A new class of inhibitors of human leucocyte elastase

Cedric H. Hassall, William H. Johnson, Anthony J. Kennedy and Noel A. Roberts*

Roche Products Ltd, Welwyn Garden City, Herts, England

Received 13 February 1985

Studies of the inhibition of elastases at a molecular level have resulted in the identification of protected dipeptides which are reversible and highly specific inhibitors of human leucocyte elastase (HLE). These have been further developed by increasing their hydrophilicity and potency to give a new family of elastase inhibitors, typicaly N^{α}-(1-adamantanesulphonyl)-N^{ϵ}-(4-carboxybenzoyl)-L-lysyl-L-alanyl-L-valinal. These compounds are active in pharmacological models designed to detect compounds of potential therapeutic value in the treatment of emphysema.

Elastase *Inhibitor* *Design* *Emphysema*

1. INTRODUCTION

There is a good basis for suggesting that specific inhibitors of human elastase may have value in medicine. It has been shown that pulmonary emphysema results from degradation of alveolar wall elastin during periods when human leucocyte elastase (HLE, EC 3.4.2.11) released in the lung is inadequately controlled by endogenous inhibitors [1,2,3]. In these circumstances, further lung degradation could be prevented by prophylactic replacement therapy with a low-M_r synthetic inhibitor of HLE. Elastase occurs in lysosomes and probably plays an important role in inflammatory processes involving other than lung tissues [4].

In [5,6] we described specific inhibitors of porcine elastase (PPE) and presented evidence suggesting that two distinct modes of binding to the active site of the enzyme were available to these protected dipeptide inhibitors. Here we have extended the investigation of the enzyme inhibitor interactions involved in those binding modes and have used the further knowledge to enable the design of specific inhibitors of HLE. The most interesting compounds with potential for in vivo application are potent, water-soluble, reversible inhibitors of HLE.

* To whom correspondence should be addressed

2. EXPERIMENTAL

The synthesis of the typical inhibitor, N^{α}-(1-adamantanesulphonyl)-N^{ϵ}-(4-carboxybenzoyl)-L-lysyl-L-alanyl-L-valinal is outlined in fig.1. Experimental details relating to the chemistry, biochemistry and pharmacology of this and related compounds will be provided in later publications.

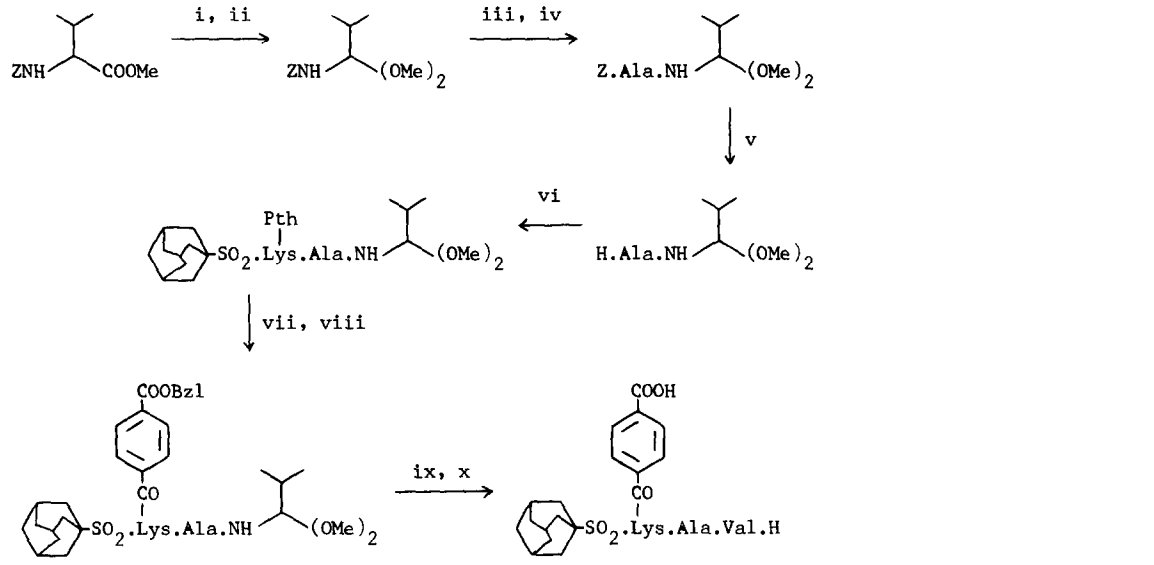
Computer graphics utilised a Megatek 7000 display processor interfaced to a VAX 11/750 computer and a Calcomp 81 plotter.

