THE UNIVERSITY of MISSISSIPPI

DEPARTMENT OF BIOMOLECULAR SCIENCES

Divisions of Environmental Toxicology, Medicinal Chemistry, Pharmacognosy, and Pharmacology

Graduate Student Handbook

Departmental Faculty Approval - August 24, 2015

SOP Vision

We are a highly respected community of learners, educators, scientists, and practitioners whose innovative achievements position us as leaders in improving health and wellness.

SOP Mission

The mission of the University Of Mississippi School Of Pharmacy is to improve health, well being, and quality of life of individuals and communities through educating students, pharmacy practitioners and pharmaceutical scientists, conducting research, and engaging in service.

We will accomplish this by providing:

- Innovative models of practice, with an emphasis on underserved populations and those with health disparities.
- Quality education for current professional and graduate students.
- Quality post-graduate training opportunities.
- Quality continuing professional development opportunities.
- An environment that promotes the generation and dissemination of new biomedical knowledge and technologies through collaborative and interdisciplinary research.
- Opportunities for discovery and dissemination of knowledge of natural products and novel pharmaceuticals.
- Leadership in the development and implementation of advanced pharmacy practice models.
- Service to internal and external stakeholders and the general population.
- Opportunities to conduct practice-based and translational research to address health disparities.

Members of the BMS Faculty

Division of Environmental Toxicology

Dr. Deborah Gochfeld
Dr. John Rimoldi
Dr. Marc Slattery
Dr. Kristie Willett

Division of Medicinal Chemistry

Dr. Stephen Cutler, Chair
Dr. David Colby
Dr. Robert Doerksen
Dr. Christopher McCurdy
Dr. John Rimoldi

Division of Pharmacognosy

1° Appointment	2° Appointment
Dr. Mark Hamann	Dr. Xing-Cong Li
Dr. Dale Nagle	Dr. Alice Clark
Dr. Marc Slattery	Dr. Ikhlas Khan
Dr. Jordan Zjawiony	Dr. Shabana Khan
	Dr. David Pasco
	Dr. Samir Ross

Division of Pharmacology

1° Appointment	2° Appointment
Dr. Tracy Brooks	Dr. David Allen
Dr. Zia Shariat-Madar	Dr. Ameeta Agarwal
Dr. Joshua Sharp	Dr. Asok Dasmahapatra
Dr. Kristine Willett	Dr. Mark Hamann
	Dr. Christopher McCurdy
	Dr. Babu Tekwani
	Dr. Karen Sabol
	Dr. Kenneth Sufka
	Dr. Larry Walker

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INTRODUCTION

This Handbook is intended to acquaint graduate students with applicable policies and requirements relating to study for the Doctorate and Master's degrees in the Department of BioMolecular Sciences and to inform them of the procedures that must be followed in accordance with Graduate School and Departmental Regulations.

This Handbook summarizes the general requirements for the Doctoral and Master's Degrees of Pharmaceutical Sciences with an emphasis in Environmental Toxicology, Medicinal Chemistry, Pharmacognosy, or Pharmacology to serve as a guide for those students who desire to obtain these advanced degrees.

It is in the best interests of the student to acquaint themselves with the location of the Graduate School, and **develop and maintain a friendly and professional relationship with the staff therein**. It is the student's responsibility to keep up-to-date on changes in Graduate School requirements and policies and procedures relating to the degree he/she is pursuing. The Department's Graduate Coordinators can also be of assistance in many instances. Please see http://gradschool.olemiss.edu/ for resources and information.

This Handbook is to be used as a supplement to the Bulletin of the Graduate School and other applicable information, which may be obtained from the Graduate School office.

ACADEMICS

GENERAL INFORMATION

REGISTRATION

Each semester, each student needs to register for courses during the registration period. The student should secure approval for the schedule of courses by either their Major Professor (if one has been selected) or Graduate Coordinator (if a Major Professor hasn't been selected). New graduate students should obtain registration instructions from their Graduate Coordinator prior to, or at the beginning of, their first period of enrollment. Continuing or re-admitted students are encouraged to take advantage of the priority registration periods.

Guidelines for Graduate Student Support

The faculty of the Department of BioMolecular Sciences is committed to financially supporting all graduate students at the highest level of support. Financial assistance is dependent upon the availability of funds for this purpose and the student's satisfactory progress toward fulfillment of departmental requirements for the degree sought.

Departmental faculty review all graduate students at least once a year and always at the end of each academic year to determine if satisfactory progress is being made and to guide students towards meeting all requirements and expectations.

As a general rule, financial support will be provided to those students making satisfactory progress for a maximum period of three years for students in the M.S. program and of five years for students in the Ph.D. program.

There are a limited number of graduate teaching assistantships and graduate research assistantships available. Entering students are awarded such assistantships on a competitive basis, while the departmental faculty members base the continuing students' receipt of an award on a review of their scholarly activity and progress toward the degree's objective. Awards are generally made for one calendar year. Students awarded teaching or research assistantships are required to be present during normal working hours at a minimum (weekdays from 8:00 am to 5:00 pm). Students with multiple absences are subject to the loss of their awards.

Students awarded teaching or research assistantships are considered full-time students and may not hold jobs outside of the department. Students taking employment outside of the department automatically forfeit their assistantship.

TIME IN RESIDENCE

The average doctoral student requires four to five years to complete the Ph.D. degree requirements (no student may exceed seven years in their attempt to attain the Ph.D.). As a general rule, after seven years a doctoral student will be dismissed from the graduate program. The average M.S. student requires two to three years for completion of degree requirements (may not exceed four years). As a general rule, after four years a Master's student will be dismissed from the graduate program. Departmental financial support will

be terminated at the end of the 5th year for Doctoral students and the end of the 3rd year for Master's students.

APPLICATION FOR A DEGREE

As described in the Graduate School catalog, a student is expected to submit an application for a degree during the last semester of resident enrollment. A student's application must be formally approved by the Departmental faculty and the Graduate School prior to the beginning of the semester in which the degree is awarded and must meet the requirements of the University catalog under which the student was admitted or readmitted to the degree program. All students planning to receive their graduate degrees must be enrolled for at least 3 hours during the fall or spring semester in which they successfully defend their theses or dissertations. Students planning to graduate during the summer must be enrolled for at least one hour. It is the responsibility of the student to apply for the degree in a timely fashion.

VACATION AND WORK SCHEDULE

Students receiving assistantships from the department are expected to be present at their assigned desks/laboratories each workday when not in classes (during normal 8 am to 5 pm work hours) as required in the terms of the financial support. Most students find it helpful to work extended hours including nights and weekends, and the department provides encouragement towards such activities by providing 24 hour safe access to departmental facilities to those wishing to take advantage of the opportunity.

When it is required for a student to be absent from duties for an extended period during the day, please notify the appropriate faculty advisor. It is requested that all students notify their advisor when they expect to be away from the department for extended periods.

Holidays are set by the University and can be located online:

http://www.olemiss.edu/hr/_files/benefits/holidays.pdf

Graduate students are required to work normal staff hours and days, including spring break, working days before the Thanksgiving break, after fall and spring semesters, and the summer breaks.

Students awarded a departmental assistantship are provided with a two-week vacation period each year. It is vital that students inform the appropriate faculty advisor of their plans to take vacation time, and discuss it with them, as early as possible to ensure that their progress to completion of degree requirements not be impeded. Students out for >4 weeks in a 1 year period will have to seek re-admission through departmental faculty – prior approval can be obtained, and we suggest that it be sought prior to finalizing travel plans.

Any personal or sick leave must be recorded on Annual Leave Forms and filed with the administrative assistant for students. Failure to submit such forms can result in a forfeiture of a student's assistantship. All University and federal guidelines on leave (including FMLA, maternity http://gradschool.olemiss.edu/wp-content/uploads/sites/36/2015/04/Parental-Leave-Policy.pdf, etc.) will be followed.

ACADEMIC AND RESEARCH PERFORMANCE

A graduate student must maintain an overall GPA of at least 3.0 to graduate. The Graduate School will place a student whose GPA falls below 3.0 in any given semester on academic probation. A student on probation who earns less than a 3.0 GPA the following semester will be dismissed from the program.

Students have the initial responsibility to recognize when they are having academic difficulties and are expected to initiate steps to resolve the problem. A student can be dismissed from the program not only for failure to maintain an adequate grade point average, but also for such reasons as unsatisfactory progress toward a degree as defined by the department or division, inability to pass a comprehensive examination, failure to prepare or to defend a thesis or dissertation in a satisfactory manner or complete thesis or dissertation work in an acceptable amount of time. Termination due to inadequate academic progress is a decision made by the department

GRADUATE PROGRAM COORDINATORS

Each Division in BioMolecular Sciences has a Graduate Program Coordinator (GPC). The GPC will be your first point of contact with our graduate program, and will continue to serve in an advising capacity throughout your tenure. GPCs also serve as liaisons between you and the UM Graduate School. The GPC for each of the divisions are:

Dr. Zia Shariat-Madar, Pharmacology

Dr. John Rimoldi, Medicinal Chemistry

Dr. Marc Slattery, Pharmacognosy

Dr. Kristie Willett, Environmental Toxicology

SELECTION OF A MAJOR PROFESSOR

Students will be required to select a major professor by the end of their first semester in the program.

CURRICULUM

Each Division has curricular requirements: See Graduate Catalog

M.S. AND PH.D. CORE CURRICULA

a. A core BMS601 class titled “Graduate Student Survival Strategies”, to be taken by all first year graduate students in BMS (offered in the Fall semester).
b. Seminar BMS641 (taken by students presenting) or BMS643 (taken by students NOT presenting). Graduate students must be enrolled for one of these courses each academic semester (Fall and Spring).

c. Two core classes are required within each division as detailed below.

i. Environmental Toxicology

- PHCL 675 (Principles of Pharmacology and Toxicology I, 4 credits)
- PHCL 547 (Introduction to Environmental Toxicology, 2 credits)

ii. Medicinal Chemistry

- MEDC 501 (Advanced Medicinal Chemistry I, 3 credits)
- MEDC 502 (Advanced Medicinal Chemistry II, 3 credits)

iii. Pharmacognosy

- PHCG 620 (Bioassays in Natural Products Research, 3 credits)
- PHCG 627 (Natural Product Chemistry I, 3 credits)

iv. Pharmacology

- PHCL 563 (Fundamentals of Pharmacology I, 4 credits)
- PHCL 564 (Fundamentals of Pharmacology II, 4 credits)

d. Classes beyond those to complete the 24 (M.S.) or 36 (Ph.D.) credit hours are to be decided by the GPC in the first semester/year, and then by the advisor and committee past that.

Division Classes available to complete 24-hour (M.S) or 36-hour (Ph.D.) requirements

1. PHCL 676 (Principles of Pharmacology and Toxicology II, 4 credits)
2. PHCG 628 (Natural Product Chemistry II, 3 credits)
3. PHCG 635 (Introduction to Molecular Cell Biology, 3 credits)
4. PHCL 661 (Advanced Physiology, 4 credits)
5. PHCL 669 (Physiological Chemistry, 4 credits)
6. PHCL 675 (Principles of Pharmacology and Toxicology I, 4 credits)
7. BISC 504 (Biometry, 3 credits)
8. PSY 603 (Quantitative Methods, 3 credits)
9. MEDC 711 (Drug Action and Design I, 3 credits)
10. MEDC 712 (Drug Action and Design II, 3)
11. MEDC 713 (Drug Action and Design III, 3)
12. MEDC 714 (Drug Action and Design IV, 3)
13. MEDC 709 (Drug Action and Design V, 3)
14. MEDC 718 (Drug Action and Design VI, 3)
15. MEDC 720 (Drug Action and Design VII, 3)

Elective Requirements: Each division has its own requirements for additional elective hours.

ENROLLMENT IN CLASSWORK

Students are responsible for enrolling in classes each semester and summer term.

