New potent inhibitors of angiotensin converting enzyme

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Using an earlier model of the favoured orientation of binding functions of angiotensin converting enzyme (ACE) inhibitors, it has been possible to postulate a new, 7,6-bicyclic system, based on hexahydropyridazine, which might be expected to have high potency. Some members of this system which have been synthesised have been shown to be very active ACE inhibitors, in vitro and in vivo.

Angiotensin-converting enzyme *Inhibitor* *Design* *Antihypertensive*

1. INTRODUCTION

Recent evidence relating to the compounds captopril [1], enalapril (MK 421) [2] and others [3] has confirmed the potential of inhibitors of angiotensin converting enzyme (ACE, EC 3.4.15.1) as agents for the treatment of hypertension [4,5] and congestive heart failure in man [6,7]. This has prompted considerable interest in the search for alternative ACE inhibitors which could have enhanced value in human therapy. In an earlier publication [8] we outlined a basis for designing compounds related to captopril but incorporating a bicyclic feature as an alternative to the pseudo-alanylproline unit. This provided an indication of the spatial requirements of the 3 functional groups of captopril (the C-terminal carboxyl, amide carbonyl and thiol) essential for good binding to the ACE active centre. In a continuation of that study, we have sought to replace the thiol by alternative binding functions suggested by evidence of substrate-ACE interactions [9], and to optimise the orientations of the C-terminal carboxyl and amide carbonyl for binding to the enzyme. This had led to the identification of a new family of very potent thiol-free ACE inhibitors with favourable characteristics for in vivo applications.

2. EXPERIMENTAL

Computer graphics utilised a Megatek 7000 display processor interfaced to a VAX11/750 computer and a Calcomp 81 plotter. NMR spectra were determined on Varian XL-100 and Bruker WM-300 spectrometers. X-ray diffraction data were provided by Dr J.J. Daly (F. Hoffmann-La Roche, Basle). Full descriptions of the methods used in chemistry, biochemistry and pharmacology will follow in later publications.

Rabbit lung and hog kidney enzymes were prepared from acetone powders [10]. Human enzyme was from plasma. The enzyme activities were determined [10,11] by measuring the release of hippuric acid from the substrate Hip-His-Leu. Assays were conducted with 2 mM substrate, 100 mM phosphate buffer (pH 8.3) containing 300 mM NaCl. Reactions initiated by addition of enzyme to a mixture of substrate and inhibitor at 37°C were allowed to proceed for 25 min (rabbit lung and hog kidney enzymes) or 60 min (human plasma). In the case of the diluted (40-fold) rabbit lung enzyme assay the incubation time was extended to 24 h.

A modified radiometric rabbit lung ACE/Hip-His-Leu assay [10,12] was used for measurement of in vivo plasma ACE activity.

The ability of selected compounds to block ACE-mediated release of histidylleucine from angiotensin I was examined. Assays were conducted in 100 mM phosphate buffer (pH 7.5) containing 30 mM NaCl, the substrate concentration being 0.4 mM. Reactions, initiated by addition of substrate to a pre-incubated mixture of enzyme

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and inhibitor, were allowed to proceed at 37°C for 30 min. Appearance of the dipeptide product was monitored fluorimetrically [13].

Selected compounds were also examined quantitatively for their ability to inhibit angiotensin I-induced contractions of the guinea pig ileum in vitro. In vivo ACE inhibitory potency was assessed in the anaesthetised rat from the decrease in angiotensin (1 μg/kg) pressor response at 15 min after intravenous administration of inhibitor. Systolic arterial pressure was recorded from a carotid arterial cannula connected to a pressure transducer. Data were controlled by relating them to the pre- and post-dose angiotensin II (1 μg/kg) pressor responses [14]. Potency and duration of action were also assessed for plasma ACE inhibition following oral administration of selected inhibitors.

The extent of oral absorption was determined from the relative urinary recovery of radioactivity after oral and intravenous doses of ^{14}C-labelled compound.

