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KETOMETHYLDIPEPTIDES I. A NEW CLASS OF ANGIOTENSIN CONVERTING ENZYME INHIBITORS

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Summary: The design rationale for a new series of angiotensin-converting enzyme (ACE) inhibitors which incorporate a ketone substituent into a peptide backbone is described. Molecular regions which were expected to mimic the binding of an N-acyl tripeptide substrate at secondary binding sites S_1 and S_1' were systematically varied in order to study the specificity of inhibitor binding and optimize inhibition against ACE. The most effective ketomethyldipeptides inhibit ACE in the 10^{-9} M range.

Introduction: Nonhydrolyzable ketomethylene tripeptide analogs of substrates[1] N-benzoyl-Phe-Gly-Pro (1) or N-benzoyl-Phe-Ala-Pro (2), in which the amide nitrogen of the sissile linkage is replaced by a methylene group, are unique as inhibitors of angiotensin converting enzyme (ACE) in that they possess no apparent ionizable zinc binding function. Nevertheless, substances of this type (*i.e.* 3, 4) are among the most potent of reported ACE inhibitors (for 4, I_{50} = 3 nM).[2,3,4] Unfortunately, the use of ketomethylene inhibitors as potential therapeutic agents for hypertension has been limited by a lack of the useful expression of their potent activity *in vivo*.[3] Our continued interest in nonhydrolyzable tripeptide analogs[5] led us to investigate new types of ketone containing ACE inhibitors which possess functional groups potentially capable of participating in additional enzyme/inhibitor interactions, and

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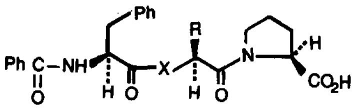
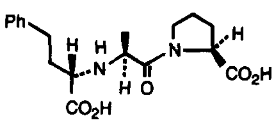


1 R = $-H$, X = $-NH-$
2 R = $-CH_3$, X = $-NH-$
3 R = $-H$, X = $-CH_2-$
4 R = $-CH_3$, X = $-CH_2-$



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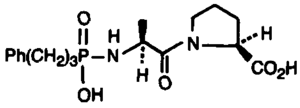

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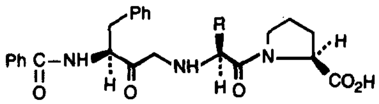
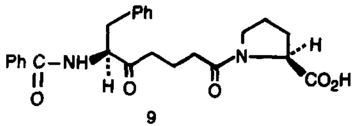


7 R = $-CH_3$
8 R = $-H$



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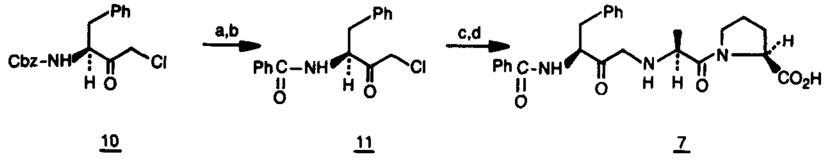
which might prove to demonstrate more desirable pharmacological properties.

Carboxyalkyldipeptides (5)[6] and phosphonamidates (6)[7,8] are two recently reported classes of tripeptide modeled ACE inhibitors which exhibit extremely potent intrinsic inhibitory activity. A common feature shared by both of these otherwise quite different substances is the presence of a weakly basic secondary nitrogen atom which occupies a position approximating that of amido nitrogen in the scissile bond of the substrate. The role of nonamide -NH- in these two series has been proposed[7,9] to involve donation of a hydrogen bond to an enzyme carbonyl of ACE. This hypothesis is derived by analogy to the X-ray structure of a thermolysin-phosphoramidon complex which is consistent with donation of the phosphoramide -NH- to the carbonyl of Ala 113.[10] The inhibitor nitrogen substituent has been shown to be a critical factor for achieving high inhibitory potency in both series. Our plans were directed toward incorporating a similar basic nitrogen into a ketone containing inhibitor. By utilizing the N-terminal amine of a suitable dipeptide as the basic nitrogen residue, we hoped to conserve peptide backbone continuity and maximize the general nature of this approach. Hence, ketomethyldipeptide 7 was selected for synthesis. This modification formally amounts to the interposition of a methylene group within the scissile amide of N-benzoyl-Phe-Ala-Pro a known substrate for ACE.

Several additional points are noteworthy: Meyer *et al.*[3] have shown that introduction of a linear three methylene chain between the ketone and C-terminal amide linkage (as in 9), while somewhat detrimental to activity compared with 3, yields a compound possessing substantial inhibitory properties.[11] Such latitude for a functional group that is critical for good inhibition is unusual, but suggests that at least spacially, the additional atom imposed within the central chain of aminoketone 7 might be well tolerated in the binding process. This observation also led the same workers to prepare a series of heteroatom replacements of this type, which was reported during the course of our work.[13] This report included derivative 8, a substance which was claimed to be 300 times less active than the parent ketomethylene ACE inhibitor (3). In contrast, we have independently prepared 7, 8, and numerous other ketomethyldipeptides, and find these interesting substances to be among the most potent ACE inhibitors yet uncovered. The balance of this report describes our efforts to define the scope and limitations of ketomethyldipeptides as inhibitors of ACE.

