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KETOMETHYLUREAS. A NEW CLASS OF ANGIOTENSIN CONVERTING ENZYME INHIBITORS

S. NATARAJAN*, E.M. GORDON*, E.F. SABO, J.D. GODFREY, H.N. WELLER, JELKA PLUSCEC, M.B. ROM, J. ENGEBRECHT, D.W. CUSHMAN, J.M. DEFORREST and J. POWELL

The Squibb Institute for Medical Research, P.O. Box 4000, Princeton, New Jersey 08540, USA

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The design rationale for a new series of tripeptide derived angiotensin converting enzyme (ACE) inhibitors, which we term "ketomethylureas", is described. Analogs of tripeptide substrates (i.e. *N*-benzoyl-Phe-Ala-Pro) in which the nitrogen atom of the scissile amide bond and the adjacent asymmetric carbon atom of the penultimate amino acid residue are formally transposed give rise to this novel class of inhibitors. The most potent ketomethylureas inhibit ACE wtih I_{50} values in the nM range.

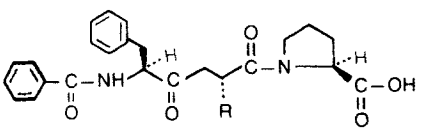
KEY WORDS: Tripeptide substrate derived ketomethyl ureas, angiotensin converting enzyme inhibition.

INTRODUCTION

In recent years various structural classes of very potent, rationally designed, inhibitors of angiotensin converting enzyme (ACE, EC 3.4.15.1) have been developed in several laboratories.[1] Ketomethylene inhibitor **1** first described by Almquist,[2] mimics the tripeptide substrate *N*-benzoyl-Phe-Gly-Pro, but introduces a hydrolytically resistant ketomethylene unit in place of the scissile amide linkage. Later studies conducted in our laboratories[3] indicated that the ketone function contained in **1**, and in the related ketomethyldipeptides (i.e.**2**)[4,5] was an essential component for the observation of high levels of inhibitory activity. Although compound **1** and its analogs exhibit very high *in vitro* inhibitory activity, only the analog bearing a P_1' methyl residue (**3**) inhibited the angiotensin I pressor response *in vivo*.[6,7] In a continuing investigation of tripeptide modelled ACE inhibitors, we undertook a program directed toward exploring several chemical modifications of the molecular backbone of ketomethylene derivative **1**, by incorporating a P_1'-residue while retaining the critical features required for ACE inhibition.

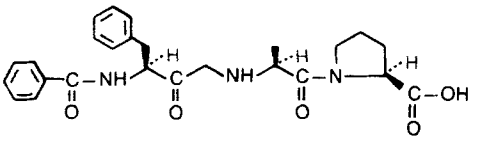
Glutaryl proline derivatives in which the C-2 carbon of glutaric acid has been replaced by a nitrogen atom (i.e. **5**) are members of a recently reported class of ACE inhibitors.[8] In these compounds the relative positions of important inhibitor functionality are conserved with respect to the glutaryl compound **4**, whereas the C-terminal amide bond and adjacent chiral center have been replaced by a urea group. While compound **5** maintains the amide carbonyl grouping of compound **4** in a

*To whom requests for reprints should be sent.

