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INHIBITION OF HUMAN LEUKOCYTE ELASTASE AND CATHEPSIN G BY ISOXAZOLINE DERIVATIVES

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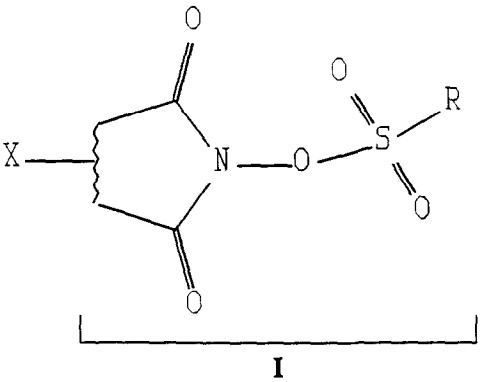
Abstract - The interaction of a series of derivatives of cis-N-hydroxy-3-phenyl-2-isoxazoline-4,5-dicarboximide toward human leukocyte elastase and cathepsin G was investigated. Both enzymes were rapidly acylated and the corresponding acyl enzymes exhibited variable stability.

In the preceding paper we have described the biochemical rationale underlying the design of inhibitors of serine proteinases that incorporate in their structures a heterocyclic recognition component (X) tethered to a reactive center I (1). We report herein the results of preliminary studies related to the interaction of isoxazoline-derived inhibitors **1-4** with human leukocyte elastase (HLE) and cathepsin G (Cath G).



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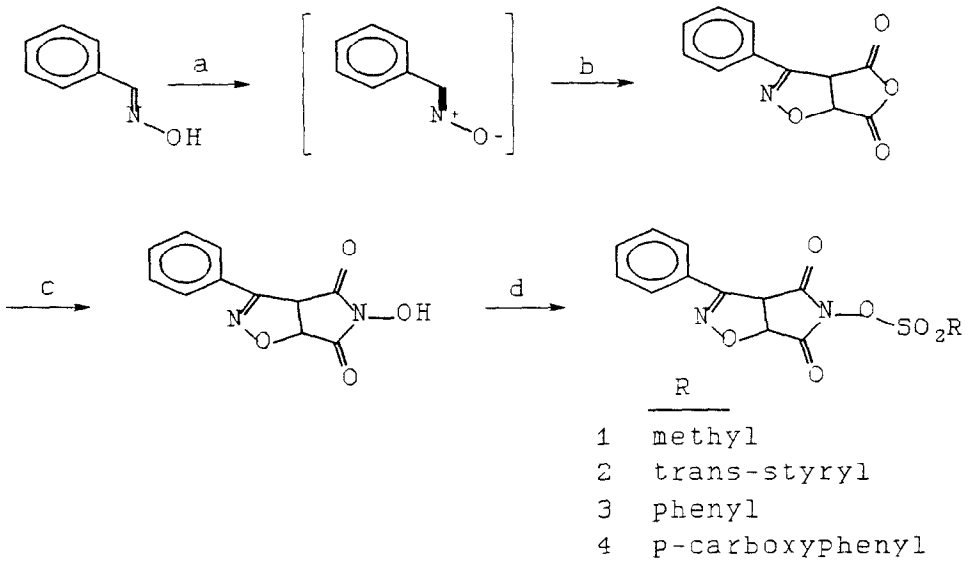
Materials Compounds **1-4** were synthesized according to Scheme **I** (2-3). Enzyme assays and inhibition studies were carried out as described previously (4-5).

SCHEME I.



[a]Cl_2/NaOH; [b]Maleic anhydride; [c]$NH_2OH.HCl/Na_2CO_3$; [d]RSO_2Cl/Pyridine

Biochemical Studies. Molecular modeling studies and other considerations (2) suggested to us that isoxazoline-derived heterocycles such as compounds **1-4** would likely bind productively to the active site of HLE and Cath G. Thus, compounds **1-4** were incubated with HLE and aliquots were withdrawn at different time intervals and assayed for enzymatic activity. The plot of % (remaining activity) vs time (Figure 1) indicates that the interaction of these compounds with HLE involves a rapid acylation-deacylation process. These compounds appear to function as alternate substrates of the enzyme. An almost total regain in enzymatic activity was observed for all four compounds after a 24-hr incubation period. The faster deacylation rates observed here contrast sharply with the much slower deacylation rates of the corresponding succinimide derivatives (4-5) and likely reflect effective stabilization of the transition state of the deacylation process (6).

