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BENZOLACTAMS. A NEW CLASS OF CONVERTING ENZYME INHIBITORS

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A series of potent inhibitors of angiotensin-converting enzyme (dipeptidyl carboxypeptidase, E.C. 3.4.15.1) derived from benzofused 1-carboxyalkyl-3-(1-carboxy-3-phenylpropylamino) lactams (III) is described. In the most effective inhibitors (I_{50} 2-4X10^{-9}M) the lactam is 7 or 8 membered and the N-1 side chain is carboxymethyl or carboxyethyl. Conformational and steric factors pertinent to binding to the enzyme are discussed.

The development of therapeutically effective inhibitors of angiotensin-converting enzyme (ACE) has led to significant advances in the treatment of hypertension (1-3). Continuing research in this field has led *inter alia* to a series of lactams (I) and (II) devised by Thorsett and coworkers (4-6). The lactams were derived by introducing conformational restrictions into the captopril (1) and enalapril (MK-421) (2) designs guided by the active site-inhibitor binding mode of captopril described by the Squibb group (1,3) and our own for MK-422 (IVa) as illustrated in Figure 1.

In this communication we report a series of benzofused lactams (III) which extends the lactam design. By inclusion of a fused aromatic ring we hoped to attain increased hydrophobic binding at subsite S_2'. Introduction of methyl groups at X_1, or X_2 in (I) and (II) has led to increased inhibition (4-6). Effective analogs of captopril and enalapril with bulky proline surrogates have also been described (3). An additional consequence of this modification is a possible favorable alteration of the lactam ring conformation, which is an important factor in the binding of these inhibitors to ACE (6).

We also report the effect of extending and modifying the N-1 substituent in (III). The acetic acid side chain was originally a result of the design of the lactam

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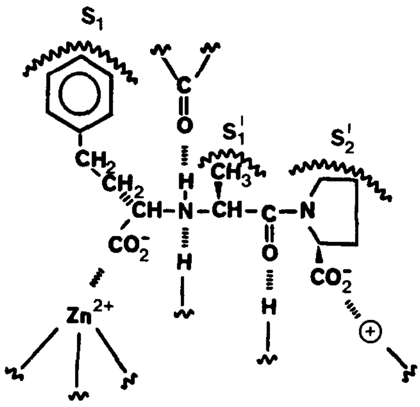
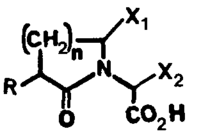


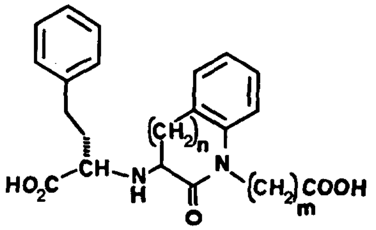
Figure 1. Proposed Binding of MK-422 in ACE

series (I) and (II). We have found that potent inhibitory activity is also compatible with N-1 propionic acid and butyric acid side chains in (III). Comparative compounds were prepared in the simple lactam (II) and enalapril series (7).