HLE was purified from leucocyte granules by a method employing acetone fractionation, gel filtration and ion-exchange chromatography.

HLE-α_2M complex was prepared by incubation of human α_2-macroglobulin with a 3.4-fold molar excess of purified HLE. The complex was purified by gel filtration. Human bronchial lavage was kindly donated by Dr T. Tetley (Charing Cross Hospital, London). Assays for elastase activity using the substrates succinyl(.Ala.)$_3$*p*-nitroanilide [7], methoxysuccinyl(.Ala.)$_3$.Pro.Val.*p*-nitroanilide [8] and bovine ligamentum elastin [9] were performed using standard procedures. Inhibition constants were determined from Dixon plots.

Lung retention was determined by the recovery of ^{14}C-labelled compound from excised lungs at intervals following intratracheal (i.t.) administration.

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/85/$3.30



Reagents: i, $[(CH_3)_2CHCH_2]_2AlH$; ii, $CH(OCH_3)_3$, Tos.OH, MeOH; iii, H_2, Pd-C; iv, Z.Ala.OH, DCCI; v, H_2, Pd-C; vi, Ad.SO_2.Lys(Pth)OH, DCCI; vii, $NH_2NH_2.H_2O$; viii, $C_6H_5CH_2O.CO.C_6H_4.COCl$; ix, H_2, Pd-C; x, Amberlyst 15, $(CH_3)_2CO$, room temperature.

Fig.1. Synthesis of N^{α}-(1-adamantanesulphonyl)-N^{ϵ}-(4-carboxybenzoyl)-L-lysyl-L-alanyl-L-valinal (compound 3).

Acute (24 h) lung haemorrhage was induced in hamster lungs by intratracheal administration of purified HLE and the haemorrhage quantitated by assay of the total haemoglobin content of the excised and homogenised lungs. Emphysema in hamsters induced by chronic administration of purified HLE was assessed histologically.

3. RESULTS AND DISCUSSION

In 1970 Thompson and Blout [10] and Atlas et al. [11] showed that PPE possesses an extended active site composed of 6 or 7 binding subsites. Occupation of subsite S_4 (nomenclature of Schechter and Berger [12]) by a substrate is particularly important for substrate binding and catalytic activity. We have previously reported studies using PPE inhibitors of general structure P_4.Ala.Pro.P_1 which emphasise the P_4-S_4 interactions [5]. We also demonstrated that P_4 and P_1 are interdependent in determining inhibitory potency. Relatively large groups can be accommodated at either position providing the other group is small. When both groups are large the compounds are inactive. We have related this structure activity data for these inhibitors to the information on the active site of PPE obtained by X-ray diffraction studies. Moreover, comparative X-ray crystallographic studies, using propionyl.Ala.Pro.ethylamide (1) and propionyl.Ala.Pro.cyclopentylamide (2) separately bound to PPE, provided direct evidence for two binding modes.

We have examined possible enzyme inhibitor interactions for each of the binding modes, using computerised molecular graphics techniques. This indicates that both compounds may bind through 3 hydrogen bonds to enzyme atoms, N.214, N.216 and O.216 (fig.2, details to be published, N. Borkakoti and A. Krohn). Similar binding has been observed for other inhibitors of serine proteases [13]. The differences in the conformations of the bound compounds (1,2) are attributed to the adjustment required for the cyclopentylamide (2) to enable the cyclopentyl group to avoid collision with the side-chain of Val.216. In this conformation (fig.2b) the putative hydrogen bond between the N-terminal amide and O.216 fixes P_4 close to residues around .Arg^{217A}.; this limits the size of

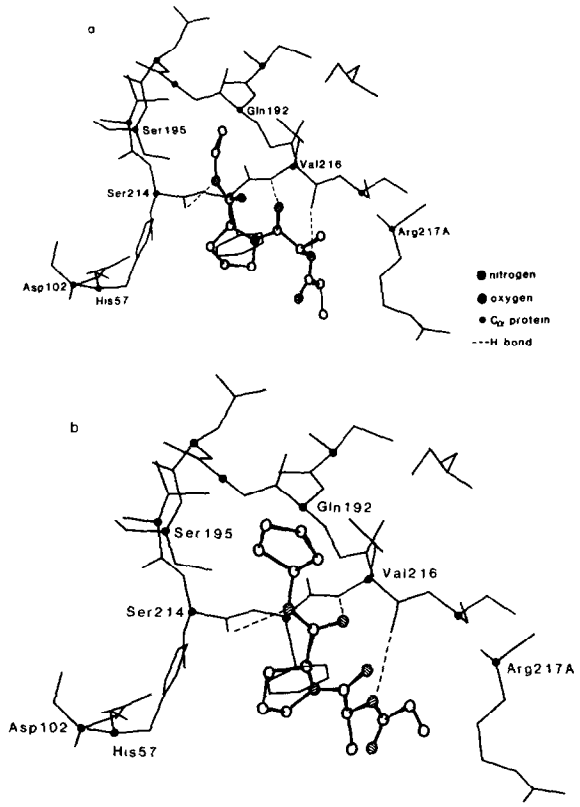


Fig.2. Binding modes of inhibitors to porcine pancreatic elastase, (a) compound 1; (b) compound 2.

acceptable P_4 residues. In comparative studies of HLE and PPE inhibition we have demonstrated that the interdependence of P_4 and P_1 for PPE inhibitors does not apply to HLE. Effective inhibition of this enzyme is obtained with protected dipeptides in which both P_4 and P_1 are large groups [5]. This suggests that in HLE residues around 216 have more space for P_1 binding than in the case of PPE; this could arise from residue 216 having a smaller side chain. This is consistent with the wider P_1 substrate specificity of HLE [14]. In addition we have synthesised compounds lacking the P_4-P_3 amide group to assess its contribution to inhibitor binding to HLE. In contrast to PPE, we find that inhibitory potency against HLE is enhanced when this amide function is replaced by sulphonamide or by a methylene bridge, but the other replacements which were investigated were less effective. Hydrogen bonding between the P_4-P_3 amide and the enzyme is clearly of less importance for inhibitor binding to HLE.

These concepts produced relatively potent, reversible, HLE-specific inhibitors such as N^{α}-(1-adamantanesulphonyl).Ala.Pro.anilide (K_i 5.2 μM). However, these compounds, like most other HLE inhibitors which have been described, are lipophilic.

An HLE inhibitor with potential for use in human therapy must have not only good potency in vitro, but also suitable characteristics for in vivo activity. This requires a reasonable prospect for retention without rapid degradation in the lung. Earlier work by Schanker [15] has shown that lipophilic compounds deposited in the lung are cleared rapidly. This is consistent with our observations with adamantane sulphonyl.Ala.Pro.anilide, a strongly lipophilic compound; the lung-retention half-life in rodents was approx. 2 min, following intratracheal (i.t.) administration. We have sought to increase the lung retention time by increasing the hydrophilicity and the molecular size of the HLE inhibitor [15]. This was achieved by replacing the alanine residue in the dipeptide series by acylated-lysyl functions (table 1); by utilising dicarboxylic acids, compounds with good potency and water solubility were obtained.

The potency of these water-soluble inhibitors has been enhanced by replacing the C-terminal amide with a valinal residue. The aldehyde function was clearly suggested by earlier work on inhibitors of serine proteases; the interaction of the serine hydroxyl with this aldehyde group is presumed to give a tetrahedral analogue of the transition state intermediate involved in peptide bond cleavage [16]. The choice of valinal was suggested by HLE substrate studies but other aldehydes corresponding to neutral amino acids can be substituted. As in the case of our earlier studies with PPE inhibitors [5], we found that the proline residue in the P_2 position could be replaced by an alanine residue in the HLE inhibitor, without significant change in potency. The alanine residue had the advantage, however, of facilitating the preparation of crystalline intermediates.

Two compounds, N^{α}-(1-adamantanesulphonyl)-N^{ϵ}-(4-carboxybenzoyl)-L-lysyl-L-alanyl-L-valinal (compound 3) and N^{α}-(1-adamantanesulphonyl)-N^{ϵ}-succinyl-L-lysyl-L-prolyl-L-valinal (compound 4), have been selected for further investigations. They inhibit not only HLE itself but also the enzyme complexed with human α_2-macroglobulin

Table 1

Modification of protected dipeptide inhibitors to obtain water solubility and increased potency

Inhibitor structure	I_{50} value (μM) against HLE. Succ(Ala)$_3$pNA as substrate
$AdSO_2$.Ala.Pro.NHPh	7[a]
$AdSO_2$.Lys.Pro.NHPh	54
Z $AdSO_2$.Lys.Pro.NHPh $CO(CH_2)_2COOH$	9
$AdSO_2$.Lys.Pro.NHPh	14
$AdCH_2CH_2$.Ala.Pro.NH^{t}Bu	10
H.Glu.OH $AdCH_2CH_2$.Lys.Pro.NH^{t}Bu $CO(CH_2)_2COOH$	10
$AdSO_2$.Lys.Pro.Val.H $CO(CH_2)_2COOH$	0.08
$AdSO_2$.Lys.Ala.Val.H (compound 4)	0.1
CO⟨⟩COOH $AdSO_2$.Lys.Ala.Val.H (compound 3)	0.08