Students should register for 12-semester credit hours in the fall and the spring semesters, of which only 9 credit hours can be graded with the other 3 as un-graded courses, e.g., dissertation or thesis hours (per Graduate School requirement or there will be a loss of financial scholarships). Students should consult with the Graduate Coordinator as whether to register for the full summer session each year. In general students should not register for intersession or the individual summer sections. Students are also required to register for 6 hours of dissertation or thesis in the full summer term.

A student's advisor should approve all coursework before registration. For students without advisors, the graduate coordinator should approve all coursework.

When possible, students should always check with the "instructor of record" of a course before enrolling. In some instances, the course may not be offered or the student may not have the appropriate background for enrolling in the course.

SEMINAR REQUIREMENTS

a. Students are to enroll in one of these two courses each semester:
    - Students presenting: BMS 641 (graded)
    - Students not presenting: BMS 643 (P/F)
b. All BMS students are expected to attend both the student seminars and the outside speaker seminars (as their class schedule allows)
    - If a non-presenting student (enrolled in BMS 641 or BMS 643) is late for or misses a student seminar, penalties will be ascribed according to the course syllabus.
c. Students will be scheduled according to seniority (more senior students go first), alphabetically by last name within each year's students.
d. Titles and 250 word abstracts are due to the departmental secretary no later than **one week** before the scheduled seminar to the departmental secretary, along with a title. This abstract must also be submitted to the course instructor via Blackboard (each seminar course will have a BBV page).
e. First year students give a 20-25 minute literature presentation following the format chosen by the faculty seminar organizer. Two first year students will present in a one-hour slot.
f. From year 2 and until defense, seminars will be on student's research progress.
    - In year 2, the research presentation will be 30 minutes.
    - Two students will present in a one-hour slot.
g. One presentation (generally in the third year) should be the prospectus defense.
h. The only valid excuse for not presenting a seminar in a semester for which a student is scheduled is if they are defending their thesis that semester, or if they are not on campus (e.g., participating in an internship or off-site field work).
i. BMS 641 grades are calculated as follows:
    - The average grade from all faculty members present are calculated
        1. A = 4.0, A- = 3.7, B+ = 3.3, etc.

- Grades from staff and students that fall within 2 standard deviations of the faculty average above are then added into the grade calculation and a new grade average is calculated. The overall percentage will be assigned a letter grade according to the course syllabus.

THESIS AND DISSERTATION PROSPECTUS/ADVISORY COMMITTEES

a. A thesis/dissertation committee should be identified, and a first committee meeting held as soon as possible. This is to be no later than the end of the student's second semester for an M.S. student, and no later than the end of the first full academic year for a Ph.D. student.
b. A Ph.D. committee consists of the student's advisor, **two** other faculty members from the student's major *division* (adjunct faculty included) and **one** member from an external division/department. A M.S. committee consists of the student's advisor, **one** other faculty member from the student's major division, and **one** external member. Additional faculty members can be added to the committee if needed.
    - Membership of the committee is recommended by the student, in consultation with his or her major advisor, and then submitted to the departmental chair for approval.
    - For the thesis and dissertation committees the student's major professor will serve as chair (or co-chair if the major professor is at the assistant professor rank).
    - Of the divisional faculty, one must have a primary appointment in that division (see page 3 for a comprehensive list).
c. Once a committee is appointed, the student is responsible for filling out the required form and obtaining signatures by the major professor and the departmental chair. All sections of the form are to be completed. Copies of the completed form should be submitted to the major advisor, the graduate coordinator, the departmental secretary, and the original should be submitted to the graduate school. The form can be found at: http://gradschool.olemiss.edu/current-students/forms-and-manuals-library/
d. The committee will receive the Annual Student Activity Reports (see above), and they will minimally meet with the once per academic year, recommended to be early each summer within one month of the annual report submission.
e. The students must have a committee meeting within 6 months of their defense date.
f. Committee meetings are minimally to include:
    - 10-15 minute student presentation with a research update
    - Review of the Annual Report
    - Discussion of plans for the coming year, as well as problems encountered to date
g. ORP committee: This will be the student's Ph.D. committee, plus additional faculty members, as need, to ensure that each of the three other divisions will be represented. The other ORP committee members should be invited by the student and should be present for the ORP defense (described below).

ANNUAL STUDENT ACTIVITY REPORT

a. All graduate students (M.S. and Ph.D.) are required to submit annual reports (due May 15th of each year) to their advisor (to be signed within 2 weeks), and to their committee members in preparation for an annual committee meeting. Reports should be compiled according to a rubric containing:
    - Status of all degree requirements, such as:
        1. An ongoing record of classes taken (each division will have its own table of classes) and grades achieved
        2. Yearly seminar given, with a statement of plans to improve/items to address
        3. ORP status. If completed: grade achieved and passing date
        4. Prospectus projected or completed date
    - Major research accomplishments
    - Research goals for the coming year
    - Papers submitted/published
    - Posters and seminars presented
b. All signed reports, including advisor evaluations and department chair signatures, are to be left on file with the advisor, the divisional GPC, and the Department Chair.

QUALIFYING EXAMS/ORPs

a. An Original Research Proposal (ORP) is a requirement that serves as the official University of Mississippi assessment of Ph.D. candidacy qualifications.
b. By the first semester of the student's third year (or by the second semester of the third year for a student admitted in Spring), the ORP should be completed.
c. The ORP should be taken as a 1-credit class (to be setup as a **BMS** class for each division), and will be graded (A-F grading scale).
d. The format is recommended to be a full NIH-R21/NSF/EPA-STARR major grant[1], on a project not directly related to the student's research. The full and completely edited grant is due to the student's ORP committee **two full weeks** before the presentation.
    - Before embarking on writing the full grant, a ~3 pg abstract should be submitted to the major divisional faculty and department chair for comments and approval.
e. The ORP presentation (not related to a student seminar) is to be ~20-30 minutes. All departmental faculty members are requested to be present, in addition to the student's ORP committee as described above. The remainder of the "ORP time" (students should reserve a room for 3 hours) is to be a rigorous, oral exam-type questioning from the ORP committee faculty.
f. Grading of ORP should follow the appropriate organizational (NIH/NSF/EPA) rubric and guidelines
g. If the ORP is not successfully completed, it may be re-attempted within **no more than** 60 days

- The second ORP will be an extensive revision of the first, either in written form or in oral form as decided by the ORP committee, addressing all of the faculty's concerns.

ADMISSION TO CANDIDACY FOR THE DOCTORATE

Once a student passes the ORP requirement, he/she will apply to be admitted into degree candidacy.

After the successful completion of the ORP requirement, the student is responsible for filling out the required form and obtaining signatures by the major professor and the departmental chair. In this department the ORP is considered the comprehensive examination. Copies of the form should be submitted to the major advisor, the graduate coordinator, the departmental secretary, and the original should be submitted to the graduate school. The form can be found at:

http://gradschool.olemiss.edu/wp-content/uploads/sites/36/2013/11/gs5_auth_to_sit_for_exam.pdf

http://gradschool.olemiss.edu/wp-content/uploads/sites/36/2013/10/gs51_admission_to_doctorate_degree.pdf

This notification to the Graduate School that a doctoral student has successfully completed all portions of ORP (comprehensive examination) is required for admission to candidacy. After admission to candidacy, enrollment must be continuous.

PROSPECTUS

a. A "pre-prospectus" is to be completed before the full prospectus (e.g. before the start of the student's 6th semester, provided that the ORP was successfully completed). It should consist of a list of committee members and an abstract (1 page)
b. The prospectus should follow NIH-R21/NSF/EPA-STARR major grant[1] appropriate formats, including all sections, e.g. abstracts, specific aims, bigger impact, biosketch, etc., as appropriate.
c. The prospectus plan should be presented and defended in a 45-minute seminar (generally a Ph.D. student's 3rd year seminar) to be followed immediately by a committee meeting to discuss the plans and presentation.

Note: NIH grants & funding- http://grants.nih.gov/grants/funding/r21.htm
NSF funding - http://www.nsf.gov/pubs/policydocs/pappguide/nsf15001/gpg_index.jsp
EPA grants and funding- http://www.epa.gov/ncer/rfa/
Graduate School Form – http://gradschool.olemiss.edu/wp-content/uploads/sites/36/2013/11/gs6_dissertation_prospectus_procedure.pdf

DISSERTATION AND THESIS PREPARATION

The Graduate School's *Thesis and Dissertation Preparation Manual* can be found online at: http://gradschool.olemiss.edu/wp-content/uploads/sites/36/2013/11/Theses-and-Dissertations-Manual_final.pdf

The dissertation or thesis in the Department of BioMolecular Sciences will be a high quality compilation of the student's research, should include a survey or introduction of the current knowledge base in the research area, and should adhere to the format and style suggested by the Graduate School. As you write, remember the steps of the scientific method are to:

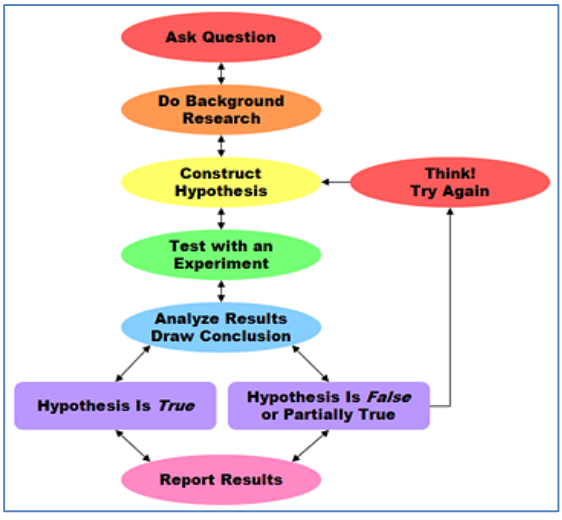
- Ask a Question;
- Do Background Research;
- Construct a Hypothesis;
- Test Your Hypothesis by Doing an Experiment;
- Analyze Your Data;
- Draw a Conclusion; and
- Communicate Your Results.

In a thesis/dissertation these points generally fit into a standard format:

1. Introduction;
2. Hypothesis;
3. Experimental;
4. Results;
5. Discussion;
6. Appendix;
7. Vitae

Copies of older thesis and dissertations are available in the departmental office and can be checked out for short periods of time.

Not only is the dissertation/thesis the compilation of your research, it is also the experiments and analyses that will always be proof of the conclusion to your hypothesis. In this vein, it greatly behooves the student to include as much raw data as possible in the dissertation. Such data is commonly inserted in the form of an appendix or set of appendices. Common data elements include ^{1}H, ^{13}C NMR spectra, UV, IR, LCMS, HRMS, elemental analysis, computer programs or macro's used, spreadsheets, Western blots, PCR gels, IHC images, etc. Your research must be able to withstand rigorous critique by the scientific community, and the standard in the business is that a thesis/dissertation/manuscript must contain all of the information needed to repeat the experiments and prove the hypotheses. A basic rule of thumb is that the more data, the better. The student should consult closely with his/her major professor and/or advisory committee on what data is necessary or optional.



A thesis/dissertation is considered a rough draft until the required number of advisory committee members officially signs it.

The final draft of the dissertation/thesis should contain all scientific data, be free of spelling, grammatical, and other errors, and meet all formatting regulations. This draft must be provided to the advisory committee members **2 full weeks in advance** of the final oral examination. The candidate should provide an electronic copy and hard copy to the members of the advisory committee, and as a courtesy, an electronic copy to all members of the departmental faculty.

Special notes:

a. Students may start writing their dissertations after their committee gives permission (majority of committee must agree, including advisor).
b. Written dissertations are due to the whole committee at least 2 full weeks prior to the scheduled defense date
c. Defense of dissertation work will be presented in a 1-hour seminar, with questioning open to the public, and then further questioning by the committee only in a closed session.

