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A GENERAL APPROACH TOWARD THE DESIGN OF MECHANISM-BASED INACTIVATORS OF SERINE PROTEINASES: INACTIVATION OF HUMAN LEUKOCYTE ELASTASE BY A PHTHALIMIDE DERIVATIVE

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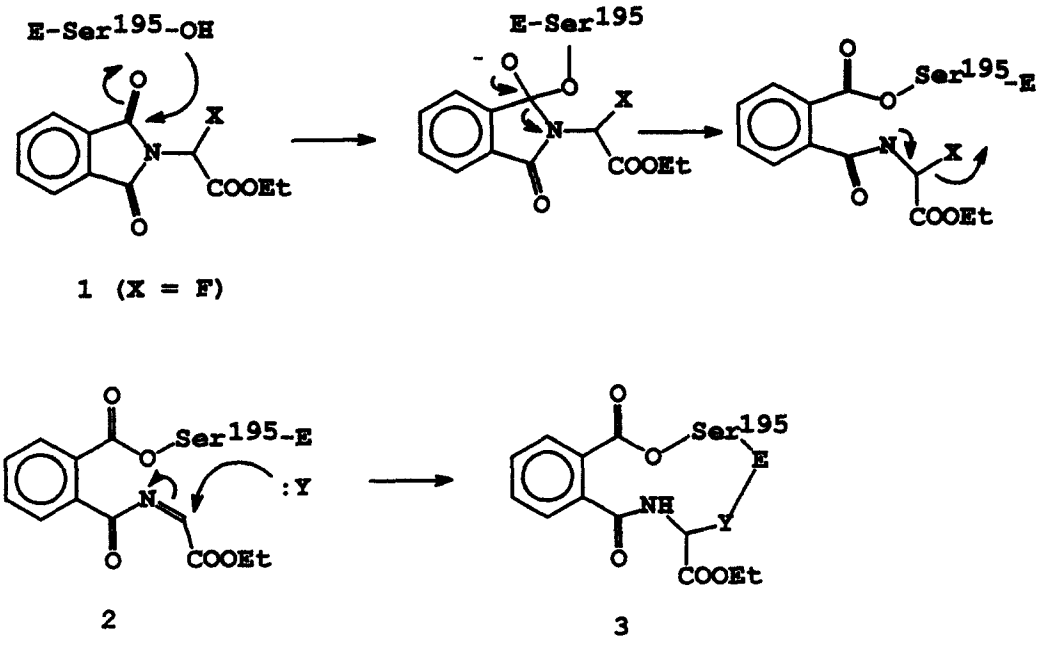
Abstract - A general approach toward the design of mechanism-based inhibitors of serine proteinases involving the linking of fragment -CHX-COOR to an appropriate recognition element is described.

An important and challenging facet of rational drug design involves the development of mechanism-based inactivators and their use in modulating the activity of a target enzyme (1). Inactivators of this type offer many potential advantages, including greater chemical stability, high specificity (since the latent reactivity embodied in a mechanism-based inactivator is unmasked following catalytic processing of the inactivator by the target enzyme), and irreversible inactivation of the enzyme (1-2). The involvement of the neutrophil-derived serine proteinases elastase, cathepsin G and proteinase-3 in inflammatory diseases (3) has provided the impetus behind the design of mechanism-based inactivators of these enzymes. These include haloenol and ynenol lactones (4-5), cephalosporin derivatives (6) and isocoumarins (7).

As part of an ongoing effort aimed at developing mechanism-based inactivators of the above-mentioned serine proteinases (8-11), we reasoned that the attachment of -CHX-COOR (X = F, R = alkyl) to an appropriate recognition element Z (Z = phthalimide, succinimide, benzisothiazole, etc.) would lead to a series of highly versatile mechanism-based inactivators of serine proteinases. Thus, it was anticipated that compound **1** would inactivate human leukocyte elastase (HLE) via enzyme-induced ring opening and tandem loss of fluoride ion to generate a Michael acceptor, which upon further reaction with an active site nucleophile (:Y = His-57, for example) would lead to irreversible inactivation of the enzyme, as illustrated in Scheme I. The soundness and validation of the rationale underlying the design of these inhibitors is exemplified herein by using phthalimide derivative **1** (12).

