Br. J. clin. Pharmac, **18** (1984), 201S–207S

The chemistry of enalapril*

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1 The design origins of the potent non-mercapto angiotensin converting enzyme inhibitors enalaprilat and its mono ethyl ester enalapril are described.
2 Lactam analogues of enalaprilat have provided some insight into the conformation of this inhibitor when it is bound to converting enzyme.
3 X-ray crystallographic studies of a related enzyme/inhibitor complex offer an explanation for the high potency and specificity of these and related inhibitors.

Keywords enalapril enalaprilat angiotensin converting enzyme inhibitors

The usefulness of angiotensin converting enzyme inhibitors in hypertension was first demonstrated with a natural product teprotide (Figure 1). The isolation of this peptide from a snake venom and its development as a pharmacological tool to probe the consequences of selective, potent inhibition of converting enzyme provides a compelling example of the importance of basic research in drug discovery. [For a review of the teprotide development see Ondetti & Cushman (1981).] Clinical studies of teprotide demonstrated efficacy in patients with both normal and high renin levels with minimal side-effects. Its only limitation was a lack of oral activity, which was not surprising since teprotide is a nonapeptide. Fortunately, the possibility of designing potent, low molecular weight inhibitors and thus of attaining oral activity was present. From analogue studies with teprotide and other snake venom peptide inhibitors (Cushman *et al.*, 1973) it was known that tripeptides as small as Phe-Ala-Pro inhibit the enzyme with $I_{50} = 3.6 \times 10^{-6}$ M. Even the dipeptide Ala-Pro is a significant inhibitor.

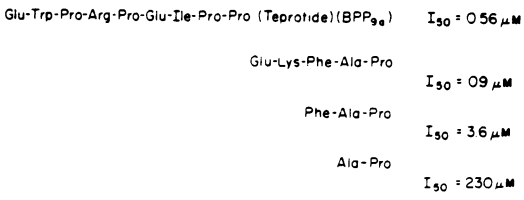


Figure 1 Teprotide and analogues (Ondetti & Cushman, 1981).

The design of both enalapril and captopril (Cushman *et al.*, 1977; Ondetti *et al.*, 1977) drew upon structural and mechanistic studies of a related Zn(II) containing enzyme carboxypeptidase A. The zinc atom in this enzyme is in the active site and is considered to play a key role in peptide bond hydrolysis by polarising the carbonyl group of the scissile bond. Furthermore, Byers & Wolfenden (1972, 1973) had designed bi-product inhibitors of this enzyme, as shown in Figure 2. It seemed reasonable to both Merck and Squibb groups that this design might be adapted to angiotensin converting enzyme after allowances were made for the specificity differences between the two enzymes. In particular, inhibitors of converting enzyme had to reflect the fact that this enzyme, unlike carboxypeptidase A, removes dipeptides rather than amino-acids from the C-terminus of substrates.

Adaptations of the Byers and Wolfenden design to converting enzyme are shown in Table 1. Although inhibitors resulted from this approach, potency was relatively low in even the

* This is a review paper, parts of which have been published elsewhere and are duly referenced. This paper itself has not been nor will be published elsewhere.

