GEORGIA INSTITUTE OF TECHNOLOGY — OFFICE OF CONTRACT ADMINISTRATION

# PROJECT ADMINISTRATION DATA SHEET

[X] ORIGINAL [ ] REVISION NO. ____

Project No. G-33-U05 Q5270-5A0 — GTRC/GIT — DATE 9/17/86

Project Director: Dr. James C. Powers — School/~~XXX~~ Chemistry

Sponsor: DHHS/PHS/NIH/NHL BI

Type Agreement: Grant No. 5 ROI HL29307-05

Award Period: From 8/1/86 To 7/31/87 (Performance) 10/31/87 (Reports)

| Sponsor Amount: | This Change | Total to Date |
|---|---|---|
| Estimated: | $ | $ 142,832 |
| Funded: | $ | $ 142,832 |

Cost Sharing Amount: $ ____ Cost Sharing No: ____

Title: Synthetic Elastase Inhibitors

ADMINISTRATIVE DATA — OCA Contact E. Faith Gleason

1) Sponsor Technical Contact:

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Defense Priority Rating: N/A — Military Security Classification: N/A

(or) Company/Industrial Proprietary: ____

RESTRICTIONS

See Attached NIH Supplemental Information Sheet for Additional Requirements.

Travel: Foreign travel must have prior approval – Contact OCA in each case. Domestic travel requires sponsor approval where total will exceed greater of $500 or 125% of approved proposal budget category.

Equipment: Title vests with GIT

COMMENTS:

Continuation of G-33-U04 (Final Report not due under prior project).


SPONSOR'S I. D. NO. 02.108.001 86.035

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FORM OCA 65:285

GEORGIA INSTITUTE OF TECHNOLOGY OFFICE OF CONTRACT ADMINISTRATION

## SPONSORED PROJECT TERMINATION/CLOSEOUT SHEET

Date Sept 17, 1987

Project No. G-33-U05 School/~~LAB~~ Chemistry

Includes Subproject No.(s) N/A

Project Director(s) Dr. James C. Power ~~GTRI~~ GIT

Sponsor DHHS/PHS/NIH/NHL BI

Title Synthetic Elastase Inhibitors

Effective Completion Date: 7/31/87 (Performance) 10/31/87 (Reports)

Grant/Contract Closeout Actions Remaining:

- [ ] None
- [x] Final Invoice or Final Fiscal Report
- [ ] Closing Documents
- [ ] Final Report of Inventions
- [ ] Govt. Property Inventory & Related Certificate
- [ ] Classified Material Certificate
- [ ] Other

Continues Project No. G-33-U04 Continued by Project No. G-33-U06

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FORM OCA 69.285

| SECTION IV<br>PROGRESS REPORT SUMMARY | GRANT NUMBER<br>HL29307-06 | |
|---|---|---|
| PRINCIPAL INVESTIGATOR OR PROGRAM DIRECTOR<br>Powers, James C. | PERIOD COVERED BY THIS REPORT<br>FROM | THROUGH |
| NAME OF ORGANIZATION<br>Georgia Institute of Technology | 5/26/86 | 5/25/87 |

TITLE *(Repeat title shown in item 1 on first page)*
Synthetic Elastase Inhibitors

*(SEE INSTRUCTIONS)*

**Publications.**

"Catalysis by Human Leukocyte Elastase. VI. Mechanistic Insights into Specificity Requirements, " R.L. Stein, A.M. Strimpler, H. Hori, and J.C. Powers (1987) Biochemistry 26, 1301-1305.

"Catalysis by Human Leukocyte Elastase. VII. The Proton Inventory as a Mechanistic Probe, " R.L. Stein, A.M. Strimpler, H. Hori, and J.C. Powers (1987) Biochemistry 26, 1305-1314.

"Mechanism-Based Inhibitors of Human Leukocyte Elastase," J.C. Powers, J.W. Harper, and H. Hori (1987) Pulmonary Emphysema and Proteolysis: 1986 (C. Mittman and J.C. Taylor, Eds.), pp. 41-48, Academic Press, New York. "Synthetic and Naturally Occuring Low Molecular Weight Protease Inhibitors/Therapy, Session Introduction," J.C. Powers, 39.

"Elastase Inhibitors for Treatment of Emphysema. Approaches to Synthesis and Biological Evaluation," J.C. Powers and Z.H. Bengali (1986) Am. Rev. Respir. Dis. 134, 1097-1100.

"Elastase Inhibitors for Treatment of Emphysema. Approaches to Synthesis and Biological Evaluation," J.C. Powers and Z.H. Bengali (1987) J. Enzyme Inhibition, 311-319.

**Patents.**

"Thioester Inhibitors of Serine Proteases," J.C. Powers (1986) U.S. Patent 4,585,793.

