contributing to the binding of **6** and **14a** to renin. The analogue containing a sulfoxide function at this position (**13**) showed one-half the potency of the ketone and sulfone congeners. Since this compound is an *R*, *S* mixture of diastereomers, one of the isomers may also be hydrogen bonding to Tyr-75 or Ser-76.

The effect of varying the hydrophobic group attached to the heteroatom was explored next, and the results are presented in Table III. For this study both thioether and

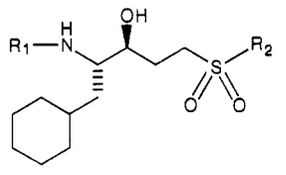
(16) Hirschmann, R. In *Peptides: Structure and Function. Proceedings of the Eighth American Peptide Symposium*; Hruby, V. J., Rich, D. J., Eds.; Pierce Chemical: Rockford, IL, 1983; pp 1–32.
(17) Skeggs, L.; Lentz, K.; Kahn, J.; Hochstrasser, J. *J. Exp. Med.* **1968**, *128*, 13.

Scheme III



15, R = $CH{=}CH_2$
16, R = CH_2CH_2OH
17, R = CH_2CH_2OMes
18a, R = $CH_2CH_2SCH(CH_3)_2$
18b, R = $CH_2CH_2SCH_2CH_3$
19a, R = $CH_2CH_2SO_2CH(CH_3)_2$
19b, R = $CH_2CH_2SO_2CH_2CH_3$

20a, R = i-C_3H_7
20b, R = C_2H_5



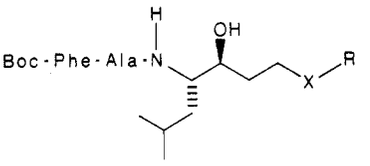
21a, R_1 = Boc-Phe-His, R_2 = i-C_3H_7
21b, R_1 = Boc-Phe-His, R_2 = C_2H_5
22a, R_1 = Boc-Phe-Ala, R_2 = i-C_3H_7
22b, R_1 = Boc-Phe-Ala, R_2 = C_2H_5
23, R_1 = Boc-Phe-Leu, R_2 = i-C_3H_7
24, R_1 = Boc-Phe-Phe, R_2 = i-C_3H_7
25, R_1 = Boc-(Me)Tyr-His, R_2 = i-C_3H_7
26, R_1 = Tba-Phe-His, R_2 = i-C_3H_7
27, R_1 = Etoc-Phe-His, R_2 = i-C_3H_7

sulfone functions were introduced at the position that is isosteric with the valine carbonyl group of angiotensinogen. The phenethyl group (**12b** and **14b**) was designed to lie in the Ile P_2' pocket like the isopentyl. In modeling compounds **12d** and **14d**, using energetics calculations,[5] we found that the terminal isopropyl group is pointing toward positions P_2' and P_3', but leaving the pockets for positions P_1' and P_2' empty. In this conformation, compound **14d** forms optimal hydrogen-bond interactions between the oxygens of the sulfone group and residues Tyr-75 and Ser-76 (Figure 3). The situation is less clear in compound **12d**, where the lack of a hydrogen bond to the α-NH's of residues Tyr-75 and Ser-76 makes it difficult to identify a unique conformation. Because of the above-mentioned hydrogen-bond interactions, as the data indicate, the sulfone analogues are more potent than the corresponding thioether congeners in each case; however, the nature of the terminal alkyl group does not seem to dramatically affect inhibitor potency. The isopropyl group was therefore selected as the "best" substituent at this position due to its smaller molecular size and the somewhat better IC_{50} value for **14d**.

Further optimization of inhibitor potency was achieved by variation of the side-chain substituents at the P_1 and P_2 positions (Table II). Replacement of the isobutyl side chain with cyclohexylmethyl produced a 20-fold increase in inhibitor potency (compare **14d** and **22a**). This striking effect for the cyclohexylmethyl side chain has previously been recognized.[14] Additional inhibitor potency could be realized by variation of the residues at subsite P_2. Both Leu and Phe were better than Ala while His at this site produced the most potent inhibitor.

The compounds tested in human plasma all displayed weaker renin inhibition as compared to the purified systems; in the His series, factors of 2.3–4.5 (**21a,b**, **25–27**) in potency were observed. Progressively larger effects, ranging from 5.5-fold to 17-fold, were noted as the His was substituted by Leu, Ala, and Phe, respectively; the reason for this loss of potency is not immediately obvious since the effect does not correlate with either the size or lipophilicity of the substituting amino acids. Large differences

Table III. Effect of Aliphatic Substituent Attached to Sulfur



compd	X	R	IC_{50},[a] μM
12a	S	$CH_2CH_2CH(Me)_2$	5.5
12b	S	CH_2CH_2Ph	4.2
12c	S	$CH_2CH(Me)_2$	4.1
12d	S	$CH(Me)_2$	4.8
14a	SO_2	$CH_2CH_2CH(Me)_2$	2.4
14b	SO_2	CH_2CH_2Ph	1.8
14c	SO_2	$CH_2CH(Me)_2$	3.2
14d	SO_2	$CH(Me)_2$	1.6

[a] Purified human renin.

in renin inhibitory activity between purified and plasma systems have been reported previously.[18]

The specificity of the more potent inhibitors for human renin was demonstrated by a corresponding weak inhibition of rat plasma renin and the other aspartic proteinases cathepsin D and pepsin (Table II).

