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A GENERAL APPROACH TOWARD THE DESIGN OF INHIBITORS OF SERINE PROTEINASES: INHIBITION OF HUMAN LEUKOCYTE ELASTASE BY SUBSTITUTED DIHYDROURACILS

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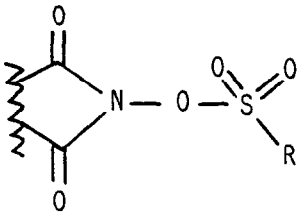
Abstract - A general approach toward the rational design of potential inhibitors of serine proteinases is described. The approach is exemplified and validated through the use of appropriate heterocyclic systems in inhibiting human leukocyte elastase (HLE).

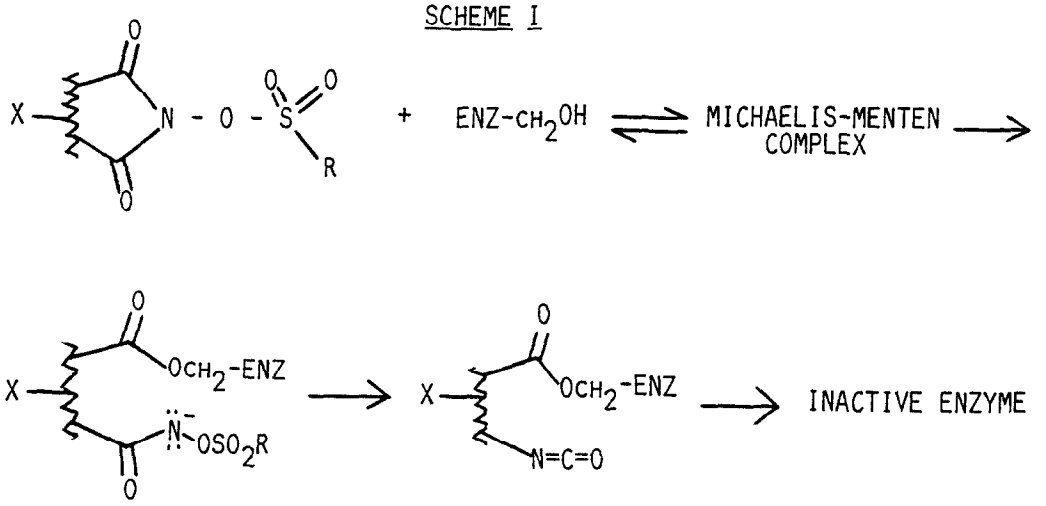
The aberrant activity of the neutrophil-derived proteolytic enzymes elastase and cathepsin G in inflammatory states can lead to the destruction of the major components of connective tissue (1-2). The intimate involvement of these enzymes in the etiology and/or pathophysiology of inflammatory diseases such as, for example, pulmonary emphysema (3), cystic fibrosis (4) and others (5), has provided the impetus behind efforts related to the development of agents capable of modulating the activity of these enzymes (6-8). In this and subsequent papers (see accompanying paper), we describe the design of a series of heterocyclic inhibitors of serine proteinases, and demonstrate the general utility of moiety I in the development of inhibitors of these enzymes.



I

We reasoned that attachment of moiety I to a recognition component X (typically a heterocyclic structure) would likely yield compounds with inhibitory activity via tandem enzyme-induced ring opening and formation of a highly rective electrophilic species. Reaction of the latter with an active site nucleophilic residue (His-57), was anticipated to lead to irreversibly inactivated enzyme (Scheme I). Assurance about the soundness of the biochemical rationale was provided by recent studies utilizing N-hydroxysuccinimide and hydantoin derivatives (9-11), and other relevant work described in the literature (12). We describe herein the results of preliminary studies related to the interaction of substituted dihydrouracils with human leukocyte elastase (HLE).

