GEORGIA INSTITUTE OF TECHNOLOGY
OFFICE OF CONTRACT ADMINISTRATION
SPONSORED PROJECT INITIATION

Date: November 9, 1978

Project Title: Synthetic Protease Inhibitors

Project No: G-33-F03

Project Director: Dr. J. C. Powers

Sponsor: DHEW/PHS/NIH - National Heart, Lung & Blood Institute
Bethesda, MD 20014

Agreement Period: From 9/1/78 Until 8/31/79 (04 Year)

Type Agreement: Grant No. 2 R01 HL18679-04

Amount: $48,401 New PHS Funds (G-33-F03)
13,566 GIT Contribution (G-33-333)
$61,967 Total

Reports Required: Annual Progress Reports with Continuation Applications
Terminal Progress Report upon Grant Expiration

Sponsor Contact Person (s):

Technical Matters

Claude Lenfant, M. D. (Dr. Eric Jurrus)
Director
Division of Lung Diseases
National Heart, Lung & Blood Institute
Betheada, MD 20014

Phone: 301-496-7332

Contractual Matters
(thru OCA)

Mr. Roger Deshaies
Grants Manager
Division of Extramural Affairs
National Heart, Lung & Blood Institute
Bethesda, MD 20014

Phone: 301-496-7255

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

Defense Priority Rating: none

Assigned to: Chemistry (School/Laboratory)

COPIES TO:

Project Director
Division Chief (EES)
School/Laboratory Director
Dean/Director—EES
Accounting Office
Procurement Office
Security Coordinator (OCA)
Reports Coordinator (OCA)

Library, Technical Reports Section
EES Information Office
EES Reports & Procedures
Project File (OCA)
Project Code (GTRI)
Other

CA—3 (3/76)

GEORGIA INSTITUTE OF TECHNOLOGY
OFFICE OF CONTRACT ADMINISTRATION

SPONSORED PROJECT TERMINATION

Date: October 18, 1979

Project Title: Synthetic Protease Inhibitors

Project No: G-33-F03

Project Director: Dr. J. C. Powers

Sponsor: DHEW/PHS/NIH - National Heart, Lung & Blood Institute
Bethesda, MD 20014

Effective Termination Date: 8/30/79 (04 year)

Clearance of Accounting Charges: ----

Grant/Contract Closeout Actions Remaining: TERMINATED

_ Final Invoice and Closing Documents
_ Final Fiscal Report
_ Final Report of Inventions
_ Govt. Property Inventory & Related Certificate
_ Classified Material Certificate
X Other Annual Report of Expenditures (05 year) due no later than 11/30/79.

NOTE: FOLLOW-ON PROJECT IS G-33-F04 (05 YEAR).

Assigned to: Chemistry (School/Laboratory)

COPIES TO:

Project Director
Division Chief (EES)
School/Laboratory Director
Dean/Director—EES
Accounting Office
Procurement Office
Security Coordinator (OCA)
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Library, Technical Reports Section
EES Information Office
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Project Code (GTRI)
Other_____

CA—4 (1/79)

SECTION IV G-33-F03/Powers Submitted

APPLICANT: REPEAT GRANT NUMBER SHOWN ON PAGE 1 →	GRANT NUMBER w/ Proposal dtd 6/20/79
SECTION IV—SUMMARY PROGRESS REPORT	HL18679-05
PRINCIPAL INVESTIGATOR OR PROGRAM DIRECTOR (Last, First, Initial)	PERIOD COVERED BY THIS REPORT
Powers, James C.	FROM: 9/1/79 — THROUGH: 8/31/80
NAME OF ORGANIZATION: GEORGIA INSTITUTE OF TECHNOLOGY	

TITLE (Repeat title shown in Item 1 on first page)
Synthetic Protease Inhibitors

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

1a. Publications

"Peptide Hydroxamic Acids as Inhibitors of Thermolysin," N. Nishino and J. C. Powers, Biochemistry, 17, 2846-2850 (1978).

"Specificity and Reactivity of Human Granulocyte Elastase and Cathepsin G, Porcine Pancreatic Elastase, Bovine Chymotrypsin and Trypsin Toward Inhibition with Sulfonyl Fluorides," M. O. Lively and J. C. Powers, Biochem. Biophys. Acta, 525, 171-179 (1978).

"Active Site Directed Irreversible Inhibition of Thermolysin," D. Rasnick and J. C. Powers, Biochemistry, 17, 4363-4369 (1968).

