The design of a new group of angiotensin-converting enzyme inhibitors

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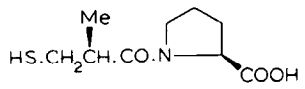
Using X-ray and NMR data relating to the conformation of the antihypertensive, angiotensin-converting enzyme inhibitor, captopril, and structure–activity relationships of analogues, it has been possible to postulate with the aid of computer graphics, the orientation of the three functions, the thiol, the terminal carboxyl and the carbonyl group which are involved in binding to the enzyme. Bicyclic mimetics of captopril, with related arrays of these functions, have been designed and synthesized. Compounds with the closest approximation to the array in captopril are the most active inhibitors of angiotensin converting enzyme, in vitro.

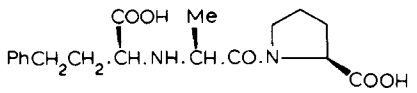
Angiotensin-converting enzyme *Inhibitors* *Design* *Antihypertensive*

1. INTRODUCTION

There is considerable interest in investigating the potential of the angiotensin–renin system for controlling blood pressure in man. Certain inhibitors of angiotensin-converting enzyme (ACE, EC 3.4.15.1) which transforms the decapeptide angiotensin I to the powerful vasoconstrictor octapeptide angiotensin II offer promise as therapeutic agents. Useful antihypertensive properties have been demonstrated, clinically, for the nonapeptide SQ 20 881 and the orally-active inhibitors captopril (SQ 14 225, I) [1] and MK 421 (enalapril II) [2]. The two latter compounds are very active inhibitors of ACE in vitro (table 2).



(I)



(II)

The thiol group in captopril was introduced by the Squibb investigators to co-ordinate with a postulated zinc atom in the enzyme while retaining a close similarity to the dipeptide Ala–Pro. The compound MK 421, which includes an Ala–Pro fragment in the molecule was the outcome of extensive structure–activity studies; it is visualised as a transition stage analogue inhibitor [2].

The molecular structure of ACE and, consequently, the details of its active site are unknown. Extensive evidence of structure–activity relationships of captopril and its close relatives is available. Using precise bond lengths and angles from the X-ray diffraction data for the captopril molecule [3] it becomes possible to postulate the favoured spatial orientation of functional groups that are important for binding. We have studied this to provide a basis for design of inhibitors with alternative molecular structures that might then be expected to show different characteristics in vivo.

2. EXPERIMENTAL

Computer graphics studies were carried out using a Megatek 7000 display processor interfaced to a PDP11/40 computer and a Midas plotter. NMR spectra were determined on Varian XL-100 and Bruker WM-300 spectrometers. X-ray diffraction data were obtained by courtesy of Dr J.J. Daly (F. Hoffmann-La Roche, Basle). Full description of the chemistry will follow in a later paper.

Enzyme was prepared according to [14]. Ac-

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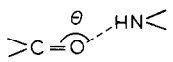
tivities were determined by monitoring the release of hippuric acid from the substrate hippuryl–histidyl–leucine (Hip–His–Leu) as in [15]. The assay medium contained Hip–His–Leu (2 mM), NaCl (300 mM), in phosphate buffer (100 mM), at a final pH of 8.3, incubated with ACE for 30 min at 37°C.

3. RESULTS AND DISCUSSION

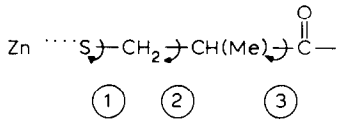
There is good evidence from the earlier studies on captopril and related compounds that the proline carboxyl, the carbonyl and thiol groups are essential [4–6] whereas the methyl function [7] and the spacing of the essential groups influence the potency of inhibition.