FINAL ORAL EXAMINATION/DEFENSE

A minimum of two hours should be set aside for the oral examination defense.

The student is asked to present a 40-50 minute overview of their hypothesis, approach, experiments, results, conclusions, future directions, and acknowledgements. Generally a few minutes for questions and answers are allowed after which the general audience will be dismissed and the advisory committee will begin the oral examination of the candidate. As a courtesy, non-advisory committee departmental faculty members in attendance for the presentation are asked if they would like to participate in the examination.

At the time of the final oral examination the advisory committee members will have already approved the candidates overall research conclusions; however, a high quality presentation and the level of defense expected of a Ph.D. are required for the successful completion of the examination.

In general, members of the advisory committee will provide the candidate with corrections to be made to the rough draft of the thesis/dissertation. In general, all corrections must be acceptable to the committee members before they will sign a final copy of the thesis/dissertation.

The student must bear the expense of reproduction of the dissertation or thesis

Arrangements must be made by the student for copies of the final dissertation/thesis to be provided to the committee members.

It is the student's responsibility to provide the advisor and the Department with a hardbound copy of the dissertation/thesis.

A fee for binding and microfilming theses/dissertations is required of all candidates/graduates; current fees can be found at:

http://gradschool.olemiss.edu/current-students/the-end-game-preparing-to-graduate/

HOODING POLICY

Getting a Master's or Doctoral hood is the pinnacle symbol of successfully completing a graduate dissertation. Thus, hoods will only be donned in graduation ceremonies after the successful defense of one's thesis, as described above. Under no circumstances is a student to submit their name to the Graduate School for hooding ceremonies. They must request that their names be sent on their behalf by the department, subject to approval. If a student wants to be hooded in the spring graduation ceremonies, documentation of a successfully completed oral dissertation must be submitted to the department chair no later than 5 pm on the last day of the spring semester.

ACADEMIC & RESEARCH ETHICS

Ethical behavior is an integral part of any professional status. Graduate students at the University are governed by the academic code of ethics, which can be found in the *University's M-Book*.

http://conflictresolution.olemiss.edu/m-book/

In addition, all members of the department must observe the highest research integrity. There are many publications related to research integrity; however, probably the most standard guide is Sigma Xi's *The Responsible Researcher: Paths and Pitfalls*, which can be found at:

https://www.sigmaxi.org/docs/default-source/Programs-Documents/Ethics-and-Research/responsible-researcher.pdf?sfvrsn=2

The ethical behavior of students is not an issue that is taken lightly in this department and those found guilty of such behavior will be summarily dismissed from the program. .

DEPARTMENTAL BUSINESS

GRADUATE STUDENT TRAVEL ON OFFICIAL BUSINESS

From time to time, students travel to scientific meetings. In general, travel is limited to the student presenting their scientific results (e.g., MALTO and ACCP) at regional conferences. Major advisors may choose to reimburse students' presenting research at a professional meeting. In addition, students should fill out a request for travel assistance from the Graduate School when appropriate. A sample of this form can be found online at:

http://gradschool.olemiss.edu/wp-content/uploads/sites/36/2013/10/2013_travel_form.pdf

Whenever a student is traveling to present a research paper, the travel request should be accompanied by an abstract of the work and evidence that the abstract has been accepted for presentation.

Such travel time does not count as vacation; however, the appropriate travel forms must be submitted at least two weeks prior to the travel date to ensure that the University approves all travel time and related expenses.

All travel requests must be approved prior to the trip. Reimbursement for expenses will not be made if travel is undertaken prior to receiving written travel request approval.
The departmental secretary can help with travel related issues and questions; however, the student should prepare well in advance of the trip.

The University can generally provide advances on some percentage of the expected expenses. In general, travel expense reimbursement is a relatively fast process (usually no more than one week). Reimbursement for expenses will not be made if expense statements and receipts are not turned in within thirty days.

TELEPHONE USAGE

Telephones are available for local calls only. An attempt has been made to have a phone where each student can be reached. For students, the Departmental number (915-7101) should be considered an emergency contact. No long distance calls will be made without permission from the Department Chair or Research Advisor (when applicable). Incoming calls should be kept to an absolute minimum. Personal cell phones should be kept with one's personal items in their desk area and should not be worn or used while at the hood or while engaged in other laboratory experiments.

COMPUTER USAGE

The Department maintains many computers in the laboratories, which should be treated with care and respect. These computers are NOT one's personal computer and should not be cluttered with personal data/programs unrelated to research activities. Although not required, it is suggested that students obtain a personal computer, preferably a laptop, for their class work and personal use. The Department does not take responsibility for damage or theft of personal computers.

The University provides a free web service to all students, staff and faculty on campus. There are many rules of use and limitations of this service. While most computer/internet rules are obvious, it is highly suggested that all students review the University's appropriate use policy at:

https://secure4.olemiss.edu/umpolicyopen/ShowDetails.jsp?istatPara=1&policyObjidPara=10642998

COPYING AND OFFICE SUPPLIES

The use of the photocopy machine for official departmental activities is open to students, faculty and staff of the department. The photocopying of a large number of documents (>50 pages) requires prior approval by a student's advisor and/or the departmental secretary.

Students must not use the departmental photocopier for personal copying.

Office supplies are available from the Administrative Office, located in Faser 413; however, students are allowed to make purchases for their lab with permission from their advisor.

PURCHASING

The Pharmacy Stockroom currently only keeps paper products and a few solvents but it is required to seek permission from your Research Advisor and get an account number in order to make a purchase there. For all other purchases, purchase recommendation forms are available in the Department Office. The student's advisor must sign all purchase recommendations before they will be processed. Written quotes must be obtained before the University will generate a purchase order. The student's advisor and/or departmental secretary can help new students get started with this procedure. It is vital that all receipts (generally found within the product shipment package) be given to the departmental secretary immediately on receipt of materials. Lost or misplaced packing slips will create significant problems so please pay close attention to this matter.

MAIL

The University prohibits the use of personal mailing and receipt through the campus system. The new post office service for all students is called Pak Mail at Crosby Hall, http://www.pakmailolemiss.com/

The Departmental secretarial staff on a daily basis will deliver official departmental mail. Student mail will be placed in their assigned boxes found in Faser room 436. Packages will be held in the departmental office for pick up to allow for the receivers to immediately provide the secretary with the afore-mentioned receipts.

SHIPPING HAZARDOUS MATERIAL

Mailing and shipping of hazardous and potentially hazardous materials is highly regulated, and can be a federal crime if abused. There are specific procedures for the mailing of ANY chemical or biological samples. Personnel from the department of Health and Safety will provide assistance with package selection, material classification and documentation. It is your responsibility to notify Health & Safety that you will be shipping a hazardous material, and to make an appointment for assistance in advance of shipping. Directions for such transport of potentially hazardous materials can be found on the University's Health and Safety website:

http://safety.olemiss.edu/

SHIPPING WITH DRY ICE

Dry ice represents a potential safety hazard. In particular, shipments involving dry ice are carefully monitored by the University of Mississippi for compliance with Federal Laws. If you plan to ship dry ice outside of UM, you must work with the Health and Safety office on campus. Keep in mind, this often requires 48 hours prep time, so plan accordingly.

RESEARCH

INTRODUCTION

Research is the search for knowledge or any systematic investigation to establish facts. Those who are able to use existing knowledge to conceptualize original scientific ideas for investigation to produce new scientific knowledge are known as scholars. Those who perform the investigations to produce the knowledge are researchers. The Ph.D. is a sacred degree given to those few who have the skills of a researcher and the potential to become a scholar.

Scientific research relies on the application of the scientific method to provide scientific information and theories for the explanation of the nature and the properties of the world around us. It makes practical applications possible.

This section of the handbook will provide merely the very basic information concerning the research rules and procedures in the Department.

SAFETY & SECURITY

Safety Training Requirements

The University mandates that no student may enter a chemical and/or biological research laboratory before successfully completing chemical and/or biological safety training. The training course must be completed within the first two weeks in residences. The departmental secretary can help schedule this training.

The laboratory can be a place of discovery and learning. However, by the very nature of laboratory work, it can be a place of danger if proper common-sense precautions aren't taken. While every effort has been made to eliminate the use of explosive, highly toxic, and carcinogenic substances from the experiments, which you will perform, there is a certain unavoidable hazard associated with the use of a variety of chemicals and glassware.

Most questions that one will have can be found online at the University's Health and Safety Department:

http://safety.olemiss.edu/safety-programs/chemical-safety/chemical-safety-manual/
http://safety.olemiss.edu/safety-programs/biological-safety/biological-safety-manual/

You are expected to learn and adhere to the following general safety guidelines to ensure a safe laboratory environment for both yourself and the people you may be working near.

The following safety training requirements apply to faculty, staff and graduate students working with biological, chemical, radiological materials or ionizing radiation producing devices on the Oxford Campus, as well as visiting investigators working with these materials under the supervision of trained University personnel on the campus.

- These training requirements will also apply to undergraduate students

when they are working in these areas in other than a regularly scheduled University course.

- Use of any of the materials/devices listed below requires the signing of the appropriate "Safety Agreement" form.
- You must present Proper Identification (Student ID, Social Security Number, Passport/Visa) at the beginning of the training sessions.
- Please call Health & Safety to schedule a date and time for your training session(s).
- Training classes start promptly at the Scheduled Time. If you are late, you will have to reschedule.
- http://safety.olemiss.edu/safety-training/safety-training-information/

1. **General Biological Safety Training**: All faculty, staff or graduate students working with biological materials are required to take the biological safety-training program and pass a written quiz on biological safety. Annual retraining is not required. Time required for the training is approximately 1.5 hours

2. **General Chemical Safety Training:** All faculty, staff and graduate students working with chemicals on the Oxford campus are required to take the chemical safety-training program and to pass a written examination on chemical safety. Annual retraining is not required. A special safety-training program is required for Physical Plant Personnel. Time required for the training is approximately 3 hours.

3. **Carcinogenic Safety Training:** All University personnel and all students are required to have special safety training in the handling and use of carcinogenic materials and written authorization prior to starting any work with carcinogenic compounds. A laboratory with a Class II or better Fume Hood is required for the handling of carcinogenic materials. A pre-requisite to this training is completion of the General Chemical Safety Training. Application to use carcinogens is made through the Department of Health & Safety. Annual retraining is not required for continued authorization. Training is part of the Chemical Safety Training.

4. **Radiological Safety Training Materials:** All University personnel including all students working with, or in areas that use or store, radioactive material are required to have completed the Radiation Safety Training program for Materials including passing a written examination and have obtained written authorization prior to working with, or in areas that house, radioactive isotopes. Authorization of personnel to work in University laboratories where personnel use or store Radiological Materials is contingent upon their direct supervisors and Department Heads receiving and maintaining similar authorization. A pre-requisite to this training is completion of the General Chemical Safety Training program and Carcinogen approval. Annual retraining is required for continued authorization. Annual retesting is not required. Time required for the training is approximately 3 hours.

Emergency Procedures

Familiarize yourself with the emergency procedures to adopt in the event of accident or fire, etc. When the fire alarm sounds you must exit the building immediately. Personnel

are requested to wait on the northeast side of University Avenue until the “all clear” sign is given. Do not interfere with emergency personnel.

When you come to work in a new laboratory, check out and ask the laboratory supervisor or more senior students about the location of fire extinguishers, fire alarms, first-aid equipment, eyewash facilities and emergency spill kits.

Any questions concerning these safety-training requirements should be referred to the Department of Health and Safety at 662-915-5433.

Emergency Telephone Numbers

FIRE 9-911

UNIVERSITY POLICE 7234

STUDENT HEALTH SERVICES 7275

BAPTIST HOSPITAL NORTH MISSISSIPPI 9-232-8100

DEPARTMENT OF HEALTH AND SAFETY 5433

Chemical & Biological Spills/Emergency Spill Procedures

Minor spillages should be dealt with as indicated here. Major spills may require the immediate evacuation of an area.

