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Design of Tripeptide Modeled Inhibitors of Angiotensin-Converting Enzyme: Studies on the Role of the N-Terminal Acylamino Group

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Several series of tripeptide modeled angiotensin-converting enzyme (ACE) inhibitors have been reported which contain an N-terminal acylamino substituent as an essential structural component for conservation of biological activity. The results of a study aimed at defining the role served by this group in enzyme/inhibitor binding by examining the consequences of systematic chemical modifications are described. The benzamido N atom is shown to play a critical function in enzyme binding of ketomethylene ACE inhibitors. Evidence is also presented to implicate the benzamido carbonyl and phenyl ring in productive enzyme binding interactions. © 1986 Academic Press, Inc.

INTRODUCTION

Recently we reported the synthesis and biological activities of several new types of angiotensin-converting enzyme (ACE) inhibitors (*1–3*) whose design was based on elaborations of the known *N*-acyl tripeptide substrate *N*-benzoyl-Phe–Ala–Pro (*4*). A common structural feature shared by both the "ketomethyldipeptide" (*1, 2*) and "tripeptidyl aminoalcohol" (*3*) ACE inhibitors, as well as the ketomethylene analogs introduced by Almquist (*5–7*), is the presence of an N-terminal benzamido substituent. The data in Table 1 demonstrate that the *N*-benzamido group is an essential structural component of these tripeptide modeled inhibitor classes in order to observe high levels of ACE inhibition. Thus deletion of the *N*-acylamino appendage in ketomethylene inhibitor (**3**) results in a 10^6-fold loss of inhibitory potency (*8*). Similar results are noted with the tripeptidyl aminoalcohols and the ketomethyldipeptides (Table 1). In contrast, captopril (**7**) (*9*), carboxyalkyldipeptides (**8**) (*10*), and phosphonamidates (**9**) (*11*), which all contain strong zinc-binding ligands, express very potent ACE inhibition without requirement of an N-terminal benzamido substituent.

Recently reported attempts by Greenlee and co-workers to further enhance the enzyme-binding affinity of enalaprilat (**8**), by introduction of an N-terminal acylamino group, did not lead to more potent inhibitors, and the conclusion was

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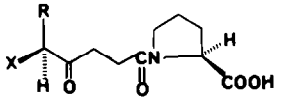
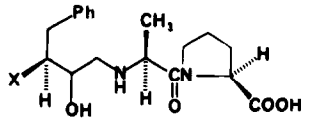
TABLE I

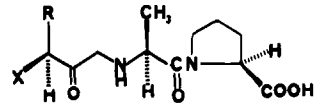
In Vitro ANGIOTENSIN-CONVERTING ENZYME INHIBITORY ACTIVITIES OF TRIPEPTIDE MODELED INHIBITORS: IMPORTANCE OF THE BENZAMIDO GROUP



No.	X	R	I_{50} (nM)
3	PhCONH-	$PhCH_2$-	12.
10	H-	$PhCH_2$-	2,600,000.
11	PhCONH-	H-	9,000.



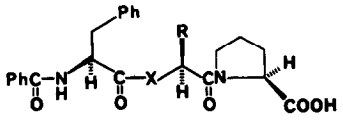
No.	X	I_{50} (nM)
12	PhCONH-	35.
13	H-	66,000.



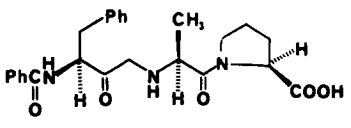
No.	X	R	I_{50} (nM)
5	PhCONH-	$PhCH_2$-	6.
14	H-	PhO-	66,000.

reached that such analogs of **8** possess geometry which does not permit simultaneous contributions to enzyme binding from both a side chain directed to the S_1 subsite of ACE and an acylamino substituent (*12, 13*).

Herein we report the results of an investigation aimed at identifying the consequences of chemical modification of the benzamido group of ketomethylene derivatives on their ability to inhibit ACE. The objectives of this study were directed toward defining the role served by an *N*-acylamino group as a fundamental component of the tripeptide (substrate)-derived ACE inhibitors, and determining what latitude, if any, is available for structural modification of this unit without significantly compromising high inhibitory potency.



