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KETOMETHYLDIPEPTIDES II. EFFECT OF MODIFICATIONS OF THE α-AMINOKETONE PORTION ON INHIBITION OF ANGIOTENSIN CONVERTING ENZYME

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Summary: Results of an investigation aimed at identifying the consequences of chemical modifications of the α-aminoketone moiety of ketomethyldipeptides on angiotensin converting enzyme (ACE) inhibition are reported. These studies lead to the conclusion that within this series, the optimal structural backbone formulation for inhibition of ACE is represented by 1. Introduction of a Sar-Pro C-terminal dipeptide in this system, in contrast to other inhibitor classes, is compatible with potent inhibitory activity. Other structure-activity relationships for ketomethyldipeptides and related derivatives are presented, and speculations on possible modes of binding of these inhibitors to ACE, and on the question of ketone rehybridization are offered.

Introduction: Our previous report described a design rationale for synthesis of ketomethyldipeptides (1), a new class of potent inhibitors of angiotensin converting enzyme (ACE).[1] Molecular regions of these modified tripeptides which were expected to mimic the binding of an 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. Ketomethyldipeptides as well as the previously reported ketomethylene inhibitors (2,3)[2,3,4] are unique as ACE inhibitors in that they apparently lack a ligand capable of strongly interacting with the enzymic active-site zinc atom. This paper reports further investigations into the mode of interaction of ketomethyldipeptides

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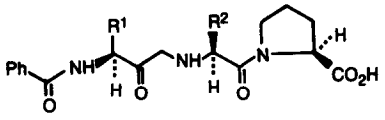

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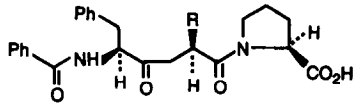
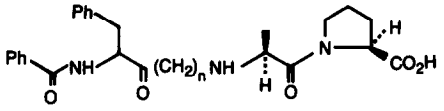
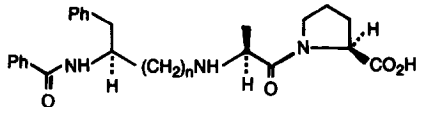


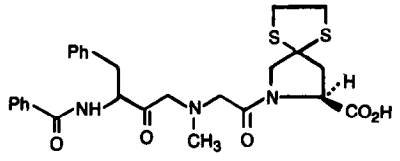
2, R = -H, I_{50} = 12 nM
3, R = $-CH_3$, I_{50} = 3 nM



4, n = 0, I_{50} = 3,200 nM
5, n = 1, I_{50} = 12 nM
6, n = 2, I_{50} = 9,100 nM



7, n = 1, I_{50} = 145,000 nM
8, n = 2, I_{50} = 7,000 nM

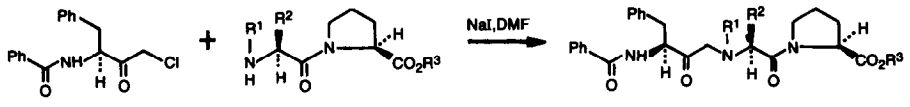


12, I_{50} = 1 nM

with ACE, in which attention was specifically directed at chemical modifications of the α-aminoketone group; that portion of the inhibitor molecule expected to occupy the position normally assumed by the scissile amide bond of the substrate.

Materials and Methods: The ketomethyldipeptides described were in general synthesized by alkylation of an N-alkylated dipeptide derivative with a chloromethylketone (Scheme 1). Complete details of the synthetic chemistry will be published elsewhere.[5] Purifications were effected by silica gel chromatography or by chromatography on Mitsubishi, HP-20 resin using gradient elution from 0.01 N aqueous HCl to methanol containing HCl. Procedures used to evaluate the *in vitro* ACE inhibitory activity of final products have been previously reported.[6]

Results: The ketomethyldipeptide modification incorporated in 5 may be viewed as the interposition of a single methylene unit within the scissile amide bond of N-benzoyl-Phe-Ala-Pro (4), a

