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DESIGN OF CONFORMATIONALLY CONSTRAINED ANGIOTENSIN-CONVERTING ENZYME INHIBITORS

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SUMMARY: Modification of alanyl proline by introduction of both zinc coordinating and S_1 subsite binding interactions affords potent new carboxy- and mercapto- acyl dipeptide angiotensin-converting enzyme (ACE) inhibitors. Design of these inhibitors was guided by an extension of the hypothetical ACE active site model originally used to derive captopril. Significant increases in ACE inhibitory activity were observed by introduction of conformational constraint into acyclic acyl dipeptides, thus further defining the three dimensional structure of the ACE active site.

Progress in the design of angiotensin-converting enzyme (ACE, EC 3.4.15.1) inhibitors has been guided by a hypothetical model of enzyme substrate interactions (1,2). For example, the model was used to aid development of potent ACE inhibitors such as captopril (I_{50} = 0.023 M), which binds to enzyme S_1' and S_2' regions (1,2). We have recently prepared various acyl dipeptides which were designed to incorporate potential new binding interactions in the S_1 region while simultaneously placing a suitable ligand (X) in the proper orientation for zinc binding (Figure 1). Results of those studies are described herein.

MATERIALS AND METHODS: Acyl dipeptides were prepared by DCC acylation of alanyl-proline esters (t-butyl, benzyl, or benzhydryl) with the appropriate carboxylic acids or dicarboxylic acid monoesters (benzyl, benzhydryl), followed by deprotection (trifluoroacetic acid for t-butyl and benzhydryl esters, catalytic hydrogenolysis for benzyl and benzhydryl esters). Products were converted to the corresponding lithium salts, purified by chromatography on Mitsubishi HP-20 resin, and lyophilized. Diastereomeric carboxy-acyl dipeptides 35-a and 35-b were prepared as described above from the individual enantiomers of trans-cyclohexane-1,2-dicarboxylic acid (3,4,5). Mercapto-acyl dipeptides 39-a and 39-b were prepared from trans-2-(4-methoxybenzylthio)cyclohexane carboxylic acid and alanyl-proline t-butyl ester. Isomers were separated by chromatography

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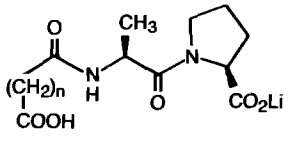
prior to deprotection (6). Trans-2-(4-methoxybenzylthio)-cyclohexane carboxylic acid was prepared by conjugate addition of 4-methoxybenzyl mercaptan to cyclohexene carboxylic acid (7), followed by fractional crystallization of the trans isomer as its dicyclohexylammonium salt. Satisfactory spectroscopic and micro-analytical data were obtained for all final compounds. Enzyme inhibition was measured by the method of Cushman and Cheung (8).

RESULTS: Alanyl-proline has been successfully employed as the C-terminal dipeptide portion of various ACE inhibitors (9,10). Use of a carboxyl group as the zinc-coordinating ligand in such compounds is also well precedented (1,2,10). We thus prepared carboxy-acyl dipeptides of varying chain length. Results suggest that a linkage of two methylene units between carboxyl and amide groups is optimal for ACE inhibition by these compounds (Table 1). While conserving a spacing of two methylene units, we then determined the effect of various potential zinc-binding groups on ACE inhibition (Table 2). Sulfhydryl and carboxyl functions appear to be effective zinc-binding groups in this series.

Table 1

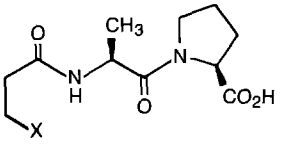
The Effect of Varying Chain Length on ACE Inhibition by Carboxy-Acyl Dipeptides



No.	n	$I_{50}(\mu M)$
1	1	3,000
2	2	37
3	3	580
4	4	400

Table 2

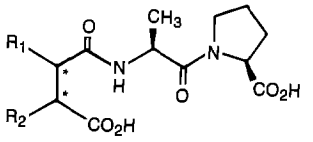
The Effect of Various Zinc-binding Groups on ACE Inhibition by Substituted Propanoyl Dipeptides



No.	X	$I_{50}(\mu M)$
5	SH	2.6
2	CO_2H	37
6	N(OH)CHO	39
7	COPh	71
8	CONHOH	92
9	$CO_2C_2H_5$	160
10	Ph	200
11	CH_2Ph	250
12	NHCOPh	260
13	N(OH)COPh	600
14	NH_2	>3,900

