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POTENTIAL MECHANISM-BASED INHIBITORS OF PROTEOLYTIC ENZYMES

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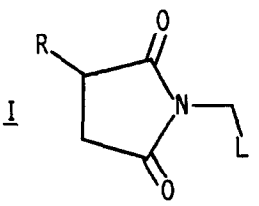
Abstract - The design, synthesis, and inhibitory activity toward human neutropil elastase, of a series of potential mechanims-based inhibitors is described.

Neutrophil-derived proteolytic enzymes have been implicated in the pathophysiology of connective tissue diseases such as, for example, pulmonary emphysema (1-2), rheumatoid arthritis, glomerulonephritis (3), and others (4-5). Of the three known serine proteinases released by activated neutrophils, the 29-kD single-chain glycoprotein human leukocyte elastase (E.C.3.4.21.37) has received the greatest attention, primarily because of its ability to attack all major connective tissue matrix components (6). Thus, research endeavors in this area have focused on the development of potent and specific inhibitors of human leukocyte elastase (HLE) (7).

We have recently described the use of 3-alkyl-N-hydroxy-succinimide derivatives as highly effective mechanism-based inhibitors of HLE (8-10) and, when appropriately modified, as potent phosphorylating agents (11). We now wish to describe the biochemical rationale underlying the design of some potential mechanism-based inhibitors represented by structures **I-III** and the results of relevant biochemical studies using HLE.



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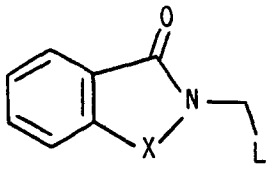
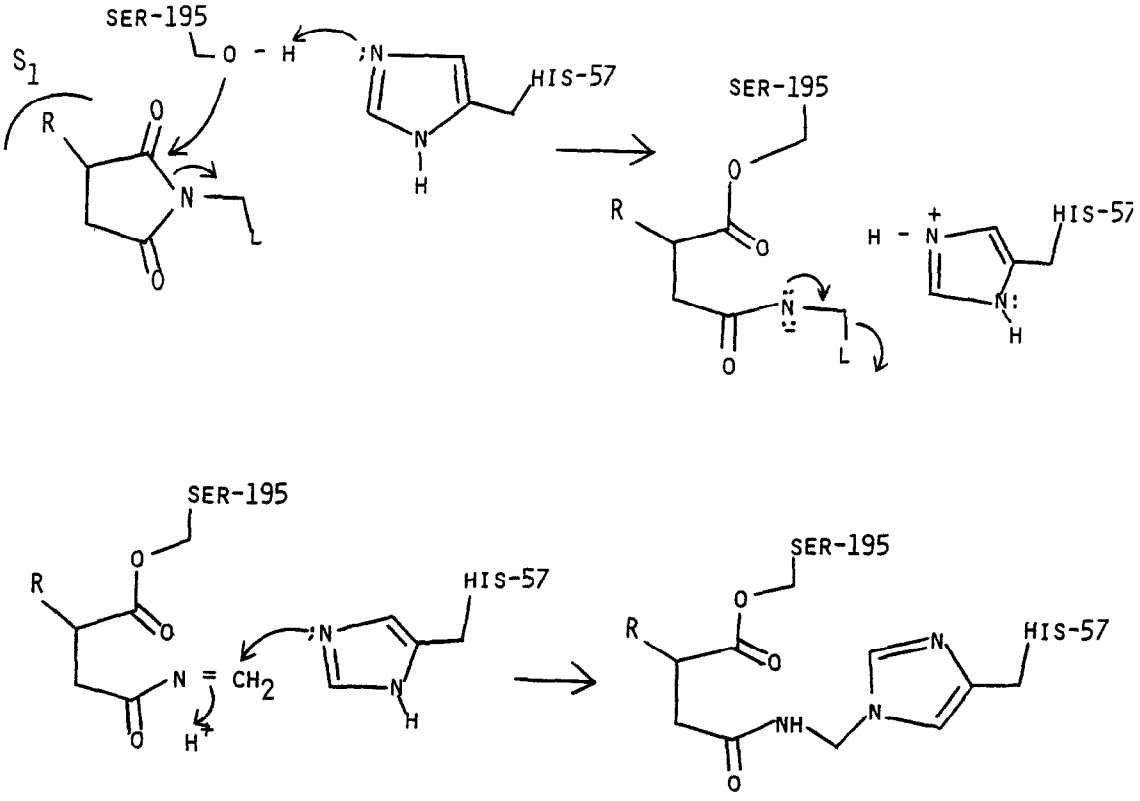
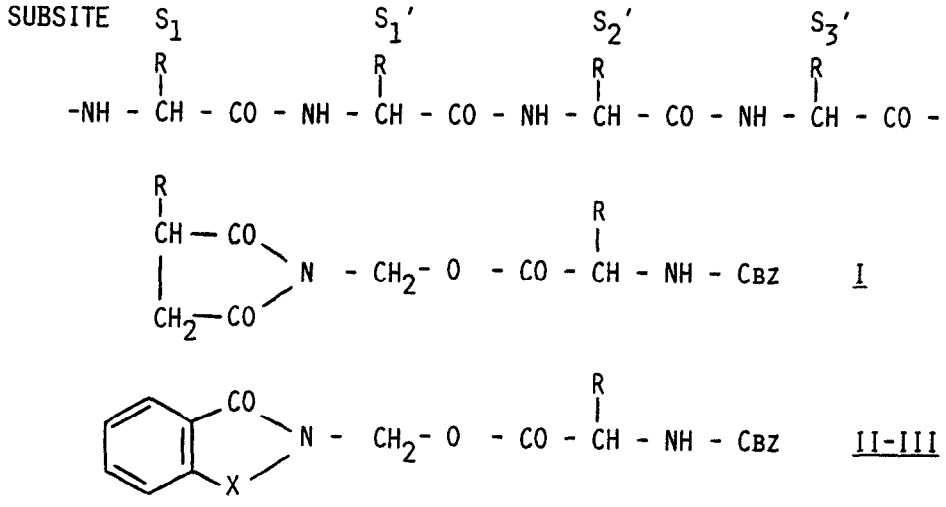


