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DIPEPTIDE MIMICS. CONFORMATIONALLY RESTRICTED INHIBITORS OF ANGIOTENSIN-CONVERTING ENZYME

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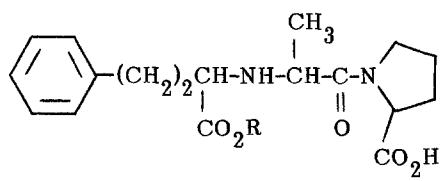
A series of potent inhibitors of angiotensin-converting enzyme (dipeptidyl carboxypeptidase, E.C. 3.4.15.1) is described which addresses conformational aspects of the enzyme-inhibitor interaction. Conformational probes were derived from simple lactams containing the required recognition and binding elements. The inhibitor potencies vary with lactam ring size in a manner predicted from molecular modeling studies and help map the active site of this important enzyme.

Our earlier reports (1,2) described efforts leading to the design of the potent angiotensin-converting enzyme (E.C. 3.4.15.1) inhibitors MK-421 (R = Et) and MK-422 (R = H) (1). This communication reveals some of our initial inhibitor design results based on consideration of some of the conformation aspects of the enzyme-inhibitor interaction.



1

Ondetti and coworkers (3,4) propose that captopril (2) binds to the active site of angiotensin-converting enzyme as shown in Figure 1. This model utilizes three primary binding groups (a zinc binding function, a hydrogen bond accepting carbonyl group and a carboxylate) as well as secondary binding sites interacting with substrate or inhibitor side chains such as the methyl group or the proline ring.

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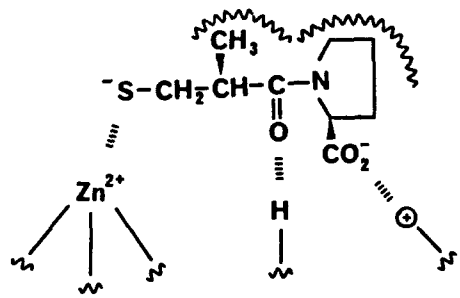
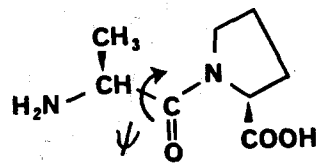


Figure 1. Binding of Captopril (2) in ACE

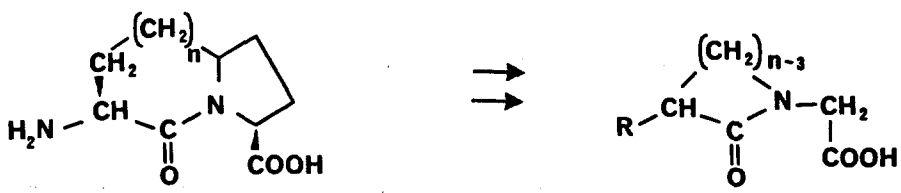
Although the methyl group present in 1 and 2 is not required for binding to the enzyme, its presence does contribute significantly to inhibitory activity (3). We interpreted this as not only a possible methyl group-enzyme interaction, but as quite likely a conformational effect. To study the latter we utilized the Merck Molecular Modeling System (5) which uses semi-empirical force field calculations to generate energy minimized conformations of a number of inhibitors searching for conformation characteristics which might be useful in subsequent design work.

Since these calculations are carried out on molecules in vacuo, there was implied in this work two assumptions, neither of which was necessarily correct. First, we assumed that calculated in vacuo conformations are representative of solution state conformations. Second, we assumed that the conformation of the bound inhibitor belonged to the family of low energy conformations. The alanyl-proline bond is a case in point. Our calculations and the reports of others (6-8) assign the lower energy to the trans-dipeptide. We successfully modeled inhibitor structures from this conformation as described below, although the outcome was by no means certain since acyl proline derivatives have a high probability of existing in the cis-conformation (6-8).