The co-ordinates of the 3 main binding sites may be deduced by analogy with known examples of substrate or inhibitor binding. In the case of the carboxyl group, it is assumed that it interacts with a positively charged centre in the enzyme ($-NH_3^+$ or $-NH-C\overset{NH_3^+}{\underset{NH_2}{}}$), lying in the vicinity of the loop represented to scale in fig.1. There is good evidence of hydrogen bonding to the carbonyl group [5]; this is postulated as favouring a *trans* orientation and interacting with an NH-group of the receptor:

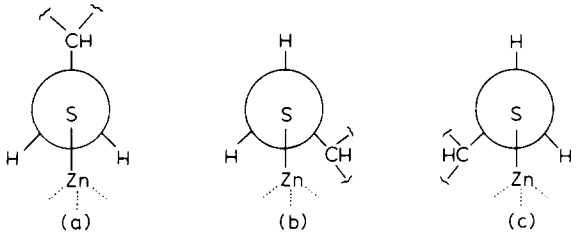


with an optimum angle Θ of 150–180°C. The hydrogen bond donator of the enzyme is anticipated to lie within the sector of a sphere of radius ~ 1.8 Å from the oxygen atom [8].

To postulate the volume of space within which the zinc–sulphur interaction may occur it has been assumed, in the first instance, that for the array:



there should be free rotation about bonds ② and ③ but for bond ① the rotamer (a), which minimises the interaction of the inhibitor with the bulky enzyme-bound zinc atom, is very much more likely than the *gauche* forms (b) and (c):



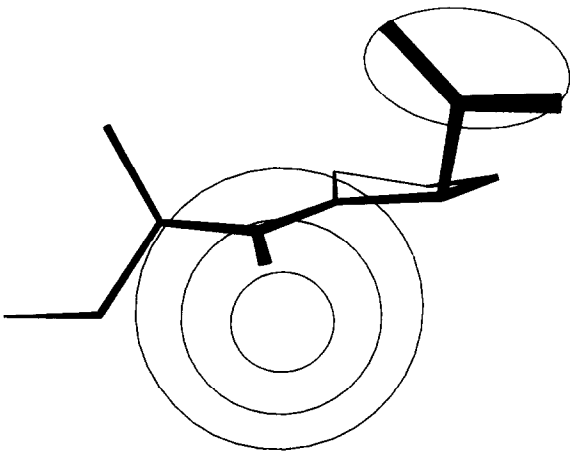


Fig.1. The hoop generated for the carboxyl group of captopril by rotation about the C–CO_2 bond. The positively charged group in the receptor binding site will lie some 2.8 Å from a point on the circumference and the spherical sectors within which the H-bonding group of the receptor must lie for angles Θ of 150°, 160° and 170°: $C = \overset{\Theta}{\widehat{O}} \ldots\ldots H-N$

On this basis, and allowing free rotation about bonds ② and ③, a rim-shaped mesh plot is produced for all possible positions of the zinc atom, as shown in fig.2; this indicates the surface of interaction with the thiol ligand.

For the rotation about bonds ② and ③ we deduced initially the locus of the sulphur atom, assuming no restriction in rotation. Using conventional molecular mechanics calculations (modified Lennard-Jones [9–11]), excluding all high energy rotational forms, a mesh plot (fig.3) was obtained for the locus with total energy < 50 kcal/mol. When this data is combined with the earlier deductions involving the zinc atom in a *trans* position the mesh plot (fig.4) is obtained for the postulated conformation of captopril bound to the 3 subsites of the enzyme.

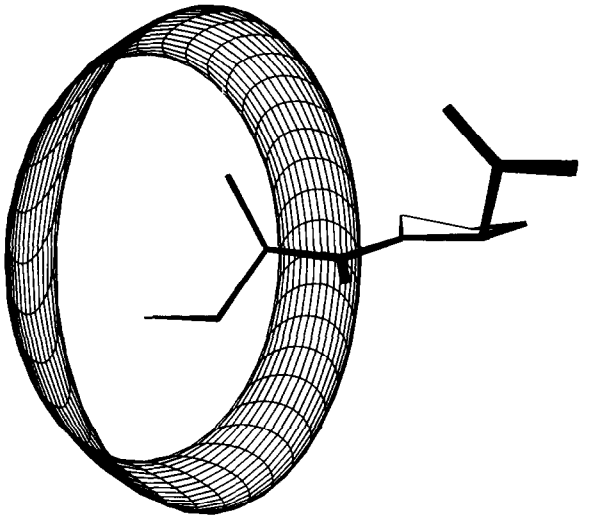


Fig.2. Allowing free rotation about the single bonds:

$$-CH_2\overset{②}{-}CH(Me)\overset{③}{-}C=O$$

and *trans* orientation about the CH_2–S bond (rotamer a), the zinc receptor atom will lie in the rim-shaped mesh as shown. (CH_2–$\widehat{S}$–Zn assumed to be 124°).