"Synthetic Inhibitors of Elastase and Cathepsin G," James C. Powers, B. F. Gupton, M. O. Lively, N. Nishino and R. J. Whitley, Chap. in K. Havemann and A. Janoff (eds), "Neutral Proteases of Human Polymorphonuclear Leukocytes," "Urban and Schwarzenberg, Baltimore-Munich, pp 221-233, 1978.

"Albumin Microsperes as Carrier of an Inhibitor of Leukocyte Elastase: Potential Therapeutic Agent for Emphysema," R. R. Martodam, D. Y. Twumasi, I. E. Liner, J. C. Powers, N. Nishino and G. Krejcarek, Proc. Nat. Acad. Sci., 76, 2128-2132 (1979).

1b. "Virus-specified Protease in Poliovirus-infected Hela cells", B. Korant, N. Chow, M. Lively and J. Powers, Proc. Nat. Acad. Sci., 76, June 1979.

"Inhibition of Thermolysin and Carboxypeptidase A by Phosphoramidates", C. Kam, N. Nishino, and J. C. Powers, Biochemistry, 18, July 1979.

2. Dr. Norikazu Nishino returned to Japan to begin an academic career and is no longer working on the project. He will be replaced in June by Dr. Tadashi Teshima who received his Ph.D. degree in 1976 with Dr. Shiba at Osaka University. At present he has over 15 research publications in the area of peptide chemistry.

3. Progress Report

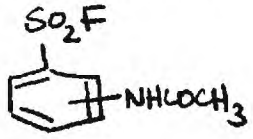
a. Overall Objectives for Total Project. A number of proteolytic enzymes such as elastase and collagenase have shown to be involved in diseases such as pulmonary emphysema and arthritis which involve tissue destruction. The goal of this proposed research is to design and synthesize specific and effective inhibitors for these proteolytic enzymes. The inhibitors should be invaluable in the study of the normal biological function and the role of these

proteases in disease. In addition, synthetic protease inhibitors should find use in the clinical treatment of pulmonary emphysema, rheumatoid arthritis and other diseases.

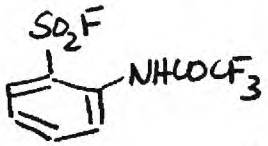
b. Goals for the Current Year. Our goals for the current year were to develop new types of inhibitors for human leukocyte elastase (a serine protease) and to continue work on developing specific metalloprotease inhibitors.

c. Studies with Human Granulocyte Enzymes. Proteolysis by enzymes released from human PMN leukocytes, macrophages and other sources are involved in several major diseases which involve tissue destruction. In the case of pulmonary emphysema, elastase seems to be principally responsible for lung damage with collagenase, cathepsin G and other proteases carring out secondary digestions. We have previously synthesized a number of specific peptide chloromethyl ketone inhibitors of both human leukocyte elastase and cathepsin G. Two of the best elastase inhibitors, Meo-Suc-Ala-Ala-Pro-ValCH_2Cl and Ac-Ala-Ala-Pro-ValCH_2Cl, have been shown to be effective at preventing emphysema by two research groups (Dr. P. Stone, Boston University and Dr. J. Kleinerman, Mt. Sinai Medical Center). In both cases the hamster emphysema animal model was utilized. In addition, preliminary experiments indicate the inhibitors will reduce the severity of the disease if they are applied to animals after disease has been allowed to progress. Even though our peptide chloromethyl ketones are effective, there has been considerable concern about their toxicity. Chloromethyl ketones are alkylating agents and would be expected to exhibit some carcinogencity. Thus most investigators believe that these compounds may not have utility for the treatment of human disease. Therefore we have begun a search for specific elastase inhibitors which have properties which would allow their utilization in humans.

One type of compound which we are investigating are amino sulfonic acid derivatives. In particular we have synthesized sulfonyl fluorides such as 1 and 2. The trifluoroacetyl derivative 2 was an extremely effective inhibi-



1 o, m, and p isomers



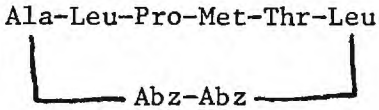
2

tors of both human leukocyte elastase ($k_{obs}/[I]$ = 500 M^{-1} s^{-1}) and porcine pancreatic elastase ($k_{obs}/[I]$ = 2000 m^{-1} s^{-1}). It was quite specific since the corresponding rates with human cathepsin G and bovine chymotrypsin were 10 and 50 m^{-1} s^{-1} respectively. The acetyl compounds (1) were in contrast quite unreactive toward all the enzymes studied. Sulfonyl fluorides are not generally considered to be toxic and thus elastase inhibitors such as 2 may find utility in the treatment of human disease.