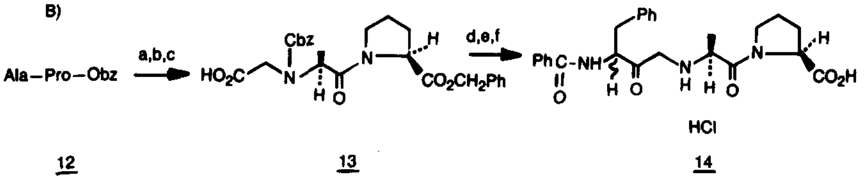
Materials and Methods: Ketomethyldipeptides were synthesized by the two general methods outlined below (A, B). Complete details of the chemistry involved will appear elsewhere. Alkylation of a dipeptide ester by chloromethylketone 11 (derived from 10), leads to diastereomerically pure products (7),[14] whereas implementation of a modified Dakin-West procedure (B)[14] necessarily affords diastereomers epimeric at what corresponds to the phenylalanine α carbon.[14] Following deprotection, the resulting aminoketones were obtained as their hydrochloride or methanesulfonic acid salts. When necessary, purification of the final

A)



a) HBr/HOAc; b) $PhCOCl/Et_3N/NaHCO_3$; c) Ala–Pro–t–butyl ester/$NaI/NaHCO_3/DMF$; d) HCl/HOAc

B)

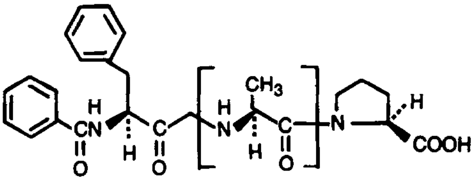


a) t–butyl bromoacetate/Et_3N/THF; b) Cbz–Cl/Et_3N; c) CF_3CO_2H; d) $(COCl)_2$/THF/cat. DMF;
e) 3–benzyl–5–phenyloxazolin–2–one, modified Dakin-West; f) H_2/Pd/C, EtOH/HCl

products was effected by chromatography on LH-20 using methanol/water elutants or on Mitsubishi HP-20 resin using gradient elution from 0.01 N aqueous HCl to methanol. *In vitro* assay procedures have been previously published.[15]

Results and Discussion: Ketomethyldipeptide 7, a close mimic of the substrate N-benzoyl-Phe-Ala-Pro (2), proved to be a potent inhibitor of converting enzyme (I_{50} = 6 nM). A program was consequently initiated with the aim of defining the scope of this observation and relevant structure-activity relationships within this inhibitor class. Of primary interest was the identification of optimal structural and stereochemical requirements at subsites S_1 and S_1'. In order to identify the optimal P_1' substituent, a series of alanine replacement analogs was synthesized (see Table 1). Inspection of the *in vitro* I_{50} values reveals that a good deal of tolerance exists at the enzyme S_1' subsite in this class of inhibitors. Analogs containing Lys, Phe, Orn, and Gly at this position were all potent inhibitors. On the other hand, insertion of a penultimate proline residue was extremely deleterious, presumably because of conformational or steric reasons and not due

Table 1: *In Vitro* Converting Enzyme Inhibitor Activities of Ketomethyldipeptides. Variation of the Penultimate Amino Acid Residue

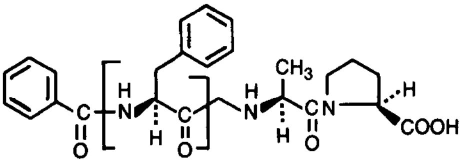


No.	[Residue]	I_{50} (nM)
7	Ala	6.
8	Gly	4.
15	Orn	4.2
16	Lys	9.
17	Phe	10.
18	Pro	282.
19	D-Ala	617.

to the fact that this substance is a tertiary, rather than a secondary amine.[16] An amino acid of natural L configuration is clearly necessary at P_1', since D-Ala analog 19 was 100-fold less active than the corresponding L derivative (7). Noteworthy is the result observed with Gly-Pro derivative 8. This compound is the most active ketomethyldipeptide prepared in this series, a somewhat surprising finding, since other major ACE inhibitor classes suffer substantial loss of inhibitory potency when the methyl group corresponding to a penultimate alanine side chain is removed.[12,17]

A second series of modifications involving replacement of the phenylalanine derived portion of 7 served to more clearly define P_1 structural requirements (see Table 2). The results of these inhibition studies clearly show that aromatic residues at P_1 are preferred to hydrophobic (Nleu) or basic (Lys) substituents. Thus, a close correspondence between optimal P_1 inhibitor structure in ketomethyldipeptides and peptide inhibitors is observed.[18]

Table 2: *In Vitro* Converting Enzyme Inhibitory Activity of Ketomethyldipeptides. Variation of the N–Terminal Amino Acid Derived Residue



No.	[Residue][1]	I_{50} (nM)
14	Phe	12.
20	3–Pyridylalanine	7.
21	Trp	8.
22	Tyr	10.
23	Nleu	27.
24	Lys	92.

[1] Compounds are approx. 1:1 mixture of diastereoisomers at bracketed positions.

Conclusions: In conclusion, our findings show that ketomethyldipeptides are potent inhibitors of ACE. Optimal structure-activity relationships in this series indicate that aromatic amino acid sidechains at the P_1 position are preferred, whereas considerable latitude exists at P_1', since numerous C-terminal penultimate L-amino acid residues are compatible with potent enzyme inhibition. In contrast to certain other classes of ACE inhibitors (*i.e.* mercaptopropanoyl amino acids, carboxyalkyldipeptides), a derivative containing a penultimate glycine residue is equivalent or marginally superior to one containing penultimate alanine. This may reflect a difference in binding conformation of the inhibitor compared to a normal substrate.

The fact that such aminoketone modifications may be conveniently embedded in the form of a dipeptide-like surrogate within various peptide chains, offers the possibility of extending this class of inhibitors to different peptidase enzymes. The results of further investigations directed at evaluating new molecular modifications of 7 and speculations on potential modes of enzyme binding are presented in the following paper.

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