If you have been properly trained, you may clean up a small chemical spill with the assistance of other personnel in your area. For small spills, wear appropriate protective clothing, particularly gloves. Be certain to wash out any cloths/paper towels etc. contaminated with chemicals before disposal. Take corrective action to neutralize toxic or hazardous materials. Wash out any contaminated clothing. Remember to wash protective gloves before disposing of them.

A small spill is defined as a spill where:

1. There is little threat to human health personnel property or to the environment, and;
2. There are no injuries beyond what simple first aid can manage, and;
3. The characteristics and the hazards of the material are known, and;
4. You have both the supplies and the knowledge necessary to clean up the materials.

If your spill does not fit all of the specifications above, you have a Large Spill.

1. Report injuries to the University Police at (915)-4911 immediately.

2. Contact Health and Safety at (915)-5433.
3. Tend to injured personnel if you can do so without

Food & Drink in the Laboratories

Eating, drinking, chewing (including chewing gum and all sweets), smoking and the application of cosmetics are strictly forbidden in all laboratories, office areas within laboratories, preparation rooms and washing-up rooms. This includes the preparation of such items. Food and drink, as well as cups, etc., must not be stored in the laboratories, or in refrigerators, cold rooms, deep freezers or ice storage bins provided for chemical or microbiological use.

Personal Electronics in Laboratories

The use of personal electronics (e.g., cell phones, iPods, laptops, televisions) in laboratory areas may constitute a safety hazard as well as annoyance to other users. The wearing of headphones is a particular problem (especially in possible failure to hear alarms) and is therefore asked to be limited in the general area of the School. The use of mobile phones, radios, etc. may be prohibited in certain laboratories or other facilities.

Security

In general, students must assume responsibility for their own personal safety and security of their personal property by practicing common sense and good judgment.

While the pharmacy complex is a relatively safe environment, from time to time personal property is reported stolen. It is incumbent on the student to lock and secure their possessions. If there is a need for special secure storage, the student should consult with their major professor.

All laboratory doors should be left closed and locked from 5:00 pm to 8:00 am on weekdays, and 24 hours a day on weekends and holidays.

For safety reasons, the laboratory door windows may not be obstructed or covered in any manner.

No student is allowed to perform research experiments alone in the building. If a student must work alone in the laboratory, they are required to notify another individual of their intention, when they will be in the lab and when they leave the lab.

The outside doors of Faser Hall and the Natural Products Center must not be left ajar at night or on weekends for any reason, and can be considered a criminal offense by the University. On days of football games be especially cautious if you have to enter the building that you make sure the outside door is locked behind you. Do not allow access to the building to the public when the building is locked.

Clean Laboratories

The cleaning of laboratory space is assigned to individual workers. Cleaning items in the lab is not the same as cleaning at home.

The cleaning problems that commonly occur in the laboratory are so varied that they cannot generally be solved using standard household cleaners. Everything that comes in contact with chemicals or biologicals must to be free from contaminates before and after use in order to eliminate interferences and to ensure personal safety and to protect the environment. Good clean science can only happen if laboratory and production facilities are well cleaned.

For example in the case of glassware the minimal procedure is: Rinse the glassware with the appropriate solvent. Use deionized water for water-soluble contents. Use ethanol for ethanol-soluble contents, followed by rinses in deionized water. Rinse with other solvents as needed, followed by ethanol and finally deionized water. If the glassware requires scrubbing, scrub with a brush using hot soapy water, rinse thoroughly with tap water, followed by rinses with deionized water.

Dirty glassware piled in sinks, countertops, and/or hoods is unacceptable.

HAZARDOUS MATERIALS

Any person introducing new equipment, substances or microorganisms into the department which may have any hazardous implications, must address storage, transport, circumstances of use and disposal before the substance or microorganism is introduced into the Department. University rules and regulations may be found at:

http://safety.olemiss.edu/

ABSOLUTE ALCOHOL 100 & ETHANOL 96 & ETHYL ETHER

Absolute Alcohol 100 and Ethanol 96 are covered under regulations set forth by the Bureau Alcohol, Tobacco, & Firearms, which allow their relief from taxes provided they are used solely for scientific or medical research, or teaching purposes.

These alcohols are normally issued in quantities up to 250 cm^3 and, in general, quantities are expected not to exceed one day's requirements.

Due to the manufacturing of illegal drugs, ethyl ether has become highly regulated. When possible this material should be kept out of sight when at all possible.

Research workers and technical staff are responsible for ensuring that no improper use is made of these alcohols. Containers should be kept in a cabinet when not in use.

FLAMMABLE LIQUIDS

Detailed guidance on the use of flammable liquids is provided in the Chemical Safety guidelines. In particular note that the use of ethanol is subject to legal constraints, and that stocks of flammable liquids in the laboratory should be kept to a minimum.

Always use solvents with the highest possible flashpoints to reduce the risk of creating flammable mixtures of solvent vapor and air. Solvents with flashpoints below ambient temperatures should only be used where absolutely necessary and in as reduced a quantity as possible.

Due to the manufacturing of illegal drugs, ethyl ether has become highly regulated. When possible this material should be kept out of sight.

In addition, solvents should be kept in flammable lockers except when in use under the hoods.

WASTE SOLVENT DISPOSAL

Employing the detailed procedures involving the hazardous waste disposal process at the University is extremely important. Laboratories and/or individuals may be cited for improper disposal and an individual can lose all access to research facilities in some cases. Procedures are given in detail at:

http://safety.olemiss.edu/safety-programs/chemical-safety/chemical-safety-manual/
http://safety.olemiss.edu/safety-programs/biological-safety/biological-safety-manual/

FUME HOODS

The fume hoods are one of the most important safety devices in the Department. All users should be thoroughly familiar with their safe operation. Fume hoods should be used when handling hazardous substances in order to reduce the risk of exposure. The performance of each cupboard is maintained and tested by the Department of Health and Safety.

The following is a brief guideline only and does not replace proper instruction in fume hood use.

- Always make sure that the fume hood is on before using.
- The sash height should always be as low as possible and in any case never greater than 600 mm gap when in operation.
- Always place apparatus as far back as possible in the hood.
- Do not obstruct the extract slots at the rear of the hood. Take great care that tissues and scraps of paper or metal foil do not get sucked into the ducting as they can damage the fan blades.
- Fume hoods are valuable and expensive pieces of equipment. Never use fume cupboards for storage.
- Always close the sash when you have finished working at the hood.
- The fume hood sash may give some limited protection against minor explosions. It must never be used as a blast shield.
- Each fume hood is fitted with a device which monitors the airflow, so should an alarm sound and a light flash, do not use the cupboard. The audible alarm can be turned off.

HAZARDOUS CHEMICALS AND BIOLOGICAL MATERIALS

Research workers should familiarize themselves with the properties of, and the emergency arrangements for, the substances with which they are working.

HYGIENE & LABORATORY TIDINESS

- Do not put your fingers in or near your mouth or eyes.
- Do not chew the ends of pens or pencils.
- Do not scratch, or bite your nails.
- Wash your hands frequently, and always before leaving the laboratory.
- Keep your working area clean and tidy; there must be sufficient bench space to allow safe working procedures.
- Keep notebooks, reference books and all other paperwork separate from areas where cultures or chemicals are being handled.
- Keep aisles and hallways clear.
- In order to give clear visibility and thus avoid collisions, posters and notices must not be attached to the glass of corridor and laboratory doors.
- Equipment and furniture, etc., must not be discarded into corridors or stairwells, which must be kept clear for escape in an emergency.

THE OVERNIGHT RUNNING OF EQUIPMENT

In general, experiments and apparatus should not be left running overnight, or outside normal working hours, unless it is absolutely necessary and then only subject to the following conditions:

The user has checked that the apparatus is functioning normally and that all the safety regulations have been complied with. Water pressure may rise at night, so all tubing must be properly secured with plastic or Jubilee clips. Check that waste outlets are clear of obstruction.

The windows in the laboratory door must not be obscured, so that during night patrols the Security staff can see easily into the laboratories.

Unauthorized experiments left running are liable to be terminated and the person concerned held responsible for any damage that might be caused.

PERSONAL PROTECTIVE EQUIPMENT

Suitable protective clothing must be worn when working in the laboratories. At the minimum, this means a laboratory coat, properly fastened, and adequate footwear. Open sandals and open-toed shoes are not permitted.

Laboratory coats must not be worn in refreshment areas and offices in the department or in amenity areas of the School or University.

Depending upon the nature of the work undertaken, additional items of protective wear (gloves, plastic aprons, safety spectacles, visors, dust masks, respirator masks, etc.) are available in laboratories or from the storeroom.

One of the most common accidents that can occur is injury to the eyes through splashing of reagents; therefore, appropriate eye protection must always be worn at all times in the laboratory (with the exception of the desk areas).

Gloves are available and the appropriate type should be worn when handling hazardous substances; always check their integrity before use. One must never touch surfaces with contaminated gloves; especially those that receive public use (phones, door handles, etc.) and remove gloves when leaving the work area.

AUTOCLAVING

Autoclaves may only be operated by competent, named operators, who have received adequate and suitable instruction both in the normal operation of the machine and in emergency actions and procedures. No operator may carry out any procedure other than those set down in the operating instructions. Autoclaves are too dangerous for unqualified tinkering.

Autoclaves use superheated steam to sterilize materials and supplies for laboratory use and to prepare contaminated items for disposal. Because there are several brands and types of machines, it is the responsibility of the laboratory supervisor to properly train all of the personnel in the safe operation of the specific type of autoclave they are using. As a general rule the following table can be used as a guide for preparation and disposal.

COMPRESSED GASES

Cylinders of compressed gas must carry the correct regulator and must be secured by the bench strap.

When not in use the cylinder valve should be turned off. Cylinder valves, that are stuck, should not be forced open, but should be returned to the manufacturer. Oil or grease must never be used to ease stuck valves or lubricate threads as it can cause an explosion in contact with oxidizing agents.

Cylinders of hydrogen or other flammable gases should only be opened using a bronze spanner to avoid the risk of sparks causing ignition. PTFE tape must not be used.

Remember that the valve outlets of combustible gases have a left-hand thread.

Whenever more than one gas is being used, a special non-return valve must be incorporated into the system to prevent the contamination of one cylinder by another.

Gases that are toxic (e.g. carbon monoxide) must not be used without approval from the Department of Health and Safety.

REFRIGERATOR & FREEZER SPACE

Only materials which are unstable at room temperature, reaction mixtures, or samples being recrystallized or which are liable to be contaminated should occupy cold space.

Drinks and foodstuffs must not be stored in any cold space designated for laboratory use.

Flammable solvents or reagents may only be stored in spark proof refrigerators.

All containers must be clearly labeled with the name of the contents, the hazard warning sign, the name of the research worker and the date. Solutions should not be stored in volumetric apparatus as this impairs the accuracy of the glassware and prohibits their use by others.

Cold space must be regularly cleaned and defrosted and advance notice of this is given. On occasions, failure of cold spaces, etc. may necessitate the clearance of the space.

Any materials not clearly labeled will be discarded.

Freezer space should not be used for material, which could be accommodated in a refrigerator, and likewise -70°C/-80°C freezer space should not be occupied with material, which could be stored at higher temperatures.

Since water and chemical vapors can initiate many potentially hazardous chemical reactions, containers in refrigerators and freezers should be tightly sealed and appropriately labeled. Freezers must be defrosted at least once per year and preferably whenever the buildup is more than ½ inch thick.

Cold space within the Department is assigned.

SHARPS/BROKEN GLASSWARE

Glassware must not be disposed of with normal trash. Building Services Personnel and others have been injured when carrying trash bags with broken glassware in them.