Our goals for next year are to synthesize analogues of 2 to see if it

might be possible to improve its reactivity and specificity. In addition, work will be directed toward the synthesis of Ac-Ala-Ala-NH-CH(R)SO_2F. Such peptide amino sulfonyl fluorides can be made to more closely imitate the structure of natural elastase substrates. Thus they may exhibit considerable specificity for elastase.

d. Small Peptide Analogs of the α_1-Protease Inhibitor. Another approach to elastase inhibitors is to make analogs of the α-protease inhibitor (α_1-antitrypsin) active site. The sequence at the active site has recently been determined by Dr. J. Travis at the U. of GA. We were then able to design and synthesize small cyclic peptides such as 3 which have the α_1-PI active site sequence. This peptide is a reversible inhibitor of human leukocyte elas-



3 Abz = 3-aminobenzoyl

tase (K_I = 0.38 mM) and is not a substrate. Although 3 is only a moderate inhibitor, the peptide is a good lead compound for the development of new inhibitors.

Our goals for the next year are to prepare analogs of 3 in order to improve binding to leukocyte elastase. Our first goal will be the synthesis of an analogue of 3 where the Met-Thr unit is replaced by a Val-Ser unit. Leukocyte elastase prefers Val over Met at its primary substrate binding site and this change should increase the potency of the inhibitor. Cyclic peptide analogs of α_1-PI are likely to be not toxic due to their close resemblance to the natural inhibitor. Synthetic elastase inhibitors seem to offer the best hope at present for the treatment of the majority of emphysema since natural α_1-PI is difficult to isolate and purify.

e. Studies with Metalloproteases. A number of metalloproteases are involved in diseases which involve connective tissue destruction. Collagenase has been found in rheumatoid synovium and has been implicated in the destruction of joints in rheumatoid arthritis. Collagenase may also be involved in periodontal disease, corneal ulceration, and several other diseases. Invasive tumors have been shown to secrete collagenase and the ability of this enzyme to attack connective tissue may allow such tumors to expand into the surrounding tissue.

Excellent progress has been made in the development of general classes of inhibitors for the metalloproteases family. Using thermolysin and carboxypeptidase as model systems in our initial experiments, we have investigated phosphoramidates, hydroxamic acids, and thiols as competitive inhibitors and haloacetyl hydroxamic acids as irreversible inhibitors. Phospho-

ramidates such as P-Leu-NH_2 and P-Phe-O^-K^+ are excellent inhibitors of thermolysin and carboxypeptidase A respectively. The hydroxamic acid NONH-BMZ-Ala-Gly-NH_2 (BZM=-$COCH(CH_2C_6H_5)CO$-) is a specific inhibitor of thermolysin (K_I = 0.7 μM) and has been attached to agarose and used in the affinity purification of thermolysin. A number of irreversible thermolysin inhibitors such as $ClCH_2CON(OH)CH(CH_2CH(CH_3)_2)CO_2CH_3$ have been designed and synthesized. The site of reaction has been determined. Several hydroxamic acids thiols, and phosphoramidates with the appropriate sequence to inhibit collagenase have been synthesized. Some were observed to be moderate inhibitors.

Our goals for next year include the synthesis of compounds such as 4 and 5.

$HSCH_2CH_2CO$-Pro-NH_2 (4)

$HO_2CCH_2\overset{OH}{N}$-CH_2CO-Pro-NH_2 (5)

Both will be tested as collaginase inhibitors.

f. Pseudomonas aeruginosa Elastase. Pseudomonas aeruginosa elastase is an infectous organism which is resistant to many antibiotics. This organism causes hemorrhagic pneumonia in mink and corneal ulcers in man. The major cause of morbidy and mortality in cystic fibrosis is the severe, chronic persistent pulmonary infection with bacteria particularily P. aeruginosa. Many strains of P. aeruginosa produce an elastase. Those strains with elastase have been shown to be more pathogenic than those without. The Elastase is likely the factor responsible for the destruction of corneal tissue and hemorrhages of the lung observed in P. aeruginosa infections.