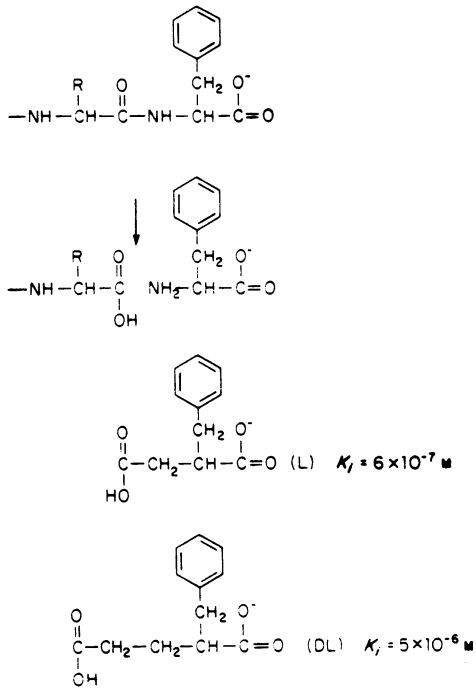
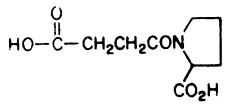
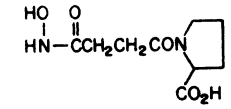
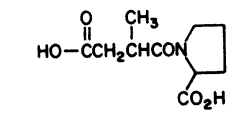
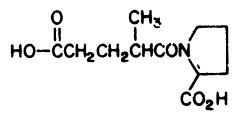
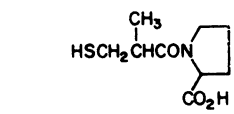


Figure 2 Bi-product inhibitors of carboxypeptidase A synthesised by Byers & Wolfenden (1972, 1973).

best of them, compound 4. The Squibb group led by Ondetti and Cushman reasoned that if the alkanoyl carboxyl group was formally derived from the scissile bond and, therefore, located at the Zn(II) atom, it should be possible to enhance activity by replacing this carboxyl group with a strong ligand to zinc. The most effective monodentate ligand to Zn(II) is the mercapto group and its substitution for the carboxyl group of compound 3 afforded captopril (compound 5). Potency was enhanced 1000-fold relative to compound 3, and as hoped captopril showed good oral activity. Its mode of binding to the enzyme is believed to be as shown in Figure 3. Captopril has been extensively studied in animals and in the clinic (Ondetti & Cushman, 1981; Antonaccio, 1982; Petrillo & Ondetti, 1982; Vidt *et al.*, 1982).

Early clinical reports indicated that rash and loss of taste were the most common side-effects seen with captopril. Because similar side-effects are experienced with penicillamine (Klaassen, 1980), our group hypothesised that an inhibitor devoid of the mercapto group might have less tendency to produce such side-effects. There was also the possibility that such an inhibitor, if it could be designed, would have a greater duration of action *in vivo* since the mercapto function easily undergoes oxidation and disulphide exchange reactions.

Table 1 Carboxyalkanoyl and mercaptoalkanoylproline inhibitors of angiotensin converting enzyme

No.		Inhibition
1		43% at 3.3×10^{-4} M*
2		84% at 6.7×10^{-5} M*
		I_{50}
3		2.2×10^{-5} M*
4		4.9×10^{-6} M*
5		2.3×10^{-8} M*

*The assay method is described by Patchett *et al.* (1980)

The approach taken by our group (Patchett *et al.*, 1980) was to try to enhance the potency of compound 4 by perfecting its presumed bi-product inhibitor design. As illustrated in Table 2, this might involve the addition of RCONH, R_1 or NH groups to compound 4 to afford additional binding interactions with the enzyme. The first of these possibilities to be tried was to add an NH group to complete an Ala-Pro unit in compound 6. To our disappointment compound 6 was not, within experimental error, more active than

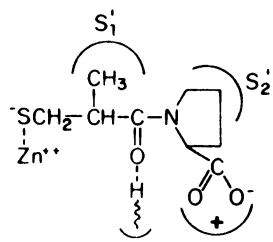
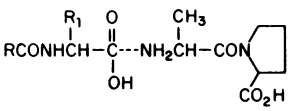
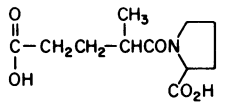
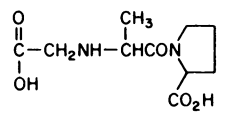
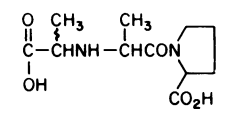


Figure 3 The proposed binding of captopril to angiotensin converting enzyme, adapted from Petrillo & Ondetti (1982).