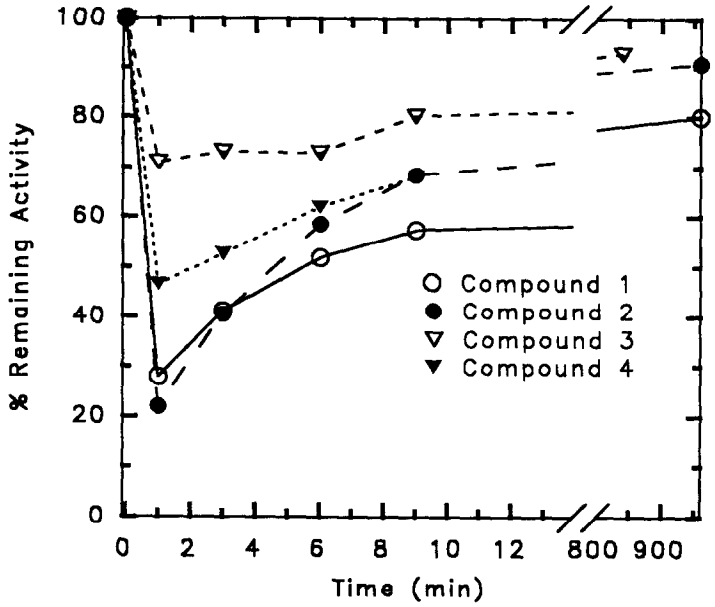


Figure 1. Time dependence of enzymatic activity. Human leukocyte elastase (269nM) was incubated with 3 μM each compound (**1-4**) in 0.1M HEPES buffer, pH 7.2, 0.5 M NaCl, and 1% DMSO.

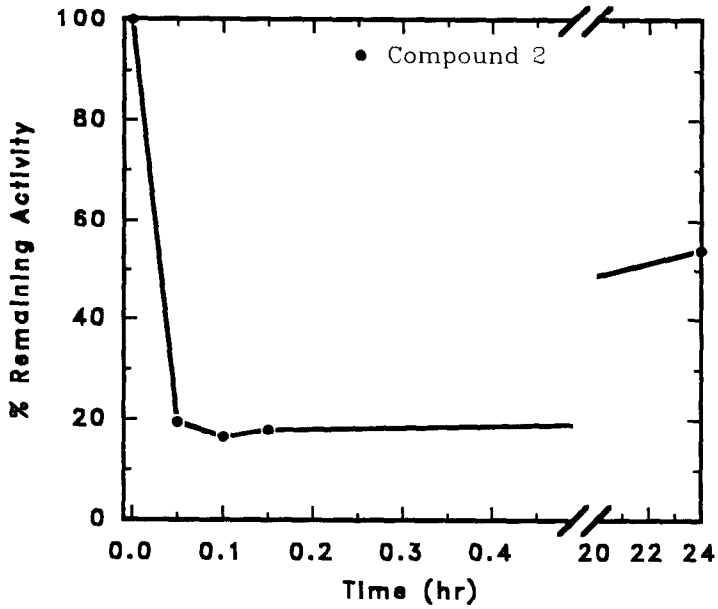


Figure 2. Time dependence of enzymatic activity. Cathepsin G (1.46μM) was incubated in 0.1M HEPES buffer, pH 7.2, 0.5 M NaCl, and 1% DMSO with inhibitor **2** (36.6μM).

Based on the known preference of Cath G for aromatic residues at its S_1 subsite (7), it was hypothesized that these compounds might also act as effective inhibitors of Cath G. Indeed, incubation of compound **2** with Cath G led to rapid formation of the corresponding acyl enzyme, followed by incomplete regain of enzymatic activity. The enzyme regained about 50% of its activity after 24 hours (Figure 2). In a separate experiment, when Cath G was incubated with a 50-fold excess of compound **2**, there was a rapid and near total inactivation of the enzyme. The addition of hydroxylamine (0.5 M) to the inactivated enzyme lead to a regain in enzymatic activity of about 15%. These observations suggest that the mechanism of inactivation of Cath G by these compounds is probably similar to that established for the succinimide series (8). The reason(s) for the observed differences in stability of the acyl enzymes formed between these isoxazoline derivatives and the two enzymes is not intuitively obvious. Studies currently in progress aim at elucidating the mechanism of action of these compounds and probing the effect of structure on bioactivity and specificity.

In summary, the incorporation of moiety **I** into the isoxazoline nucleus has lead to the development of a novel class of inhibitors and useful probes of human neutrophil cathepsin G and elastase.

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