X = SO_2 (II), CO (III)

We reasoned that compounds **I-III** would inactivate HLE via an enzyme-induced process leading to the formation of a reactive electrophilic species. Subsequent Michael reaction with an active site nucleophilic residue (His-57, for example) would ultimately lead to an irreversibly inactivated enzyme (Scheme I). Other design considerations included (a) tailoring the specificity and strength of binding of **I-III** for the target proteinase by manipulating R (assumed to be accomodated at the primary specificity site S_1 of the enzyme) (12), (b) using an amino acid-derived component for L in order to optimize S_n' sub-site binding interactions (Scheme II) and, (3) since connective tissue diseases are associated with elevated levels of oxidants and are inflammatory in nature (13), a known anti-inflammatory agent was introduced into structures **I-III** for release into the surrounding milieu during the inactivation process.



SCHEME I



SCHEME II

Materials. Compounds **Ia-c**, **IIa-c**, and **IIIa-c** were synthesized by using procedures analogous to those described in the literature (14-15). Racemic ibuprofen was used in the synthesis of **Ia-b**, **IIa**, and **IIIa**. All synthesized compounds gave satisfactory elemental analyses and spectral data. Enzyme assays and inhibition studies were carried out as described previously using methoxysuccinyl Ala-Ala-Pro-Val p-nitroanilide as the substrate (8-9).