SCHEME I



Incubation of HLE with compound **1** (X = F) led to time-dependent loss of enzymatic activity (Figure 1). The magnitude of the bimolecular rate constant $k_{obs}/[I]$ M^{-1} s^{-1} was determined to be 100. The interaction of **1** with the enzyme involves the active site, as evidenced by an observed decrease in the $k_{obs}/[I]$ M^{-1} s^{-1} value (40 M^{-1} s^{-1}) when the experiment was repeated in the presence of substrate.

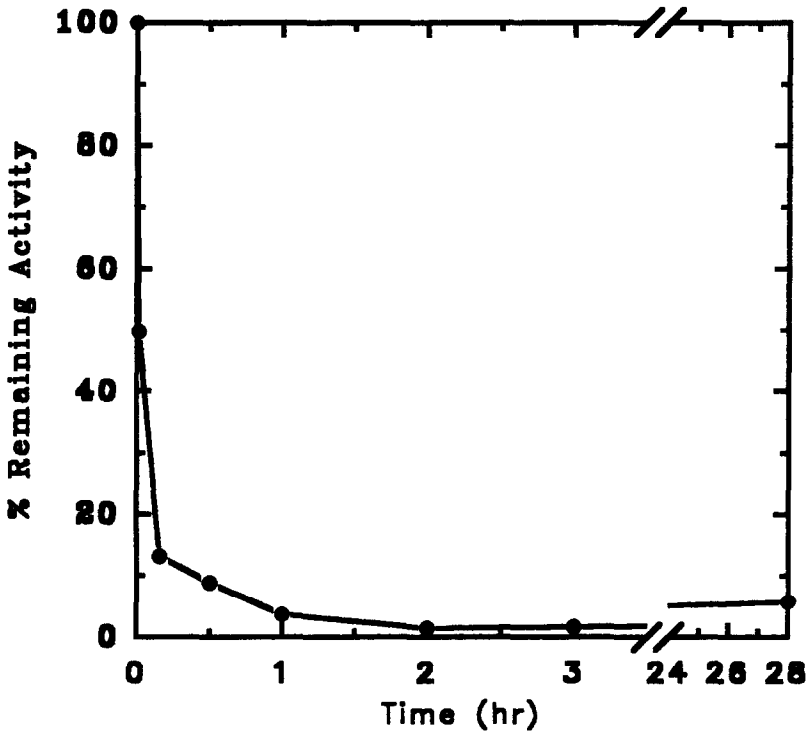


Figure 1. Time dependent loss of enzymatic activity. Human leukocyte elastase (302 nM) was incubated with compound **1** (90.6 μM) in 0.1 M HEPES buffer, pH 7.25, 0.5 M NaCl, and 1% DMSO. Aliquots were withdrawn at different time intervals and assayed for remaining enzyme activity using methoxysuccinyl Ala-Ala-Pro-Val p-nitroanilide.

Centricon 10 filtration of a fully-inactivated enzyme did not result in any regain of enzyme activity. In a separate experiment, hydroxylamine (0.5 M, 0.1 M HEPES buffer, pH 7.25) was added to fully-inactivated enzyme, and the regain in enzymatic activity was monitored over a 24 h period. The enzyme rapidly regained about 38% of its activity and remained at that level throughout the period of observation. This suggests the presence of at least two inactive forms of the enzyme, one having a labile acyl linkage that leads to active enzyme upon treatment with hydroxylamine, and a second form that is unaffected by hydroxylamine. The latter is tentatively proposed as being **3**, depicted in Scheme I. These preliminary results also indicate that the electrophilic species **2** partitions between a pathway leading to **3** and other pathways. Interestingly, compound **1** showed no inhibitory activity toward human leukocyte cathepsin G (200-fold excess inhibitor over enzyme, 10 minute incubation period).