Broken glassware should immediately be cleaned up. Forceps or duct tape can be used to pick up the smaller pieces of broken glass.

Discarded glassware must not contain any hazardous wastes, Medical Waste, Pathological Waste or Radiological Wastes.

If the glassware contains hazardous wastes or Radiological Wastes, please call Health & Safety (5433) for disposal instructions.

Details on the proper disposal of glassware can be found at:

http://safety.olemiss.edu/safety-training/safety-training-information/disposal-of-laboratory-glassware/

STORAGE & LABELING

All containers used to hold chemical or biological substances must be securely closed and clearly labeled to show precisely what each container holds.

All manufacturer labeled chemical and solvent containers must show the date in which they were opened, and preferably the dates and amounts after each use.

Labels must not be written over and one label should not be placed upon another.

The label must be written in "plain English"; the chemical formula alone is not acceptable. The concentration of solutions and the solvent used must be stated. In addition the name of the owner and a date should be given plus any hazard warning appropriate.

Toxic and highly toxic chemicals must be stored in locked cabinets or refrigerators, or kept in a secured laboratory.

Always secure the tops of reagent bottles immediately after use and return stocks to their storage safety cabinet.

Solvent bottles and other large glass vessels should not be stored above waist height or left unprotected on the floor.

Flammable liquids must be stored in the fireproof cabinets provided.

Correct storage and labeling of biologicals and chemicals is a legal requirement.

VESSELS UNDER VACUUM

There is always a risk of implosion of any vessel under vacuum.

Vessels under vacuum must be approved for such use and within the manufacturers tolerance range.

All vacuum applications must be performed under a fume hood and/or behind a safety shield.

SYNTHETIC CHEMICAL REACTIONS

All synthetic chemical reactions must be performed in a chemical fume hood in which the sash is appropriately pulled down to still allow for proper airflow. Synthesis performed on a bench top is unacceptable. Violations of this rule can endanger the lives of many laboratory personnel and those found in violation of this rule will be expelled from the graduate program.

POTENTIALLY EXPLOSIVE REACTIONS

Many chemical reactions pose the possible risk of explosion. Always know the risk of any reaction before it is attempted.

Potentially explosives reactions must be performed under a fume hood and behind a safety shield. Violations of this rule can endanger the lives of many laboratory personnel and those found in violation of this rule will be expelled from the graduate program.

All laboratories are equipped with safety shield, if one is unsure whether a reaction requires special safety consideration use a shield.

RESEARCH DATA COLLECTION

Research data should be collected in a method suitable for publication in an internationally recognized journal such as one published by the American Chemical Society (ACS), the inclusion of such data be required for publication in a journal and your thesis/dissertation. Students can examine in detail the requirements as described in the instructions to authors section of a journal appropriate to the scientific discipline the student works within. Students should become familiar with these requirements as they gather scientific data. Of course, one's major professor can answer and specific questions.

The student must keep a record of all computer programs, scripts and macros that are written during their degree. The copyrights for all such written materials belong to the University of Mississippi.

Each student is responsible for backing up all of his/her electronic data and notes. The student's supervisor is responsible for providing the backup resources, but the student must make sure that regular backups are performed.

THE LABORATORY NOTEBOOK

The technical notebook is one of the basic tools for any experimental work, whether it is basic research, product development, or engineering design. It is primarily for the experimenter's own use, but another person with similar technical background should be able to understand and duplicate any experiment, data, and conclusion, or to prepare a technical report by following only the lab notebook details.

Your laboratory notebooks MUST contain all the information that would be required for you or someone else to completely reproduce your experiment.

A good reference to consult in these regards is the American Chemical Society's (ACS) publication, *Writing the Laboratory Notebook*. Copies of this book are kept in the departmental office for loan.

Various electronic notebook programs are now available and students should consult with their major advisor before using these formats.

The research notebook is the property of the Department of BioMolecular Sciences and the University of Mississippi. Upon completion of the degree program, the students may furnish themselves with a photocopy of his/her research notebook.

While each major professor may require somewhat different formats and media, most notebooks contain certain commonalities, which will be discussed here.

There are many reasons to keep an accurate and complete record of experimental work. Among these are:

1. To establish the authenticity of the work.
2. To defend patents.
3. To act as a basis for technical reports and articles.
4. To avoid duplication of effort.
5. To avoid repetition of erroneous procedures.

The nature of the work and the purpose of the experimenter will influence the content and format of the laboratory notebook. Many companies have rigid internal requirements tailored to their specific needs.

Notebooks should be bound, never loose-leaf, and the pages numbers consecutively, preferably by the manufacturer.

A neat, organized, and complete lab notebook record is as important as the investigation itself. The lab notebook is the record of what was done. You must use ink, and write directly in the notebook as the experiment is done. You will have to date and sign each entry. Sometimes you may be required to make an entry of no progress made today, just to show that you were working on the project. If a mistake is made, you should X it out and start over. This leaves the original entry readable and keeps a permanent record of all your work, which can be used as evidence in a patent court.

Use all the pages of a notebook to prevent accusations of adding data after the fact. If pages are left bank after your graduate career, you should draw a large X on each page. In addition; if blank spaces are on a page, these areas should also have an X drawn through them.

Date and initial each page at the top as it is used.

In industry it is very important to sign and date all work and leave no spaces where additions might be added later in order to preserve the legal integrity of the notebook. Your major professor may require his/her signature in your notebook at the end of each day.

Leave several pages blank at the beginning of the notebook so that they may be used as a table of contents upon completion of experiments.

For each experiment, you should adhere to following format. Each experiment should be started on a new page with the following information at the top of every page:

1. date
2. experiment number
3. experiment title
4. your name

On the first page of each experiment list the names of any partners (postdocs, graduate students, undergraduate students, etc.) that worked on this experiment with you. You may start with a data page to include all the data for the experiment. Depending on your graduate advisor, this data page may need to be initialed and dated by the major professor before you leave the lab. Make sure you include all information that will be necessary for use in the final write up. Then start on the following page with:

1. Objectives
   Briefly state the major goals of the experiment.
2. Preliminary

State your approach to the experiment, i.e., how you intend to achieve the objectives.

3. Equipment and Supplies List
   The manufacturer, chemical formula, purity, and lot number must be used in the identification of all the supplies employed in the experiments. All instruments used must be identified by make, model, and serial number.
4. Procedure
   Give essential details on how the experiment was conducted. Make sure that this information is complete. Data should be inserted in the procedure description so that it appears near the corresponding procedure.
5. Results
   These are the physical observations, whether it is the temperature of a water bath, a melting point, an explosion (lets hope not), or an NMR spectrum. Record as much detail as possible, there is no such thing as too much observation only too little. Try not to put yourself in the situation of having to repeat an experiment because “I forgot what happened”.
6. Conclusions
   Write scientific conclusions about the results of the experiment. Base your conclusions on what you actually did, not on what you think you should have done. Be factual and concise. Do not conclude something unless your results actually support that conclusion. Remember a scientific conclusion is a statement about the behavior of some physical system based on the observation of facts.

The laboratory notebook must answer the following questions (the 5 Ws) in at least one part of the report. You can use this as a checklist.

1. What Was Done
2. Who Did It
3. When Was It Done
4. What Were The Results
5. What Does It Mean

EQUIPMENT

Scientific laboratory equipment can be extremely expensive to purchase, replace, and maintain; therefore, it is vital that all equipment be kept in working order.

A student should never use ANY equipment on which they have not been trained by an appropriate person in this department regardless of whether they have been trained on the equipment at another institution. A student should never use, move, adjust, or modify any instrumentation without prior approval of the person in charge of that equipment. Students should also be vigil the reporting individuals who do misuse equipment. A student must immediately report any malfunctioning equipment to the person in charge of the equipment. After use, a piece of equipment should be left in the condition in which it was found, that is operational, clean, and ready for the next user.

Failure to obey the above rules will result in the dismissal of a student from the Ph.D./M.S. program.

INSTRUCTIONS FOR FINAL CHECK OUT

The general rule of thumb when checking out, leave the laboratory as you found it when you arrived. Remember, you are leaving the space to a new student to begin their studies; please don't make them start out having to clean up your mess.

Each advisor will direct what you are expected to do and how and where you are to place valuable samples, and original lab notebooks & electronic files.

Most importantly, make sure that toxic and dangerous materials are either disposed of or safely stored. You should make your advisor aware of any such materials left in the lab.

Make sure to return all keys to your advisor!

Finally, make sure to clean your desk area. Papers, manuscripts, and personal items have a very high disappearance rate once the student/graduate leaves the lab.

APPENDIX MATERIALS

APPROXIMATE TIMELINE FOR COMPLETION OF THE PH.D.

First Year

Emphasis on Coursework
Selection of Research Advisor *(must be completed before the end of the first semester)*
Selection of Dissertation Committee
Present Topic or Literature Departmental Seminar (30 min)

Second Year

Continue Coursework: Should Be Completed or Near Completion by the End of Year 2
Well into Research Project
Present First Research-Based Departmental Seminar (30 min)
Completion of ORP Requirement and Admission to Candidacy

Third Year

Coursework Should Be Completed or Near Completion
Emphasis on Research Project
Present Research-Based Departmental Seminar (45-50 min)
Approval of Dissertation Prospectus

Fourth/Fifth Year

Completion of Research Project
Complete Dissertation and Dissertation Defense
Completion of Graduate School Requirements
Laboratory Checkout

APPROXIMATE TIMELINE FOR COMPLETION OF THE M.S.

First Year

Emphasis on Coursework
Selection of Research Advisor *(must be completed before the end of the first semester)*
Selection of Thesis Committee
Present First Research-Based Departmental Seminar (30 min)

Second/Third Year

Complete Coursework
Complete Research Project

Present Research-Based Departmental Seminar
Complete Thesis and Thesis Defense
Completion of Graduate School Requirements
Laboratory Checkout

BIOMOLECULAR SCIENCES CORE COURSES AND DESCRIPTIONS

- BMS 641, BMS 643. Seminar on Current Pharmaceutical Topics. (1, 1). (A-F, Z grade)□

Medicinal Chemistry Courses

- MEDC 501. Advanced Medicinal Chemistry I. Advanced study of organic medicinal agents with emphasis on names, synthesis, chemical properties, and pharmacological properties. Readings in the current literature required. Prerequisite: Consent of the department (3). □

- MEDC 502. Advanced Medicinal Chemistry II. Continuation of Medicinal Chemistry 501. Readings in the current literature required. Prerequisite: MEDC 501. (3) □

- MEDC 503. Medicinal Chemistry Research Methodology. Lecture and hands-on laboratory in various methods used in medicinal chemistry research. (3) □

- MEDC 507. Organic Chemistry of Drug Synthesis. Discussion of the synthetic approaches to many of the therapeutic classes of drugs studied in medicinal chemistry courses with an accent on the relationship of chemical structure to improved efficacy. Prerequisite: MEDC501 or equivalent and consent of department.□

- MEDC 541, MEDC 542. Problems in Medicinal Chemistry. Investigation of individual problems. Prerequisite: Consent of instructor. (1-4,1-4)□

- MEDC 697. Thesis (1-12)

- MEDC 710. Selected Topics in Medicinal Chemistry. Recent advances emphasizing mechanisms of drug action and other new concepts. (May be repeated for credit. (3).□

- MEDC 730. Pharmaceutical Protein Design and Development. This course focuses on the chemical and structural characteristics of protein pharmaceuticals, which make them different from conventional pharmaceutical products. (3)□

- MEDC 711. Drug Action and Design I. Introduction to Computer-Aided Ligand Design. Modern molecular modeling methods and techniques as they pertain to molecular design and the simulation of molecular properties and interactions. Examples include modeling of small molecules at the level of mechanics calculations up to *ab initio* calculations; homology modeling of proteins and related validation methods; docking interactions of ligands and receptors. (3)□

- MEDC 712. Drug Action and Design II. Quantitative Structure-Activity Relationships in Drug Design. Introduction to simple mathematical models of drug action (2D-QSAR) and application of the concepts to the use of computer-aided drug design to develop 3D pharmacophore models based on quantitative structure-activity relationships (3D-QSAR) (3)

- MEDC 713. Drug Action and Design III. Drugs Affecting the Central and Peripheral Nervous System. Discussion and application of the design, synthesis, and biological activities of drugs affecting both the central and peripheral nervous system. Prerequisite: 501 or equivalent. (3)

- MEDC 714. Drug Action and Design IV. Chemotherapy of Cancer and Infectious Diseases. Overview of anticancer, antimicrobial and antiviral chemotherapy as related to drug design, chemical synthesis, structural classes, mechanisms of pharmacological action, toxicities, resistance mechanisms and clinical usefulness (3).