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



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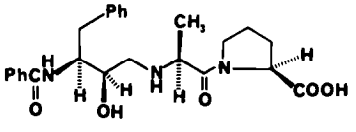

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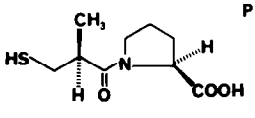

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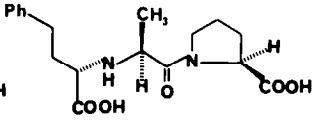

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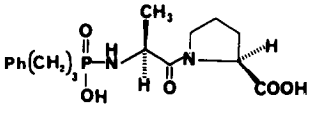

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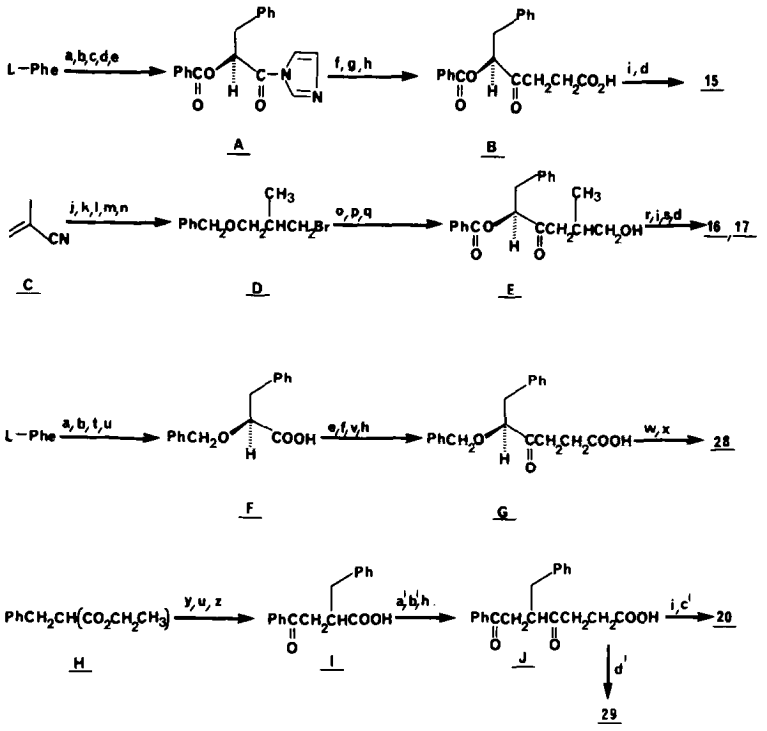
EXPERIMENTAL

Ketomethylene derivatives **15**, **16**, **17**, **20**, **28**, and **29** were synthesized as outlined in Scheme 1. The details of these syntheses will be published elsewhere. Compounds **26**, **32**, **33**, and **35–39** were prepared using methodology similar to that found in Ref. (*6*). Syntheses of **18** and **19** have been previously published (*7*). Purifications were effected by silica gel flash chromatography. Procedures used to evaluate the *in vitro* ACE inhibitory activity of final products have been previously reported.[2]

RESULTS AND DISCUSSION

The initial phase of our investigation was directed toward evaluating the role and importance of the N-terminal acylamino NH group. We thus synthesized ketomethylene and substrate (*N*-benzoyl-Phe–Ala–Pro) analogs in which the nitrogen atom in question was replaced by oxygen or a methylene unit. A previous report by workers at Warner–Lambert Company indicated that N-methylation of ketomethylene inhibitor **3** led to an "inactive" product (**21**) (*15*). In contrast we

[2] I_{50} values were determined by the method of Cushman and Cheung (*14*).