I, $R=HS-CH_2$; n=1-4

II, $R=C_6H_5-(CH_2)_2-\underset{\underset{COOH}{|}}{CH}-NH$; n=1-5



III n=1-3

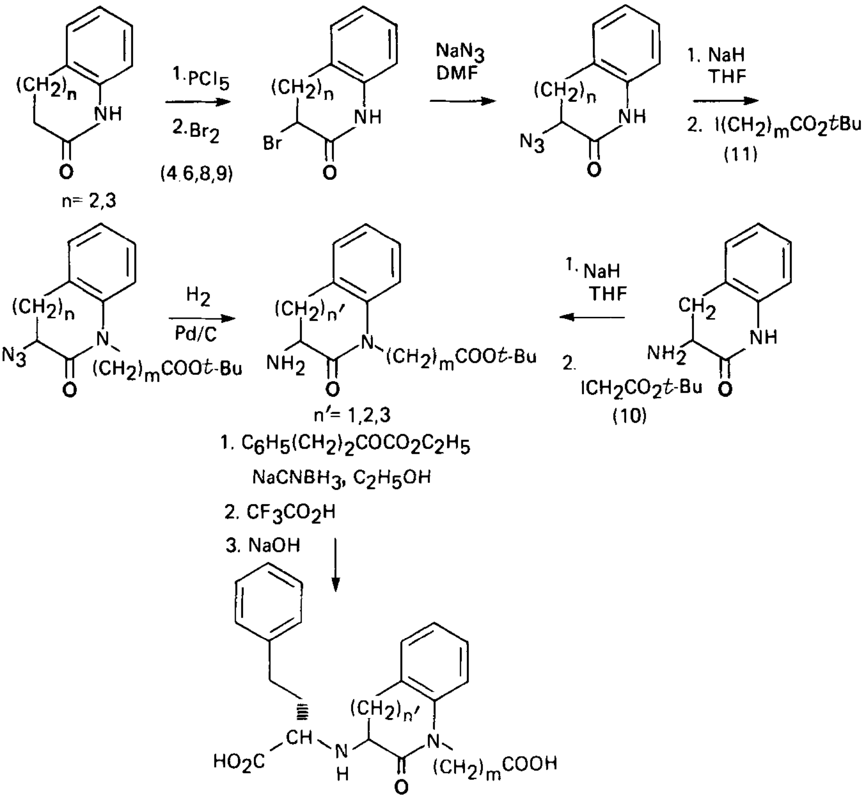
m=1-3

Materials and Methods

The synthetic procedures employed to prepare the benzolactams (III) are outlined below. Lactam (IIi) (Table 2) was prepared similarly from octahydroazocin-2-one. Preparation of (IVa) (Table 2) is described in reference (2). Compound (IVb) was prepared analogously from ethyl(S)-1-t-Boc-pyrrolidin-2-yl acetate. The enzyme assay procedure has been described (2).

Results

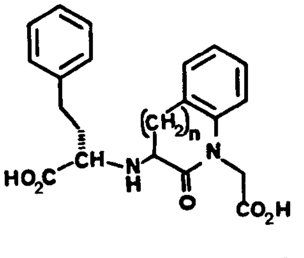
Of the three benzolactams in Table 1, the benzperhydroazepinone (IIIb) and benzperhydroazocinone (IIIc) are three orders of magnitude more effective than the tetrahydroquinolinone (IIIa). The marked superiority of ring sizes 7 and 8 over 6 also obtains in the simple lactam series (I) (5) and (II) (4,6). Both (IIIb) and (IIIc) are potent inhibitors with I_{50} values in the low nanomolar region similar to that of (IIc).

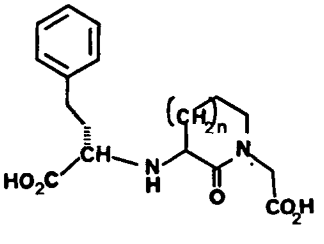


III

The effects of variation in the N-1 side chain are shown in Table 2. In the benzperhydroazepinone series (III) (n=2) no loss in activity occurs on extension of the side chain from acetic acid (IIIb) to propionic acid (IIId). Only a slight fall off occurs

Table 1: Effect of lactam ring size; *In Vitro* ACE inhibition[a,b]

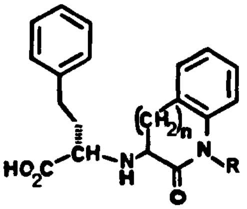


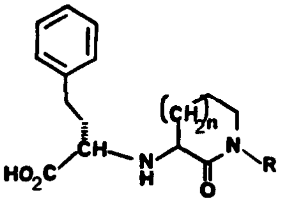


III	$I_{50}(10^{-9}M)$	II	$I_{50}(10^{-9}M)$
(a) n=1	1200		
(b) n=2	2.8	(b) n=2	19[c]
(c) n=3	4.0	(c) n=3	4.8[c]

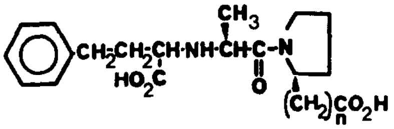
a. Results are for the more active racemate.
b. The benzofused-5-membered lactam was synthesized but was not stable.
c. Ref. 6

Table 2: Variation in N-1 side chain: In Vitro ACE inhibition





III	$I_{50}(10^{-9}M)$	II	$I_{50}(10^{-9}M)$
(b) n=2, R=-CH_2COOH	2.8		
(d) n=2, R=-$(CH_2)_2COOH$	2.9		
(e) n=2, R=$(CH_2)_3COOH$	7.9		
(f) n=2, R=-CH_2-CH=CH-COOH	65		
(g) n=2, R=-$(CH_2)_4COOH$	9,400		
(h) n=2, R=CH_3	130,000		
(c) n=3, R=-CH_2COOH	4	(c) n=3, R=-CH_2COOH	4.8
(i) n=3, R=-$(CH_2)_2COOH$	28	(i) n=3, R=$(CH_2)_2COOH$	57



	$I_{50}(10^{-9}M)$
IVa, MK-422 (n=0)	1.2
IVb (n=1)	32

in the butyric acid (IIIe) homolog. However, the upper limit is exceeded in the next higher homolog (IIIg) which is 1000 fold less active. Incorporation of a trans double bond (IIIf) into the butyric acid side chain results in an eight fold activity loss. The absolute requirement for carboxyl functionality is shown by the N-methyl analog (IIIh) in which activity has dropped precipitously relative to (IIIb).

