Project #: G-33-550  Cost share #:  
Center #: 10/11-6-P5238-2A0  Center shr #:  
Contract#: 5 F31 AG05614-02  Mod #:  
Prime #:  
Subprojects ?: N  
Main project #:  
Project unit: CHEMISTRY  Unit code: 02.010.136  
Project director(s): POWERS J C CHEMISTRY  (404)894-4038  
Sponsor/division names: DHHS/PHS/NIH  
Sponsor/division codes: 108  
Award period: 931001 to 940930 (performance) 941230 (reports)  
Sponsor amount  
Contract value  11,491.00  
Funded  11,491.00  
New this change  
Total to date  11,491.00  
Cost sharing amount  
0.00  
Does subcontracting plan apply ?: N  
Title: ACETYLCHOLINESTERASE INHIBITORS AND DEMENTIAS  
Sponsor technical contact  
DR. CARL BANNER  
(301)496-9350  
NATIONAL INSTITUTE ON AGING  
National Institutes of Health  
5333 WAESTBARD AVE  
BETHESDA, MD 20816  
Sponsor issuing office  
JOANNE COLBERT  
(301)496-1472  
NATIONAL INSTITUTE ON AGING  
Grants & Contracts  
Gateway Building  
7201 WISCONSIN AVE., SUITE 2N-212  
BETHESDA, MARYLAND 20892  
Security class (U,C,S,TS) : U  
Defense priority rating :  
Equipment title vests with:  
Sponsor  
GIT X  
NONE AUTHORIZED  
Administrative comments -  
INITIATION OF INDIVIDUAL NATIONAL RESEARCH SERVICE AWARD - 2ND YEAR.
GEORGIA INSTITUTE OF TECHNOLOGY
OFFICE OF CONTRACT ADMINISTRATION

NOTICE OF PROJECT CLOSEOUT

Closeout Notice Date 10/24/94

Project No. G-33-550__________
Center No. 10/11-6-P5238-2A0_

Project Director POWERS J C__________
School/Lab CHEMISTRY____

Sponsor DHHS/PHS/NIH/NATL INSTITUTES OF HEALTH____________________

Contract/Grant No. 5 F31 AG05614-02__________ Contract Entity GTRC

Prime Contract No. ________________________________

Title ACETYLCOLINESTERASE INHIBITORS AND DEMENTIAS____________________

Effective Completion Date 940930 (Performance) 941230 (Reports)

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Comments

***NOTE: USE DHHS FORM FOR PATENT ***

Subproject Under Main Project No. _________________

Continues Project No. _________________

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NOTE: Final Patent Questionnaire sent to PDPI.
**ID: NATIONAL RESEARCH SERVICE AWARD CONTINUATION APPLICATION**

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**TOTAL AWARD PERIOD**

- From: 10/01/92
- Through: 09/30/96

**REQUESTED BUDGET PERIOD**

- From: 10/01/93
- Through: 09/30/94

**APPLICANT** (Name and address, street, city, state, zip code)

STARKS, KENNETH M
GEORGIA INST OF TECHNOLOGY
SCH OF CHEMISTRY & BIOCHEM
ATLANTA, GA 30332-0400

**SPONSORING INSTITUTION** (Name and address, street, city, state, zip code)

GEORGIA TECH RES CORP
GEORGIA INST OF TECH
ATLANTA, GA 30332

**TITLE OF RESEARCH TRAINING PROPOSAL**

ACETYLCHOLINESTERASE INHIBITORS AND DEMENTIAS

**HUMAN SUBJECTS**

- Exemption #
- IRB Approval Date

**VERTEBRATE ANIMALS**

- Yes... IACUC Approval Date

**RAINING SITE(S)** (Organizations and Addresses)

- 3546 Heritage Valley Rd.
- Atlanta, Georgia 30331

**APPLICANT CERTIFICATION AND ACCEPTANCE**

I certify that the statements herein are true, accurate, and complete to the best of my knowledge, and I agree to comply with Public Health Service terms and conditions if an award is issued as a result of this application. I certify that I have read the National Institutes of Health Assurance, and that I will abide by the Assurance if an award is made, and that the award will not support residency training. Willful violation of false information is a criminal offense (U.S. Code, Title 18, Section 1001). I am aware that any false, fictitious, or fraudulent statement may, in addition to other remedies available to the Government, subject me to civil penalties under the Program Fraud Civil Remedies Act of 1986 (45 CFR Part 79).

**SIGNATURE**

DATE 07-15-93

**COMPLETE AND RETURN APPLICATION BY 1 AUGUST 1993**
13. APPLICANT SUMMARY OF ACTIVITIES

A. CHANGES

There have not been any alterations in the focus of the research or the training plan of the student since the original proposal.