P. aeruginosa is a zinc metalloprotease and we have developed a new substrate to assay the enzyme. Specific inhibitors for this elastase have been designed and synthesized. In particular, the hydroxamic acid HONH-$COCH_2CH(CH_2C_6H_5)CO$-Ala-Gly-NH_2 was a potent reversible inhibitor (K_I = 0.044 μM) and $ClCH_2CO$-HO Leu-Ala-Gly-NH_2 was an irreversible inhibitor. Both compounds may find utility in the treatment of infections due to P. aeruginosa elastase.

g. Significance. It is the belief of the author that reagents which control the activity of proteolytic enzymes can be used in a number of clinically sitiations. Diseases involving tissue destruction such as emphysema and arthritis have been shown to involve enzymes such as elastase, cathepsin G and collagenase. Invasive tumors secrete collagenase possibility accounting for their ability to expand into the surrounding connective tissue. Viral protein processing requires a protease. Organisms like Neisseria Gonorrhoeae and N. meningitidis secrete proteases which cleave the principal mucosal antibody, immunoglobulin A. And P. aeruginosa produces an enzyme which destroys lung tissue.

The basic goal of our research is to develop new classes of inhibitors for the two major families of proteases: serine and metalloproteases. Within this framework our emphasis have been directed toward inhibitors for granulocyte elastase and cathepsin G, and collagenase since these enzymes are involved in two major chronic diseases: emphysema and arthritis. In the course of this work we are learning new information about these specific enzymes and about the two general classes of proteases. In addition we are discovering ways to increase the specificity of inhibitors both for a specific enzyme within a class of proteases and for an enzyme when it is located in its natural environment which may contain a multitude of other reactive groups. The information should be useful to other investigators who desire specific inhibitors for other proteases.

At present some of our elastase inhibitors are being tested in animals for the treatment of emphysema. There is a good possibility that the course of emphysema can be arrested by use of the appropriate inhibitor. At present better elastase inhibitors are desired. Our studies with synthetic protease inhibitors are leading us closer to clinically useful drugs.

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

June 18, 1979
Date

James C. Powers
Principal Investigator of Program Director

☆ U.S. GOVERNMENT PRINTING OFFICE 1976 - 623-890

Department of Health, Education, and Welfare

Grant No.
2 R01 HL18679-04

DATE OF THIS REPORTING PERIOD
FROM 9/1/78 TO 8/30/79

PROJECT PERIOD
FROM 9/1/78 TO 8/31/81

☐ CHECK IF FINAL REPORT

NAME AND ADDRESS OF GRANTEE INSTITUTION
Georgia Institute of Technology
Atlanta, Georgia 30329

TRANSACTION NO.
(08)R1HL18679B

INSTITUTIONAL ID NO.
G-33-F03

1. Expenditures of DHEW Funds for this Reporting Period

a. Personnel	$ 18,583.04	h. Alterations and renovations	
b. Consultant services		i. Other Retirement	272.26
c. Equipment	3,593.00		
d. Supplies	8,539.76	j. Total direct costs	31,260.81
e. Travel, domestic	272.75	k. Indirect costs: Rate 76 % ☒ S&W ☐ TDC	
f. Travel, foreign		Base $ 18,583.04	14,123.11
g. Patient care costs		l. TOTAL	$ 45,383.92

2. Expenditures from Prior Periods (previously reported)	191,274.99
3. Cumulative Expenditures	236,658.91
4. Total Amount Awarded – Cumulatively	242,621.00
5. Unexpended Balance (Item 4 less Item 3)	5,962.09
6. Unliquidated Obligations	-0-
7. Unobligated Balance (Item 5 less Item 6)	5,962.09
8.a. Cost Sharing Information – Grantee Contribution This Period	13,565.59
b. % of Total Project Costs (Item 8a divided by total of Items 1 and 8a)	% 23.0
9.a. Interest/Income (enclose check)	-0-
b. Other Refundable Income (enclose check)	-0-

10. Remarks

I hereby certify that this report is true and correct to the best of my knowledge, and that all expenditures reported herein have been made in accordance with appropriate grant policies and for the purposes set forth in the application and award documents.

James C. Powers
Dr. J. C. Powers — Professor — 2/13/80
(Date)

David V. Welch
SIGNATURE OF INSTITUTION OFFICER — DATE

David V. Welch, Manager, Grants & Contracts Acctg.
404/894-4624

HEW-489 (REV. 10/73)

REPORT OF RESEARCH GRANT EXPENDITURES

OMB 85R0219.