When the peptide bond in alanylproline is set in the trans-configuration, the major remaining conformational freedom is in the ψ-angle (Figure 2). A connecting link of varying length between the alanine methyl and the proline C-5 methylene allows restrictions of this angle and leads to structure 4. For subsequent computational and synthetic simplicity we reduced the target molecule to 5 since this structure embodies the major interactions of interest. The use of lactams such as 5 as conformationally restrained dipeptide units has been independently developed and elaborated by Freidinger,



3



4 5

Figure 2. ψ-Restriction via Lactam Formation

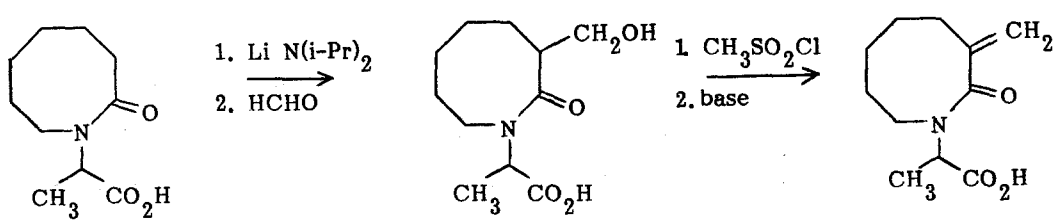
Veber and coworkers (10). Their efforts have been successful but interestingly their optimal lactam ring sizes differ from ours.

Further, we chose R = CH_2SH because it is the simplest of the zinc ligands for angiotensin-converting enzyme. It also allowed us to compare our results with 2 which we assumed was bound in the same or closely related conformation.

Materials and Methods

The synthesis of lactam inhibitors was based on the α-methylene lactam rearrangement as the key step (9). Synthetic procedures for lactam syntheses analogous to those used by us have been published (11,12).

The α-methylene lactam precursor to 5d was synthesized by standard procedures using the following route:

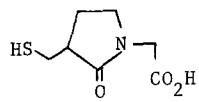
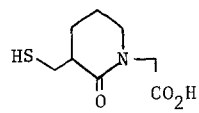
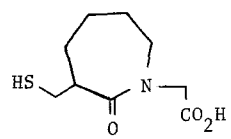
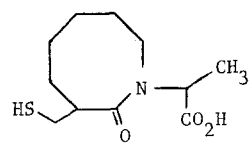
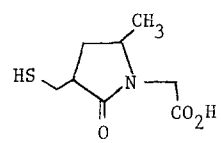
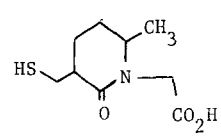
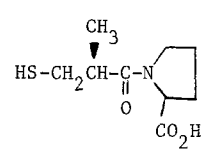


The enzyme assay procedure has been published (1).

Results

After modeling the lactams (5) we found there were low energy conformations only in the 7-9 membered lactams which corresponded to those calculated for 3. Based

Table 1: Mercaptomethyl lactams: In Vitro Biological Results.

No.	Compound[a]	I_{50} (μM)
5a		3.7[b]
5b		3.4, 1.0[b], 10[c]
5c		0.17
5d		0.053[d]
5e		4.9[d]
5f		0.6[d]
2		0.023

a. All lactams tested as racemic mixtures
b. Ref. 11
c. Ref. 12
d. Most active racemate

on these results and our initial assumptions, we predicted the most active inhibitors would have n ≥ 7 while the nonoptimal geometry of n = 5,6 would result in less potent compounds.

In the Table are listed the compounds synthesized to test our hypothesis along with two examples from the recent literature (11). Inspection of the IC_{50} data reveals

a wide range of inhibitor potencies. Based on our initial assumptions, the prediction of low activities for the 5 and 6 membered lactams was borne out while the 7 and 8 membered lactams showed very good inhibitory activities. Noteworthy is the considerable enhancement of activity in passing from n = 6 to n = 7. Also, introduction of pieces of the proline ring (i.e., suitably placed methyl groups) leads to enhancement of activity.

Discussion

From a predictive point of view our results were very gratifying. Based on conformational arguments, our modeling studies led us to believe 5 and 6 membered lactams should be the least active. Indeed, in a recent report by Klutchko and coworkers (11), 5a and 5b were reported to have only modest activity. Our result for 5b was similar as was another recently reported activity for 5b by Condon and coworkers (12). However, larger ring sizes were apparently overlooked by other investigators. It is only in the larger rings ($n \geq 7$) that we begin to find accessible conformations approximating the low energy conformations of 3.

The addition of a suitably placed methyl group (cf. 5b and 5f) enhances activity, an effect which very likely contributes to the high potency of 5d. From modeling studies these added methyl substituents produce a favorable rotational restriction of the carboxymethyl side chain. They may also contribute hydrophobic binding and preferred ring conformer stabilization.

Conclusions

Our results establish that the acyl-proline bond is trans in the enzyme-bound inhibitors. We have also shown that there is a distinct conformational element in the enzyme-inhibitor interaction, and we have made progress in defining the conformational preference. In addition, these results along with those of Freidinger, Veber and coworkers (10) demonstrate the feasibility of designing peptide mimics based on conformational considerations.

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