In Vitro Stability Studies. A major obstacle in developing therapeutically useful drugs from peptide lead structures has been the propensity of peptides to undergo proteolytic degradation in the intestinal tract and in the serum. In a previous paper[6] we showed that renin inhibitors containing a Boc-Phe-His fragment in their structure were susceptible to facile cleavage between Phe and His by chymotrypsin in vitro. It was further demonstrated that substitution of (Me)Tyr for Phe in these compounds prevented this degradation. In order to evaluate the enzymatic stability for the current series of compounds, we subjected several analogues to a battery of in vitro preparations (Table IV).

The four compounds evaluated for their stability to enzymatic degradation (**21a**, **22a**, **25**, **26**) were found not to be susceptible to cleavage by liver, kidney, or intestinal enzymes. However, specific post-phenylalanine hydrolysis was catalyzed by both purified chymotrypsin and crude pancreatic protease for **21a**, **22a**, and **26**. In each case, the two expected products were identified by their HPLC retention times and were subsequently stable in the incubation. The Phe-Ala bond of **22a** was cleaved somewhat more rapidly than the Phe-His bond of **21a** and **26**. Surprisingly, the purified commercial enzyme and the crude preparation were equally active on a weight basis on their hydrolysis of compounds **21a**, **22a**, and **26**. The derivative in which L-Phe was substituted with *O*-methyl-L-tyrosine (**25**) was found not to be cleaved by chymotrypsin or pancreatic protease, consistent with the published specificity requirements of the enzyme.[6,19]

Cardiovascular Effects. The in vivo actions of **21a** are described in Figure 4, and the corresponding base-line values (mean ± SE) are as follows for the 0.1, 1.0, and 10 mg/kg doses: blood pressure 54 ± 4, 58 ± 7, and 63 ± 12 mmHg; plasma renin activity (PRA) 52.9 ± 6.7, 25.0 ± 8.5, and 74.1 ± 32.7 ng/mL per h; heart rate 133 ± 9, 140 ± 15, and 150 ± 14 beats/min. Although the base-line blood pressure values were similar to each other (P = not sig-

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(19) Kundu, N.; Roy, S.; Maenza, F. *Eur. J. Biochem.* **1972**, *28*, 311.

Table IV. Incubation of Selected Renin Inhibitors with Commercial Enzymes and Tissue Homogenates[a,b]

compd	purified chymotrypsin, Sigma C 4129	crude pancreatic protease, Sigma P 4630	liver homogenate	intestinal homogenate	kidney homogenate
21a	5.0 min	4.5 min	stable[c]	stable	stable
22a	2.5 min	1.0 min	stable	stable	stable
25	stable	stable	stable	stable	stable
26	3.5 min	3.0 min	stable	stable	stable

[a] $t_{1/2}$ for degradation. [b] Incubation conditions detailed in Experimental Section. [c] Less than 10% degradation of compound in 90 min.

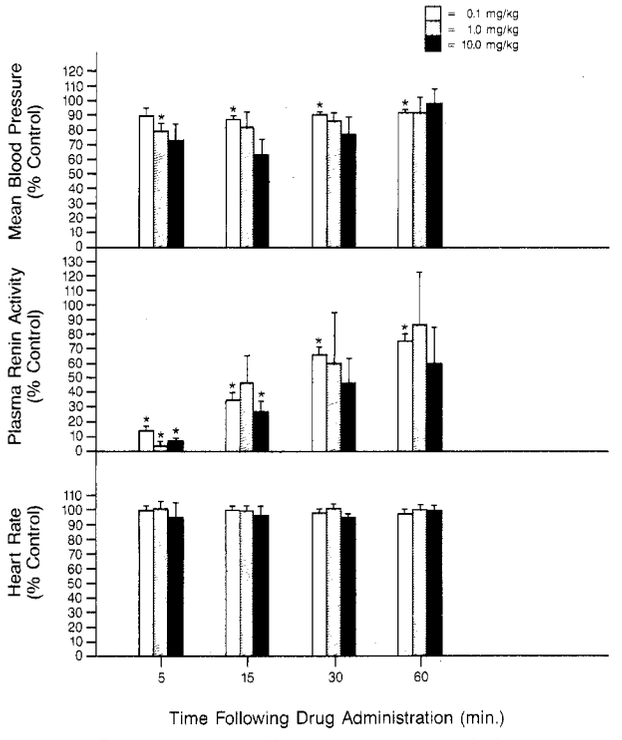

Figure 4. Dose–response effects of intravenous bolus injections of **21a** in the sodium-depleted anesthetized monkey. Values are shown as mean ± SE (n = 5, 0.1 mg/kg; n = 3, other groups). (*) Significance was accepted if $P < 0.05$.

nificant among groups for all parameters), they were lower than the average figures that we normally obtain in this preparation. We attribute these low base-line pressures to furosemide-induced volume depletion reflected by loss of body weight and/or a deep level of anesthesia. Compound **21a** tended to cause a dose-related fall in blood pressure. However, near-maximal and maximal suppression of PRA were achieved with the lowest and middle doses of **21a**, respectively. (Statistical significance is not always achieved due to variance in the data.) Thus, our data suggest that **21a** may be inhibiting, in addition to PRA, other pools of renin.[20] This may account, at least in part, for the dissociation between PRA inhibition and blood pressure reduction.[21] Note that the hypotensive effect of **21a** was not accompanied by a reflex increase in heart rate. There were no significant differences among treatment groups in any of the measured parameters based on percent control analyses of absolute control values. The cardiovascular effects of **25**, which shows stability to chymotrypsin in vitro, were similar to **21a** (data not shown).