Interestingly, this mesh plot accomodates the conformation for captopril in the solid state (X-ray diffraction data [3]) which is also the major rotamer in solution (^{1}H-NMR studies [12]).

We have used fig.4 as a basis for designing new analogues of captopril which satisfy the criteria for binding to the active site. From earlier knowledge of the conformation of derivatives of piperazic acid [13] it was deduced that the critical subsites for binding in bicyclic compounds such as III would fall largely within the captopril mesh plot (fig.4). The preparation of typical 5,6- and 6,6-bicyclic compounds is illustrated in table 1. A comparison of ACE-inhibiting activities is in table 2. The conformations of key compounds III (as *S*-acetyl-methyl ester) and IX in the solid state have been determined by X-ray diffraction studies and in solution using high field ^{1}H-NMR studies. When these conformations were related to the energy mesh plot for captopril there was a good correlation between the degree of fit and the observed activity for ACE inhibition. This is illustrated by the results for the pair of inhibitors VIII and IX. It appears from these results that for good binding to the enzyme the CH_2SH and COOH groups should

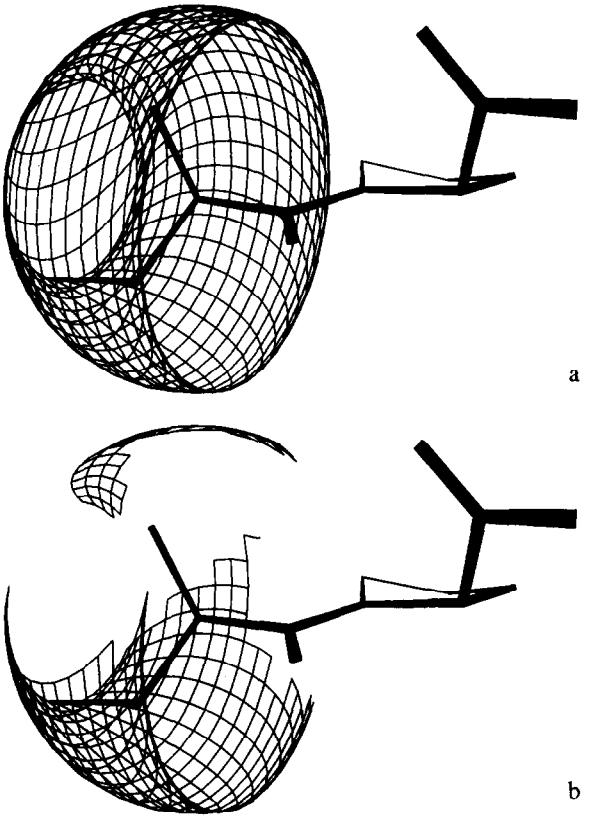


Fig.3. (a) The locus of the sulphur atoms allowing free rotation about bonds ② and ③. (b) The reduced locus excluding high energy rotational forms.

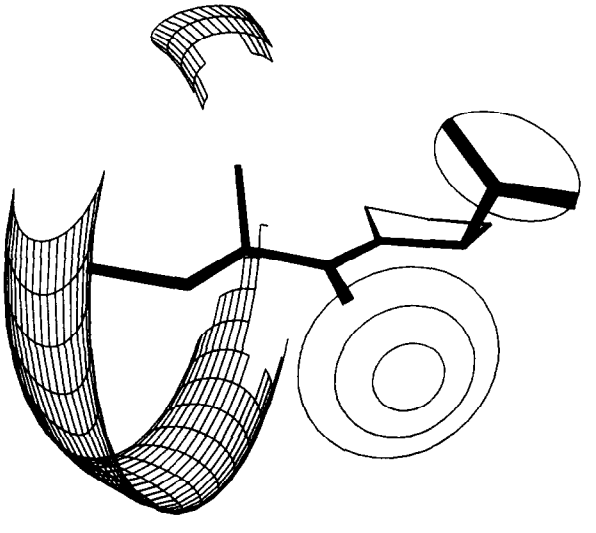
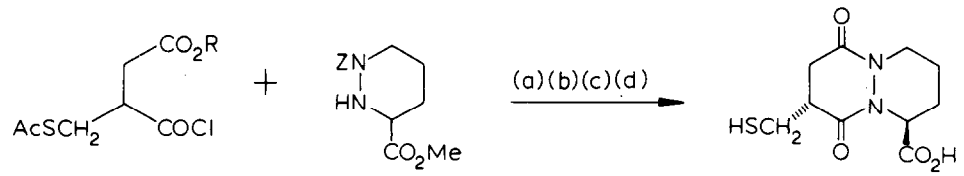