Biochemical Studies. The synthesized compounds showed variable inhibitory characteristics towards HLE. For example, incubation of compound **Ia** with HLE lead to time-dependent loss of enzymatic activity (Figure 1). The enzyme was inhibited inefficiently ($k_{obs}/[I]$ 45 M^{-1} s^{-1}) and regained 80% of its activity after 24 h. The results suggest that **Ia** may simply act as an alternate substrate inhibitor (16), although this remains to be established. Based on the known preference of HLE for a three- or four-carbon hydrophobic P_1 residue (12), compound **Ib** was investigated for its inhibitory activity toward HLE. Surprisingly, **Ib** was found to be less effective in inhibiting HLE ($k_{obs}/[I]$ 16 M^{-1} s^{-1}), while **Ic** was found to be marginally active.

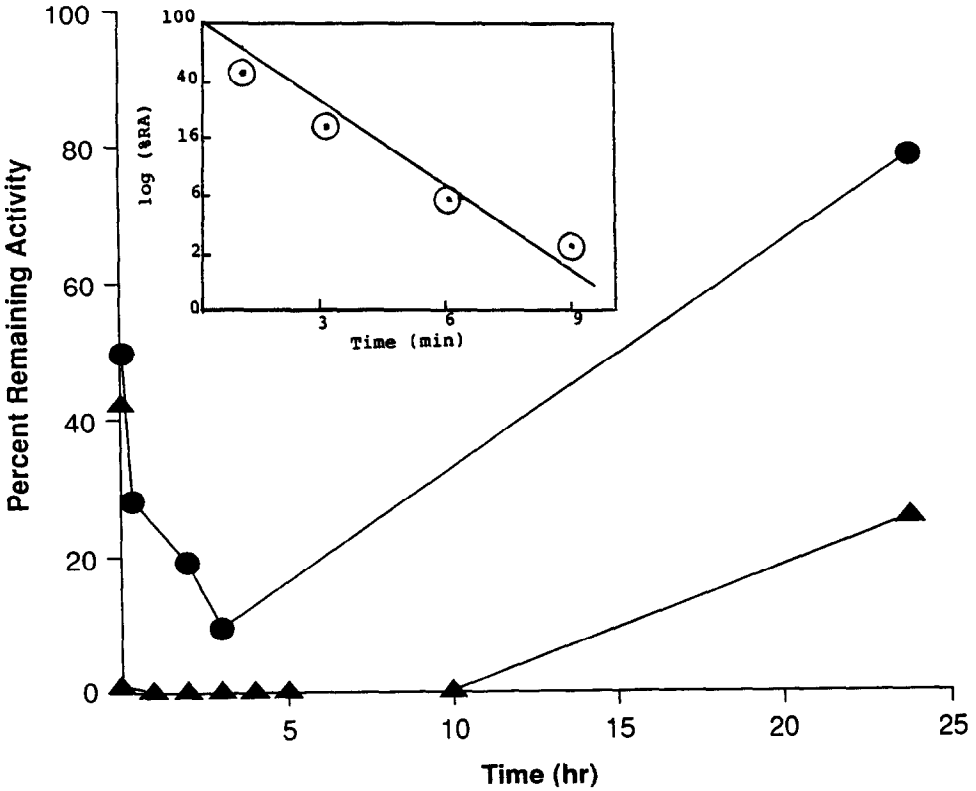


Figure 1. Time dependent loss of enzymatic activity. Human leukocyte elastase (235 nM) was incubated with compound **Ia** (47 uM) (●) or compound **IIa** (47 uM) (▲) in HEPES buffer, pH 7.2, 0.5 M NaCl, and 1% DMSO. Insert: Log (% remaining activity) vs time plot for compound **IIa** showing pseudo first-order inactivation is occurring.

The corresponding saccharin-derived compounds **IIa-b** were found to inhibit HLE much more efficiently ($k_{obs}/[I]$ 160 and 420 M^{-1} s^{-1}, respectively) in a time-dependent manner (17). Incubation of **IIa** with HLE lead to progressive and total loss of enzymatic activity (Figure 1). The enzyme regained 25% of its activity after 24 h. It was observed earlier that the presence of an aromatic residue in some compounds related to **Ia-c** yielded highly effective inhibitors of HLE and this was ascribed to a favorable binding interaction with an aromatic residue located in the vicinity of the active site (8). The marginal activity of **IIc** may reflect differences in modes of binding (12).