- MEDC 709. Drug Action and Design V. Heterocyclic Compounds. Methods of synthesis of medicinally important compounds that contain a heterocyclic ring system. (3).

- MEDC 718. Drug Action and Design VI. Bioorganic Chemistry. The study of chemical interactions and catalytic strategies fundamental to drug design and development, using the principles of organic chemistry as the intellectual framework for addressing biological problems at the molecular level. (3)

- MEDC 720. Drug Action and Design VII. Combinatorial Chemistry: Theory and Practice. A combined lecture and laboratory experience in the field of high-throughput synthesis including background, theory, and specific solid-supported or solution-phase chemistries as applied to the generation of drug libraries for drug discovery and lead optimization. (3)

- MEDC 797. Dissertation (1-12)

Pharmacognosy Courses

- PHCG 620: Selected Topics in Pharmacognosy (Bioassays In Natural Products Research) Selected Topics in Pharmacognosy. An in-depth discussion of recent advances in knowledge of plant and animal materials with biological properties of interest to pharmaceutical scientists. (May be repeated once for credit). (3 hours).

- PHCG 627: Natural Products Chemistry I Natural Product Chemistry. A comprehensive consideration of the chemistry and pharmacology of those plant constituents important because of their biological activity. Included are the bread classes, the alkaloids, the terpenoids, the steroids, the flavanoids, and other related groups. (3, 3 hours).

- PHCG 628: Natural Products Chemistry II Natural Product Chemistry. A comprehensive consideration of the chemistry and pharmacology of those plant constituents important because of their biological activity. Included are the bread

classes, the alkaloids, the terpenoids, the steroids, the flavanoids, and other related groups. (3, 3 hours).

- PHCG 635: Molecular Cell Biology for Pharmacognosy (CHEM 580: Molecular Biochemistry I can be substituted for this course as needed

- PHCG 321: Pathogenesis & Etiology of Infectious Diseases

- PHCG 422: Natural Product Derived Pharmaceuticals. This course covers all aspects of natural products used as pharmaceuticals including both plant derived and microbial derived (antibiotics). Prerequisites: PHCG 421, MEDC 324, PHCL 343. (4 hours).

- PHCG 541/542: Problems in Pharmacognosy Problems in Pharmacognosy. Individual investigation of problems of current interest in Pharmacognosy. (1-4, 1-4 hours).

- PHCG 545/546: Individual Study in Pharmacognosy Research

- PHCG 632: Analysis of Natural Products II (Isolation Theory and Practice) Analysis of Natural Product Drugs. A discussion of techniques used for identification and determination of structure of substances of natural origin. Included for discussion are physical methods and spectroscopic techniques of structure elucidation. (3 hours).

- PHCG 633: Analysis of Natural Products III Analysis of Natural Product Drugs. A discussion of Fourier-transform nuclear magnet resonance techniques including 2D-NMR for the determination of structure of substances of natural origin. Prerequisite: 632. (3 hours).

- PHCG 697. Thesis (1-12)

- PHCG 797. Dissertation (1-12)

Pharmacology Courses

- PHCL 501. Principles of Life Science Research. This course consists of facilitated discussions of the topics in the syllabus. Students are assigned to be discussion facilitators for one or two topics. (1).

- PHCL 505. Modern Pharmacology. Novel drugs in clinical trials. An in-depth discussion of topics of current importance in pharmacology of commonly occurring diseases are emphasized. Prerequisite: graduate standing or consent of instructor. (2).

- PHCL 541. Problems in Pharmacology. Investigation of individual problems. Prerequisite: consent of instructor. (May be repeated for credit). (1-3).

- PHCL 547. Introduction to Environmental. (2).

- PHCL 563. Introductory Pharmacology I. General principles of pharmacodynamics; drugs affecting central nervous system. Prerequisite: 361, 362, 373. (4).

- PHCL 564. Introductory Pharmacology II. Continuation of 563. Autonomic, cardiovascular, and renal drugs; endocrinological and chemotherapeutic agents. Prerequisites 563. (4).

- PHCL 569. Drug Abuse Education. Pharmacological, legal, and sociopsychological aspects of drug abuse. Prerequisite: fourth-year standing, graduate standing with nonpharmacy major, or consent of instructor. (2).

- PHCL 581. Introduction to Toxicology. (3).

- PHCL 586. Receptors and Channels. (3).

- PHCL 651, 652. Directed Studies n Pharmacology and Toxicology. Research tutorials requiring individual conferences, literature assignments, and laboratory experiences with departmental faculty members. (1, 1).

- PHCL 661. Advanced Physiology**.** Physiology of those systems, organs, and physiological mechanisms of special significance to pharmacology, including a comparative cross-species emphasis for selected organ systems. Prerequisites: PHCL 361-364 or equivalent, or consent of instructor. (Lecture and lab). (4).

- PHCL 668. Externship in Pharmacology. Credit given for participation in pharmacological screening procedures carried out in the laboratories of a pharmaceutical manufacturer. (1-8).

- PHCL 669. Physiological Chemistry. Carbohydrate, protein, and nucleic acid structure and function, enzyme catalysis, intermediary metabolism, biochemical endocrinology, membrane structure, mechanisms of solute transport, and molecular genetics. (4).

- PHCL 675. General Principles of Pharmacology and Toxicology I. General principles of toxicology; biotransformation of toxicants; chemical carcinogenesis, mutagenesis, teratogenisis; systemic toxicology. Prerequisite: PHCL 669 or consent of instructor. (4).

- PHCL 676. General Principles of Pharmacology and Toxicology II. Toxicity of organic and inorganic compounds; toxins of animal and plant origin; food additives and therapeutic agents; environmental toxicology; risk assessment. (4).

- PHCL 677. Advanced Topics. Lectures, readings, and discussions of special areas of experimental pharmacology and allied subjects. (May be repeated for credit). (2).

- PHCL 681. Selected Topics in Pharmacology and Toxicology. Topics may include pharmacokinetic, pharmacodynamics and receptor selectivity of biologically active agents, food additives, drug toxicity, toxicology of agricultural and industrial

chemicals, clinical toxicology, toxicity of plastics; naturally occurring toxins. Prerequisite: consent of instructor. (May be repeated for credit) (2).

- PHCL 685. Externship in Toxicology. Credit given for research performed in toxicology at other academic institutions or private industrial concerns. (1-8) (Z grade).

- PHCL 697. Thesis (1-12)

- PHCL 797. Dissertation (1-12)

Annual Report Format

STUDENT ACTIVITY REPORT

Prepared by Student Name
May 1, 2014 to April 30, 2015

I. Degree Requirements

A. Courses (example for current PHCL requirements

Class	Title	Semester completed	Grade

Address any grades <B, including remediation plans:

B. Seminar

Title:
Date:
Grade:

C. Original Research Proposal

Circle one: Completed Projected

Date:

If completed, grade:

D. Prospectus

Circle one: Completed Projected

Date:

Approved on:

II. Research

A. Major Accomplishments for previous year

B. Plans for the next year

III. Papers published
A. Complete Bibliographic Citations for Journal Articles.
B. Posters Presented at Professional Meetings.
C. Seminars and oral presentations given, not including department seminar
D. Papers Submitted and Accepted for Publication or Presentation.

IV. Other notable mentions (e.g. grants, awards, teaching, service)

Student: _____

Date: _____

Advisor: _____

Date: _____

Advisor Evaluation and comments:
Progress is (circle one): Satisfactory Not Satisfactory

Department Chair: _____

Date: _____

Department of Biomolecular Sciences Student Seminar Series Eval Sheet

Student Name: _____
Division: _____
Date: _____

	Poor Quality	Needs Improvement	Average	Very Good	Exceptional
Visual Aids					
Clarity					
Voice Quality/Variation					
Organization					
Pace					
Information content					
Comprehensibility to a broad audience					
Did the speaker make the material interesting?					
Depth of speaker's knowledge of the subject					
Ability to answer questions					
Overall – please give an overall letter grade (A, B, etc.; including +/- scale) for the seminar					

Strengths of the seminar:

Suggestions to improve future seminar presentations:

What have you learned from this seminar?

General comments:

SEMINAR TIPS

The Golden Rule of any presentation is:

"Tell 'em what you are going to tell 'em.
Tell 'em.
Then tell 'em what you told 'em."

Audiences tend to absorb very little information at first exposure.
Hence, multiple exposures are the best way for information to sink in.

1) Prepare your material carefully and logically.
Tell a story. The story should have four sections:

(a) The Introduction to the subject,
(b) The Overview,
(c) The Subject Matter, and
(d) The Conclusion/Summary.

The *Introduction* should provide the audience with the information leading up to present day knowledge of the subject matter. This usually includes a description of the problem, a history of the means by which a solution to the problem has been addressed, and an introduction to the most recent solution attempts. You should strive to convince the audience that the problem is important to them. The *Introduction* should be no more than three or four slides.

The *Overview* should be a single slide describing what you will be covering in your presentation. These are major points that you want to emphasize, and there shouldn't be more than four or five. This will orient the audience with what to expect. You will want to come back these points in your *Conclusion/Summary* as well.

The *Subject Matter* is the "meat" of the presentation and should focus on only the most recent advances in the field. The most recent advances include seminal publications and recent journal articles usually within the past two or three years. Remember, a seminar is only a summary in itself, include only the most salient details. In order words, don't get bogged down in minutiae such as "busy" slides of tables and graphs. Such materials will usually bore your audience. More in-depth details will emerge as people ask questions.

The *Conclusion/Summary* section should connect with the *Overview* statements. Again, don't have too many points – four or five is usually the maximum – and one slide should be sufficient.

Good speakers may also add fifth item: *Future Research*.

2) Practice your talk.
There is no excuse for a lack of preparation. The best way to familiarize yourself with the material and get the talk's timing right is to practice your talk. Many scientists believe that they are such good speakers, or so super-intelligent that practice is beneath them. This is an arrogant attitude. Practice never hurts and even a quick

run through will produce a better talk. Even better, practice in front of a small audience.

3) Speak proper English.

Everyone speaks with some kind of an accent; however, it is unacceptable to speak with an accent that is not understandable by your audience! Most international students from non-English speaking backgrounds will require a great deal of practice. Poor grammar is a no-no as well. Avoid slang and addressing the audience as you may address your peers.

4) Don't put in too much material.

Good speakers will have one or two central points and stick to that material. How many talks have you heard where the speaker squanders their time on unessential details and then runs out of time at the end? The point of a talk is to communicate scientific information, not to show people how smart you are (in case they can't figure it out for themselves). *Less is better for a talk.* Here is a good rule of thumb - each slide should take about 1.5 – 2.0 minutes to cover, thus for a 45-minute presentation on should have somewhere between 23 and 30 slides. One should leave approximately 10 minutes for Q&A, hence a 45-minute talk is what to “shoot” for. The seminar host will generally end the Q&A session after 10 minutes. Remember, it is considered rude for a presenter to exceed their allotted time.

5) Know your audience.