Table 3

Effect of Substitution on ACE Inhibition by Succinyl Dipeptides



No.	R1	R2	$I_{50}(\mu M)$
2	H	H	37
15	$PhCH_2$	H	11
16	H	$PhCH_2$	110
17	$PhCH_2CH_2$**	H	61
18	$PhCH_2CH_2$***	H	1,200
19	H	OH	530
20	H_2N (D)	H	1,000
21	H_2N (L)	H	100
22	H	H_2N (D)	660
23	H	H_2N (L)	52
24	PhCONH (D)	H	340
25	PhCONH (L)	H	88
26	H	PhCONH (D)	1,200
27	H	PhCONH (L)	>2,400

*Diastereomeric mixtures unless noted, **Isomer A, ***Isomer B

Substituted derivatives of succinyl alanyl-proline were subsequently prepared in an attempt to incorporate new interactions in the S_1 subsite region (Table 3). Among those compounds, only the benzyl substituted analog 15 has improved ACE inhibitory potency relative to the unsubstituted parent 2. As evident from comparison of 17 and 18, absolute configuration has an important influence on activity.

Our proposed model (Figure 1) assumes that enzymic zinc is located proximate to the inhibitor N-terminal amide carbonyl

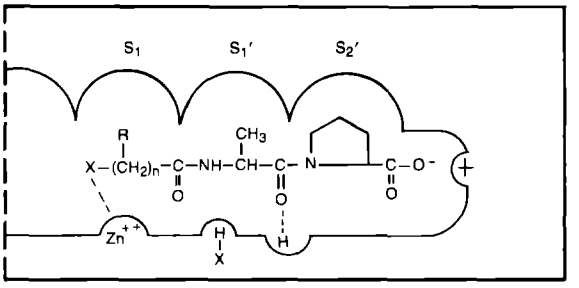
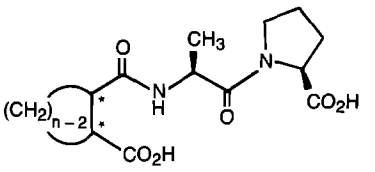


Figure 1: Model for Binding of Prototype Acyl Dipeptides to ACE

Table 4

Conformationally Constrained Carboxy-Acyl Dipeptides



	CIS*			TRANS*		
n	No.	α**	I_{50} (μM)	No.	α**	I_{50} (μM)
3	28	0	2,000	29	150	1,200
4	30	33	58	31	99	1,700
5	32	45	4.9	33	75	38
6	34	60	42	35	60	5.2
				35-a (R,R)*	60	2.8
				35-b (S,S)*	60	77
7	36	non-rigid	28	37	non-rigid	4.1

*Stereochemistry, except as noted all compounds are diastereomeric pairs

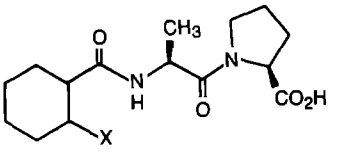
**See text

group. We thus prepared a series of compounds in which conformational constraint was used to efficiently orient the zinc binding carboxyl ligand (Table 4). The results suggest that carboxyl group orientation is directly related to good ACE inhibition. Suitably constrained compounds (e.g., 32, 35, 37) are more potent inhibitors than the acyclic analog 2. Absolute configurations of the individual diastereomers of 35 were assigned by independently preparing isomers 35-a and 35-b. The more active isomer (35-a) has the R,R configuration about the cyclohexane dicarboxylate centers. The significance of proper stereochemistry is highlighted by a 25-fold difference in activity between the isomers 35-a and 35-b, while importance of the carboxyl group in 35 is illustrated by comparison with the carboxyl deletion analog 38 (Table 5).

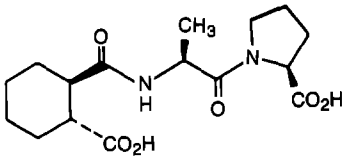
Since sulfhydryl is a more effective zinc binding group than carboxyl in the acyclic series (5 vs. 2, Table 2), conformationally constrained mercapto-acyl dipeptides (39) were

Table 5

Variation of the Zinc Coordinating Group in Cyclic Acyl-Dipeptides



No.	X	Stereochemistry	$I_{50}(\mu M)$
35-a	CO2H	trans, R,R	2.8
38	H	---	2,000
39-a	SH	trans, A	0.003
39-b	SH	trans, B	0.14



35-a

also prepared (Table 5). Introduction of conformational constraint in this series results in nearly a 1000-fold increase in ACE inhibitory potency (39-a vs. 5), highlighting the role played by proper functional group orientation in achieving potent inhibition. Although absolute configurations have not yet been assigned, this orienting effect is reflected in the large activity difference between isomers 39-a and 39-b. The results are consistent with a strong zinc-sulfhydryl interaction at the ACE active site, which is sensitive to proper orientation.