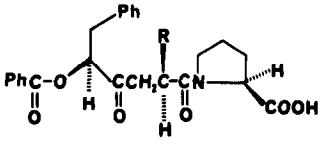
(a) H_2SO_4, $NaNO_2$, H_2O
(b) $PhCH_2Br$, $NaHCO_3$, DMF
(c) PhCOCl, Et_3N, DMAP, CH_2Cl_2
(d) H_2, Pd/C, EtOAc
(e) CDI, THF, 0°C
(f) BrMg $(CH_2)_2CHO(CH_2)_3O$, THF, −50°C
(g) CH_3CHO, acetone, *p*-TSA
(h) Jones oxidation
(i) L-Pro-OBzl HCl, DCC, DIPEA, HOBt, THF
(j) NaH, $PhCH_2OH$
(k) HCl, CH_3OH
(l) $LiAlH_4$, ether
(m) mesyl chloride, DIPEA, CH_2Cl_2
(n) LiBr, acetone
(o) Mg, THF, ultrasound
(p) compound "A," THF, −35°C
(q) $Pd(OH)_2$, H_2, ethyl acetate, acetic acid
(r) PDC, DMF
(s) separate isomers by chromatography
(t) NaH, $PhCH_2Br$, $(n\text{-bu})_4N^+I^-$, THF
(u) NaOH, H_2O, THF
(v) HCl, acetone
(w) 2-morpholinoethyl isocyanide, HOBt, THF, *t*-butyl-L-Pro
(x) TFA, CH_2Cl_2
(y) NaH, $PhCOCH_2Br$
(z) HCl, dioxane, heat
(a′) DCC, 2-hydroxypyridine
(b′) $I(CH_2)_2CHO(CH_2)_3O$, Zn, $NiCl_2$, DMF
(c′) LiOH; (d′) H_2, Pd/C ethanol

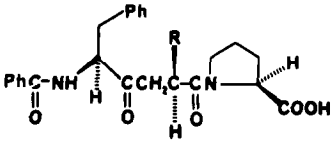
SCHEME 1

found that replacement of benzamido NH in **3** by an oxygen atom (**15**) caused only a fourfold decline in inhibitory potency (Table 2). We also investigated *O*-benzoyl/*N*-benzamide replacement in the ketomethylene series which mimics benzoyl-Phe–Ala–Pro. In order to evaluate effectively the biological activity of benzoyloxy analogs **16** and **17** with respect to the corresponding benzamido-containing substances we independently synthesized the previously

TABLE 2

In Vitro ANGIOTENSIN-CONVERTING ENZYME INHIBITORY ACTIVITIES OF KETOMETHYLENE TRIPEPTIDES: COMPARISON OF TERMINAL *N*-BENZAMIDO AND *O*-BENZOYL SUBSTITUENTS





No.	R	I_{50} (nM)	No.	R	I_{50} (nM)
15	-H	49.	1	-H	12.(3.2)[a]
16	$-CH_3$ (R)	2.3	18	$-CH_3$ (R)	2.6(1.0)[a]
17	$-CH_3$ (S)	342.	19	$-CH_3$ (S)	468.(46)[a]

[a] Values in parentheses are taken from Ref. (*7*).

reported *R*- and *S*-methyl substituted ketones **18** and **19** (*7*). Replacement of benzamido NH in diastereoisomers **18** and **19** by an oxygen atom led to two diastereoisomeric *O*-benzoyl substances (**16, 17**) which were nearly equipotent to the parent compounds (Table 2). Hence it may be inferred that the benzamido NH group does not serve a critical role in enzyme binding by hydrogen bond donation. In contrast to these results, insertion of a methylene group in place of acylamino NH (i.e., **20**),[3] afforded a material which was 30,000 times less active than parent ketomethylene inhibitor **3** (Table 3). The observation that benzamido NH can be adequately replaced by an oxygen atom, but not by a carbon atom, demonstrates that an unsubstituted heteroatom must be conserved at this position.[4] This suggests that the heteroatom participates directly or indirectly in an important inhibitor/enzyme interaction, and/or that the inductive effect exerted by this atom is highly significant, possibly enhancing carbonyl polarization and thus facilitating enzyme-induced ketone rehybridization (*2*). The effects of such CH_2/NH replacement in the tripeptidyl aminoalcohols (**6**) have not yet been studied.