HLE remained unaffected when incubated with a 200-fold excess of compound **4** (X = H). The lack of reactivity of **4** and several other phthalimide derivatives (13) is tentatively ascribed to the lower electrophilicity and, hence, the lower susceptibility of the phthalimide carbonyl to attack by the active site serine (14). Lastly, the stability of **1** in 0.1 M HEPES buffer (pH 7.25, 0.5 M NaCl) was monitored by high-pressure liquid chromatography over a 24 h period. No changes in the structural integrity of the compound were observed.

In conclusion, the first example of a mechanism-based inactivator of HLE by a phthalimide derivative is reported. This is a specific case of general approach involving the design of mechanism-based inhibitors of serine proteinases via the attachment of moiety -CHX-COOR to an appropriate heterocycle, or other recognition element.

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References

1. Silverman, R.B. Mechanism-based Enzyme Inactivation: Chemistry and Enzymology; CRC Press: Boca Raton, FL, 1988; Vols. I and II.

2. Advances in Detailed Reaction Mechanisms (Coxon, J.M., ed.), Jai Press, Inc., Greenwich, CT, 1992, Vol. 2.

3. Inflammation: Basic Principles and Clinical Correlates (Gallin, J.I., Goldstein, I.M., Snyderman, R., eds.), Raven Press, New York, 1992, 2nd ed.

4. Dai, W., Katzenellenbogen, J.A. J. Org. Chem. 1993, 58, 1900-1980 and references cited therein.

5. Copp, L.J., Krantz, A., Spencer, R.W. Biochemistry 1987, 27, 169-178.

6. Knight, W.B., Maycock, A.L., Green, B.G., Ashe, B.M., Gale, P., Weston, H., Finke, P.E., Hagmann, W.K., Shah, S.K., Doherty, J.B. Biochemistry 1992, 31, 4980-4986.

7. Hernandez, M.A., Powers, J.C., Glinski, J., Oleksyszyn, J., Vijayalakshmi, J., Meyer, E.F. J. Med. Chem. 1993, 35, 1121-1129.

8. Groutas, W.C., Brubaker, M.J., Stanga, M.A., Castrisos, J.C., Crowley, J.P., Schatz, E.J. J. Med. Chem. 1989, 32, 1607-1611.

9. Groutas, W.C., Stanga, M.A., Brubaker, M.J. J. Am. Chem. Soc. 1989, 111, 1931-1932.

10. Groutas, W.C., Venkataraman, R., Brubaker, M.J., Stanga, M.A. Biochemistry 1991, 30, 4132-4136.

11. Groutas, W.C., Huang, H., Epp, J.B., Brubaker, M.J., Keller, C.E., McClenahan, J.J. BioMed. Chem. Lett. 1992, 2, 1565-1570.

12. Racemic 1 was synthesized by stirring phthalimide potassium salt (0.93 g; 5 mmol) and ethyl bromofluoroacetate (0.46 g; 2.5 mmol) in 10 mL dry DMF at room temperture for 14 h. Work up yielded 0.5 g (80% yield) of 1, mp 93-4°C: ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.3-1.4 (t,3H), 4.4 (q,2H), 6.35 (d,1H), 7.8-7.9 (q,2H), 7.9-8.0 (q,2H); ^{13}C NMR (300 MHz, $CDCl_3$) δ 13.9, 63.0, 79.7, 82.5, 123.5, 124.3, 131.3, 134.3, 134.9, 135.0, 164.0, 164.4, 165.5. Anal. Calcd for $C_{12}H_{10}FNO_4$: C, 57.37; H, 3.98; N, 5.68. Found: C, 57.60; H, 4.10; N, 5.71.

13. Phthalimidyl-CH_2X derivatives with X = -F, -OH, -CN, -NO_2, -SO_2-Ph, -CO-Ph, etc., were found to be inactive when incubated with HLE (200-fold excess inhibitor over enzyme) for 10 minutes.

14. Teshima et coworkers (J. Biol. Chem. 1982, 257, 5058-5091) have described the use of two N-substituted phthalimide derivatives as competitive inhibitors of elastase.