This result suggests that in vitro enzymatic stability is not the sole requirement for a long duration of action in vivo. Other factors such as biliary excretion and/or metabolic degradation by unidentified enzymes may also contribute to a short in vivo half-life.

Summary. In this study we have described a series of relatively small analogues of angiotensinogen, certain members of which inhibit purified human renin at the nanomolar level. This high level of enzyme inhibition was obtained by optimization of hydrophobic and hydrogen-bonding interactions between the inhibitor molecules and the active site region of renin. Key structural features contributing to these interactions include a lipophilic cyclohexylmethyl side chain at the P_1 subsite, a hydroxyethylene isostere replacing the scissile bond, and an isopropylsulfonyl group in place of the Val-10 residue. The more potent congeners of this series show a high specificity toward human renin and very weak affinity for rat plasma renin, cathepsin D, and pepsin. Finally, hypotensive effects in cynomolgus monkeys were produced following iv injection of **21a**. The small size of the compounds reported herein coupled with their high potency and specificity offers the potential for further development into an orally active antihypertensive agent.

Experimental Section

Solvents and other reagents were reagent grade and used without further purification. Anhydrous tetrahydrofuran was distilled from sodium/benzophenone ketyl. Proton magnetic resonance spectra were measured on a Nicolet QE-300 (300 MHz). Chemical shifts are reported as δ values (parts per million) relative to Me_4Si as an internal standard. Elemental analyses and the above determinations were performed by the Analytical Research Department, Abbott Laboratories. Column chromatography was performed on silica gel 60, 0.04–0.063 mm (E. Merck), eluting with 5–10 psi of air pressure. Melting points were determined on a Thomas-Hoover capillary apparatus and are uncorrected. Boc-(Me)Tyr was purchased from Bachem.

(4*S*,5*S*)-4-Isobutyl-5-(4-butenyl)oxazolidin-2-one (1). To a rapidly stirred −78 °C solution of Boc-leucinal[10] (14 g, 65.2 mmol) in anhydrous THF (150 mL) was added a 0 °C solution of 4-butenylmagnesium bromide[22] (160 mL of a 0.8 M solution in Et_2O) dropwise over the course of 15 min; 2 h later the mixture was acidified to pH 7. The organic phase was separated, washed with brine, and dried (Na_2SO_4). Filtration and evaporation provided in quantitative yield a 3/2 mixture of diastereomeric alcohols, which was acetylated to facilitate purification. A 25-g (0.092 mol) sample of the above product was dissolved in CH_2Cl_2 (200 mL) containing 10 mL of pyridine. Ac_2O (11.74 g, 0.115 mol) was added by dropwise addition, and the resulting mixture was stirred for 24 h at room temperature. The mixture was washed successively with aqueous $NaHCO_3$, aqueous citric acid, and brine solution. After drying over $MgSO_4$, the solvent was evaporated to a residue. Flash chromatography on silica gel gave a 42% yield of (5*S*,6*S*)-5-acetoxy-6-[(*tert*-butyloxycarbonyl)amino]-8-methyl-1-nonene as an oil. Anal. ($C_{17}H_{31}NO_4$) C, H, N.

A solution of the above product (33 g, 0.105 mol) dissolved in 150 mL of DMF and 50 mL of THF was treated with 11 g (0.204 mol) of $NaOCH_3$ in one portion. The reaction mixture was stirred overnight at room temperature and then poured into an acidified solution of aqueous NaCl with cooling. The resulting mixture

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(21) Blaine, E. H.; Schorn, T. W.; Boger, J. *Hypertension (Dallas)* **1984**, *6* (Suppl. I), I–III.
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was extracted with Et_2O. The ethereal extract was washed three times with brine solution and dried over $MgSO_4$. Evaporation of the solvent gave 19.6 g (95%) of liquid product: NMR (300 MHz, $CDCl_3$, ppm) 0.91 (d, 3 H, J = 6 Hz), 0.94 (d, 3 H, J = 6 Hz), 2.25 (m, 2 H), 3.55 (ddd, 1 H), 4.15 (ddd, 1 H, J = 4.8, 6.0, 7.6 Hz), 5.00–5.10 (m, 2 H), 5.81 (m, H), 6.35 (br s, 1 H). Anal. ($C_{11}H_{19}NO_2$) C, H, N.

(4*S*,5*S*)-4-Isobutyl-5-(2-formylethyl)oxazolidin-2-one (2). To a rapidly stirred solution of olefin **1** (2 g, 10.1 mmol) in THF (70 mL) was added OsO_4 solution (3.18 mL of a 2.5 w/v % solution in *t*-BuOH) followed by $NaIO_4$ (5.4 g, 25.2 mmol) in water (70 mL). After 24 h the mixture was partitioned between CH_2Cl_2 and aqueous Na_2SO_3. The aqueous phase was further extracted with CH_2Cl_2, and the combined extracts were washed sequentially with 10% Na_2SO_3 and brine. Drying (Na_2SO_4), filtering, and evaporating gave the crude product, which was flash chromatographed on silica gel eluting with EtOAc/hexane (50/50). There was obtained 1.1 g (54%) of **2** after trituration with hexane, mp 30–31 °C. Anal. ($C_{10}H_{17}NO_3$) H, N; C: calcd, 60.28; found, 59.41.

(4*S*,5*S*)-4-Isobutyl-5-(6-methyl-3-oxoheptyl)oxazolidin-2-one (3). A 2-g (0.01 mol) portion of **2** was dissolved in THF (50 mL) and treated at 0–5 °C with 37.5 mL of an 0.8 M solution of isopentylmagnesium bromide in THF. The reaction mixture was stirred for 2 h at room temperature and then poured into ice water that contained 6.5 mL of 6 N HCl. The mixture was extracted with CH_2Cl_2. Evaporation of the dried CH_2Cl_2 solution gave a quantitative yield of the Grignard adduct. This material was dissolved in 300 mL of acetone and treated by dropwise addition with Jones solution[23] until the orange color persisted. The chromium salts were filtered, and the filtrate was evaporated. The residue was diluted with Et_2O, and the resulting solution was washed successively with aqueous $NaHCO_3$ and brine solution. After drying over $MgSO_4$, the solvent was evaporated to give 1.7 g (66%) of **3** as an oil: NMR (300 MHz, $CDCl_3$, ppm) 0.86–0.96 (m, 12 H), 2.41 (m, 2 H), 2.67 (m, 2 H), 3.5 (m, 1 H), 4.15 (m, 1 H).

(4*S*,5*S*)-4-Isobutyl-5-(6-methyl-3-oxoheptyl)oxazolidin-2-one Ethylene Ketal (4). A mixture of **3** (2.5 g, 9.3 mmol), ethylene glycol (7.5 mL), and *p*-toluenesulfonic acid (60 mg) in toluene (100 mL) was heated at reflux with a Dean–Stark trap for 8 h. The cooled mixture was washed with aqueous $NaHCO_3$ and dried over $MgSO_4$. Evaporation of the solvent gave a residue, which was flash chromatographed on silica gel eluting with 65/35 hexane/EtOAc to give 2.3 g (79%) of **4** as an oil: NMR (300 MHz, $CDCl_3$, ppm) 0.85–0.95 (4 d, 12 H), 3.5 (m, 1 H), 3.95 (s, 4 H), 4.15 (m, 1 H).

(4*S*,5*S*)-4-Amino-2,11-dimethyl-5-hydroxy-8-oxododecane Ethylene Ketal (5). A solution of **4** (1.45 g, 4.63 mmol) and barium hydroxide octahydrate (2.92 g, 9.25 mmol) in dioxane (162 mL) and water (108 mL) was heated at reflux under N_2 for 21 h. The solid barium carbonate was filtered, and the filtrate was partially evaporated. The residue was diluted with water, and the resulting solution was extracted with Et_2O. The organic extract was washed with brine solution, dried over $MgSO_4$, and evaporated to a residue. Trituration with cold hexane gave 991 mg (73%) of product: mp 26–26.5 °C; NMR (300 MHz, $CDCl_3$, ppm) 0.87 (d, 6 H), 0.88 (d, 3 H), 0.94 (d, 3 H), 2.62 (m, 1 H), 3.22 (m, 1 H), 3.96 (s, 4 H).

(4*S*,5*S*)-4-[(Boc-L-phenylalanyl-L-alanyl)amino]-2,11-dimethyl-5-hydroxy-8-oxododecane (6). To a stirred −12 °C solution of Boc-Phe-Ala-OH[24] (468 mg, 1.39 mmol) in anhydrous THF (3 mL) were added *N*-methylmorpholine (153 μL, 1.39 mmol) and isobutyl chloroformate (181 μL, 1.39 mmol) sequentially. After 3 min, a −12 °C solution of **5** (400 mg, 1.39 mmol) in anhydrous THF (2 mL) was added. Ten minutes later, the mixture was allowed to warm to room temperature for 2 h, at which time the solvent was evaporated, and the resulting residue was partitioned between EtOAc and saturated $NaHCO_3$. The organic phase was washed sequentially with 0.01 M H_3PO_4 and brine. Drying (Na_2SO_4) and evaporating provided 675 mg (80%) of the corresponding ethylene ketal as a glass. Anal. ($C_{33}H_{55}N_3O_7$) C, H, N.

The desired deketalized product was obtained as follows. A solution of the ketal (400 mg) was stirred for 24 h in 35 mL of HOAc/water/THF (3/1/1). The solvents were evaporated under reduced pressure, and the residue was triturated with hexane/Et_2O (65/35) to give 280 mg (76%) of **6**, mp 137–138 °C. Anal. ($C_{31}H_{51}N_3O_6$), C, H, N.

(4*S*,5*S*,8*RS*)-4-[(Boc-L-phenylalanyl-L-alanyl)amino]-5,8-dihydroxy-2,11-dimethyldodecane (7). To a 0 °C solution of **6** (200 mg, 0.356 mmol) in EtOH (5 mL) was added $NaBH_4$ (27 mg, 0.712 mmol) all at once. After being stirred at 0–5 °C for 2.5 h, the reaction mixture was distributed between brine solution and CH_2Cl_2. The organic phase was dried and evaporated to a residue. Crystallization from Et_2O/hexane gave 160 mg (80%) of **7**, mp 129–133 °C. Anal. ($C_{31}H_{53}N_3O_6$) C, H, N.

(4*S*,5*S*)-4-Isobutyl-5-vinyloxazolidin-2-one (8). To a rapidly stirred −78 °C solution of Boc-leucinal[10] (14 g, 65.2 mmol) in anhydrous THF (250 mL) was added a −78 °C solution of vinylmagnesium bromide (135 mL of a 1 M solution in THF) dropwise over the course of 15 min, and 2 h later the mixture was acidified to pH 7. The organic phase was separated, washed with brine, and dried (Na_2SO_4). Filtration and evaporation provided a 3/2 mixture of diastereomeric alcohols, which was purified by flash chromatography on silica gel eluting with 7/3 hexane/Et_2O. There was obtained 4.5 g (41%) of product, mp 57–59 °C. Anal. ($C_{13}H_{25}NO_3$) C, H, N.

To a solution of the above Grignard adduct (10.5 g, 0.043 mmol) in DMF (100 mL) was added NaH (2.4 g of 50% dispersion) portionwise at 0–5 °C. After being stirred for 20 h at room temperature, the reaction mixture was poured into cold aqueous NaCl solution. The resulting mixture was extracted with CH_2Cl_2, and the organic phase was washed several times with brine solution. Drying over $MgSO_4$ and evaporation gave a residue, which was flash chromatographed on silica gel eluting with hexane/EtOAc mixtures. There was obtained 5.7 g (78%) of the desired compound as an oil: NMR (300 MHz, $CDCl_3$, ppm) 0.9 (2 d, 6 H), 1.35–1.75 (m, 3 H), 3.6 (m, 1 H), 4.55 (t, 1 H), 5.4 (m, 2 H), 5.9 (m, 1 H).

(4*S*,5*S*)-4-Isobutyl-5-(2-hydroxyethyl)oxazolidin-2-one (9). To a 0 °C solution of **8** (4.7 g, 0.028 mol) in THF (20 mL) was added 9-BBN (75 mL, 0.0375 mol in THF) by dropwise addition. After the mixture was stirred for 5 h at room temperature, the reaction was quenched by the addition of water (1 mL). A solution of NaOH (6.7 g) in water (21 mL) was then added followed by careful addition of H_2O_2 (18 mL of a 30% solution). The resulting mixture was heated at 65 °C for 1 h, the THF was partially evaporated, and the residue was distributed between EtOAc and brine solution. The organic phase was washed with brine solution and dried over $MgSO_4$. Evaporation of the solvent gave a residual oil, which was flash chromatographed on silica gel eluting with 5% MeOH in CH_2Cl_2. The pure fractions were combined and evaporated to give 4.62 g (88%) of **9**: NMR (300 MHz, $CDCl_3$, ppm) 0.92 (2 d, 6 H), 3.58 (ddd, 1 H), 3.85 (m, 2 H), 4.37 (ddd, 1 H, J = 4.8, 6.0, 7.6 Hz), 6.14 (br s, 1 H).

(4*S*,5*S*)-4-Isobutyl-5-[2-(isoamylthio)ethyl]oxazolidin-2-one (10a). A solution of **9** (3.95 g, 0.021 mol) and triethylamine (3.2 g, 0.032 mol) in CH_2Cl_2 (40 mL) was cooled to 0 °C and treated by dropwise addition with mesyl chloride (2.89 g, 0.025 mol). After being stirred for 1 h at 0–5 °C, the CH_2Cl_2 solution was washed successively with 0.5 N HCl, aqueous $NaHCO_3$, and brine solution. The organic solution was dried and evaporated to a solid product. Recrystallization from hexane/CH_2Cl_2 gave 3.9 g (70%) of product, mp 99–100 °C. Anal. ($C_{10}H_{19}NO_5S$) C, H, N.

To a 0 °C solution of the above mesylate (500 mg, 1.88 mmol) and isoamyl mercaptan (0.25 mL) in THF (6 mL) was added NaH (95 mg, 1.98 mmol of a 50% dispersion) all at once. The reaction mixture was stirred for 3 h at room temperature and then distributed between CH_2Cl_2 and brine solution. The organic layer was washed with brine solution, dried over $MgSO_4$, and evaporated. The residue was chromatographed on silica gel eluting with 65/35 hexane/EtOAc to give 503 mg (98%) of **10a** as an oil. Anal. ($C_{14}H_{27}NO_2S$) C, H, N.

(4*S*,5*S*)-4-Isobutyl-5-[2-(phenethylthio)ethyl]oxazolidin-2-one (10b) was prepared from **9** in a fashion analogous to that for the preparation of **10a** in 67% yield. Anal. ($C_{17}H_{25}NO_2S$)

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C, H, N. Intermediates **10c** and **10d** were prepared by the above procedure.

(3*S*,4*S*)-4-Amino-3-hydroxy-1-(isoamylthio)-6-methylheptane (11a). A solution of **10a** (0.825 g, 3 mmol) and barium hydroxide octahydrate (1.9 g, 6 mmol) in dioxane (105 mL) and water (70 mL) was heated at reflux under N_2 for 21 h. The solid barium carbonate was filtered, and the filtrate was partially evaporated. The residue was diluted with water, and the resulting solution was orated. The residue was diluted with water, and the resulting solution was extracted with Et_2O. The organic extract was washed with brine solution, dried over $MgSO_4$, and evaporated to a residue. Trituration with cold hexane gave 560 mg (75%) of **11a**, mp 64–65 °C. Anal. ($C_{13}H_{29}NOS$) C, H, N.

(3*S*,4*S*)-4-Amino-3-hydroxy-6-methyl-1-(phenethylthio)-heptane (11b) was obtained from **10b** in the same manner as **11a** in 72% yield, mp 94–95 °C. Anal. ($C_{16}H_{27}NOS$) C, H, N. Products **11c** and **11d** were prepared by the above procedure.

(3*S*,4*S*)-4-[(Boc-L-phenylalanyl-L-alanyl)amino]-3-hydroxy-1-(isoamylthio)-6-methylheptane (12a) was obtained from **11a** and Boc-Phe-Ala-OH in 68% yield by using the mixed anhydride procedure described for compound **6**, mp 137–138 °C. Anal. ($C_{30}H_{51}N_3O_5S$) C, H, N. Products **12b**, **12c**, and **12d** were prepared by the above protocol (see Table I).

(3*S*,4*S*)-4-[(Boc-L-phenylalanyl-L-alanyl)amino]-3-hydroxy-1-(isoamylsulfinyl)-6-methylheptane (13). To a solution of **12a** (80 mg, 0.141 mmol) in CH_2Cl_2 (3 mL) and cooled in an ice bath was added 28 mg (0.141 mmol) of *m*-chloroperoxybenzoic acid. After being stirred for 1 h at 0–5 °C, the CH_2Cl_2 solution was washed successively with aqueous $NaHSO_3$ and aqueous Na_2CO_3. The organic layer was dried and evaporated to a solid residue. Trituration with Et_2O/hexane gave 65 mg (80%) of **13**, mp 150–153 °C. Anal. ($C_{30}H_{51}N_3O_6S$) C, H, N.

(3*S*,4*S*)-4-[(Boc-phenylalanyl-L-alanyl)amino]-3-hydroxy-1-(isoamylsulfonyl)-6-methylheptane (14a). To a solution of **12a** (200 mg, 0.35 mmol) in CH_2Cl_2 (4 mL) was added 150 mg (0.7 mmol) of *m*-chloroperoxybenzoic acid. After being stirred for 1 h at room temperature, the CH_2Cl_2 solution was washed successively with aqueous $NaHSO_3$ and aqueous Na_2CO_3. The organic layer was dried and evaporated to a solid product. Trituration with hexane gave 0.19 g (90%) of **14a**, mp 165–166 °C. Anal. ($C_{30}H_{51}N_3O_7S$) C, H, N. Products **14b**, **14c**, and **14d** were prepared by the above procedure (see Table I).

(4*S*,5*S*)-4-(Cyclohexylmethyl)-5-vinyloxazolidin-2-one (15). To a stirred −78 °C solution of Boc-cyclohexylalanine methyl ester[13] (10.2 g, 35.8 mmol) in dry toluene (60 mL) was added diisobutylaluminum hydride (34 mL of a 1.5 M solution in toluene). After 30 min, vinylmagnesium bromide (108 mL of a 1 M solution in THF) was added at −78 °C. After being stirred for 15 h at 0 °C, the mixture was quenched with MeOH and then treated with 10% aqueous citric acid. The organic layer was isolated from the two-phase mixture and was washed successively with aqueous citric acid and brine solution. Drying and evaporating furnished a crude product, which was chromatographed on silica gel eluting with hexane/EtOAc mixtures to give 6.1 g (60%) of the Grignard adduct. A 2.8-g (9.88 mmol) sample of this material in dry DMF (50 mL) was added to a stirred suspension of NaH (593 mg of a 60% oil dispersion, 14.8 mmol, Et_2O washed) in dry DMF. After 3 h, the mixture was quenched with excess brine solution and then extracted with Et_2O. Drying and evaporating gave the cyclized product as an 82/18 mixture of 5*S*/5*R* diastereomers. Flash chromatography on silica gel led to 1.65 g (80%) of pure **15**. Anal. ($C_{12}H_{19}NO_2$) H, N; C: calcd, 68.87; found, 68.44.

(4*S*,5*S*)-4-(Cyclohexylmethyl)-5-(2-hydroxyethyl)oxazolidin-2-one (16) was obtained from **15** as described for **9** in 72% yield, mp 109–111 °C. Anal. ($C_{12}H_{21}NO_3$) C, H, N.

(4*S*,5*S*)-4-(Cyclohexylmethyl)-5-[2-(mesyloxy)ethyl]oxazolidin-2-one (17) was prepared analogously to the corresponding isobutyl congener (see **10a**) in 96% yield, mp 122–123 °C. Anal. ($C_{13}H_{23}NO_5S$) C, H, N.

(4*S*,5*S*)-4-(Cyclohexylmethyl)-5-[2-(isopropylthio)-ethyl]oxazolidin-2-one (18a) was obtained from **17** in the same manner as **10a** in 86% yield, mp 87–88 °C. Anal. ($C_{15}H_{27}NO_2S$) C, H, N.

(4*S*,5*S*)-4-(Cyclohexylmethyl)-5-[2-(ethylthio)ethyl]oxazolidin-2-one (18b). By use of the procedure described for **10a**, **18b** was prepared in 85% yield from **17**, mp 85–86 °C. Anal. ($C_{14}H_{25}NO_2S$) H, N; C: calcd, 61.95; found, 62.46.

(4*S*,5*S*)-4-(Cyclohexylmethyl)-5-[2-(isopropylsulfonyl)-ethyl]oxazolidin-2-one (19a). The oxidative procedure described for **14a** was used to prepare **19a** in 96% yield from **18a**, mp 133–134 °C. Anal. ($C_{15}H_{27}NO_4S$) C, H, N.

(4*S*,5*S*)-4-(Cyclohexylmethyl)-5-[2-(ethylsulfonyl)-ethyl]oxazolidin-2-one (19b) was prepared as described for **19a** in 95% yield, mp 121–122 °C. Anal. ($C_{14}H_{25}NO_4S$) C, H, N.

(2*S*,3*S*)-2-Amino-1-cyclohexyl-3-hydroxy-5-(isopropylsulfonyl)pentane (20a) was prepared from **19a** in the same manner as **11a** in 82% yield, mp 142–143 °C. Anal. ($C_{14}H_{29}NO_3S$) H, N; C: calcd, 57.70; found, 58.20.

(2*S*,3*S*)-2-Amino-1-cyclohexyl-5-(ethylsulfonyl)-3-hydroxypentane (20b) was obtained from **19a** in the same manner as **11a** in 84% yield, mp 139–140 °C. Anal. ($C_{13}H_{27}NO_3S$) C, H, N.

(2*S*,3*S*)-2-[(Boc-L-phenylalanyl-L-histidyl)amino]-1-cyclohexyl-3-hydroxy-5-(isopropylsulfonyl)pentane (21a). To a stirred 0 °C solution of Boc-His-OH (0.84 g, 3.3 mmol) in anhydrous DMF (7.5 mL) was added a solution of **20a** (1 g, 3.09 mmol) in DMF (7.5 mL). Hydroxybenzotriazole (0.51 g, 3.77 mmol) and DCC (0.64 g, 3.1 mmol) were then added sequentially. After 2.5 h, the mixture was warmed to 25 °C, stirred for 12 h, filtered, and evaporated in vacuo. The residue was distributed between $CHCl_3$ and saturated $NaHCO_3$. The organic phase was then washed separately with saturated $NaHCO_3$ and brine. Drying (Na_2SO_4) and evaporating provided a white solid, which was crystallized from EtOAc/hexane to give 1.25 g (73%) of the Boc-histidyl intermediate. A 0.195-g (0.35 mmol) sample of the Boc-protected intermediate was stirred in 4 M HCl/dioxane (1 mL) for 2 h and evaporated to dryness. The residue was dissolved in DMF (3 mL) and treated with triethylamine (0.07 g, 0.7 mmol). This amine solution was coupled to Boc-Phe-OH by using the HOBT/DCC procedure described above to give 77 mg (33%) of **21a**, mp 145–147 °C. Anal. ($C_{34}H_{53}N_5O_7S$) C, H, N.

Final products **21b**, **25**, **26**, and **27** were prepared by the same procedure described for **21a** (see Table I).

(2*S*,3*S*)-2-[(Boc-L-phenylalanyl-L-alanyl)amino]-1-cyclohexyl-3-hydroxy-5-(isopropylsulfonyl)pentane (22a) was obtained from **20a** in the same manner as **12a** in 75% yield, mp 92–94 °C. Anal. ($C_{31}H_{51}N_3O_7S$) C, H, N.

Final products **22b**, **23**, and **24** were prepared by using the same procedure (see Table I). Boc-Phe-Leu-OH[25] and Boc-Phe-Phe-OH[26] for these coupling reactions were prepared as described.

Inhibition Studies. Purified human renal renin[27] was assayed by utilizing pure human angiotensinogen[28] at pH 6.0 in maleate buffer. Test compounds were dissolved in Me_2SO and diluted so that prior to addition to the assay system the solutions were 10% in Me_2SO and 0.5% in BSA. The final incubation mixture (100 μL) contained the following: maleate buffer, pH 6.0, 0.135 M; EDTA, 3 mM; PMSF, 1.4 mM; angiotensinogen, 0.21 μM; renin, 0.24 mGU;[29] BSA, 0.44%; Me_2SO, 1%. At least three different concentrations of inhibitor that bracketed the IC_{50} were preincubated with renin for 5.0 min at 37 °C, substrate was added, and the incubation was allowed to proceed for 10.0 min. The reaction was stopped by freezing the solution in a methanol–dry ice bath, and after thawing at 4 °C, an aliquot was analyzed for angiotensin I by radioimmunoassay utilizing a commercial kit (NEN Research). The percent inhibition of the reaction was determined, and the IC_{50} (the concentration causing 50% inhibition) was calculated by regression analysis. The reaction time of 10 min was on the linear portion of the incubation time–angiotensin I generation curve, and at the highest concentrations tested, none of the compounds cross-reacted with the antibody

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to angiotensin I. The presence of 1% Me_2SO in the final incubation mixture caused no statistically significant effect on the renin activity.

Plasma assays utilized the endogenous renin and angiotensinogen. The final incubation mixture (100 μL) contained the following: plasma, 40 μL; maleate buffer, pH 6.0, 0.135 M; EDTA, 3 mM; PMSF, 1.4 mM; Me_2SO, 1%; 8-hydroxyquinoline, 3.4 mM (rat plasma only). Samples were incubated at 37 °C for 2 h and then placed in ice; an aliquot was analyzed for angiotensin I as before.