B. PROGRESS REPORT

During the past academic year at Georgia Tech I have been working diligently to complete both the synthesis of specific low molecular weight inhibitors of acetylcholinesterase as well as the curriculum of required courses set forth by Georgia Tech. I have satisfied the course requirements for matriculation by successfully completing courses in Advanced Physical Organic Chemistry, Macromolecular Structure, and Immunochemistry and I am a student in good standing.

The research plan that I proposed called for the synthesis of derivatives which would improve the specificity, potency and hydrophobicity of compound 1 which has been previously synthesized by the Powers group. I have made significant progress towards completing the goals that I have set and I expect to finalize the synthesis of several compounds for testing before the end of the summer quarter 1993. Compound 2 has been synthesized and shown to inhibit acetylcholinesterase in vitro.

The first step in the synthesis of these analogs requires a 3-hydroxypicolinaldehyde (4) which can be synthesized by a variety of methods. I have made this compound several times by using a large excess of MnO₂ and acetone at room temperature. The yields for this reaction are typically low with the highest yield being approximately 51%. This reaction has however proven to be unreliable at times because of the sensitivity to inactivation of the MnO₂ catalyst. I am interested in carrying out other literature procedures to synthesize this aldehyde that do not require MnO₂ but proceed by rearrangement and hydrolysis of 3-hydroxypicolinaldehyde triacetate (3). This alternative should provide a better yield as well as improve the repeatability of the experiment.

Phenyl semicarbazide derivatives (6) have been synthesized by reacting the appropriately substituted phenyl isocyanate (5) with a 10 fold excess of hydrazine monohydrate in methylene chloride at zero degrees. The products are easily isolated by suction filtration. This method has produced several substituted phenylsemicarbazides which include: 2,6-dichloro, 4-chloro, 2,6-dimethyl, 2,6-difluoro, 4-methoxy, 4-nitro, and 3-nitro. The yields for these reactions are typically in the low eighties with the highest yield being 88% obtained from the 2,6 dimethyl derivative.
The synthesis of pyridine semicarbazones (7) and hydrazides (compound 2 is an example of a hydrazide) have been carried out by reacting the appropriate phenylsemicarbazide or phenylhydrazide with the pyridine aldehyde in ethanol at reflux temperature for one hour. The clean products are isolated by suction filtration. Yields for this reaction are generally 70-75%. The Schiff base analogs are then carbamylated by using pyridine and dimethylcarbamyl chloride at room temperature for 14 hours with subsequent methylation of the pyridinal nitrogen by methyl iodide then ion exchange by silver chloride. The carbamylation reaction yields up to 85% of its products while the yield for the salt ion exchange has not been determined.

C. RESEARCH TRAINING PLAN

During the upcoming budget period I will be synthesizing more derivatives of compound 1 as potential acetylcholinesterase inhibitors. I will also be testing my compounds for their effectiveness by in vitro assays that have been worked out by the Power's group. All of the compounds that are deemed successful will be tested in animal models by a team of behavioral pharmacologists at The Medical College of Georgia. In the future we plan to submit the results of our joint investigations for publication.
Proposed reaction scheme:

\[ \text{OH} \xrightarrow{\text{H}_2\text{O}_2} \text{O} \]

\[ \text{Ac}_2\text{O} \]

\[ \xrightarrow{\text{H}_2\text{O}} \]

\[ \text{OAc} \]

\[ \text{OH} \]

\[ \text{CH}_2\text{Cl}_2 \]

\[ \text{EtOH} \]

\[ \text{DMF} \]

\[ \text{CH}_3 \text{Cl} \]

\[ \text{PHS 416-1 (Rev. 4/89) Page 4} \]
A. Evaluate the quality of the training (including academic work) and research progress made by the fellow during the past year. Include performance on cumulative and qualifying examinations, if applicable.

B. Human subjects and vertebrate animals (see instructions).

15A. Ken received low grades in his courses in the fall quarter of 1992 and was placed on probation during the winter quarter of 1993. During the spring quarter of 1993, he received good grades in several courses and returned to normal graduate student status (good standing). He has decided to complete a Masters degree in chemistry before continuing with his graduate studies. He has now completed all of the necessary course work and must simply complete enough research work for a Masters thesis. I expect Ken to complete sufficient research for a Masters thesis sometime during the next year.