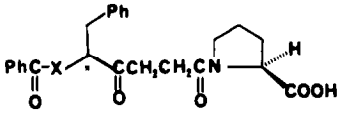
The effect on ACE inhibition of changes in substrate (*N*-benzoyl-Phe–Ala–Pro)

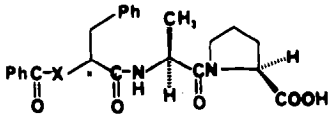
[3] This material is reminiscent of a related ketone employed by Kaiser and Lipscomb in the study of carboxypeptidase A (*16*).

[4] The evidence which suggests that an unsubstituted heteroatom must be present in the acylamino portion of the ketomethylene inhibitors is the inactivity of the Warner–Lambert *N*-methyl analog. This is not the case for other ACE inhibitor/substrate classes. For example, bradykinin (Arg–Pro–Pro–Gly–Phe–Ser–Pro–Phe–Arg), with an imino group at P_1, is a substrate for ACE, demonstrating that an N-substituted amino acid is tolerated in this position. The peptide *p*-Glu–Lys–Pro–Ala–Pro is a potent inhibitor of ACE (*17*). Other inhibitor classes possess imino acid elements in this position (*12*). Thus it is unclear whether a ketomethylene inhibitor possessing an N-acylated pyrrolidine would be an active ACE inhibitor. The effect of the *N*-methyl in the Warner–Lambert compound may be predominantly conformational. We thank a referee for addressing the above points.

TABLE 3

In Vitro ANGIOTENSIN-CONVERTING ENZYME INHIBITORY ACTIVITIES OF KETOMETHYLENE INHIBITOR ANALOGS AND RELATED TRIPEPTIDES: IMPORTANCE OF THE BENZAMIDO NITROGEN ATOM





X	No.	*	I_{50} (nM)	No.	*	I_{50} (nM)
-NH-	1	R	12.	2	R	3,200.
-O-	15	R	49.	22	R	28,500.
$-CH_2-$	20	R,S	360,000.	23	R,S	22,000.
$-N(CH_3)-$	21	R,S	"Inactive"[a]	24	R	21,000.

[a] Data taken from Ref. (*15*).

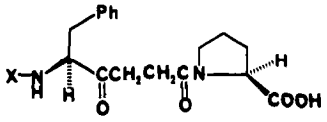
analogs, where benzamido NH has been replaced by either an oxygen, a methylene, or a N-methyl unit, is summarized in Table 3. The modified substrate analogs are of roughly equal inhibitory activity—about sevenfold less potent than substrate **3**. Interestingly, both ester **22** and ketone **23** were found to serve as viable substrates for ACE (*18*). Heretofore, an *N*-acyltripeptide has been considered the minimal competent substrate unit for this enzyme (*19*). This result demonstrates that an N-terminal acylamino function is not an absolute requirement for ACE cleavage of tripeptide-sized systems and indicates that this group may play a more profound role in the tripeptide inhibitors than it does in substrate binding and catalysis.

We next examined the role of the N-terminal benzamido carbonyl group in ACE inhibition. Previous work has demonstrated that aminoketones such as **25** are weak ACE inhibitors (Table 4) (*8*). These results may reflect incompatibility of a basic amine function in this active-site region with good inhibitor/enzyme binding. On the other hand, elimination of the amino group (i.e., **10**, Table 1) is even more deleterious, eradicating virtually all inhibitory activity. Comparison of **28** and **29** in which the carbonyl groups of ester **15** and diketone **20** have been replaced by methylene groups again illustrates the crucial importance of retaining a heteroatom at the α-ketone position. Retention of the ether oxygen confers a >650-fold increase in inhibitory potency on **28**, compared to alkyl analog **29**. Comparison of ether **28** with *O*-benzoyl ester **15** suggests a positive binding influence of the ester carbonyl group in **15** which confers a 16-fold enhancement in inhibitory potency of **15** over **28**.[5] Ketoether analog **28** proved to be the most potent nonamide or ester derivative of **3** prepared in our study.