Bovine cathepsin D (Sigma) and porcine pepsin (Sigma) activities were assessed by the hydrolysis of hemoglobin at pH 3.1 and 1.9, respectively, at 37 °C, and measurements of the absorbance at 280 nm of the supernatant after precipitation with trichloroacetic acid.[30]

Stability Studies. Chymotrypsin (bovine pancrease, Sigma C 4129) and protease (pancreatic, Sigma P 4630) were dissolved at 0.1 mg/mL in 30 mM sodium phosphate/100 mM sodium chloride, pH 6.9. Fresh rat liver, kidney, and intestinal mucosa homogenates were prepared by homogenization of the tissues in 4 volumes of phosphate-buffered saline followed by low-speed centrifugation. The concentrated homogenate was diluted 10-fold in phosphate-buffered saline for incubation with the compounds.

Test compounds **21a**, **22a**, **25**, and **26** were introduced at 0.1 mg/mL by dilution of a 5 mg/mL methanol stock solution (20 μL/mL) into the enzyme solution or homogenate suspension. Aliquots were removed at intervals, quenched by addition of acetonitrile, then centrifuged, and filtered. Analyses of the incubation were carried out by HPLC on a Waters μBondapak analytical C-18 column (30 cm) eluted with a gradient from 20% CH_3CN/80% H_2O/0.1% TFA to 80% CH_3CN/20% H_2O/0.1% TFA over 20 min. Peak detection was by UV absorbance at 214 nM with quantitation using a Perkin-Elmer LCI-100 computing integrator.

Blood Pressure Measurement. The cardiovascular effects of **21a** were assessed in salt-depleted male cynomolgus monkeys (*Macaca fascicularis*) weighing 4–6 kg. Enhancement of base-line plasma renin activity was achieved by feeding the monkeys a low-salt chow and fruit diet in conjunction with two furosemide treatments (5 mg/kg, po; each dose) given 1 week and 1 day before the experiment. On the morning of the experiment, the monkeys were sedated with ketamine (10 mg/kg, im) and anesthetized with an intravenous bolus 15 mg/kg and a 0.1 mg/kg per min maintenance infusion of sodium pentobarbital (Abbott Laboratories, North Chicago, IL) through a leg vein catheter. Blood pressure and heart rate were monitored noninvasively at 2-min intervals by using an arm cuff with microphone and a sphygmomanometer (Narco Biosystems, Houston, TX). Blood samples were collected before and 5, 15, 30, and 60 min following drug administration for the determination of plasma renin activity. Compound **21a** was given as an intravenous bolus in three concentrations in a constant volume. Each animal received only one dose. Data were analyzed by using paired "*t*" test to examine within-group differences, and ANOVA was used to compare differences among groups.

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1,3-Diarylpyrazolo[4,5-*c*]- and -[5,4-*c*]quinolin-4-ones. 4. Synthesis and Specific Inhibition of Benzodiazepine Receptor Binding

Giovanna Palazzino,† Lucia Cecchi,† Fabrizio Melani,† Vittoria Colotta,† Guido Filacchioni,*† Claudia Martini,‡ and Antonio Lucacchini‡

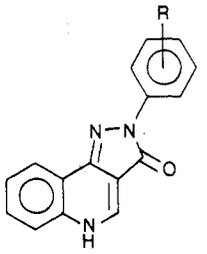
Dipartimento di Scienze Farmaceutiche, Università di Firenze, 50121 Firenze, Italy, and Istituto Policattedra di Discipline Biologiche, Università di Pisa, 56100 Pisa, Italy. *Received February 2, 1987*

A series of 1,3-diarylpyrazolo[4,5-*c*]- and -[5,4-*c*]quinolin-4-ones were prepared and tested for their ability to displace [^{3}H]flunitrazepam from bovine brain membranes. While the 1,3-diarylpyrazolo[4,5-*c*]quinoline derivatives showed affinity for the receptor site, their [5,4-*c*] isomers were devoid of binding activity.

The great structural differences among non-benzodiazepine compounds with affinity for the benzodiazepine receptor make it difficult to generalize about the molecular requirements of the recognition site of the receptor itself. Consequently, in an effort to elucidate these requirements, numerous compounds have been synthesized and tested.[1]

Most of the reported structures displayed a fused nitrogen-containing heteroaromatic system as a common feature. In particular, some 2-arylpyrazolo[4,3-*c*]-quinolin-3-ones (CGS series[2]) have been reported to have an extremely high affinity for the benzodiazepine receptor site (see Table I). Following this finding, the synthesis and binding activity of some 2-aryl- and 1-aryl-3-methylpyrazolo[4,5-*c*]quinoline derivatives[3–5] have been reported. In order to provide a better insight into the molecular requirements of the recognition site of the receptor and to provide further structure–activity relationships in our series of pyrazoloquinolines, we are now reporting the synthesis and binding activity of some 1,3-diarylpyrazolo[4,5-*c*]quinolin-4-ones. The 3-methyl was replaced by the phenyl group to provide information on how a bulkier group than methyl would affect the binding potency.

Table I. Benzodiazepine Receptor Binding of CGS Series[2]



CGS a-c

compd	R	IC_{50},[a,b] nM
CGSa	H	0.4
CGSb	4-Cl	0.6
CGSc	4-OCH_3	0.1

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† Dipartimento di Scienze Farmaceutiche.
‡ Istituto Policattedra di Discipline Biologiche.

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0022-2623/87/1830-1737$01.50/0