"Heterocyclic Inhibitors of Serine Proteases," J.C. Powers and W. Harper (1986) U.S. Patent 4,596,822.

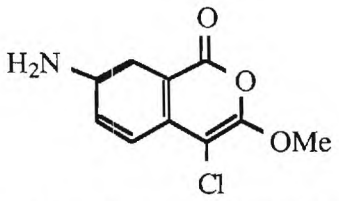
"Aryl Sulfonyl Fluoride Inhibitors of Elastase and Chymotrypsin-like Enzymes," J. C. Powers (1987) U.S. Patent 4,659,855.

**Report.**

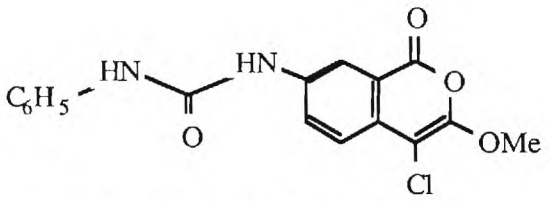
The primary goal of this research is to develop a synthetic elastase inhibitor which would be useful for the treatment of human emphysema. A variety of structures are being investigated including heterocyclic mechanism-based inhibitors and peptide transition state analogs. All of the inhibitors are being tested for specificity with other cellular proteases such as cathepsin G and the mast cell chymotrypsin-like enzymes (chymases). A secondary goal of this

research is the extension of any potent inhibitor structures to cathepsin G and mast cell chymases. Any promising elastase inhibitors will be provided to other investigators for studies in animal models of emphysema. This research should lead to a better understanding of the active site structures of the serine proteases involved in connective tissue turnover, may produce clinically useful drugs for the treatment of emphysema and related diseases, is stimulating the research of medicinal chemists in pharmaceutical companies, and will provide new tools for the in vivo and in vitro study of the role of leukocyte and mast cell proteases in variety of physiological processes.

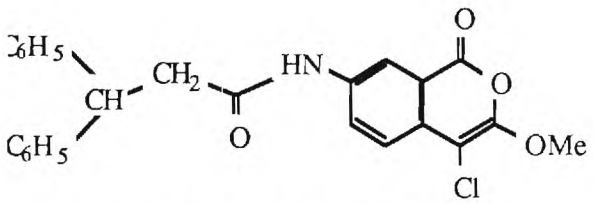
**Isocoumarin Mechanism-Based Inhibitors.** Much of our recent work has been centered around 7-aminoisocoumarins. Substituted 7-amino-4-chloroisocoumarins are potent inactivators of HL elastase and a number of other serine proteases. In order to increase the solubility of these inhibitors or animal studies, we have investigated various 7-acylamino derivatives. We discovered that compounds such as the 7-glutarylamino and 7-phthaloylamino were more reactive than the 7-aminoisocoumarin. This led us to investigate other acyl derivatives and we found that the most reactive compounds were derivatives were the acyl derivative was fairly hydrophobic. Representative inhibition constants are listed below and in the table.



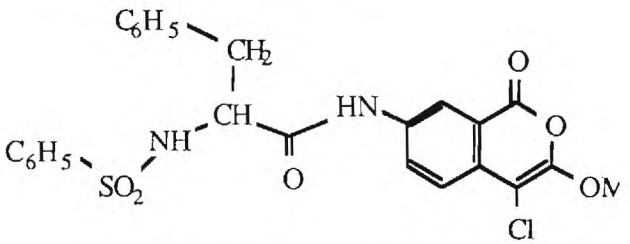

HLE, 10000; PPE, 1000 $M^{-1}s^{-1}$




HLE, 49000, PPE, 8000




HLE, 133000; PPE, 2400




HLE, 200000; PPE, 6500

Inactivation of Serine Proteases by 7-Acylaminoisocoumarins.

| isocoumarin inhibitor | HLE | PPE | Cat G | ChyT |
|---|---|---|---|---|
| 3-MeO-4-Cl-7-$NH_2$ | 10000 | 1000 | 17 | 110 |
| 7-Acyl derivatives of above | | | | |
| 7-Glutaryl-NH | 52000 | 2700 | | |
| 7-Phthaloyl-NH | 51000 | 870 | | |
| 7-PhNH-CO-NH- | 49000 | 8000 | | |
| 7-Tos-PhGly-NH- | 84000 | 1500 | | |

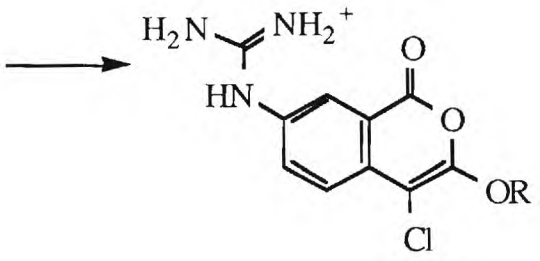
0.1 M Hepes, 0.5 M NaCl, pH 7.5, 10 % $Me_2SO$. Second order inhibition constants ($k_{obsd}/[I]$) are in units of $M^{-1}s^{-1}$. HLE = human leukocyte elastase, PPE = porcine pancreatic elastase, Cat G = cathepsin G, ChyT = chymotrypsin.