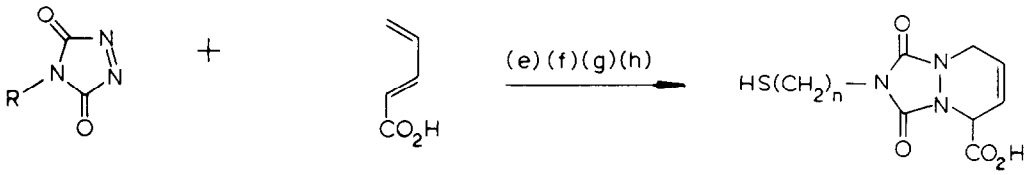
Fig.4. The total composite picture of the proposed binding site for captopril.

Table 1

The synthesis of representative examples of bicyclic ACE inhibitors

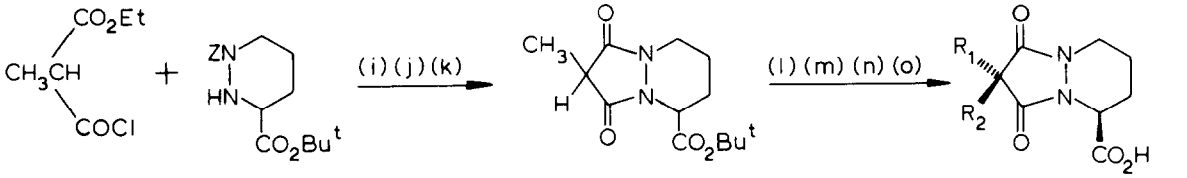


R = CH_3, CH_2Ph III



R = $(CH_2)_nCl$

IV n = 1
V n = 2
VI n = 3



	R_1	R_2
VII	CH_2SH	CH_3
VIII	CH_3	CH_2CH_2SH
IX	CH_2CH_2SH	CH_3

Reagents and conditions: (a) CH_2Cl_2, NaOH, 16 h, RT; (b) HBr/HOAc, 1.5 h, RT; (c) PCl_5/DMF, 2 h, 0°C; (d) MeOH, 1 M NaOH, 2 h, RT; (e) dioxan, ~ 1 h, RT; (f) MeOH/HCl, 16 h, RT; (g) AcSK, NaI, acetone, reflux, 18 h; (h) MeOH, NaOH, 2 h, RT; (i) CH_2Cl_2, 1 M NaOH, RT; (j) H_2/Pd, MeOH; (k) HOAc, 2 h, 100°C; (l) NaOH, DMF, RT; (m) $AcS(CH_2)_nBr$, 5 h, 75°C; (n) TFA, 1 h, RT; (o) NH_4OH, 2 h, RT.

Table 2

Inhibition, in vitro of angiotensin-converting enzyme from rabbit lung

Compound	I	II	III[a]	IV	V	VI	VII	VIII	IX
I_{50} (μM)[b]	0.007	0.004	0.038	0.7	0.48	6	1	43	0.1

[a] Compounds III → IX racemic mixtures
[b] See section 2 for details

be held *trans* to each other about the mean molecular plane, e.g., III, in line with the proposed binding areas suggested in fig.4. The arrangement in space of the important molecular groups in the most active bicyclic compound III is not yet optimal but is better than for the compounds IV–IX.

We are extending this study to other structures designed as ACE inhibitors. Evidently, this use of computerised molecular graphics could be applied to assist in other cases of drug design where there is a similar basis for utilising evidence of molecular conformation and biological activity measurements.

ACKNOWLEDGEMENTS

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