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Design of Peptide Derived Amino Alcohols as Transition-State Analog Inhibitors of Angiotensin Converting Enzyme

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Summary: A new amino alcohol modification designed to mimic the putative transition-state of amide bond cleavage by proteolytic enzymes has been incorporated into the scissile bond position of N-benzoyl-Phe-Ala-Pro, a known substrate of angiotensin converting enzyme (ACE). The resulting modified tripeptides (i.e. 4) are shown to be a new class of potent inhibitors of converting enzyme. © 1985 Academic Press, Inc.

Introduction : Enzyme-mediated proteolytic cleavage of peptides (i.e. 1)is assumed to proceed via a transition-state which may be formally represented by 2 . It is well established that chemically stable mimics of 2 may possess far greater enzyme binding affinity than do related ground state analogs [1-3] , and consequently we were intrigued by the possibility of applying this principle to the identification of new inhibitors of angiotensin-converting enzyme (ACE) [4].In the course of new inhibitor design ,we set out to test the hypothesis that an amino alcohol modification of the scissile amide bond, as depicted in 3, might satisfactorily mimic putative transition state 2, and hence when embedded within an appropriate peptide backbone could confer the elements required for enzyme inhibition. Integration of amino alcohol design element 3 into the penultimate amide bond of N-benzoyl-Phe-Ala-Pro (5), a known substrate for ACE,

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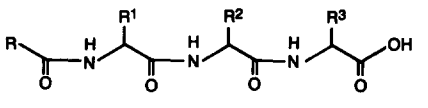

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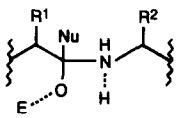
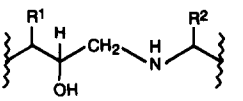


2 Nu = nucleophile
E = electrophile



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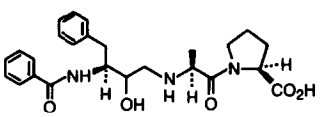

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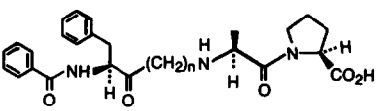


5, n=0, I_{50} = 3,200 nM
6, n=1, I_{50} =6 nM

suggested structure 4 as an appropriate model to evaluate the validity of our hypothesis .

Methods and Materials: Peptide modified amino alcohols were synthesized by two general methods . Reduction ($NaBH_4$/THF/ H_2O) of ketomethyldipeptide esters [5,6] or their corresponding N-Cbz derivatives ,afforded (after deprotection) aminoalcohols usually as a mixture of diastereomeric alcohols . In the case of 9,10, and 11 , the compounds were prepared by reductive amination ($NaBH_3CN$) of an appropriate aldehyde and an Ala-Pro ester .In both cases , resulting alcohol derivatives were deprotected by standard means (trifluoroacetic acid or catalytic hydrogenolysis). Complete details of the chemistry involved, including preparation and characterization of pure diastereomers 7 and 8 will appear elsewhere [7]. All products gave satisfactory NMR,IR, Mass spectra and elemental analyses. In vitro assay procedures for measurement of ACE inhibition have been previously published [8].

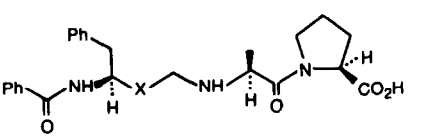
Results and Discussion:

Aminoalcohol 4, a 1:1 mixture of R,S diastereoisomers , epimeric at the hydroxyl center, proved to be a potent inhibitor of ACE (I_{50} = 35 nM). This substance bears a close structural relationship to ketomethyldipeptide 6, a compound formally derived by interposition of a methylene group within the scissile linkage of N-benzoyl-Phe-Ala-Pro (5) and member of an inhibitor class which we have recently identified to possess strong affinity for ACE (Ref. 5 ,6). However,in contrast to 6, and all other potent ACE inhibitors ,substances (i.e. 4)which rely upon

a hydroxyl moiety to forge essential inhibitor/enzyme binding interactions (vide infra)have heretofore not been reported. The balance of this paper summarizes the results of our investigations into defining which structural and stereochemical features of aminoalcohols such as 4 are required for inhibition of angiotensin converting enzyme.