The Tos-Phe-NH derivative is thus far the most reactive of the isocoumarin inhibitors. We have spent several days at the laboratory of Ed Meyer (Texas A & M) modeling this compound with both PP and HL elastase and have not yet come up with a unique binding mode. Ed Meyer is planning to do crystallography on several of the isocoumarins to determine the structures of the inhibitors bound to PP elastase.

The proposed inactivation mechanism for isocoumarin inhibitors involves acylation of the active site serine by the isocoumarin carbonyl. Enzyme acylation is followed by formation of a 4-quinone imine methide which can react with an enzyme nucleophile (probably His-57) to give an irreversibly inhibited enzyme structure or with a solvent nucleophile such as acetate to give a stable acyl enzyme.

Another isocoumarin structure which is very reactive with HL elastase is 7-guanidino-4-chloro-3-methoxyisocoumarin. This is quite surprising since HL elastase doesn't hydrolyze substrates with basic side chains. However, Wolfram Bode (Munich) has recently solved the structure of HL elastase and finds that the $S_1$ pocket contains an Asp residue (Asp 229). Although this residue points away from the substrate it may account for the fact that the 7-guanidino isocoumarin is more reactive than expected. Interestingly, cathepsin G has a Glu residue at this position.

Does this react with
Asp-229 of HL elastase

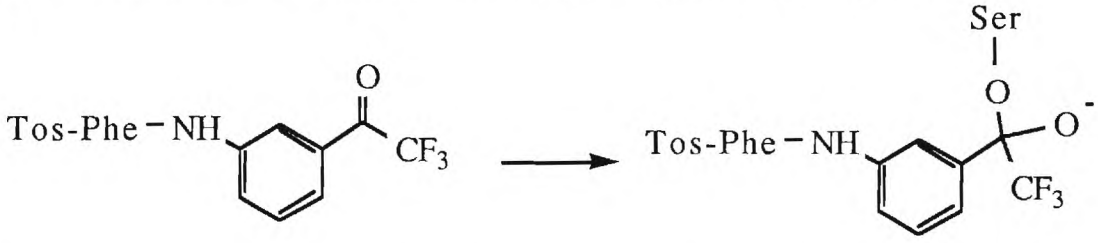



One of our goals for the next year is to investigate the significance of these acidic residues in the $S_1$ pockets of HL elastase and cathepsin G using synthetic substrates and inhibitors.

**Transition State Inhibitors.** Peptide aldehydes, peptide trifluoromethyl ketones, and peptide boronic acids are three types of transition state analogs which have been reported as potent serine protease inhibitors. In the enzyme-inhibitor complex, the active site serine has added to the aldehyde, ketone or boronic acid moiety to form a tetrahedral-like adduct.

We have recently synthesized the trifluoromethyl ketone shown below and find that it is a moderate inhibitor of HL elastase with a

$K_I$ value of 40 μM. This inhibitor structure was based on the finding that the Tos-Phe derivative of the isocoumarin was an excellent inhibitor. We simply placed the trifluoromethylketone moiety in the location of the isocoumarin carbonyl group. The proposed mechanism of inhibition is shown below. We expect that the carbonyl group is forming a tetrahedral adduct with the active site serine.




A specific aim for next year is to make analogs of this structure in order to increase its potency. In particular, placement of a methyl group on the ring adjacent to the trifluoromethyl ketone should interact with the $S_1$ pocket and improve the inhibitory potency.

**Specific Objectives.** We have several objectives for next year. We plan to use molecular modeling of the active sites of HL elastase and PP elastase for the improvement of our isocoumarin inhibitors and for the design of new types inhibitors. We have recently obtained the coordinates for the active site of HL elastase and are one of only two groups in the U.S. that have them available at present. Based on the crystal structure of HL elastase, we plan to investigate the role of Asp 229 in the $S_1$ binding pocket on the interaction of substrates and inhibitors with HL elastase. We plan to synthesize a number of aromatic trifluoromethyl ketone inhibitor structures in order to improve their inhibitory potency. We are trying to extend the aminoisocoumarin inhibition to simpler heterocyclic compounds and are trying to synthesize some oxazoles which may also be potent inhibitors. And finally, we plan mechanistic studies of the mode of inhibition of some of the inhibitors. These will include both C-13 NMR investigations and careful measurement of inhibitor deacylation rates. Mechanistic information is extremely valuable in the design of the next generation of inhibitors.