[a] This compound at 100 μM does not inhibit PPE

N- and C-terminal groups are not restricted to those examples shown above. Here they are limited to facilitate comparison of the effect of other changes within the molecule

and the form(s) of elastase in human bronchial lavage (table 2). They are inactive at 100 μM against trypsin, chymotrypsin, cathepsin G, angiotensin converting enzyme and guinea pig complement.

Intratracheal administration of ^{14}C-labelled compound (4) to the hamster showed a lung retention half-life of 2 h. An intratracheal dose of 50 mg/kg of either compound was tolerated in the hamster. No overt toxicity was observed following

Table 2

Inhibition of elastases by compounds 3 and 4

Elastase	Inhibition constant K_i (μM)	
	Compound 3	Compound 4
Human leucocyte elastase	0.06	0.11
HLE-α_2M complex (residual elastase activity)	0.1	0.15
Elastase activity of human bronchial lavage	0.03[a]	0.15[a]
HLE-elastin as substrate	I_{50} 1.4 μM	I_{50} 1.2 μM

[a] This value varies several-fold between different lavage samples. The two compounds were assayed against different samples

Substrate for K_i determination was MeOSucc(.Ala.)$_2$.Pro.Val.pNA

a single intraperitoneal dose of 3.2 g/kg compound (4) to mice. Acute (24 h) lung oedema and haemorrhage can be induced in the hamster by a single i.t. instillation of purified HLE. Both compounds block this response. Doses of compound (3) of 10 μg and 1 mg/animal i.t. given concomitantly with, or 40 min prior to the enzyme respectively, inhibited lung haemorrhage by 90 and 100%. Emphysema can be generated in the hamster by repeated i.t. instillation over several weeks of lower doses of purified HLE. Concomitant i.t. administration of compound (3), 200 μg/animal, significantly inhibits development of the emphysema assessed both histologically and by quasi-static lung compliance measurements.

This new family of water-soluble reversible inhibitors of HLE appears to have potential for use in the treatment of pulmonary emphysema. Further details of the chemistry, biochemistry, pharmacology and pharmacokinetics of this new family of inhibitors will be reported in later publications.

REFERENCES

[1] Janoff, A. and Carp, H. (1983) Lung Biol. Health Dis. 19, 173–208.
[2] Stone, P.J. (1983) Clin. Chest Med. 4, 405–412.

[3] Stockley, R.A. (1983) Science 64, 119–126.
[4] Baggiolini, M., Bretz, U., Dewald, B. and Feigenson, M.E. (1978) Agents Actions 8, 3–10.
[5] Hassall, C.H., Johnson, W.H. and Roberts, N.A. (1979) Bioorg. Chem. 8, 299–309.
[6] Hassall, C.H., Johnson, W.H. and Roberts, N.A. (1980) in: Enzyme Inhibitors (Brodbeck, V. ed.) pp.259-260, Verlag Chemie, Weinheim.
[7] Bieth, J., Spiess, B. and Wermuth, C.G. (1974) Biochem. Med. 75, 350–357.
[8] Castillo, M.J., Nakajima, K., Zimmerman, M. and Powers, J.C. (1979) Anal. Biochem. 99, 53–64.
[9] Ohlsson, K. and Olsson, I. (1974) Eur. J. Biochem. 42, 519–527.
[10] Thompson, R.C. and Blout, E.R. (1970) Proc. Natl. Acad. Sci. USA 67, 1734–1740.
[11] Atlas, D., Levit, S., Schechter, I. and Berger, A. (1970) FEBS Lett. 11, 281–283.
[12] Schechter, I. and Berger, A. (1967) Biochem. Biophys. Res. Commun. 27, 157–162.
[13] Segal, D.M., Powers, J.C., Cohen, G.H., Davies, D.R. and Wilcox, P.E. (1971) Biochemistry 10, 3728–3738.
[14] Zimmerman, M. and Ashe, B.M. (1977) Biochim. Biophys. Acta 480, 241–245.
[15] Schanker, L.S. (1978) Biochem. Pharmacol. 27, 381–385.
[16] Thompson, R.C. (1973) Biochemistry 12, 47–51.