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HETEROCYCLIC INHIBITORS OF HUMAN LEUKOCYTE ELASTASE: 3-HYDROXYPYRIDAZOPYRIMIDINE, 3-HYDROXYPYRIDOPYRIMIDINE AND 3-HYDROXYQUINAZOLINE-2,4(1H,3H)DIONE DERIVATIVES

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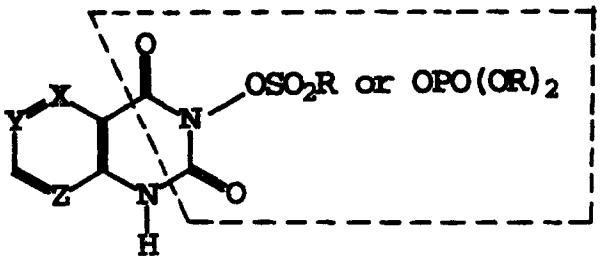
Abstract - Several heterocyclic compounds derived from 3-hydroxypyridazopyrimidine-(I), 3-hydroxypyridopyrimidine-(II) and 3-hydroxyquinazoline-2,4(1H,3H)diones-(III) have been found to be time-dependent irreversible inhibitors of human leukocyte elastase.

Activated neutrophils release several lysosomal enzymes, including the serine proteinases elastase (HLE), cathepsin G (Cath G) and proteinase 3 (PR 3) (1). The activity of HLE is modulated by the plasma glycoprotein alpha-1-proteinase inhibitor (2-3) and the secretory leukocyte protease inhibitor (SLPI) (4-5). In disease states, such as, for example, pulmonary emphysema and cystic fibrosis, the presence of an elastase/antielastase imbalance in the lungs may result in lung damage due to the degradative action of HLE on connective tissue components (6). Synthetic low molecular weight compounds that inhibit HLE may be of value in redressing this imbalance (7-8).



X = Z = N, Y = C (I)
X = N, Y = Z = C or
Y = N, X = Z = C (II)
X = Y = Z = C (III)

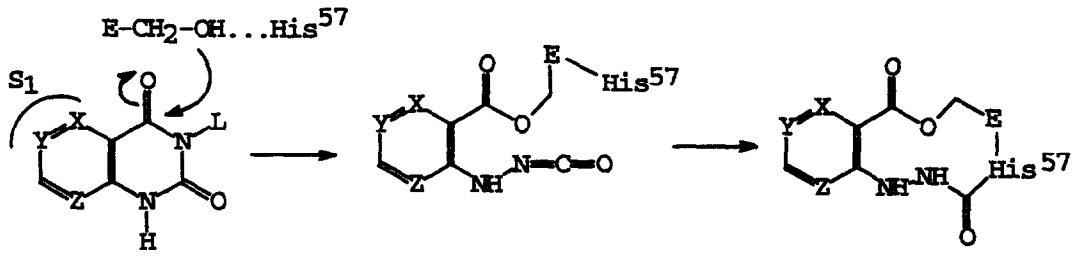
This report describes the results of preliminary studies related to the inhibition of HLE by heterocyclic compounds **I-III**. The design of the inhibitors is based on the known chemistry of compounds **I-III** with nucleophiles (9-11), and recent studies by our group involving the general utility of the moiety circled in structures **I-III** in the design of inhibitors of proteolytic enzymes (12-15). We reasoned that ***fusing*** this moiety with an appropriate fragment would yield derivatives of compounds **I-III** that would bind to HLE productively, ultimately leading to irreversible inactivation of this enzyme in accordance with the tentative mechanism shown in Scheme I.

Materials. Compounds **1-12** were synthesized using literature procedures with minor modifications (9-11). Enzyme assays and inhibition studies were carried out as described in detail elsewhere (13). The apparent pseudo first-order inactivation rate constants were determined from the slopes of the semilogarithmic plots of enzymatic activity remaining *vs.* time, using $\ln(E_t/E_o) = k_{obs}\, t$, where E_t/E_o is the amount of active enzyme remaining after time t. The bimolecular rate constants, $k_{obs}/[I]$ $M^{-1}\, s^{-1}$, reflecting the ability of the synthesized compounds to inhibit HLE, are listed in Table I.

Biochemical Studies. Incubation of HLE with excess compound **3** led to time-dependent loss of enzymatic activity and followed pseudo first-order kinetics (Figure 1). The rest of the active compounds behaved similarly. When HLE was mixed with **3** and the % remaining activity was determined by assaying for enzymatic activity over a 48 h period, the enzyme was found to be totally inactivated (100%) within 10 minutes, and remained totally inactive throughout the period of observation.

It is evident from Table I that inhibitory activity is greatly influenced by the nature of the R group, with a 3 or 4 carbon alkyl chain yielding the best inhibitors (compounds **3, 5, 11, 12**). This