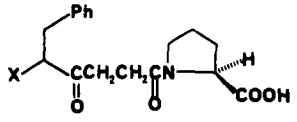
[5] This point is best made in the oxygen series rather than by comparing corresponding benzamido vs *N*-benzylamine analogs in view of the potential for misinterpretation due to a possibly charged nitrogen.

TABLE 4

In Vitro ANGIOTENSIN-CONVERTING ENZYME INHIBITORY ACTIVITIES OF KETOMETHYLENE ANALOGS: NONAMIDE REPLACEMENTS OF THE *N*-TERMINAL BENZAMIDO GROUP



No.	X	I_{50} (nM)
3	PhCO-	12.
25	H-	25,000.[a]
26	p-CH_3PhSO_2-	28,000.
27	HO_2CCH_2-	9,700.



No.	X	I_{50} (nM)
10	H-	2,600,000.
28	$PhCH_2O$-	775.
29	$PhCH_2CH_2$-	530,000.
30	$PhCONHCH_2$-	81,000.
31	HO_2CCH_2-	1,700,000.

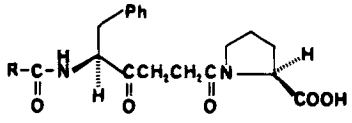
[a] Data taken from Ref. (*8*).

Replacement of the benzamido group in **3** by a sulfonamide (**26**) led to a marked drop in activity. Since a *p*-aromatic substituent is apparently compatible with good binding (Table 5), it is clear that the sulfonyl group performs unsatisfactorily as a surrogate for the carbonyl function in these inhibitors. This result most likely implicates the carbonyl group in an important enzyme-binding role (such as hydrogen bond acceptance), or alternatively may indicate that the preferred conformation of **26** improperly positions the aromatic ring or NH group for productive enzyme binding.

The effects on ACE inhibition by ketomethylene inhibitors containing a variety of *N*-benzamido phenyl ring replacements and modifications of **3** are summarized in Table 5. Inspection of these data and those of Meyer and co-workers (*6*) indicates that the aromatic ring makes a positive contribution to binding. Compound **3** is measurably more potent than either the corresponding cyclohexanoyl analog **32** or acetyl derivative **34** (*6*) (Table 5). The extent of electronic influence

TABLE 5

In Vitro ANGIOTENSIN-CONVERTING ENZYME INHIBITORY ACTIVITIES OF KETOMETHYLENE INHIBITOR ANALOGS: EFFECT OF PHENYL RING REPLACEMENT AND MODIFICATION IN THE *N*-TERMINAL BENZAMIDO SUBSTITUENT



No.	R	I_{50} (nM)
3	Ph-	12.
32	cyclohexyl-	58.
33	CF_3-	1,700.
34	CH_3-	330.[a]
35	p-CH_3OPh-	11.
36	p-HOPh-	29.
37	p-NCPh-	4.
38	p-O_2NPh-	24.
39	p-PhCH_2OPh-	214.
40	2-furanyl	1.4[a]
41	2-tetrahydrofuranyl	20.[a]

[a] Data taken from Ref. (*6*).

exerted by the phenyl group on enzyme binding is uncertain. Although trifluoroacetyl ketone **33** is a substantially poorer inhibitor than acetyl analog **34**, the inhibition results of a collection of compounds (**35–39**) designed to modify phenyl ring electronegativity did not suggest a trend in either direction. Overall, aromatic (**3**) or heteroaromatic (**40**) (*6*) ring substituents afforded the most potent inhibitors.

In conclusion we find that the role served by the *N*-benzamido group in enzyme binding of ketomethylene ACE inhibitors is complex, and appears to be based on multiple inhibitor/enzyme interactions acting in concert. The benzamido carbonyl and aromatic ring are implicated in productive inhibitor/enzyme interactions, whereas the presence of a heteroatom in the *N*-benzamido NH position is crucial to the expression of potent ACE inhibitory activity by these compounds.

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