Table 2 The development of the N-carboxyalkyl dipeptides from a bi-product inhibitor design



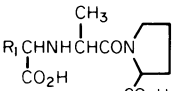
Number		I_{50}
4		4.9×10^{-6} M*
6		2.4×10^{-6} M†
7		9×10^{-8} M†

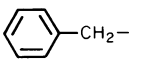
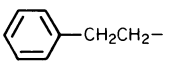
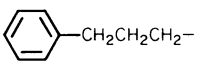
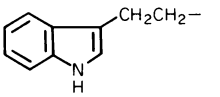
*Cushman *et al.* (1977).
†The assay method and the synthesis of these compounds are described by Patchett *et al.* (1980).

compound 4. However, we reasoned that introducing an NH in place of a CH_2 without change in potency might imply the presence of compensating factors. Since an NH group is much more hydrophilic than a CH_2 group, we decided to test the effect of hydrophobic groups in compound 6. The effect was dramatic. Introduction of a single methyl group raised potency in compound 7 to an I_{50} of 9×10^{-8} M even in an unseparated mixture of diastereomers. Clearly a route to high potency non-mercapto converting enzyme inhibitors had been uncovered and a wide variety of R_1 substituents were introduced into this design to capitalise on the breakthrough. Some of these substituents are shown in Table 3. Potency was high in compounds containing arylalkyl groups such as the phenethyl group (compound 16). This finding is in accord with the enzyme's specificity assuming that the R_1 group enters the enzyme's S_1 subsite.

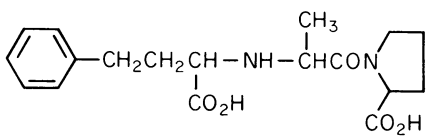
The more active, S, S, S-diastereomers of the best of these compounds was designated enalaprilat (MK-422, Figure 4). Its potency (I_{50} = 1.2 $\times$ 10^{-9} M; Patchett *et al.*, 1980) was significantly greater than that of captopril (I_{50} = 2 $\times$ 10^{-8} M). Calculation of their K_i values from a determination of steady-state velocities as a function of inhibitor concentration has given values of K_i = 2 $\times$ 10^{-10} M (enalaprilat) and K_i = 5 $\times$ 10^{-10} M (captopril) (H. G. Bull *et al.*, in preparation).

Table 3 Substituted carboxyalkyl derivatives of Ala-Pro

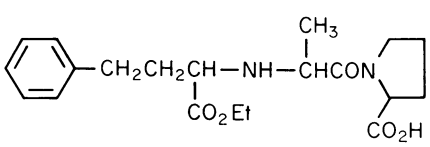


Number	R_1	I_{50}*
6	H–	2.4×10^{-6} M†
7	CH_3–	9×10^{-8} M†
8	CH_3CH_2–	1.7×10^{-8} M†
9	$CH_3CH_2CH_2$–	5×10^{-9} M‡
10	$(CH_3)_2CH$–	4×10^{-8} M‡
11	$(CH_3)_2CHCH_2CH_2$–	4.3×10^{-9} M†
12	$CH_3(CH_2)_5$–	3.7×10^{-9} M‡
13	$NH_2(CH_2)_4$–	2.2×10^{-9} M†
14	$NH_2(CH_2)_5$–	5.2×10^{-9} M†
15		3.9×10^{-8} M†
16		3.8×10^{-9} M†
17		8.3×10^{-9} M†
18		7×10^{-9} M§

*The assay method and a general synthesis of these compounds as a mixture of diastereomers are described by Patchett *et al.* (1980).
†Patchett *et al.* (1980).
§Hangauer (1981).
‡Unpublished data of E. H. Ulm. The compounds were synthesised by M. J. Wyvratt and T. Ilkeler.



Enalaprilat (MK-422)
$I_{50} = 1.2 \times 10^{-9}$ M



Enalapril (MK-421)
$I_{50} = 1.2 \times 10^{-6}$ M

Figure 4 The structures of enalaprilat and enalapril.