DISCUSSION: Differences in activity between cis and trans pairs may be explained by differences in relative orientation of the carbocyclic ring (Figure 2). For example, the dihedral angle between bonds to carbonyl groups is the same in both cis and trans cyclohexane dicarboxylate derivatives 34 and 35, yet they differ in activity. Assuming a strong orienting interaction between both the carboxyl and amide groups with ACE, then the

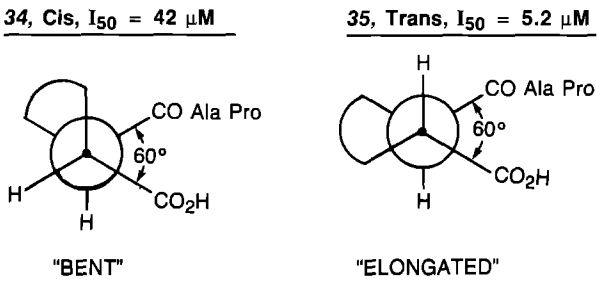


Figure 2 : Orientation of Carbocyclic Ring

observed activity difference must be due to relative orientation of the carbocyclic ring (Figure 2). For example, in the conformations drawn, an "elongated" orientation (trans) is preferred over the "bent" orientation (cis). Within each series, (cis and trans) activity differences are presumed to be due to the relative position of carboxyl and amide groups. A correlation may be drawn between approximate dihedral angles (α) and ACE inhibitory activity (Table 4). This relationship is shown graphically in Figure 3 where the optimum dihedral angle appears to be in the range 30° to 60°.

These data contribute to a further evolution of the three-dimensional ACE active site model in the previously incompletely defined S_1 region. In addition, combination of important fea-

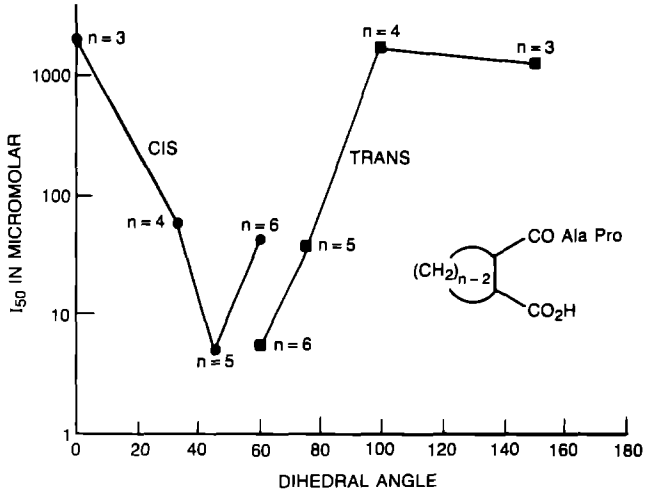


Figure 3 : Plot of I_{50} vs. Dihedral Angle for Cyclic Carboxy-Acyl Dipeptides

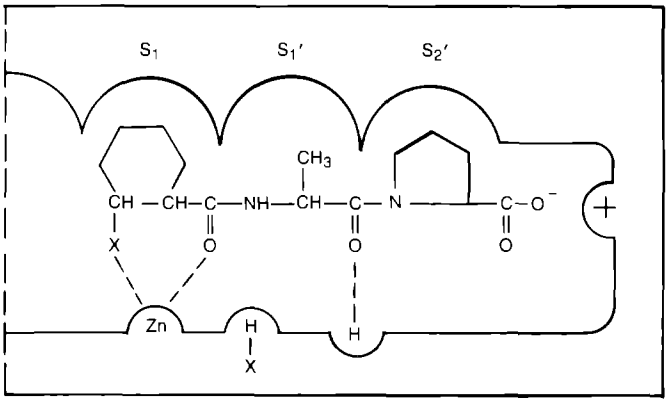


Figure 4 : Model for Binding of Cyclic Acyl-Dipeptides to ACE

tures has led to design of a new class of potent ACE inhibitors conforming to the original proposed model. An adaptation of the model for binding of these new inhibitors to ACE is shown in Figure 4. Bond angles and distances may be consistent with a bidentate zinc-binding hypothesis for interaction of these new compounds with ACE (11).

CONCLUSIONS: Functionalized acyl dipeptides conforming to our proposed model were prepared and several structural features were found to be necessary for good ACE inhibition:

1. Carboxyl- and sulfhydryl- groups are effective zinc ligands in this series.

2. A linkage of two methylene units between the zinc binding carboxyl group and the proximate amide bond is optimal.

3. Conformational constraint of the zinc binding ligand can result in substantial increases in ACE inhibitory activity.

4. Optimally constrained compounds have a dihedral angle of 30-60^{o} between bonds to zinc-binding and amide groups.

5. Preferred configuration about the conformationally constraining ring is trans-(R,R), as pictured in 35-a.

6. Combination of the above factors led to synthesis of mercapto-acyl dipeptide 39-a, which has in-vitro ACE inhibitory potency nearly ten times that of captopril (12).

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