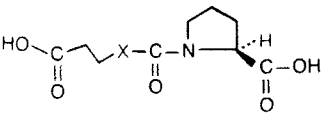


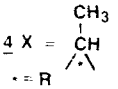
1 R = H: I_{50} = 12 nM

3 R = CH_3: I_{50} = 3 nM

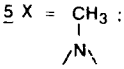


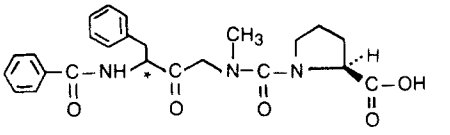
2 I_{50} = 6 nM



4 X =  : I_{50} = 4900 nM

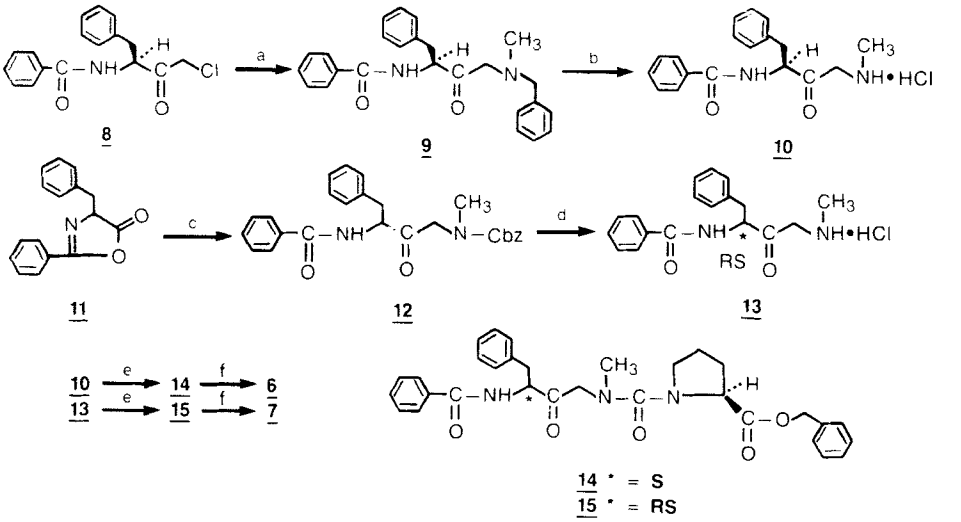
• = R

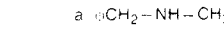
5 X =  : I_{50} = 790 nM



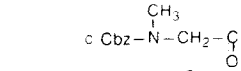
6 • = S; I_{50} = 2 nM

7 • = RS; I_{50} = 2 nM

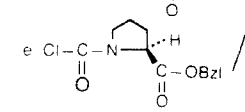


a 

b $H_2/Pd(OH)_2$–C, CH_3OH–HCl.

c 

d H_2/Pd–C, CH_3OH–HCl;

e 

f H_2/Pd–C, EtOH

SCHEME I

similar environment, it lacks chirality at the centre bearing the P_1' group. The stereochemical disposition of the P_1' substituent in compound **4** and other series of ACE inhibitors has been shown to be critical for the exhibition of potent ACE inhibition.[1] Nevertheless, the ureido analogs which lack the sp^3 character at this centre exhibited enhanced levels of inhibition.[8] Earlier work showed that the oxygen of the amide carbonyl between P_1' and P_2' residues engages in hydrogen bonding interaction with an enzymic residue.[9] The increased level of inhibition exhibited by urea analogs may arise by the ability of the oxygen of the carbonyl grouping of the urea moiety to engage in a more effective hydrogen bonding interaction with the enzyme.[8] This communication reports our efforts aimed at incorporating a similar ureido modification into a series of ketone containing ACE inhibitors. Since the asymmetric carbon atom bearing the P_1' side chain in the proposed compounds is replaced by an alkyl substituted urea nitrogen, we anticipated that their synthesis would be simpler than the methodology needed to prepare **3** and its analogs.

MATERIALS AND METHODS

Ketomethylureas **6** and **7** were synthesized by two general methods outlined in Scheme I. Alkylation of *N*-methyl benzylamine by chloromethyl ketone[4] **8** afforded a chiral product **9**, whereas adaptation of a modified Dakin-West reaction[4,10] necessarily affords racemic **12**. Following hydrogenolysis to remove the *N*-Cbz or *N*-benzyl protecting groups, the resulting aminoketone hydrochloride salts were reacted with chlorocarbonyl proline benzyl ester to afford ketomethylureido esters **14** and **15**. Final deprotection by hydrogenolysis yielded compounds **6** and **7**. Purification when necessary was performed by flash chromatography on silica gel (E. Merck, Mesh 230–400) using mixtures of ethyl acetate-benzene and chloroform-methanol-acetic acid as eluants. Details of synthesis of this new class of inhibitors "ketomethylureas" and other related compounds discussed in this paper will be published elsewhere. *In vitro* and *in vivo* assay procedures have previously been published.[11,12] *In vitro* and *in vivo* data reported for compounds **1** and **3** in this communication are our determinations.