Assume most of the audience will know very little about the subject, and will need a clear explanation – it is good to know your audience. Show only very simple tables/synthetic routes/equations if you show any at all. Ask yourself - is showing this important? Is it central to my talk? People are used to studying tables/synthetic routes/equations, not seeing them flashed on the screen for a minute or two. When showing tables/synthetic routes/equations simplify them.

6) Have only a few conclusion points.

People generally take away only a couple points from a presentation, especially if they are hearing many presentations at large meetings. For example, if a colleague asks you about someone's talk you heard, how do you typically describe it? You say something like "So and so looked at such and such and they found out this and that." You don't say, "I remember all 6 conclusions points." The fact is, people will only remember one or two things from your talk - you might as well tell them what to remember rather than let them figure it out for themselves.

7) Talk to the audience not to the screen.

One of the most common problems is that the speaker will speak to the screen. It is hard to hear the speaker in this case and without eye contact the audience loses interest. This is difficult to avoid, but the speaker needs to consciously look at the object on the screen, point to it, and then turn back to the audience to discuss the feature. Speak loudly and slowly.

8) Avoid making distracting sounds.

Everyone gets nervous speaking in public. But sometimes the nervousness often comes out as annoying sounds or habits that can be really distracting. Try to avoid "Ummm" or "Ahhh" between sentences. Don't put your hands in your pockets, and make sure to take the keys and change out so you accidently jingle them during your talk. Don't even take your cell phone to the presentation!

9) Polish your graphics.

- Use large letters (no fonts smaller than 16 pts!!)
- Keep the graphic simple.
- Don't crowd the slide.
- Don't use different fonts or type styles - it makes your slide look like a ransom note.
- Make sure the tables/synthetic routes/equations are simple and clear.
- A little professional effort on graphics can really make a talk impressive.
- Avoid red in the text - red is difficult to see from a distance.
- Careful with color schemes some look great on a small computer screen, but project poorly.
- A cartoon such as a Rube-Goldberg sort can be great for explaining complex ideas.

10) Be personable in taking questions.

Questions after your talk can be scary. But questions are very important. If there are no questions after a talk that I give, I am disappointed. It means that I failed to stimulate the audience, or that they understood nothing of what I said. I failed to communicate. Questions tell you what part of your talk the audience did not understand. Questions may also help you focus your research or help you in the write up. So what is the best way to answer questions?

- First, repeat the question. This gives you time to think, and the rest of the audience may not have heard the question. Also if you heard the question incorrectly, it presents an opportunity for clarification.
- If you don't know the answer then say "I don't know, I will have to look into that." Don't try to invent an answer on the fly. Be honest and humble. You are only human and you can't have thought of everything.
- If the questioner disagrees with you and continues to harp on the same point, a good way to move on is to de-fuse the situation e.g. "We clearly don't agree on this point, let's go on to other questions and you and I can talk about this later." A good moderator will usually intervene for you, but if not then you will have to handle this yourself.
- Never insult the questioner. He/she may have friends, and you never need more enemies.

11.) Miscellaneous.

- Thank you

  Thank the audience for their attention. **Unless the presentation is a dissertation or thesis talk or you are presenting at a scientific meeting avoid an acknowledgement slide!!!**

- Dress appropriately.

  People are there to hear your material, but when you dress up you send the message that you care enough about the audience to look nice for them.

- Check your slides and equipment before you give the talk.

  Does this equipment work? Do I know how to use it? Do I have a pointer? Is my software compatible with the projection equipment? Stopping to have to fix problems is embarrassing and will destroy your time schedule!

EXAMPLE OF A SEMINAR ABSTRACT

Title: G-quadruplex formation in the kRAS promoter: a new molecular target

Authors: Christine E. Kaiser, Chi-Fan Hockings, Vanessa C. Gaerig, Laurence H. Hurley, and Tracy A. Brooks*

Abstract:
kRAS is one of, if not the, most prevalent oncogenic aberrations identified to date. It is either upregulated or mutationally activated in a multitude of cancers, including 95+% of pancreatic adenocarcinomas. Pancreatic cancer carries the highest mortality rate of all cancers, with only a 3-6 month median survival, and a 5-year survival of <5%. There is a desperate need for new therapeutics, particularly those targeting kRAS. The presented works describe a novel target to downregulate kRAS expression – secondary structures within the proximal promoter, which contains a unique string of G-rich DNA. Negative superhelicity induced by transcription results in this region opening up to form unique secondary structures called G-quadruplexes (G4s). These G4s most often act as silencer elements, forming globular structures that mask binding sites for transcriptional factors, allowing for specific molecular targeting by small molecule drugs, modulating transcription, and protein expression. The G-rich region of the kRAS promoter is extensive, containing three separate putative G4-forming regions. Initial works have described the near G4 as a unique 'kinked' structure, as well as described two compounds that stabilize different isoforms of this structure. Further elucidation of promoter activity has highlighted new, and more biologically important, G4s exists more distal from the promoter. Stabilization of this region in a plasmid system significantly decreases luciferase expression, and initial examinations indicate that the mid-G4-forming region forms a stable, complex structure that is currently the target of further elucidation efforts with the ultimate goal of describing a new molecular structure for further drug development.

THE ORIGINAL RESEARCH PROPOSAL (ORP)

Getting Started With an Idea

Sometimes seeing what others have been funded to do can help one develop their own ideas or even provide seeds for new ideas (although be careful to avoid plagiarism and/or stealing others intellectual property). NIH provides a terrific database search engine for examining summaries of NIH-funded research projects.

http://projectreporter.nih.gov/reporter.cfm

NIH Application Information

http://grants.nih.gov/grants/oer.htm

NIH Page for Downloadable Forms

http://grants.nih.gov/grants/forms.htm

University of Mississippi Grant Proposal Information

https://www.research.olemiss.edu/proposal-development/transmittal

EXAMPLE OF ORP ABSTRACT SUBMISSION TO FACULTY

Note: Please ensure that you are following the most up-to-date guidelines for your application. Items in the following example may be no longer current.

Design, Synthesis and Biological Evaluation of Dual Anti-Diabetic Agents

Original Research Proposal Abstract

Submitted by

Pankaj R. Daga
July 5, 2007

Summary:

The objective of my research proposal would be to design and synthesize the small molecule entities which will inhibit two enzymes namely Dipeptidyl Peptidase-IV and Neutral Endopeptidase simultaneously. The newly synthesized molecules will be tested for their anti-diabetic activity.

Introduction

Diabetes Mellitus (DM) is a major debilitating disease that is caused by abnormal glucose homeostasis and is commonly associated with obesity. Approximately 5% of the world's population is suffering from this disorder.[1] Two main types of diabetes are reported. Type I diabetes mellitus (T1DM develops when body's immune system destroys pancreatic β cells, thus alternating insulin secretion from pancreas. Type 2 diabetes mellitus (T2DM) is increasingly becoming a worldwide epidemic. It accounts for about 90 percent or more cases diagnosed.[1]

For people with T1DM, the treatment of choice is insulin. For people with T2DM, primary treatment includes healthy meals and exercise program. Oral medications such as sulphonylureas, biguanides, PPAR regulators and others are prescribed in these cases.[2] Currently there is much focus on the glucagon-like peptide-1 (GLP-1) peptide hormone as the basis for a potential new treatment paradigm for type 2 diabetes.[3]

GLP-1 Based Therapies for Diabetes Mellitus

GLP-1 possesses a number of properties that make it a potentially ideal antidiabetic target. GLP-1 is released from intestinal L-cells and shows effects on pancreas, on gastro-intestinal track and in the brain. After release, GLP-1 is stimulates meal-induced insulin secretion, and helps in insulin biosynthesis. It is also found to increase pancreatic beta β mass, by stimulating β cell proliferation and differentiation and reducing the β cell apoptosis. In gastrointestinal tract, it is found to decrease gastric motility and secretions.[4] GLP-1 is rapidly degraded in vivo through action of Dipeptidyl Peptidase IV (DPP-IV) which cleaves N-terminal two amino acids to give inactive GLP-1.[5] Various approaches have been devised to generate DPP-IV resistant GLP-1 analogs. Exendin-4 (**Byetta**®), close analog of GLP-1, was approved for the treatment of T2DM in 2005.[6] Various long-acting GLP-1 mimetics have already been in clinical trials.

DPP-IV. Another approach widely used is inhibition of dipeptidyl peptidase IV. As discussed above, this enzyme is responsible for inactivation of GLP-1 by N-terminal truncation. Inhibition of DPP-IV has proved to be an excellent approach to combat GLP-1 degradation and thus increasing GLP-1 serum levels. **Januvia**® (Sitagliptin) was approved for the treatment of type 2 diabetes in the United States on October 2006.[7] Apart from Sitagliptin, many other DPP-IV inhibitors are in various stages of clinical development.

Neutral Endopepptidase (NEP). While major research has been focused on the enzyme responsible for N-terminal truncation, fewer studies have been carried out to study other enzymes involved in GLP-1 degradation. NEP, also known as neprilysin, is wide-spread membrane zinc-metallopeptidase with broad substrate specificity. Although, mainly present in the brush-border membranes of the kidney, it is also found in diverse tissues like lymph nodes, small intestine and CNS. [8] NEP cleaves peptides at hydrophobic residues. NEP is known to be involved in inactivating opioid peptides in the CNS, atrial natriuretic peptide, vasoactive intestinal peptide and many other peptides.[9] NEP is also shown be involved in degradation of β-amyloid peptides, thus showing role in Alzheimer's disease.[10] NEP was also reported to degrade

members of glucagon/secretin/glucose-dependent insulinotropic polypeptide family including GLP-1, in vitro.[11, 12]

Scientific Rationale

Plamboeck and associates recently investigated role of NEP in GLP-1 metabolism by using candoxatril, a selective inhibitor of NEP.[13] The degradation of GLP-1 was found to be reduced by candoxatril thus confirming the role of NEP in GLP-1 metabolism. The authors also found that NEP is responsible for degradation of 50% of GLP-1 in plasma. When candoxatril was co-administered with DPP-IV inhibitor, GLP-1 degradation was completely prevented, and metabolic stability of GLP-1 was improved, resulting in six-fold increase in its plasma half-life.[14]

The available evidences suggest that metformin is more effective as monotherapy than either sitagliptin, or the related DPP-IV inhibitor, vildagliptin.[7] Study carried out by Plamboeck and associates may provide the probable reason for this. So in this case designing a dual ligand will provide an effective therapy.

Specific Aims

Designing multiple ligands is becoming very popular approach, in which small molecule ligands are designed targeted towards multiple targets. The overall objective of my original research proposal would be to design and synthesize the small molecule entities which will inhibit DPP-IV and NEP simultaneously.

Analysis of available small molecule inhibitors (Scheme 1) for DPP-IV revealed that these inhibitors posses one hydrophobic group like cyclopentyl, cyclohexyl, phenyl or any other cyclic ring structure away from N-H (shown in blue and red respectively) at particular distance. Similarly, NEP inhibitors consist of one metal chelating group in close proximity of hydrophobic group like benzyl, t-butyl, sec-butyl etc (shown in orange and magenta respectively).

My approach is to combine various pharmacophoric features of different class of inhibitors to design dual inhibitor of DPP-IV and NEP. The general structure of the proposed lead molecule and representative structures of lead molecules is as shown in scheme 2. The preliminary computational studies have shown that the lead molecule fits well into the active site of DPP-IV and NEP. This is a novel approach and will be a turning point in the treatment of the T2DM.