Table 4 The oral activity deficit of MK-422*

Compound	Enzyme inhibition	Blockade of angiotensin I (in rats) ID_{50} i.v.	(± 95% CL) ED_{50} Oral
Captopril	2.0×10^{-8} M	60.5 μg/kg† (51.3–71.6)	0.33 mg/kg
MK-422	1.2×10^{-9} M	8.2 μg/kg (6.6–10.6)	2.29 mg/kg

*Patchett *et al.* (1980).
†More recent results with captopril have given I_{50} values as low as 26.1 g/kg.
CL = confidence limits.

Nonetheless enalaprilat, instead of being more potent orally than captopril was in fact considerably less effective (Patchett *et al.*, 1980; Table 4). Several solutions were developed to overcome this oral activity problem, the first of which was the ethyl ester enalapril (MK-421). As shown in Figure 5, both the activity and duration of action of enalapril as its maleate salt given orally in rats were now quite satisfactory and exceeded that of captopril. Studies in man confirmed that enalapril maleate but not enalaprilat (MK-422) was absorbed well by the oral route (Biollaz *et al.*, 1981). It is important to realise that enalapril is a prodrug. Its I_{50} is only 1.2×10^{-6} M (Figure 4) which is 1000-fold weaker than enalaprilat. Thus, *in vivo* de-esterification is necessary before enalapril becomes optimally effective as an angiotensin converting enzyme inhibitor.

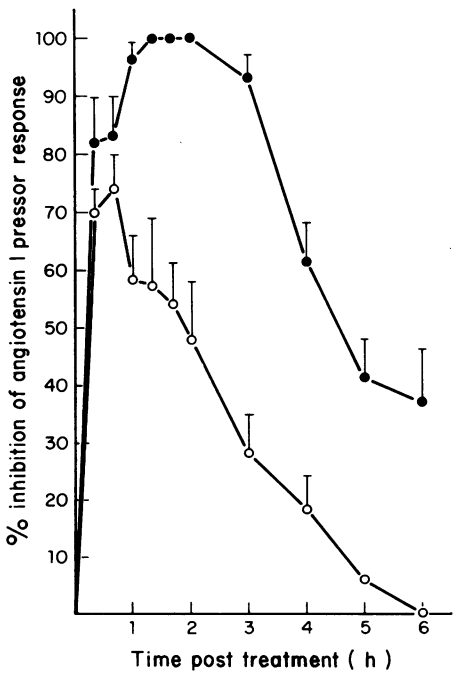


Figure 5 Comparison (mean ± s.e mean) of captopril (○) and enalapril (●) orally (3 mg/kg) inhibiting the pressor response to angiotensin I (300 ng/kg) given intravenously to Sprague-Dawley rats. The experimental methods are described by Sweet (1983) and the data shown are from his laboratory.

Enalaprilat and its analogues have been useful in mapping the active site of angiotensin converting enzyme. In the process new series on non-peptide converting enzyme inhibitors have been generated. The approach has been to evaluate the effects that conformational restrictions have on enzyme inhibitory activity. The conformation of enalaprilat as determined by X-ray crystallography is shown in Figure 6. Two features are

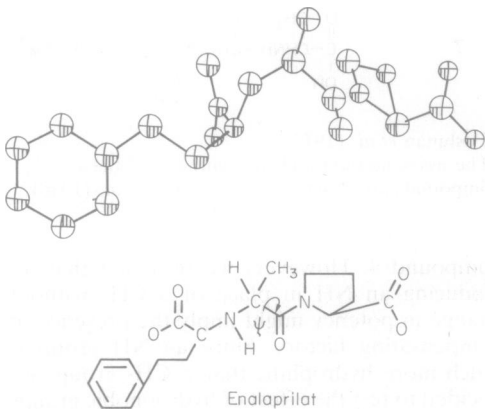
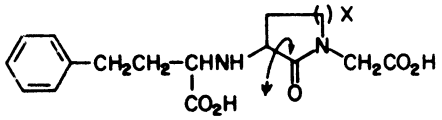