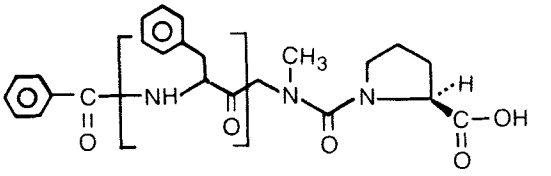
RESULTS AND DISCUSSION

Ketomethylurea **6** was found to be a potent inhibitor of ACE (I_{50} = 2 nM). We then examined the consequences of systematically varying the P_1 sidechain substituent while maintaining the balance of the inhibitor molecule constant. In general, a P_1' methyl substituent has been observed in numerous series of ACE inhibitors to amply satisfy S_1' binding requirements and consequently this substituent was employed. Inspection of the I_{50} values (Table I) reveals that aromatic (**6**, **17**, **18**, and **19**) are preferred over alkyl (**20** and **21**) or alkylamino (**22**) residues at the P_1 position. Analog **6** possessing the *S* configuration at the center corresponding to L-phenylalanine of the peptide substrate was more potent (I_{50} = 2 nM) than the related diastereoisomer of *R* configuration (**16**, I_{50} = 300 nM).

A lysine residue can be substituted for alanine at the P_1' position in several ACE inhibitor systems.[4,13] To test the usefulness of this substitution in the ketomethylurea series an analog (**23**) possessing a 4-aminobutyl N- substituent was synthesized. In contrast to what has been observed in the ketomethyldipeptides[4] and the car-

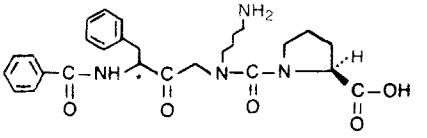
TABLE I

In Vitro Converting Enzyme Inhibitory Activity of Ketomethylureas. Variation of the N-Terminal Amino Acid Derived Residue



No.	[Residue]	I_{50}(nM)
6	L–Phe	2
7	D,L–Phe	2
16	D–Phe	300
17	D,L–3–PyridylAla	2
18	D,L–Trp	4
19	D,L–Tyr	2
20	D,L–Nle	18
21	D,L–Ala	110
22	D,L–Lys	124

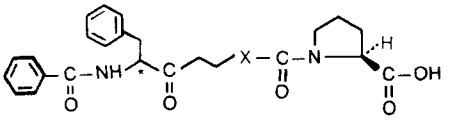
boxyalkyldipeptides,[13] substance **23** was considerably less potent than **7**. Homologation of the ketomethylurea skeleton to ketoethyl derivative **24** caused greatly diminished inhibition with respect to **7**. This result contrasts with a similar homologation of ketomethylene **1** to ketoethylene **25**, in which a significant level of inhibitory activity was retained.[14] Compound **26** exhibited a level of inhibition (I_{50} = 4300 nM) approximating that observed for the tripeptide Phe-Ala-Pro (I_{50} = 4200 nM);[3] thus, the presence of a N-terminal benzoyl substituent is a necessary structural feature in this inhibitor series, as it has been shown to be in other ACE inhibitors modelled on tripeptides.[3,6,15]



* = RS

23

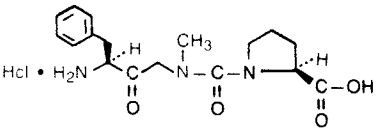
I_{50} = 120 nM



* = RS

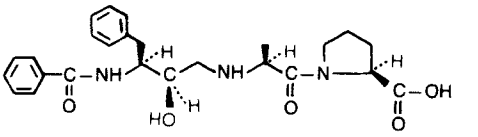
24: X = –N(CH$_3$)–; I_{50} = 5000 nM

25: X = –CH$_2$–; I_{50} = 211 nM



26

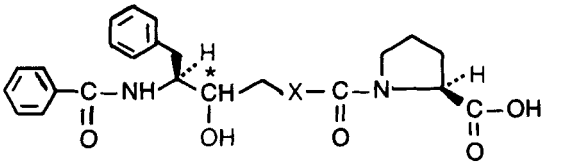
I_{50} = 7300 nM



27

I_{50} = 28 nM

TABLE II
In Vitro Converting Enzyme Inhibitory Activity of Hydroxyethylureas and Related Analogs