In contrast to the benzperhydroazepinone series homologation of the carboxyl side chain in the benzperhydroazocinone (III c,i), the perhydroazocinone (II c,i) as well as in the MK-422 series (IV a,b) results in a significant 7-16 fold loss in activity.

Discussion

For effective binding to the active site of angiotensin - converting enzyme (and related zinc peptidases) an inhibitor or substrate should possess properly oriented primary binding functionality - a zinc ligand, an H bond accepting carbonyl group, and a carboxyl group - as well as correctly oriented hydrophobic groups for subsite recognition (1-3). In the lactam series Thorsett et al showed by calculation and experiment that these features are well accommodated by the 7-9 membered lactams (I) and (II) with the N-1 acetic acid side chain (4-6).

We have shown in the present work that benzofusion to lactams (II) yields inhibitors (III) with significantly superior I_{50} in the perhydroazepinone series (Table 1-(IIb) and (IIIb)) and equivalent activity in the perhydroazocinone series cf. (IIc) and (IIIc). These results point up the accommodative steric potential of the S_2' subsite.

We also have demonstrated that the benzperhydroazepinone system can tolerate extension of the N-1 acetic acid side chain to the propionic acid with complete retention of potency and further extension to the butyric acid with only minimal effect on activity (Table 2-(IIIb), (IIId), (IIIe)). These results imply unexpected degrees of freedom at the carboxyl binding site. However, limitations are defined by the rigid crotonic acid analog (IIIf) and the valeric acid analog (IIIg). Similar findings were described recently for an analogous series of inhibitors, N-(-1-carboxy-3-phenylpropyl)-Phe-X of porcine kidney zinc endopeptidase in which X=gly, β ala, γ-aminobutyric and δ-amino valeric (12).

By contrast both eight membered lactam systems cf. (IIc), (IIi), (IIIc), (IIIi) and the MK-422 system cf. (IVa), (IVb) can not tolerate chain extension without moderate activity decrease—pointing up the subtle steric and conformational factors which operate in these systems.

Conclusion

The lactam design principle for ACE inhibitors has been extended successfully to benzofused lactams. In the benzperhydroazepinone series the N-1 acetic acid side chain can be homologated with retention of high inhibitory activity.

References

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

2. Patchett, A. A., Harris, E. E., Tristram, E. W., Wyvratt, 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. For a recent review see Petrillo, E. W. Jr. and Ondetti, M. A. (1982) Medicinal Research Reviews 2, 1-41.
4. Thorsett, E. D., Harris, E. E., Aster, S. D., Peterson, E. R., Patchett, A. A., Ulm, E. H., Vassil, T. C., (1982) 184 ACS Nat'l meeting. Abstract Medi. 78.
5. Thorsett, E. D., Harris, E. E., Aster, S. D., Peterson, E. R., Taub, D., Patchett, A. A., Ulm, E. H., and Vassil, T. C. (1983) Biochem. Biophys. Res. Commun. 111, 166-171.
6. Thorsett, E. D., Harris E. E., Aster, S. D., Peterson, E. R., Tristram, E. W., Snyder, J. P., Springer, S. P. and Patchett, A. A. (1983) Proc.-Eighth Am. Peptide Symp., Abstract 6-1.
7. Following completion of our work we learned of independent synthesis of benzolactam 1-acetic acids: Watthey, J. W. H., Desai, M., Babiarz, J. E., Gavin, T., Finn, B., and Stanton, J. L., (1983) 186th ACS Nat'l meeting, Abstract Medi. 93; U. K. Patent Appl., GB-2, 103,614 (1982).
8. Briggs, L. H., De Ath, G. C., (1937) J. Chem. Soc., 456.
9. Hjelte, N. S., Agback, T., (1964) Acta. Chem. Scand. 18, 191-194.
10. Davis, A. L., Lloyd, R., Fletcher, J., Bayliss, L., McCord, T. J., (1963) Arch. Bioch. Biophys., 102, 48-51.
11. For (IIIf) and (IIIh) methyl bromocrotonate and methyl iodide respectively were used.
12. Mumford, R. A., Zimmerman, M., ten Broeke, J., Taub, D., Joshua, H., Rothrock, J. W., Hirschfield, J. M., Springer, J. P., and Patchett, A. A., (1982) Biochem. Biophys. Res. Commun., 109, 1303-1309.