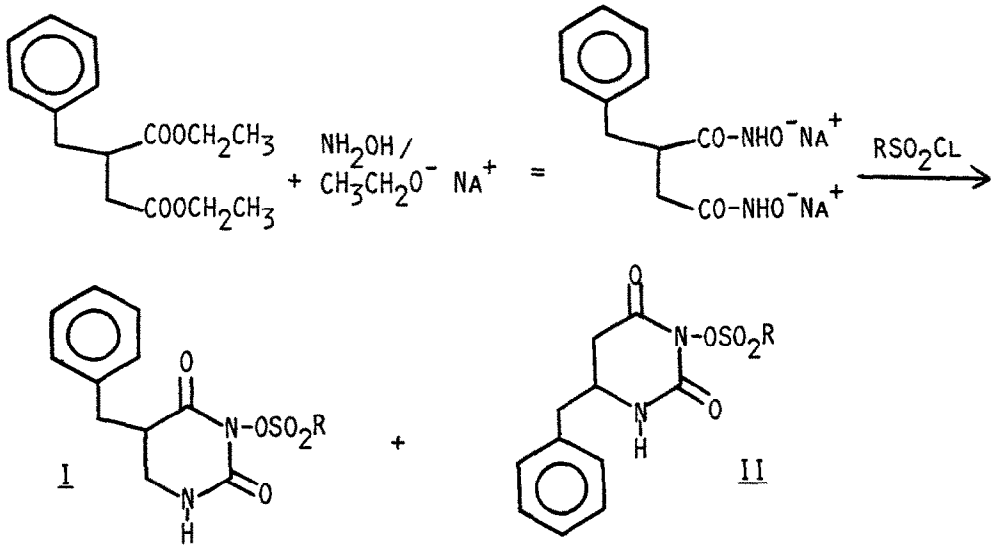
SCHEME I



Materials. Compounds 1-4 were synthesized according to Scheme II (12). The regioisomers were separated on a Chromatotron plate using silica gel and hexane/methylene chloride. Enzyme assays and inhibition studies were carried out as described previously using methoxysuccinyl Ala-Ala-Pro-Val p-nitroanilide as the substrate for HLE (9).

The pseudo first-order inactivation rate constants (k_{obs}) were obtained from plots of $\ln(v_t/v_o)$ vs t and the potency of the synthesized compounds is expressed in terms of the bimolecular rate constant $k_{obs}/[I]$ M^{-1} s^{-1} (Table I).

SCHEME II



R = METHYL (I-IIa), TRANS-STYRYL (I-IIb)

TABLE I. Inhibition of Human Leukocyte Elastase by Compounds Ia-b and IIa-b.

Compound	$k_{obs}/[I]$ M^{-1} s^{-1} (SD)
Ia	3310 (100)
Ib	480 (10)
IIa	800 (10)
IIb	1980 (20)

Biochemical Studies. Compounds **Ia-b and IIa-b** were found to inactivate HLE efficiently and in a time-dependent manner. For example, incubation of a 20-fold excess of compound **Ia** with HLE led to rapid and complete inactivation of the enzyme (Figure 1). The enzyme regained 10% of its activity over a 24-hr period. It is evident from Table I that all compounds are fairly efficient inactivators of HLE. Somewhat surprising is the observed reversal in the effect of the trans-styryl group on potency (**Ib vs IIb**), previously found to be uniformly beneficial in enhancing inhibitory activity (9). This suggests different binding modes for the two regioisomers. An apparent geometric complementarity of regioisomers **Ia** and **IIa** is evident in Figure 2, which depicts the superposition of the energy-minimized structure of **Ia** (13) on the X-ray crystal structure of **IIa**.