	*	X	I_{50} (nM)
28	ISOMER–A	$-N(CH_3)-$	160
29	ISOMER–B	$-N(CH_3)-$	270
30	ISOMER–A	$-CH_2-$	4700
31	ISOMER–B	$-CH_2-$	25000
32	RS	$-C(CH_3)(H)-$ (R)	96

(ISOMER–A and ISOMER–B are pure diastereoisomers of undetermined configuration)

We recently reported[16] that certain tripeptide derived amino alcohols (i.e. **27**) inhibited ACE, and that in these substances the hydroxyl group of *R* configuration played an essential role in inhibitor/enzyme interaction. In order to explore the substitution of a hydroxyl function for the ketone moiety in the present series, we synthesized diastereomeric alcohols **28–32** (Table II). These substances were considerably less potent ACE inhibitors than the corresponding ketone derivatives. It is therefore clear that replacement of the ketone by a hydroxyl group in both the ketomethylurea and ketomethylene ACE inhibitor series leads to a marked deterioration in inhibitory activity.

Compound **7**, when administered intraveneously to normotensive rats inhibited the pressor response induced by exogenously administered angiotensin I (Figure 1). However, when administered by the oral route, it exhibited poor inhibition of AI pressor response. This result may arise from a combination of very low oral absorption and rapid biliary excretion.[17]

CONCLUSION

Ketomethylureas such as **6** are very potent inhibitors of angiotensin converting enzyme. Structure activity relationships in this series indicate that incorporation of aromatic amino acid sidechains of a stereochemical disposition mimicking a natural L amino acid, at the inhibitor position corresponding to P_1 of substrate are preferred. Neither homologation of the ketomethyl urea unit nor replacement of the ketone

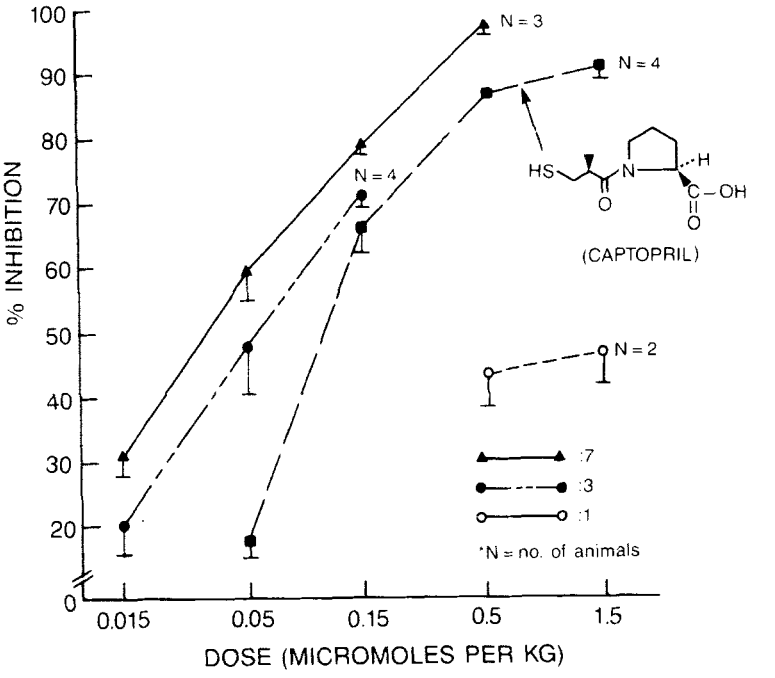


FIGURE 1 % Maximum Inhibition of AI pressor response in normotensive conscious rats after IV administration.

function by an alcohol is well tolerated. Our findings reported herein offer the possibility of utilizing, in a general way, ketomethylureas as components in the design of peptidyl inhibitors of various proteolytic enzyme systems.

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