Our initial investigations focused on evaluating the consequences of structural and stereochemical modifications of the hydroxyl group of 4 (Table 1). Separation of 4 into pure diastereomeric components 7 and 8 revealed that the R alcohol (7,) possessed approximately 400-fold greater inhibitory potency (I_{50}= 28 nM) than the corresponding S isomer (8, I_{50} = 10,000 nM). Assignment of the R configuration to the hydroxyl group in 7 was shown by its chemical correlation with a synthetic precursor whosestructure was confirmed by X-ray crystallographic analysis[7] Removal of the hydroxyl group as in analog (9) proved

Table 1
In Vitro Converting Enzyme Inhibition of Peptide Derived Amino Alcohols. Importance of the Hydroxyl Group



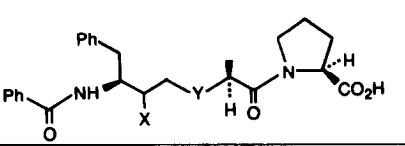
No.	X		I_{50} (nM)
4		(R, S)	35.
6			6.
7		(R)	28.
8		(S)	10,000.
9	$-CH_2-$		7,000.
10		(A)*	52,000.
11		(B)*	675,000.

*pure diastereomers of undetermined configuration, epimeric at the hydroxyl center.

extremely deleterious to activity (I_{50} = 7,000 nM), as did introduction of a methyl group to form tertiary alcohol isomers 10 and 11 (I_{50} = 52 uM, 675 uM, respectively). Hence, the structural constraints imposed on the hydroxyl group in order to satisfy enzymic requirements for optimal binding by aminoalcohol inhibitors demand a secondary alcohol function of proper stereochemical disposition.

Following identification of the critical role served by the inhibitor hydroxyl in enzyme/inhibitor binding , attention was directed to ascertaining what importance, if any, could be attributed to the secondary amino substituent. Several classes of potent ACE inhibitors such as carboxyalkyldipeptides [9] and phosphonamidates [10] possess secondary non-amide nitrogen substituents which are important to the manifestation of high inhibitor potency . In contrast , we showed earlier that a secondary amine was important, but not essential, for good inhibition by the related ketomethyldipeptides (6) [6] . Thus ketoether 14 was previously shown to be only slightly less active than aminoketone 12 (Table 2).This relationship was not conserved in the aminoalcohols since alcoholether 13 was found to be poorly active. Consequently, in contrast to the

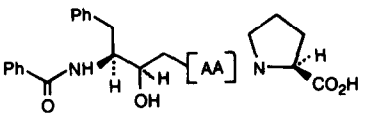
Table 2
In Vitro Converting Enzyme Inhibition of Peptide Derived Amino Alcohols. Importance of the Amino Group



No.	X	Y	I_{50} (nM)
4	−OH (R, S)	−NH−	35.
12	=O	−NH−	12.*
13	−OH (R, S)	−O−	7782.*
14	=O	−O−	21.*

*indicates a 1:1 mixture of diastereomers, epimeric at the center corresponding to α-Phe.

Table 3
In vitro Converting Enzyme Inhibition of Peptide Derived Amino Alcohols. Effect of Replacement of the Penultimate Amino Acid Residue *



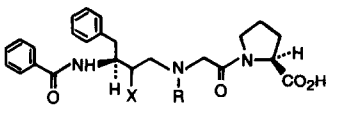
No.	[AA]	I^{50} (nM)
4	Ala	35.
15	Arg	21.
16	Lys	27.
17	Orn	65.
18	Gly	252.
19	Phe	1325.
20	Val	20,000.

*All compounds are approximately 1:1 mixtures of diastereomers, epimeric at the alcohol center.

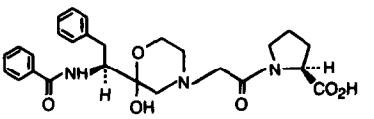
ketomethyldipeptides, aminoalcohols require the amine function in order to observe significant ACE inhibition .

Investigations into the effects of replacements of the penultimate amino acid residue of 4 are summarized in Table 3. Arg (15) and Lys (16) replacements give rise to inhibitory activities in the same range as 4, whereas Orn (17)and Gly (18) derivatives are somewhat less potent. A significant difference in structure activity relationships between ketomethyldipeptides and the corresponding alcohols is observed with 18 and the related Gly-Pro derived ketone. In the aminoketone series [5] a penultimate Gly derivative was comparable in activity (I_{50} = 4 nM) to Ala derived ketone 6. In contrast, aminoalcohol 18 has substantially diminished potency relative to 4.