Figure 6 The structure of enalaprilat determined by X-ray crystallography by J. P. Springer (personal communication).

worth noting. The amide bond to proline is in the *trans* configuration, meaning that the carbonyl oxygen and carboxyl groups are *syn* to one another. Secondly, once the amide bond is fixed, then only the ψ angle need be determined to define the conformation of the Ala-Pro part-structure of enalaprilat. To investigate the importance of these two conformational features Dr Thorsett and his colleagues synthesised a series of model lactams in which the amide conformation is that of a *trans* peptide (Thorsett *et al.*, 1983). Ring size variation permitted calculable changes in the angle. The results of part of this study are shown in Table 5. The activities of some of these compounds approached that of enalaprilat (MK-422) itself, which is remarkable since two methylenes of the proline ring are absent, as is conformational restriction of the C-terminal carboxylate. These data lead

Table 5 Inhibitory activities as a function of ψ



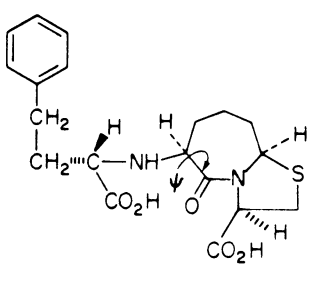
Ring size	ψ of lowest energy conformer	I_{50} (M)
5	-132°	1.3×10^{-5}
6	-138°	5.7×10^{-6} M†
7	166°	2.9×10^{-8} M*
8	145°	4.8×10^{-9} M*
9	135°	8.1×10^{-9} M*
MK-422	143°	1.2×10^{-9} M‡

*Tested as a racemic mixture of the more active diastereomer.
†Tested as a mixture of two diastereomeric compounds.
‡Determined by X-ray crystallography.

one to conclude that the Ala-Pro part-structure of enalaprilat binds to converting enzyme in a conformation very close to that found in its crystal structure (ψ = 143°). Furthermore this conformation closely corresponds to its minimum energy conformer based on MM2 calculations by Dr J. P. Snyder (personal communication). Thus, enalaprilat must bind to converting enzyme with minimal conformational change at least in respect to its Ala-Pro part-structure. Presumably the fact that very little binding energy must be expended to adjust enalaprilat's conformation contributes to its high potency.

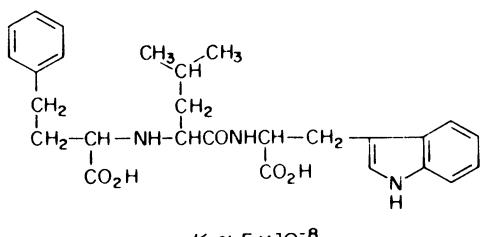
The ultimate step in this progression of analogues was to synthesise bicyclic lactams in which complete proline part-structures were present. The synthesis of one of these compounds was reported by Wyvratt and his co-workers (Figure 7). Its K_i was determined to be 7.6×10^{-11} M making it the most potent converting enzyme inhibitor yet to be described (Wyvratt *et al.*, 1984).

Finally, I would like to describe our efforts to understand some of the binding interactions which develop when carboxyalkyldipeptides interact with metallopeptidases. Since angiotensin converting enzyme has not been crystallised, an analogous enzyme thermolysin was used for X-ray crystallography studies. It is a crystalline Zn(II) peptidase which like converting enzyme is potently inhibited by carboxyalkyldipeptides. The specificity of thermolysin does not permit inhibition by enalaprilat. Instead it is inhibited by *N*-carboxyalkyl derivatives of dipeptides such as Leu-Trp (Figure 8) whose K_i value is about 5×10^{-8} M (Maycock *et al.*, 1981). The structure of this inhibitor bound to thermolysin was determined by Monzingo & Matthews



$K_i = 7.6 \times 10^{-11}$ M
ψ = 168°

Figure 7 The structure of a potent bicyclic converting enzyme inhibitor (Wyvratt *et al.*, 1983).