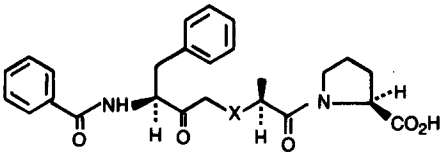


Scheme 1

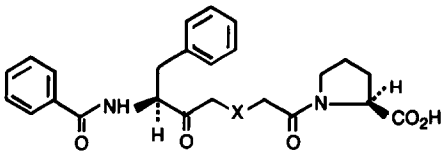
known substrate for ACE. Lengthening this linkage by a second methylene unit proved to be extremely detrimental to activity as exemplified by 6, which suffered a *ca.* 750-fold decline in inhibitory potency when compared with 5. The ketone function of ketomethylene ACE inhibitors has been shown to be essential in order to achieve high levels of inhibition;[7] this fact is reaffirmed in the aminoketones by the poor activity observed for 8, which is identical to 13 but contains a methylene in place of the ketone carbonyl. Interestingly, this substance is twenty times more potent than "reduction analog" 7,[7] a compound which is isosteric with benzoyl tripeptide, and in which the scissile amide carbonyl is replaced by a methylene group.

The consequences of replacing amino nitrogen by alternative heteroatoms are summarized in Table 1. Ketomethylether 9 possesses about half the inhibitory potency of 13 (5), whereas ketosulfide 10 and ketosulfoxide 11 (mixture of diastereomers) proved to be even less active. These results closely parallel those of Meyer *et al.*[8] who prepared similar ether and sulfide analogs lacking the α-methyl substituent, however our conclusion that

Table 1: *In Vitro* Converting Enzyme Inhibition Activities of Ketomethyldipeptides. Effect of Amine Modification



No.	X	I_{50} (nM)
9	–O–	21.*
10	–S–	38.
11	$-\overset{+}{S}-$ (O) **	38.
13	–N–H	6.
14	$-N-CH_3$	194.
15	$-N^{+}(CH_3)_2$	3239.
16	–N–CHO	1422.
17	$-N-COCH_3$	32,400.



No.	X	I_{50} (nM)
18	–NH–	4.
19	$-N-CH_3$	18.
20	$-N^{+}(CH_3)_2$	2824.*
21	$-N-C_5H_6$	23,000.
22	$-CH_2-$	210.*

*Approximate 1:1 mixture of diastereoisomers at center corresponding to α–Phe.

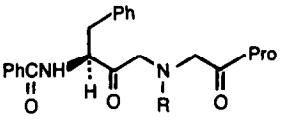
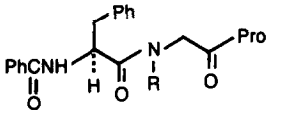
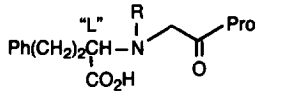
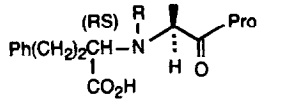
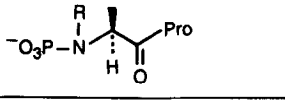
**Mixture of sulfinyl diastereomers.

amino nitrogen is the optimal substitution stands in contrast to the findings of these workers.

The effects of amino nitrogen derivatization as summarized in Table 1, details the *in vitro* inhibitory activities of several N-alkylated and N-acylated ketomethyldipeptides. N-Formylation or N-acetylation of 13 destroyed nearly all activity.[16,17] N-Methylation of 13 caused a 32-fold drop in inhibitory potency (14), while dimethylation was even more deleterious (15). On the other hand, Sar-Pro derivative (19) formally resulting from N-methylation of the Gly-Pro analog showed only a modest 4.5-fold diminuition of I_{50} with respect to 18. The corresponding dimethylated quaternary salt 20 was very poorly active. Modification of 19 by introduction of a 4-ethylenethioketal proline as the C-terminal substituent, produced an extremely potent inhibitor (12, I_{50} = 1.7 nM).[13]

Discussion: The finding that Gly-Pro terminating ketomethyldipeptide 18 shows a greater than 25-fold increase in inhibitory potency over ketone 22[1] is directly attributable to the introduction of amino nitrogen, which either must engage in a new enzymic interaction or strengthen a previously weaker interaction. Comparison of the foregoing data with similar modifications in other series makes it clear that the amino group is enhancing inhibitor binding in a manner somewhat different from the role served by its counterpart in carboxyalkyl dipeptides, N-phosphoryl dipeptides, or N-benzoyl tripeptides. Comparison of 9 or 10 with 13 demonstrates that replacements of nitrogen by oxygen or sulfur in the ketomethyldipeptides are modifications compatible with retention of high inhibitory potency, whereas such latitude is not the case in carboxyalkyldipeptides.[9,10] Additionally, the consequences of N-alkylation in the former series as well as in