We had previously shown that in contrast to other series of ACE inhibitors (i.e. carboxyalkyl dipeptides, N-phosphoryl dipeptides, and tripeptides)ketomethyldipeptides possessing a penultimate sarcosine residue retained high inhibitory activity[6] This observation also extends to the aminoalcohols since the effect of glycine N-methylation in this series is also relatively



21, X = O, R = $-CH_2CH_2OH$, I_{50} = 809 nM
22, X = OH, R = $-CH_3$ (Isomer A), I_{50} = 2,020 nM
23, X = OH, R = $-CH_3$ (Isomer B), I_{50} = 237 nM



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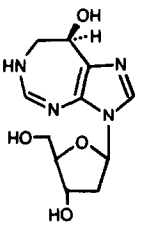

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insignificant (i.e. 18 vs 23) [6] . In an attempt to extend the amino alcohol design principle,and more closely simulate the tetrahedral array of amide bond cleavage shown in 2 an N-alkylated ketomethyldipeptide (21) was synthesized. Substance 21 is shown by C^{13} NMR to exist primarily in the hemi-ketal form (24). Unfortunately the inhibitory activity of 21 was weaker (I_{50}= 809 nM) than sarcosine containing aminoalcohol (23, I_{50}=237 nM).

In considering possible modes of binding of aminoalcohol inhibitors to the ACE active site, we envision three likely scenarios (Figure 1). Aminoalcohols such as 7 could function as transition-state analogs by either having the hydroxyl serve as a weak zinc ligand (A), or as a mimic of the putative incoming nucleophile (B). In both cases amino nitrogen could function as a hydrogen bond acceptor/donor, as shown. Alternatively, the amino and hydroxyl groups could both interact with enzymic zinc in a bidentate fashion (C). Finally we note , an interesting structural similiarity between 4 and "pentostatin" (2'-deoxycorfomycin,25) which inhibits adenosine deaminase (ADA) with a K_i =10^{-12}M and has been termed a "transition-state" analog.[11] Indeed, the chemical conversion of adenosine to

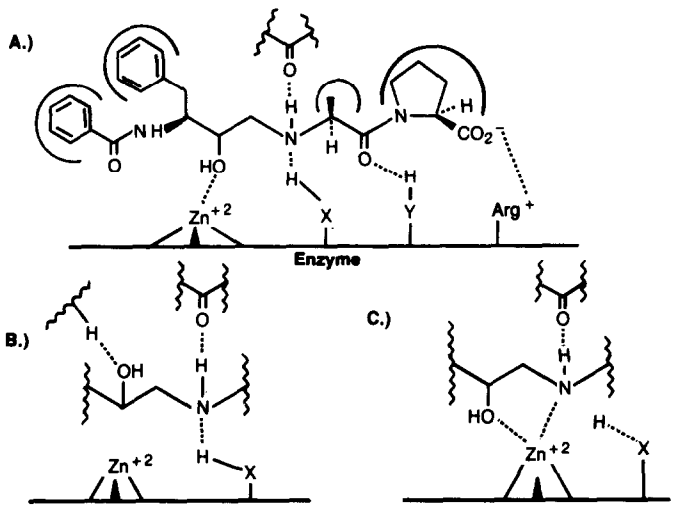

Figure 1: Hypothetical Modes of Binding of Peptide Derived Amino Alcohol Inhibitors and Angiotensin Converting Enzyme.

inosine as catalyzed by ADA bears a strong mechanistic analogy to amide bond cleavage.

Conclusion: The aminoalcohol design element represented by 3,when integrated into an appropriate peptide backbone,gives rise to a series of potent ACE inhibitors (i.e. 7) High inhibitory potency in this series demands the simultaneous presence of a secondary hydroxyl group of correct stereochemical disposition (R configuration) and a suitably positioned amine nitrogen. The fact that this aminoalcohol modification may be conveniently embedded in the form of a dipeptide-like surrogate within various peptide chains, offers the possibility of extending this inhibitor design to different peptidase enzymes.

Acknowledgements

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