TABLE 1. Inhibition of Human Leukocyte Elastase (HLE) by Compounds I-III.

Compound	R	L	$k_{obs}/[I]$ M^{-1} s^{-1}
Ia	benzyl	ibuprofen	45
Ib	isobutyl	"	16
Ic	benzyl	Cbz-L-leu	3
IIa	-	ibuprofen	160
IIb	-	Cbz-L-leu	420
IIc	-	-COOBzl	4
IIIa	-	ibuprofen	a
IIIb	-	Cbz-L-leu	a
IIIc	-	L-leu.HCl salt	b

[a]insoluble under assay conditions;
[b]reversible inhibitor (K_I 17.5 uM).

Due to solubility problems under the assay conditions employed, we were unable to assess the inhibitory activity of **IIIa-b**. Thus, **IIIb** was converted to **IIIc** and its inhibitory activity toward HLE was then investigated. Unlike the succinimide and saccharin-derived compounds, **IIIc** was found to be a good competitive inhibitor of HLE (Figure 2). The different modes of binding observed in this study are in agreement with observations related to the binding of structurally-similar low molecular weight compounds to the active site of porcine pancreatic elastase (18). Further studies aimed at ascertaining how structural variations in L affect inhibitory activity, the mode of binding and the mechanism of action of these classes of compounds are currently in progress.

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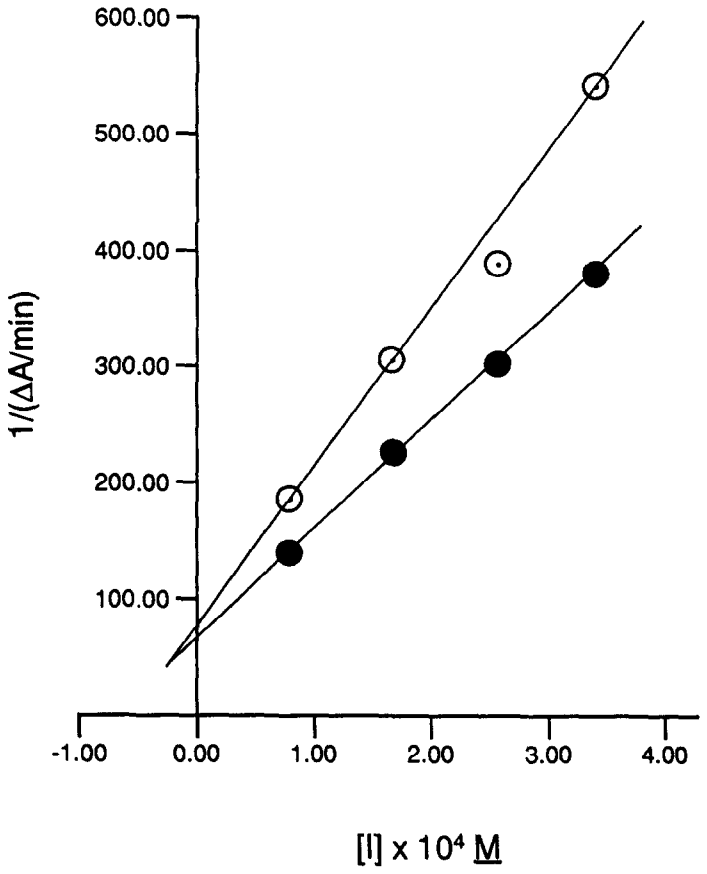

Figure 2. Dixon plot using inhibitor **IIIc**. Dixon plot using inhibitor **IIIc**. Substrate concentrations used were 10.3 uM (●) and 8.3 uM (o), respectively. Final enzyme concentration: 49.8 nM.

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