Table 2: Effect of N-Methylation on *In Vitro* Converting Enzyme Activities in Several Inhibitor Series

No.	Structure	R	I_{50} (nM)	Ref.
18		-H	.004	
19		$-CH_3$	.018	
24		-H	9.4	
25		$-CH_3$	525.	10
26		-H	.290	
27		$-CH_3$	114.	10
28		-H	.0038	
29		$-CH_3$	.100	9
30		-H	.0014	
31		$-CH_3$	29.	12

tripeptides and N-phosphoryl dipeptides[12] further serves to differentiate these systems from the aminoketones (Table 2). N-Methylation in all four other series shown, compromises activity significantly. This is not the case in Gly-Pro terminating ketomethyldipeptides where only a 4.5-fold loss of inhibitory potency is noted. Such latitude breaks down in the Ala-Pro derived ketones (13, 14) presumably due to unfavorable conformational effects now imposed by the vicinal methyl groups.

Since heteroatom replacement analogs 9, 10, and 11, as well as N-methyl derivative 19 retain most of the ACE inhibitory activity of ketomethyldipeptide 13, it is likely that hydrogen bond donation by nitrogen, as has been proposed by Patchett[18] for binding of enalaprilic acid with the ACE active-site, is not an important interaction in the inhibitor/enzyme complex involving 13. The amine nitrogen atom of substances 13 and 19 might function as hydrogen bond acceptor sites, and/or these substances

as well as 9, 10, and 11 may owe their enhanced inhibitory properties with respect to 22, to mediation of ketone carbonyl electronics (*vide infra*). Since the ketone carbonyl has been shown to be critical for potent inhibition,[7] it is tempting to speculate that the ketone containing inhibitors participate in a specific interaction between ketone carbonyl and enzymic functionality at the active-site. Bunning and Riordan state that although the enzyme zinc atom is required for ACE catalytic activity, it is not necessary for precatalytic substrate binding. That is, the zinc atom functions solely in the hydrolytic step of catalysis.[14] Hence, any essential interaction of ketone inhibitors with enzymic zinc may come at a step following initial binding, a step which may mimic normal substrate hydrolysis. Since it is well known that analogs of transition states possess far greater enzyme affinity than ground state substrate analogs[15,16,17] a postulate which could explain the high inhibitory potency of these ketone containing inhibitors is that after initial binding the ketone undergoes enzyme induced rehybridization (involving either an enzymic nucleophile or a water molecule) to produce a stabilized transition state like species.[19] One function the heteroatoms in 13, 18, 9, 10, and 11 could serve is to facilitate this process by inducing heightened ketone reactivity toward an enzymic nucleophile. Ammonium nitrogen could be especially capable in this regard due to the placement of a cationic charge (α) to the ketone. Such protonation of nitrogen is a reasonable possibility, since this event would tend to mimic actual catalysis wherein a proton is delivered to scissile nitrogen presumably by a tyrosine residue.[20] It is interesting to note that the *in vitro* activity of 9, 10, and 22 and (protonated) 13, roughly parallels the electronegativity of these atoms. A consequence of rehybridization[21] would be to afford a viable ionized zinc binding ligand in sp^3

oxygen and thus permit the analogy to be drawn between ketones and all other classes of ACE inhibitors.

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21. A further point which may bear on the above relates to the Burgi-Dunitz vector hypothesis [Burgi, H. B., Dunitz, J. D., Lehn, J. M. and Wipff, G. (1974) Tetrahedron, 30, 1563-1615] which states that productive nucleophilic attack on a carbonyl group proceeds with stringent stereochemical constraints, along a vector directed from the rear of the carbonyl (side opposite oxygen) subtending an angle of 110° with the plane of the C=O linkage. Baldwin has extended these ideas and used a simple vector summation to identify the approach vector of various carbonyl functions [Baldwin, J. (1976) J.C.S. Chem. Comm., 739-741]. Applying this analysis in the case of an N-protonated amide, one notes that an incipient nucleophile proceeds along the same approach vector as in attack on a ketone. This simple analysis might provide the basis for an explanation for why a ketone carbonyl is capable of satisfactorily mimicking the carbonyl of an amide undergoing peptidase cleavage.