3. RESULTS AND DISCUSSION

The scaled graphical representation of captopril in 3 dimensions in comparison with our earlier bicyclic analogues [8,15] suggested that the

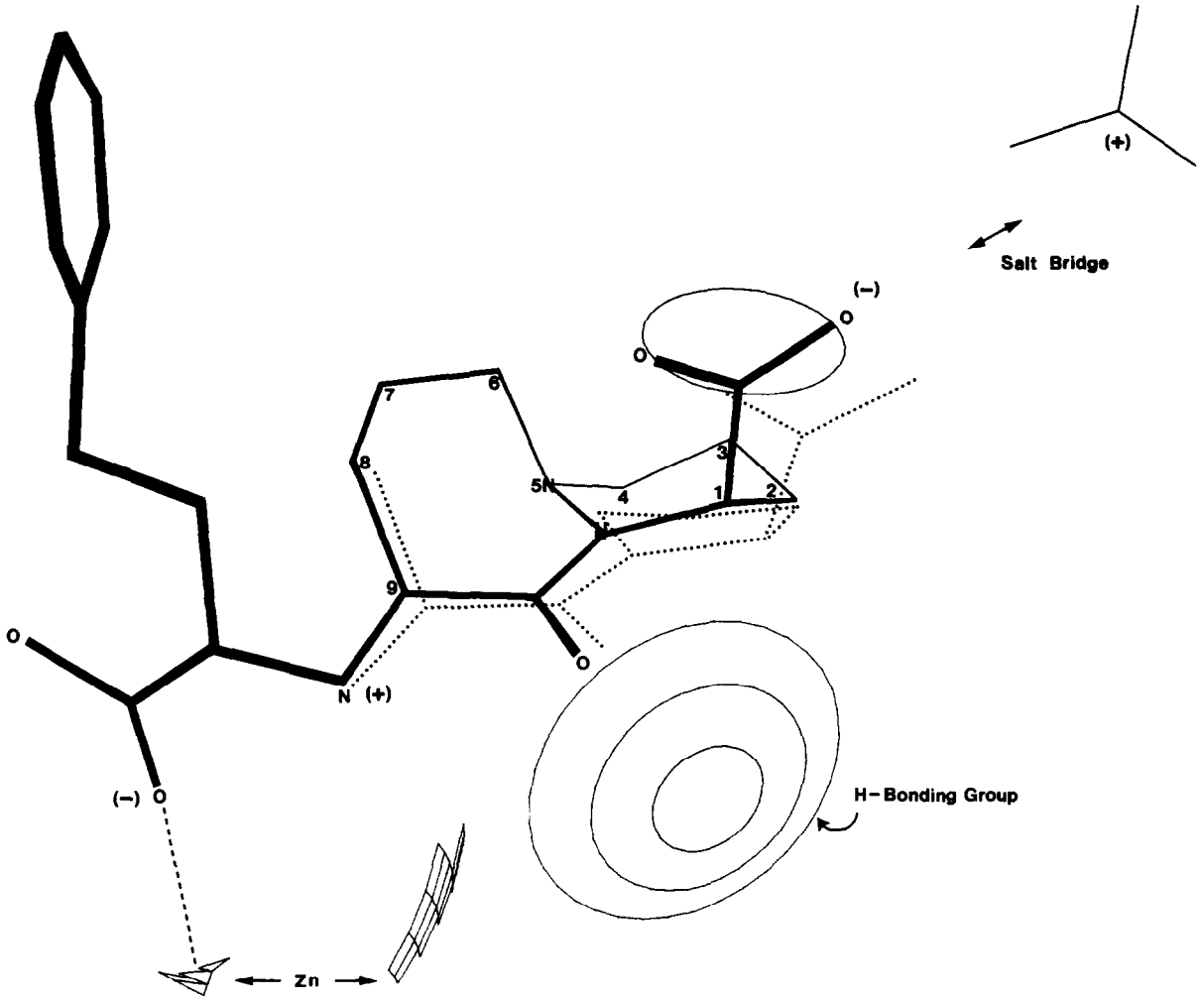


Fig. 1. The computer-drawn structures of alanylproline (dotted) line and the 7,6-bicyclic compound I (diacid form) (full line), overlaid in space and placed in the ACE active site derived previously, with the 3 binding interactions highlighted. The basic structures are based on X-ray diffraction data on closely related compounds. For clarity, the two structures are offset slightly.

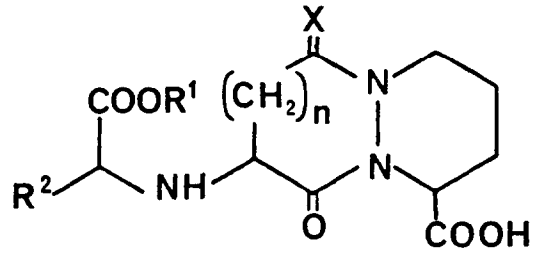
6,6-bicyclic system incorporating a hexahydropyridazine nucleus might be modified to enhance binding to the ACE active centre. In the 6,6-series the methylene group of the pseudo-alanine assumed a position corresponding to the high-energy conformation of Ala-Pro in which the methyl group almost eclipsed the proline δ-carbon. We postulated that this strain could be avoided and a more favoured position of this methylene would be produced in a compound with the related 7,6-bicyclic system (fig. 1).

The compounds retaining the favoured *S,S*-configuration for carboxyl and side chain substitutents at positions 1,9 were synthesised both with and without 6-oxo-functionality by means of the procedures indicated in table 3. The conformations of the two 7,6-bicyclic systems were indicated by NMR spectroscopy and later confirmed by X-ray diffraction. Moreover, the graphics showed (fig. 1) that this enabled the carboxyl, amide carbonyl and 8-methylene group to simulate the positions in space of the corresponding functions in a favoured conformation of L,L-Ala-Pro. We reasoned that, given this, the binding of such a rigid bicyclic system might well give rise to an entropy gain in comparison with the more flexible substrates; this would be reflected in higher potency. The C-9 substituent group $Ph(CH_2)_2CH(CO_2Et)$-NH- for the compound I (Ro 31-2848) was chosen, as in other cases [16, 17], as an alternative to the thiol in captopril to favour in vivo characteristics [2], and at the same time to provide for good binding to the receptor; this choice can be related to the structure of typical ACE cleavage products [18]. We found, like others, that the substituent R^2 attached to the glycine moiety could be varied in several cases with little change in potency (table 1).

The in vitro activities of representative 7,6-bicyclic compounds as ACE inhibitors are listed in table 1; these are compared with MK 421-diacid, SRI-20 and captopril as inhibitors of different ACE preparations, using angiotensin-1 and Hip-His-Leu as substrates. It is evident from table 1 that the new 7,6-bicyclic system favours high potency in vitro. The results in table 2 establish that the diacid derivative of compound I is an exceptionally potent inhibitor. The diluted enzyme assay was undertaken to avoid the possibility that the estimate of potency might be understated due to a mutual depletion effect; this may arise in cases where enzyme concentration and IC_{50} values are comparable [19].

Table 1

Inhibition of rabbit lung ACE-induced Hip-His-Leu cleavage in vitro



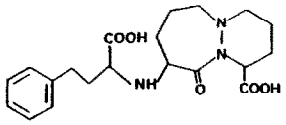
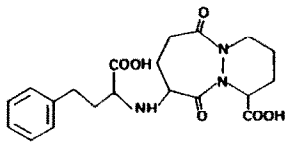
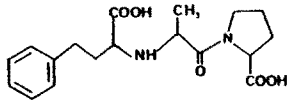
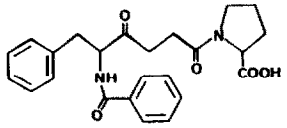
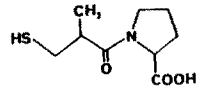
	R^1	R^2	n	X	I_{50} (nM)
	H	$PhCH_2CH_2$*	1	H_2	56
(I)	Et	$PhCH_2CH_2$	2	H_2	1.8
	Et	$H_2N(CH_2)_4$*	2	H_2	5
	H	$PhCH_2CH_2$	1	O	20
(II)	Et	$PhCH_2CH_2$	2	O	4
	H	$(CH_3)_2CHCH_2CH_2$*	2	O	12
	Et	*p*-MeO$PhCH_2CH_2$	2	O	3
	H	$PhCH_2CH_2CH_2$	2	O	11
	Et	H	2	O	560
	Et	$ZNH(CH_2)_4$	2	O	5
	Et	$ZNHCH_2$*	2	O	16
	H	$PhCH_2$*	2	O	26
	H	$ZNH(CH_2)_4$*	1	H_2	30
	H	$H_2N(CH_2)_4$*	1	H_2	100
	H	$PhCH_2CH_2CH_2$*	1	H_2	56
	H	$(CH_3)_2CHCH_2CH_2$*	1	H_2	100

When R^1 = Et, I_{50} determined after incubation with hog liver esterase

* Mixture of two isomers

The potent inhibitor II (Ro 31-2201) and the related desoxy compound I (Ro 31-2848) have been investigated in animal studies. They confirm that I is better absorbed (p.o. 80–100%) than II (p.o. 20–30%) but both have long-acting high potency in the rat for inhibition of plasma ACE when administered orally (fig.2), and high potency for antagonism of the pressor effect of angiotensin I when given intravenously as the diacids (fig.3). Detailed pharmacological studies with further evidence of the anti-hypertensive properties of these compounds in animals and in man will be reported elsewhere.

Table 2
Comparative biological evaluation of potent ACE inhibitors

Inhibitor	Structure	IC_{50} value (nM) Substrate : Hip – His – Leu				IC_{50} value (nM) Substrate: angiotensin I Rabbit lung ACE ([Enzyme] = 0.4 nM)	IC_{50} value (nM) (95% limits) guinea-pig ileum	ID_{50} value (μmol/kg i.v.) (95% limits) A1/A11 pressor inhibition
		Rabbit lung ACE ([Enzyme] = 1.5 nM)	Human plasma ACE ([Enzyme] = 0.5 nM)	Hog kidney ACE ([Enzyme] = 1.5 nM)	Diluted rabbit lung ACE = 0.04 nM)			
I Diacid		1.8	0.6	3.3	0.08	0.8	10.9 (3.1–16.2)	0.06 (0.03–0.10)
II Diacid		3.8	1.4	8.3	0.21	0.9	14.3 (12.6–16.2)	0.08 (0.06–0.17)
MK 421 Diacid		3.3	1.5	6.0	0.37	1.2	19.0 (14.7–22.9)	0.09 (0.08–0.11)
SRI-20		9.0	13	34	7.0	360	N.D.	N.D.
Cartopril		7.5	14	13	N.D.	N.D.	44.5 (31.9–67.9)	0.49 (0.35–0.76)

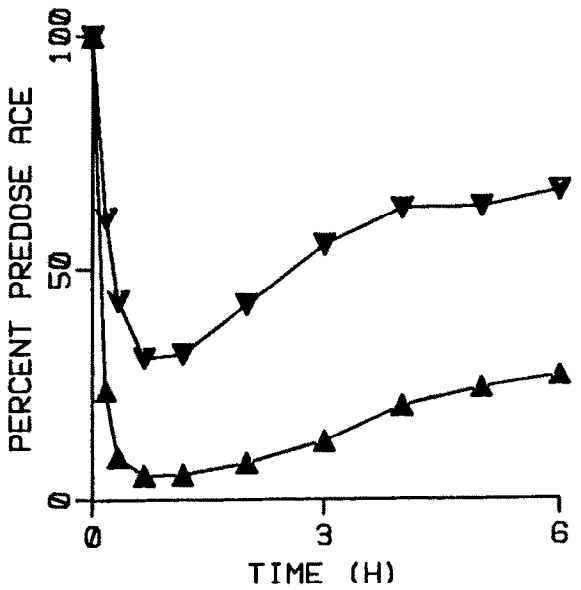


Fig. 2. The time course for plasma ACE inhibition in the rat after oral II (▼) (1 mg/kg) and I (▲) (0.25 mg/kg).

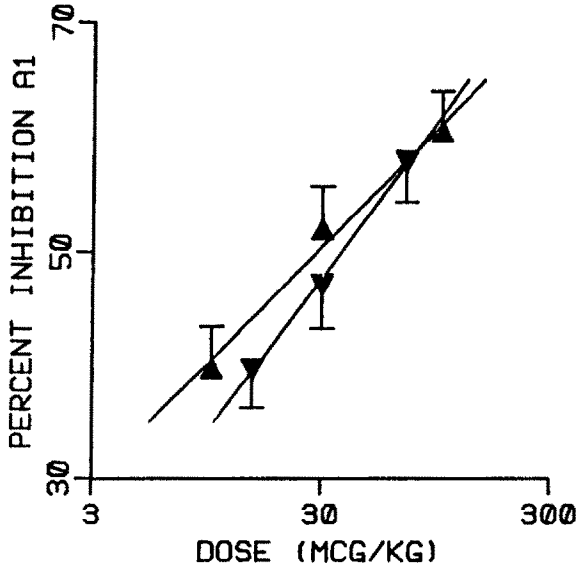
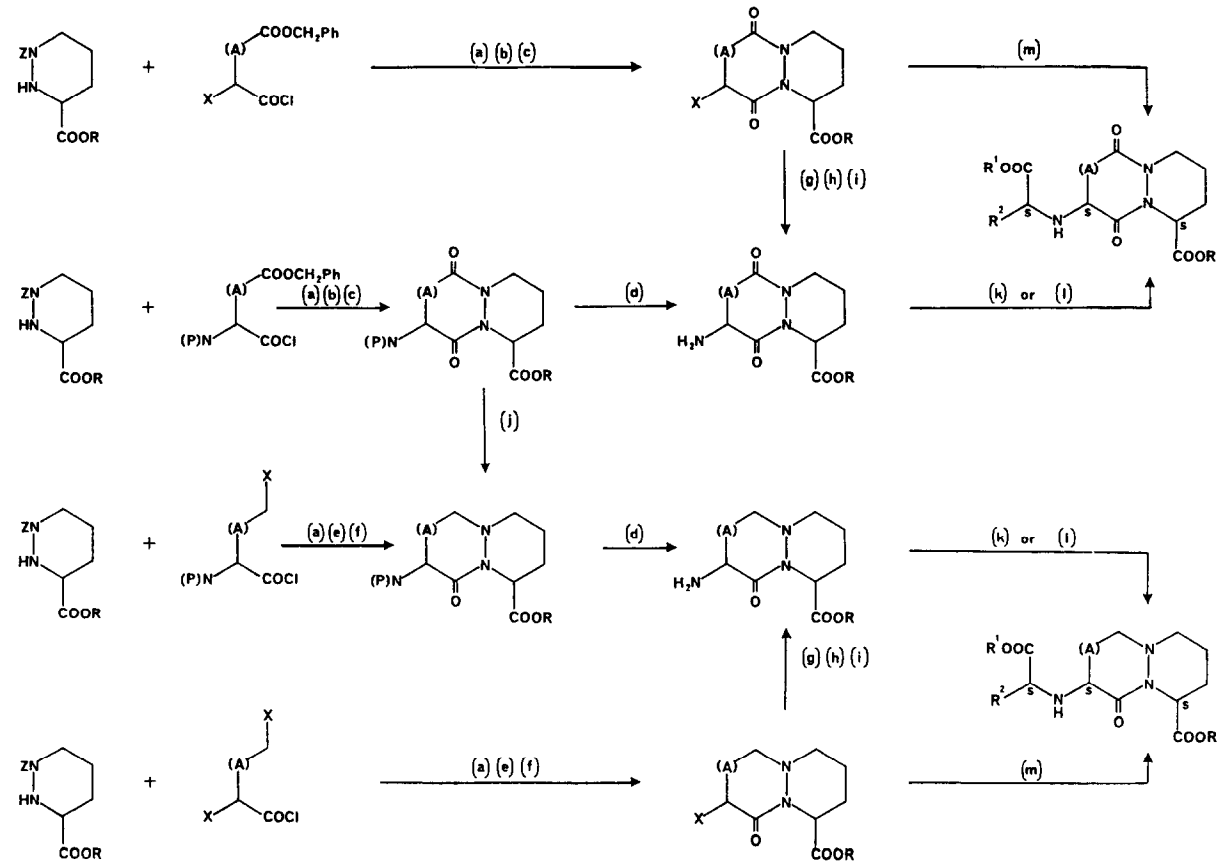


Fig. 3. The intravenous dose-response relationship for antagonism of the angiotensin I(AI) pressor effect in anaesthetised rats (i.v.). Diacids of II (▼) and I (▲).