Ken's research progress to date has been satisfactory, but quite slow. He works consistently in the laboratory and is enthusiastic and interested in his research problem. He is very interested in the research field and is reading constantly in the area. However, he had taken a long time to learn synthesis skills and is still learning how to effectively perform techniques as simple as the recrystallization of reaction products. As he progresses, Ken is slowly making more of these skills part of his repertoire. He is beginning to work out the synthetic methods for the synthesis of the proposed acetylcholinesterase inhibitors (see progress report). He has now synthesized his first final inhibitor structure. Since he plans to synthesize a number of closely related derivatives to complete his thesis work, the remainder of the compounds should be more easily and quickly synthesized now that he has worked out the methods with the first derivative.

15B. No human subjects or vertebrate animals are involved in this research.

CERTIFICATION. We, the undersigned, certify that: (a) the information herein, including involvement of Human Research Subjects, Recombinant DNA Research, and Vertebrate Animals, are true, accurate, and complete to the best of our knowledge; (b) if this application results in an award, appropriate training, adequate facilities, and supervision will be provided; and (c) we will comply with the Public Health Service terms and conditions of award. A willfully false certification is a criminal offense (U.S. Code Title 18, Section 1001). We are aware that any false, fictitious, or fraudulent statement may, in addition to other remedies available to the Government, subject us to civil penalties under the Program Fraud Civil Remedies Act of 1986 (45 CFR Part 79).

Sponsor

Department Head

Official Signing for Sponsoring Institution

James C. Powers

Laren M. Tolbert

Janis L. Goddard

894-4038

894-4002

404/894-4817

7/27/93

7/29/93

7/30/93
14. The fellow was given a supplement of $295/month from a seed grant from the Georgia Tech/Medical College of GA seed grant program. The amount is the difference between the normal stipend of $13,000/year for chemistry graduate students and the amount provided by the NIH fellowship.
INDIVIDUAL NRSA CONTINUATION APPLICATION
CHECKLIST
Applicant completes Section I. Sponsor completes Section II.

Section I—Applicant

ASSURANCES/CERTIFICATIONS

The following assurances/certifications are made by checking the appropriate boxes and are verified by your signature in Item 12 on the CE PAGE of the application. Descriptions of individual assurances/certifications begin on page 2 of the application instructions.

Debarment and Suspension: [X] No [ ] Yes (Attach explanation)
Delinquency Federal Debt: [X] No [ ] Yes (Attach explanation)

Section II—Sponsoring Institution

The following assurances/certifications are made by checking the appropriate boxes and verified by the signature of the Official Signing for Sponsoring Institution in Item 16. Descriptions of sponsoring institution assurances/certifications begin on page 7 of the application instructions.

Human Subjects (Complete Item 7 on the Face Page) [ ] Full IRB Review [ ] Expedited Review

Use of Human Subjects: [ ] Change [ ] No Change Since Previous Submission

Vertebrate Animals (Complete Item 8 on the Face Page)

Use of Vertebrate Animals: [ ] Change [ ] No Change Since Previous Submission

Debarment and Suspension: [X] No [ ] Yes (Attach explanation)

Misconduct in Science (Form PHS 6315) [X] Filed [ ] Not Filed

If filed, date of Initial Assurance or latest Annual Report: 1/14/92

Civil Rights (Form HHS 441) [X] Filed [ ] Not Filed

Handicapped Individuals (Form HHS 641) [X] Filed [ ] Not Filed

Sex Discrimination (Form HHS 639A) [X] Filed [ ] Not Filed

Age Discrimination (Form HHS 680) [X] Filed [ ] Not Filed
Final Report

National Research Service Award to

Kenneth M. Starks

F31 GM15612

October 1, 1992 to September 1, 1994

Research Supervisor

James C. Powers
School of Chemistry and Biochemistry
Georgia Institute of Technology
Atlanta, GA 30332-0400
(404)894-4038
Research Summary

The carbamate 1-(methyl-3-(N,N-dimethylcarbamoyloxy)-2-pyridylmethylene)-4-(4-phenyl)diazinecarboxamide chloride (MHP 133) is the parent for a new class of pyridinium salts which inhibit acetylcholinesterase (AChE) *in vitro* as well as *in vivo*. Fourteen new derivatives of MHP 133 have been synthesized with the intention of improving their hydrophobicity while maintaining their propensity to inhibit acetylcholinesterase. Upon prolonged incubation with AChE, the pyridinium salts exhibit progressive time-dependent inhibition according to first order kinetics with $k_{obs}/[I]$ values ranging from 3 to 345 M$^{-1}$s$^{-1}$. The enzyme didn't regain any activity after prolonged incubation with the inhibitors (1 day). The partition coefficients for each inhibitor were evaluated in octanol/water in order to determine their hydrophobic character as hydrophobicity is a key prerequisite for crossing the blood brain barrier.
Patent Applications:


Publications: