

GEORGIA INSTITUTE OF TECHNOLOGY
OFFICE OF CONTRACT ADMINISTRATION
SPONSORED PROJECT INITIATION

CEB

Date: September 23, 1976

Project Title: Synthetic Protease Inhibitors

Project No: G-33-F01 (continuation of G-33-609)

Project Director: Dr. James C. Powers

Sponsor: DHEW/PHS/NIH National Heart & Lung Institute, Bethesda, MD.

Agreement Period: From 9/1/76 Until 8/31/78*

*Only the 02 year funded (9/1/76 - 8/31/77)

Type Agreement: Grant No. 5-R01-H118679-02

Amount: \$64,761 PHS
10,497 GIT (G-33-387)
\$75,208 Total

Reports Required: Annual Progress Reports w/continuation applications.
Terminal Progress Report upon Grant expiration.

Sponsor Contact Person (s):

Technical Matters

Contractual Matters
(thru OCA)

Claude Lenfaut, M.D.
Director, Division of Lung Diseases
National Heart & Lung Institute
DHEW/PHS/NIH
Bethesda, MD. 20014

Defense Priority Rating:

Assigned to: Chemistry (School/Laboratory)

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Security Coordinator (OCA)	Other _____
Reports Coordinator (OCA) ✓	

GEORGIA INSTITUTE OF TECHNOLOGY
OFFICE OF CONTRACT ADMINISTRATION
SPONSORED PROJECT TERMINATION

Post of
CAG
OAH

Date: 9/15/77

Project Title: Synthetic Protease Inhibitors
Project No: G-33-F01
Project Director: Dr. J. C. Powers
Sponsor: DHEW/PHS/NIH National Heart, Lung, & Blood Institute;
Bethesda, MD. 20014

Effective Termination Date: 8/31/77 9end of 02 year)

Clearance of Accounting Charges: by 8/31/77

Grant/Contract Closeout Actions Remaining:

- Final Invoice and Closing Documents
- Final Fiscal Report
- Final Report of Inventions
- Govt. Property Inventory & Related Certificate
- Classified Material Certificate
- Other Annual Report of Expenditures due by 11/30/77.

NOTE: FOLLOW-ON PROJECT (03 YEAR) IS G-33-F02.

Assigned to: Chemistry (School/Laboratory)

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| Reports Coordinator (OCA) | |

SECTION IV

APPLICANT: REPEAT GRANT NUMBER SHOWN ON PAGE 1 →		GRANT NUMBER	
SECTION IV—SUMMARY PROGRESS REPORT		HL 18679-03	
PRINCIPAL INVESTIGATOR OR PROGRAM DIRECTOR (Last, First, Initial)		PERIOD COVERED BY THIS REPORT	
Powers, James C.		FROM	THROUGH
NAME OF ORGANIZATION		9/1/76	8/31/77
Georgia Institute of Technology			
TITLE (Repeat title shown in item 1 on first page)			
Synthetic Protease Inhibitors			

- List publications: (a) published and not previously reported; (b) in press. Provide five reprints if not previously submitted.
- List all additions and deletions in professional personnel and any changes in effort.
- Progress Report. (See Instructions)

- "Inhibitors of Elastase and Pulmonary Emphysema," James C. Powers, Trends in Biochemical Sciences, 1, 211 (1976).
 - "Specificity of Porcine Elastase, Human Leukocyte Elastase and Cathepsin G. Inhibition with Peptide Chloromethyl Ketones," Biochem. Biophys. Acta, in press.
 "Haloketone Inhibitors of Proteolytic Enzymes," James C. Powers, Chap. in Weinstein (ed.), "Chemistry and Biochemistry of Amino Acids, Peptides and Proteins," Vol. 4, Marcel Dekker, New York, in press.
 "Reaction of Serine Proteases with Halomethyl Ketones," James C. Powers, Chap. in W. Jakoby and M. Weilchek (eds.), Meth. in Enzymology, Academic Press, in press.
 "Reaction of Serine Proteases with Aza-Amino Acid and Aza-peptide Derivatives," James C. Powers and B. F. Gupton, Chap. in W. Jakoby and M. Weilchek (eds.), Meth. in Enzymology, Academic Press, in press.
- Dr. M. Sakamoto has returned to Japan after spending one year in my laboratory. He is an assistant professor at Meiji College of Pharmacy, Tokyo, Japan, who spent his sabbatical leave doing research on synthetic protease inhibitors.

No changes in effort are planned.

3. Progress Report

Objectives. A number of proteolytic enzymes such as elastase, collagenase and cathepsin G have been shown to be involved in diseases such as pulmonary emphysema and arthritis which involve tissue destruction. The goal of this research is to design and synthesize specific and effective inhibitors for these proteolytic enzymes. The inhibitors are proving to be invaluable in the study of the normal biological function and the role of these proteases in disease. I am hopeful that synthetic protease inhibitors will find use in the clinical treatment of pulmonary emphysema and other diseases in the near future.

Elastase and Cathepsin G-Chloromethyl Ketones. Our goals for the year were to synthesize a radiolabeled peptide chloromethyl ketone elastase inhibitor, a water soluble inhibitor and to stimulate other investigators to carry out animal studies with these inhibitors. We synthesized both [¹⁴C] and [³H] labeled Ac-Ala-Ala-Pro-ValCH₂Cl, an effective elastase inhibitor.

We are also collaborating with Aaron Janoff in a study of the effect of the inhibitors on emphysema induced by elastase in rats, and finally we plan to use a labeled chloromethyl ketone to locate the active site histidine of leukocyte elastase in collaboration with J. Travis (University of Georgia).

Elastase-Other Inhibitors. One goal for the current year was to learn how to synthesize derivative of α -amino sulfonic acids and devise methods for their introduction into peptide structures. We wish to synthesize some sulfonamide and sulfonic acid derivatives related to elastase substrates. Very little attention has been devoted to this problem during the last year and thus little progress has been made. We have learned how to synthesize dipeptide sulfonic acids such as Z-Gly-Sala-OH (Sala = $-\text{NH CH}(\text{CH}_3)\text{SO}_2-$). We have also been able to prepare sulfonic acid methyl esters such as Z-Sgly-OCH₃. In the next year we plan to continue our efforts to develop synthetic methods so we can prepare compounds such as Ac-Ala-Ala-Sala-OCH₃ and Ac-Ala-Ala-Sala-F as potential elastase inhibitors. However, we do not expect to devote much time to this problem next year since we are currently giving other areas of investigation higher priority.

In a related area we have synthesized Ac-Ala-Ala-Aala-NHC₆H₄SO₂F and shown that it inhibits elastase. We are planning to study the kinetics of reaction of this inhibitor with elastase and cathepsin G in the course of the next year.

In the area of aza-peptides, we have synthesized a number of new compounds and tested them as inhibitors of elastase. These include Ac-Ala-Ala-NCH₃-NCH₃-CO-ONp and Ac-Ala-Aala-CH₂NCH₃-CO-ONp. The second compound slowly inhibits elastase ($t_{1/2} = 1-2$ days) while the first is not an inhibitor. Thus neither compound offers any advantages over our best aza-peptide elastase inhibitor Ac-Ala-Ala-Anvl-ONp. In the future we would like to make analogs of Ac-Ala-Ala-Anvl-ONp with different leaving groups besides p-nitrophenol.

Metalloproteases-Collagenase. During the last year we have made considerable progress in the area of metalloprotease inhibitors. Our goal is to develop functional groups which would inactivate members of the metalloprotease family and then incorporate this functional group into a substrate like structure in order to obtain a specific inhibitor for mammalian collagenase. We have utilized thermolysin as a model system since mammalian collagenase is at present still difficult to isolate. In addition thermolysin will bind to smaller peptides than collagenase which simplifies synthesis of inhibitors for testing. We have investigated three classes of compounds as metalloprotease inhibitors: phosphoramides, hydroxamates and haloacetyl amino acid derivatives.

Phosphoramides such as Pi-Leu-Trp-OH and Pi-Leu-NH₂ have been synthesized and found to be excellent competitive inhibitors of thermolysin. These compounds are related to the microbial inhibitor phosphoramidon. The inhibitors bind to thermolysin due to coordination of a phosphoryl oxygen with the zinc atom of the enzyme. To test our proposed binding mode, Pi-Phe-OH was synthesized and shown to be an excellent inhibitor of carboxypeptidase. Our goal for the near future is to synthesize Pi-Ile-Ala-Gly-OEt, Pi-Ile-Ala-Gly-Gln-OEt and Pi-Ile-Ala-Gly-Gln-Arg-OEt as collagenase inhibitors. The first compound has already been synthesized but not yet tested with collagenase.

The second type of inhibitor which we have investigated are hydroxamates of peptides. We have discovered that compounds such as HONH-Bzm-Ala-Gly-NH₂ (Bzm = CO-CH(CH₂C₆H₅)CO-) are excellent competitive inhibitors of thermolysin. They are also quite specific and do not inhibit other proteases such as carboxypeptidase or chymotrypsin. We believe inhibition is due to coordination of the hydroxamate oxygen with the zinc atom of the enzyme. The HONH-Bzm-Ala-Gly-moiety has been attached to agarose and has been used for the affinity chromatography of metalloproteases. Recently we have synthesized HONH-Ibm-Ala-Gly-NH₂ and HONH-Ibm-Ala-Gly-Gln-Arg-OCH₃ (Ibm = isobutylmalonyl) as collagenase inhibitors. We are currently studying their reaction with collagenase. Jim Travis (University of Georgia) is supplying the collagenase. One urgent need in this area of research is for some decent substrates for collagenase. We are currently synthesizing some. Our goal for the future is to study the kinetics of the reaction of the above inhibitors with collagenase.

The final group of metalloprotease inhibitors which we have studied are irreversible active site directed inhibitors. Thermolysin is rapidly inhibited ($t_{1/2}$ = 5-10 min) by ClCH₂CON(OH)-CH(CH₂CH(CH₃)₂)CO-OEt and BrCH₂CON(OH)-CH(CH₂CH(CH₃)₂)-Cl at pH 7.2. At present we are investigating the kinetics of these reactions and characterizing the site of reaction. Once the reaction mechanism with thermolysin is better understood, we plan to synthesize compounds such as ClCH₂CON(OH)-CH(CH₂CH(CH₃)₂)-CO-Ala-Gly-Gln-Arg-OEt and test them as an irreversible inhibitors of collagenase.

Significance. It is the belief of the author that we are approaching the day when synthetic protease inhibitors will be utilized clinically in the treatment of diseases which involve tissue destruction such as emphysema and arthritis. Chloromethyl ketone inhibitors of elastase are being used in various in vivo studies of emphysema in animal model systems. Protease inhibitors have proven to be valuable tools for study of the role of proteases in biological processes. Even if the compounds available at present only tell us that it is possible to treat diseases such as emphysema with inhibitors, that will be a major step forward since clinically useful compounds would quickly follow.

The undersigned agrees to accept responsibility for the scientific and technical conduct of the project and for provision of required progress reports if a grant is awarded as the result of this application.

May 21, 1977
Date

James C. Powrie
Principal Investigator or Program Director