Table 3

Preparation of *N*-carboxyalkyl derivatives of bicyclic dipeptide mimetics



(A) is CH_2 or CH_2CH_2; (P) is protecting group (usually phthaloyl); R and R^1 are appropriately Me, Et, ^{t}Bu or H (interchangeable by standard procedures); R^2 - refer to table 1; X is halide or sulphonate leaving group; Z is benzyloxycarbonyl. The required *S,S,S*-stereochemistry of the products was achieved by stereoselective synthesis or separation of isomers as appropriate. (a) toluene/H_2O/$NaHCO_3$, (b) H_2/catalyst, (c) $SOCl_2/CH_2Cl_2$, (d) $N_2H_4 \cdot H_2O$/EtOH, (3) HBr/HOAc, (f) DMF/K_2CO_3, (g) NaN_3/acetone, (h) Ph_3P, (i) NH_4OH, (j) H_3B-THF, (k) $R^2COCO_2R^1/NABH_3CN$,

(l) $R^2\underset{\displaystyle X}{\underset{|}{C}}HCO_2R^1/CH_3CN/Et_3N$, (m) $R^2\underset{\displaystyle NH_2}{\underset{|}{C}}HCO_2R^1/DMF/Et_3N$

REFERENCES

[1] Cushman, D.W., Cheung, H.S., Sabo, E.F. and Ondetti, M.A. (1977) Biochem. 16, 5484-5491.

[2] Patchett, A.A., Harris, E., Tristram, E.W., Wyrratt, M.J., Wu, M.T., Taub, D., Peterson, E.R., Ikeler, T.J., Ten Broeke, J., Payne, L.G., Ondeyka, D.L., Thorsett, E.D., Greenlee, W.J., Lohr, N.S., Hoffsommer, R.D., Joshua, H., Ruyle, W.V., Rothrock, J.W., Aster, S.D., Maycock, A.L., Robinson, F.M., Hirschmann, R., Sweet, C.S., Ulm, E.H., Gross, D.M., Vassil, T.C. and Stone, C.A. (1980) Nature 288, 280-283.

[3] Burnier, M., Turni, G.A., Brunner, H.R., Porchet, M., Kruithof, D., Vukovich, R.A. and Gavras, H. (1981) Br.J.Clin. Pharmacol. 12, 893-899.

[4] Bravo, E.L. and Tarazi, R.C. (1979) Hypertension 1, 39-46.

[5] Chrysant, S.G., Brown, R.D., Kem, D.C. and Brown, J.L. (1983) Clin. Pharmacol. Ther. 33, 741-746.

[6] Romankiewicz, J.A., Brogden, R.N., Heel, R.C., Speight, T.M. and Avery, G.S. (1983) Drugs 25, 6-40.

[7] Turni, G.A., Waeber, B. and Brunner, H.R. (1983) Eur. Heart J. 4 Suppl. A, 189-197.

[8] Hassall, C.H., Kröhn, A., Moody, C.J. and Thomas, W.A. (1982) FEBS Lett. 147, 175-179.

[9] Ondetti, M.A. and Cushman, D.W. (1982) Annu. Rev. Biochem. 51, 283-308.

[10] Cushman, D.W. and Cheung, H.S. (1971) Biochem. Pharmacol. 20, 1637-1648.

[11] Hayakari, M., Kondo, Y. and Izumi, H. (1978) Anal. Biochem. 84, 361-369.

[12] Ryan, J.W., Chung, A., Ammons, C. and Carlton, M.L. (1977) Biochem. J. 167, 501-504.

[13] Almquist, R.G., Chao, W.-R., Ellis, M.E. and Johnson, H.L. (1980) J. Med. Chem. 23, 1392-1398.

[14] Natoff, I.L., Brewster, M. and Patel, A.T. (1981) J. Pharmac. Methods 5, 305-312.

[15] Hassall, C.H., Kröhn, A., Moody, C.J. and Thomas, W.A. (1983) J. Chem. Soc. Perkin Trans. I, in press.

[16] Chen, D-S., Dotson, R.A., Burrell, R.D. and Watkins, B.E. (1983) Pharmacologist 25, 240.

[17] Sybertz, E.J., Baum, T., Ahn, H.S., Nelson, S., Eynon, E., Desiderio, D.M., Pula, K., Becker, F., Sabin, C., Moran, R., Vander Vliet, G., Kastner, B. and Smith, E. (1983) J. Cardiovasc. Pharmacol. 5, 643-654.

[18] Ondetti, M.A. and Cushman, D.W. (1981) Biopolymers 20, 2001-2010.

[19] Henderson, P.J.-F. (1972) Biochem. J. 127, 321-333.