SCHEME I



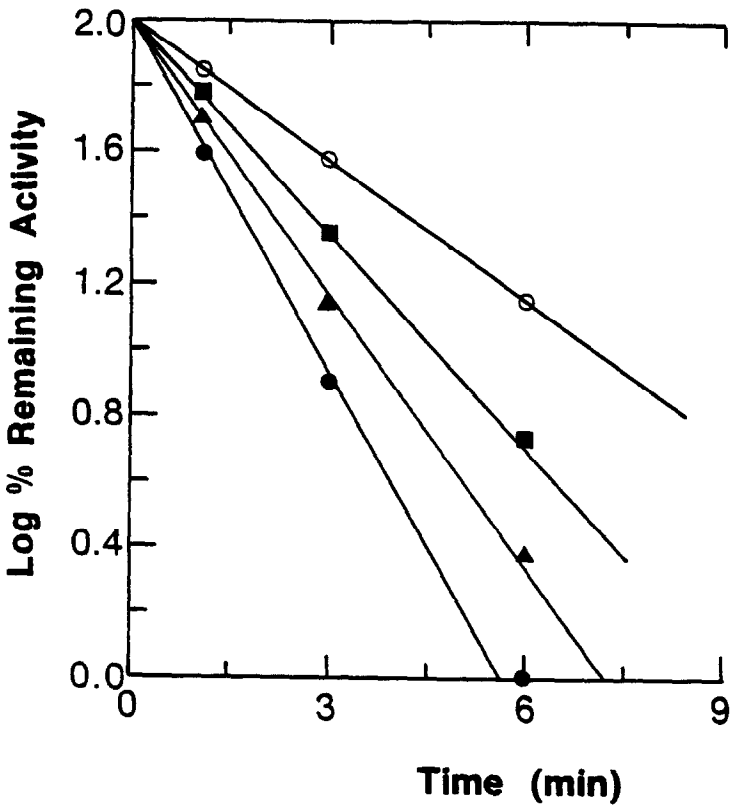


Figure 1. Kinetics of inactivation of human leukocyte elastase by compound 3. The enzyme (302 nM) was incubated with 3 (3.02 μM; 2.01 μM; 1.51 μM, 1.20 μM) in 0.1 M HEPES buffer, pH 7.2 and 1.0% dimethyl sulfoxide. Aliquots were withdrawn periodically and assayed for enzymatic activity using methoxysuccinyl Ala-Ala-Pro-Val p-nitroanilide.

Table I. Inhibition of Human Leukocyte Elastase by Compounds I-III.

Compound	X	Y	Z	L	$k_{obs}/[I]$ M^{-1} s^{-1}
1	C	C	C	$-OSO_2CH_3$	inactive
2	N	C	N	"	"
3	C	C	C	$-OSO_2$ n-butyl	180
4	N	C	C	"	64
5	C	N	C	"	58
6	N	C	N	"	20
7	C	C	C	$-OSO_2$ isobutyl	170
8	C	C	C	$-OSO_2$ n-pentyl	40
9	C	C	C	$-OSO_2$ phenyl	inactive
10	N	C	N	"	"
11	C	C	C	$-OPO(O\text{ n-propyl})_2$	3160
12	C	C	C	$-OPO(O\text{ n-butyl})_2$	3870

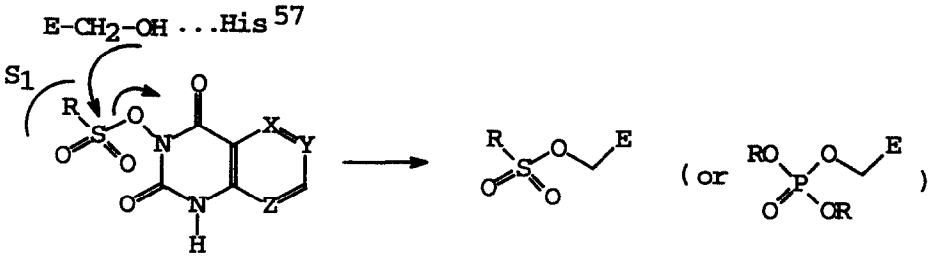
finding is in accord with the known preference of HLE for small hydrophobic side chains. Inhibitory activity was abolished with R = phenyl or methyl (compounds **9**, **10**, **1**, **2**). The corresponding phosphorus compounds were found to be highly potent inhibitors of HLE (compound **3** *vs.* **12**).

The nature of the inactivation process was probed further by conducting the following experiments: (a) when hydroxylamine (45.5 mM final concentration) was added after incubating HLE with a 150-fold excess of compound **3** for 10 minutes, the enzyme did not regain any activity, indicating the absence of any labile acyl linkages in the inactive enzyme; (b) the enzyme was found to be partially protected from inactivation ($k_{obs}/[I]$ = 43 M^{-1} s^{-1}) in the presence of substrate (methoxysuccinyl Ala-Ala-Pro-Val p-nitroanilide 42.5 mM in incubation mix), suggesting that the inactivation of HLE by **3** involves the active site; (c) the irreversible nature of the inhibition was demonstrated by extensive dialysis of the totally inactive enzyme:inhibitor mix against 0.1 M HEPES buffer, pH 7.2, over a 48 h period. No regain in activity was observed; (d) when the enzyme was incubated with **3** in the presence of dithiothreitol (226 μM; 150-fold excess inhibitor over enzyme), the apparent pseudo first-order inactivation rate constant (k_{obs}) remained unaffected, suggesting that any enzyme-induced formation of a reactive species does not lead to its release from the active site during the inactivation process.

While the precise mechanism of action of **I-III** remains to be established, when taken together these results suggest that a *reverse* mode of binding may be operative, namely, accommodation of the R group at the S_1 subsite is likely followed by sulfonylation or phosphorylation of the active site serine (Scheme II) (16).

In summary, a series of heterocyclic inhibitors of HLE has been reported. Studies with related heterocyclic systems, and further mechanistic and biochemical studies are currently in progress and will be reported in due course.

SCHEME II



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