$K_i \simeq 5\times10^{-8}$ M

Figure 8 The structure of the thermolysin inhibitor used in the X-ray crystallography studies of Monzingo & Matthews (in press).

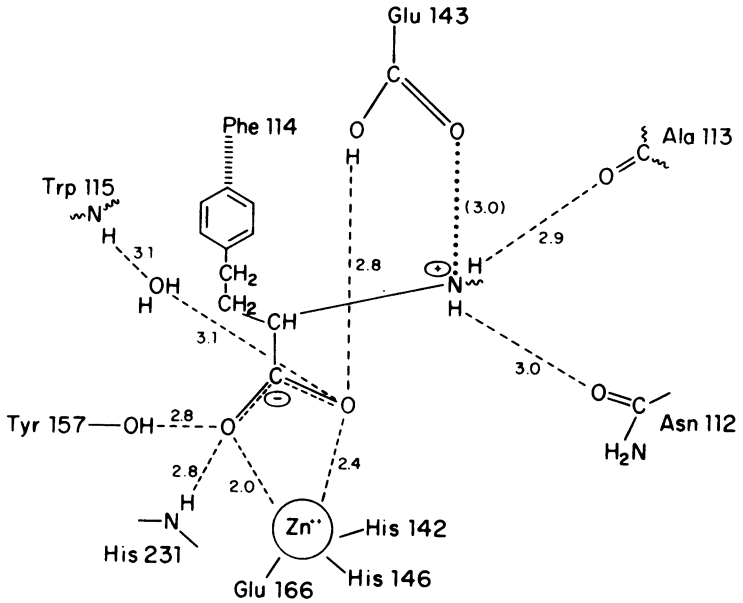


Figure 9 Active site binding interactions of *N*-[(S)-1-carboxy-3-phenylpropyl]-L-leucyl-L-tryptophan with thermolysin determined by X-ray crystallography [adapted from Monzingo & Matthews (in press)].

(in press). The multiple, sterically precise binding interactions which they uncovered were unexpected and no doubt contribute both to the potency and specificity of these inhibitors. As can be seen in Figure 9, the carboxyl group was bound as a bidentate ligand to Zn(II) and was further engaged in a network of hydrogen bonds with Glu 143, Tyr 157, His 231, and with a water molecule which it displaced from Zn(II). The phenethyl group of the inhibitor was in the S_1 subsite of the enzyme with its phenyl group approximately orthogonal to the phenyl group of Phe 114. Furthermore, the inhibitor's NH was in hydrogen bonding distance to Glu 143, the carbonyl oxygen of Ala 113, and the carboxamide of Asn 112. We assume that thermolysin and converting enzyme have closely related mechanisms and therefore, that functionally equivalent groups must be located similarly in the two enzymes. If so, we propose that enalaprilat binds to angiotensin converting enzyme as schematically indicated in Figure 10.

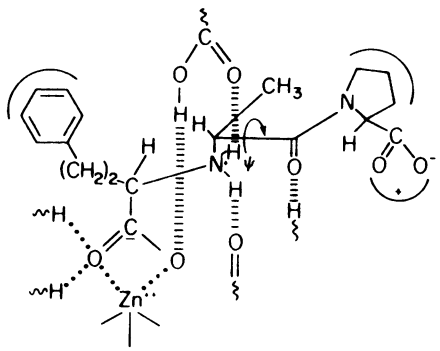


Figure 10 Proposed binding of enalaprilat to angiotensin converting enzyme. The value of ψ is probably within a range of 130–170°.

In summary, this research has led to the development of a potent new class of non-mercapto angiotensin converting enzyme inhibitors from which enalapril was selected for clinical study. These inhibitors have also been valuable tools in broadening our understanding of the structure and mechanism of Zn(II) endopeptidases.

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