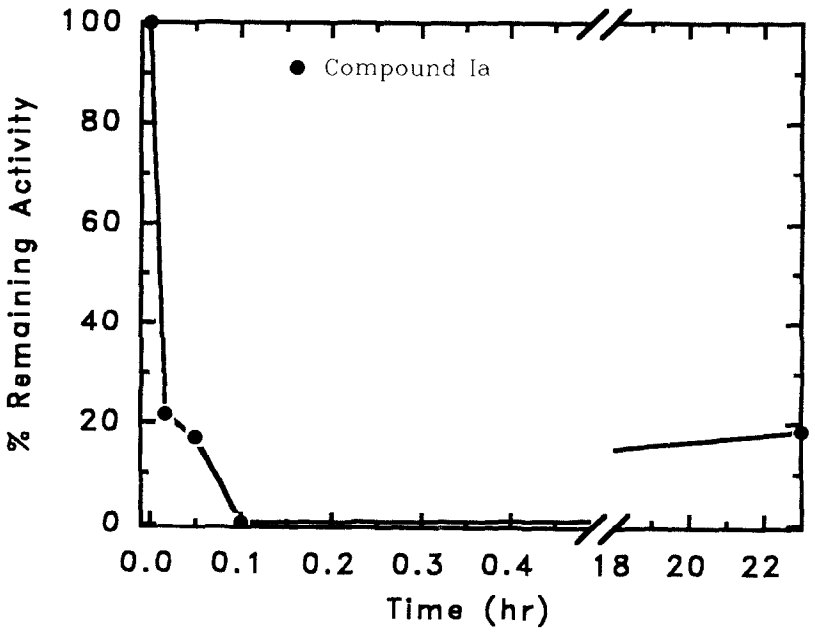


Figure 1. Time dependence of enzymatic activity. Human leukocyte elastase (323nM) was incubated with compound **Ia** (6.46uM) in 0.1M HEPES buffer, pH 7.25, 0.5M NaCl, and 1% DMSO.

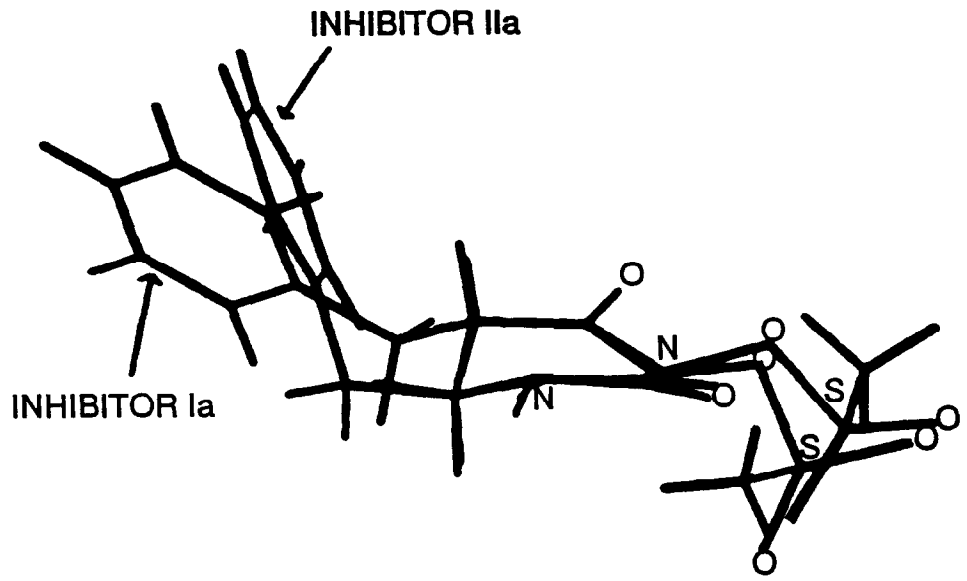


Figure 2. Superposition of the energy-minimized structure of Ia on the X-ray crystal structure of IIa.

While the mechanism by which these compounds inactivate HLE remains to be established, it is interesting to note that when hydroxylamine (0.5 M) was added to HLE that had been fully-inactivated by incubating with a 50-fold excess of inhibitor IIa, there was a net regain in enzymatic activity of only 10% after a 24-hr period. Likewise, incubation of HLE with a 20-fold excess of compound Ia led to rapid inactivation of the enzyme. There was a 16% regain in enzymatic activity after 24 hours. The irreversibility of the inhibition was demonstrated in a separate experiment where Centricon-10 filtration of a solution of HLE that had been fully-inactivated by a 20-fold excess of compound Ia, did not lead to any additional regain in activity.

In summary, the efficient inhibition of HLE by some substituted dihydrouracils is reported. Further studies with these compounds are currently in progress.

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13. The commercially available SYBYL 5.41 software program distributed by Tripos Associates, 1699 S. Hanley Road, Suite 303, St. Louis, MO 63144 was used in conjunction with a Silicon Graphics IRIS workstation.