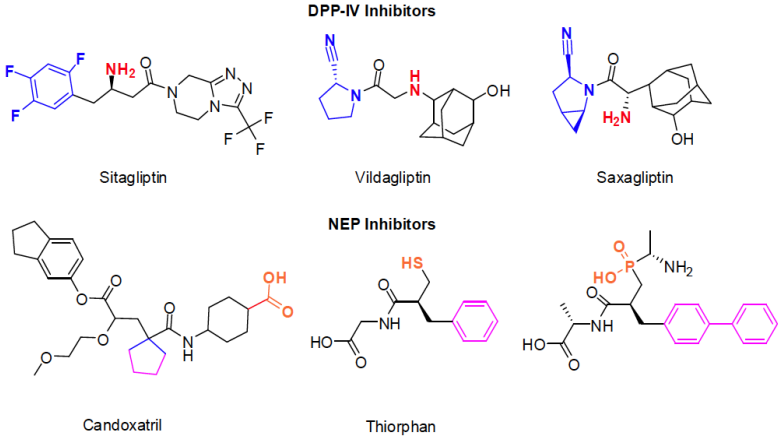
To accomplish this objective, the following specific aims will be considered.

1. To synthesize the lead compounds.
2. To perform *in vitro* and *in vivo* biological evaluation of synthesized compounds for DPP-IV and NEP inhibition.
3. To carry out synthesis of various analogs of lead compounds and test them for the biological activity.
4. To design virtual combinatorial library of compounds with various substituents and metal chelating groups. To carry out virtual screening of the library using docking studies
5. To carry out synthesis of library with available resources and test the compounds for the biological acivity.

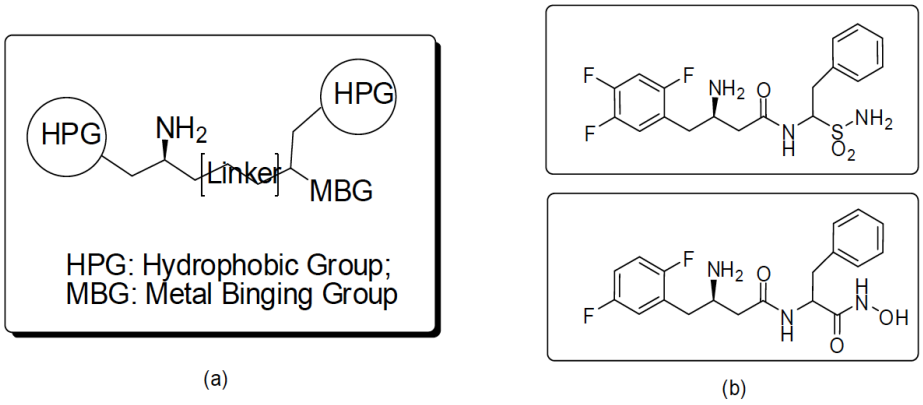
Reference:

1. American Diabetes Association. Economic Costs of Diabetes in the U.S. in 2002. *Diabetes Care* **2003**, *26*, 917-932.
2. Krentz, A. J.; Bailey, C. J. Oral Antidiabetic Agents: Current Role in Type 2 Diabetes Mellitus. *Drugs* **2005**, *65*, 385-411.
3. Knudsen, L. B. Glucagon-like Peptide-1: The Basis of a New Class of Treatment for Type 2 Diabetes. *J. Med. Chem.* **2004**, *47*, 4128-4134.
4. Nauck, M. A.; Niedereichholz, U.; Ettler, R.; Holst, J. J.; Orskov, C.; Ritzel, R.; Schmiegel, W. H. Glucagon-like peptide 1 inhibition of gastric emptying outweighs its insulinotropic effects in healthy humans. *Am J Physiol Endocrinol Metab* **1997**, *273*, E981-988.
5. Kieffer, T. J.; McIntosh, C. H.; Pederson, R. A. Degradation of glucose-dependent insulinotropic polypeptide and truncated glucagon-like peptide 1 in vitro and in vivo by dipeptidyl peptidase IV. *Endocrinology* **1995**, *136*, 3585-3596.
6. Keating, G. M. Exenatide. *Drugs* **2005**, *65*, 1681-1692.
7. Drucker, D.; Easley, C.; Kirkpatrick, P. Sitagliptin. *Nat Rev Drug Discov* **2007**, *6*, 109-110.
8. Turner, A. J.; Isaac, R. E.; Coates, D. The neprilysin (NEP) family of zinc metalloendopeptidases: Genomics and function. *BioEssays* **2001**, *23*, 261-269.
9. Rogues, B. P.; Beaumont, A. Neutral endopeptidase-24.11 inhibitors: from analgesics to antihypertensives? *Trends in Pharmacological Sciences* **1990**, *11*, 245-249.
10. Yasojima, K.; McGeer, E. G.; McGeer, P. L. Relationship between beta amyloid peptide generating molecules and neprilysin in Alzheimer disease and normal brain. *Brain Res.* **2001**, *919*, 115-121.
11. Hupe-Sodmann, K.; McGregor, G. P.; Bridenbaugh, R.; Goke, R.; Goke, B.; Thole, H.; Zimmermann, B.; Voigt, K. Characterisation of the processing by human neutral endopeptidase 24.11 of GLP-1(7-36) amide and comparison of the substrate specificity of the enzyme for other glucagon-like peptides. *Reg. Pept.* **1995**, *58*, 149-156.
12. Hupe-Sodmann, K.; Goke, R.; Goke, B.; Thole, H. H.; Zimmermann, B.; Voigt, K.; McGregor, G. P. Endoproteolysis of Glucagon-like Peptide (GLP)-1(7-36) amide by Ectopeptidases in RINm5F Cells. *Peptides* **1997**, *18*, 625-632.
13. Plamboeck, A.; Holst, J.; Carr, R.; Deacon, C., Neutral Endopeptidase 24.11 and Dipeptidyl Peptidase IV are Both Involved in Regulating the Metabolic Stability of Glucagon-like Peptide-1 in vivo. In *Dipeptidyl Aminopeptidases in Health and Disease*, **2004**; pp 303-312.
14. Plamboeck, A.; Holst, J.; Carr, R.; Deacon, C. Neutral endopeptidase 24.11 and dipeptidyl peptidase IV are both mediators of the degradation of glucagon-like peptide 1 in the anaesthetised pig. *Diabetologia* **2005**, *48*, 1882-1890.

Schemes and Figures:



Scheme 1. Known DPP-IV and NEP inhibitors in the literature. Pharmacophoric features are colored in red and blue for DPP-IV inhibitors; magenta and orange for NEP inhibitors.



Scheme 2. (a) The general structure of the proposed lead molecule (b) Representative structures of lead molecules

EXAMPLE OF AN OUTSTANDING ORP *(submitted by and intellectual property of Mr. Pankaj Daga). Please note that the length of this example may not apply to the current guidelines.*

Form Approved Through 09/30/2007 — OMB No. 0925-0001

Department of Health and Human Services
Public Health Services

Grant Application

Do not exceed character length restrictions indicated.

LEAVE BLANK—FOR PHS USE ONLY.

Type	Activity	Number
Review Group		Formerly
Council/Board (Month, Year)		Date Received

1. TITLE OF PROJECT *(Do not exceed 81 characters, including spaces and punctuation.)*
Design, Synthesis and Biological Evaluation of Dual Anti-Diabetic Agents

2. RESPONSE TO SPECIFIC REQUEST FOR APPLICATIONS OR PROGRAM ANNOUNCEMENT OR SOLICITATION ☒ NO ☐ YES
(If "Yes," state number and title)
Number: Title:

3. PRINCIPAL INVESTIGATOR/PROGRAM DIRECTOR — **New Investigator** ☐ No ☒ Yes

3a. NAME (Last, first, middle): Daga Pankaj R.
3b. DEGREE(S): Ph.D
3h. eRA Commons User Name:
3c. POSITION TITLE: Assistant Professor
3d. MAILING ADDRESS *(Street, city, state, zip code)*: 417 Faser Hall, Department of Medicinal Chemistry, School of Pharmacy, Univeristy of Mississippi, University, MS 38677
3e. DEPARTMENT, SERVICE, LABORATORY, OR EQUIVALENT: Department of Medicinal Chemistry
3f. MAJOR SUBDIVISION: School of Pharmacy
3g. TELEPHONE AND FAX *(Area code, number and extension)*: TEL: 662-915-7101 FAX: 662-915-5638
E-MAIL ADDRESS: pdaga@olemiss.edu

4. HUMAN SUBJECTS RESEARCH	4b. Human Subjects Assurance No.	5. VERTEBRATE ANIMALS ☐ No ☒ Yes	
☒ No ☐ Yes	4c. Clinical Trial ☒ No ☐ Yes; 4d. NIH-defined Phase III Clinical Trial ☒ No ☐ Yes	5a. If "Yes," IACUC approval Date	5b. Animal welfare assurance no.
4a. Research Exempt ☒ No ☐ Yes	If "Yes," Exemption No.	-	-

6. DATES OF PROPOSED PERIOD OF SUPPORT *(month, day, year—MM/DD/YY)*		7. COSTS REQUESTED FOR INITIAL BUDGET PERIOD		8. COSTS REQUESTED FOR PROPOSED PERIOD OF SUPPORT	
From	Through	7a. Direct Costs ($)	7b. Total Costs ($)	8a. Direct Costs ($)	8b. Total Costs ($)
05/01/2008	04/30/2011	$239,405	$325,593	$739,980	$1,065,573

9. APPLICANT ORGANIZATION
Name: University of Mississippi, School of Pharmacy,
Address: 417 Faser Hall
University, MS 38677

10. TYPE OF ORGANIZATION
Public: → ☐ Federal ☒ State ☐ Local
Private: → ☐ Private Nonprofit
For-profit: → ☐ General ☐ Small Business
☐ Woman-owned ☐ Socially and Economically Disadvantaged

11. ENTITY IDENTIFICATION NUMBER
64-6001159
DUNS NO.
Cong. District 01

12. ADMINISTRATIVE OFFICIAL TO BE NOTIFIED IF AWARD IS MADE
Name: Dr. Robin Buchannon
Title: Director of Sponsored Program Administration
Address: University of Mississippi
100 Barr Hall
P.O. Box 907 University, MS 38677
Tel: 662-915-7482 FAX: 662-915-7577
E-Mail: research@olemiss.edu

13. OFFICIAL SIGNING FOR APPLICANT ORGANIZATION
Name: Dr. Alice M. Clark
Title: Vice Chancellor for Research
Address: University of Mississippi
100 Barr Hall
P.O. Box 907 University, MS 38677
Tel: 662-915-7482 FAX: 662-915-7577
E-Mail: research@olemiss.edu

14. APPLICANT ORGANIZATION CERTIFICATION AND ACCEPTANCE: I certify that the statements herein are true, complete and accurate to the best of my knowledge, and accept the obligation to comply with Public Health Services terms and conditions if a grant is awarded as a result of this application. I am aware that any false, fictitious, or fraudulent statements or claims may subject me to criminal, civil, or administrative penalties.

SIGNATURE OF OFFICIAL NAMED IN 13. *(In ink. "Per" signature not acceptable.)* — DATE

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DESCRIPTION: See instructions. State the application's broad, long-term objectives and specific aims, making reference to the health relatedness of the project (i.e., relevance to the **mission of the agency**). Describe concisely the research design and methods for achieving these goals. Describe the rationale and techniques you will use to pursue these goals.

In addition, in two or three sentences, describe in plain, lay language the relevance of this research to **public** health. If the application is funded, this description, as is, will become public information. Therefore, do not include proprietary/confidential information. **DO NOT EXCEED THE SPACE PROVIDED.**

The prevalance of diabetes is increasing dramatically throughout the world. Serious complications developed due to Diabetes Mellitus (DM) have increased the total cost of this disorder in multi-billions. Of the two types, type 2 diabetes mellitus is the most common. Although, a lot of front-line agents are available for the treatment of the Type 2 Diabetes Mellitus, severe side-effects associated with these agents limit their use. Recently, glucagon-like peptide-1 (GLP-1) based therapy is gaining attention for the role in treatment of DM. Despite having many biological actions, which can regulate blood glucose level, therapeutic use of GLP-1 is limited because of its short half-life of less than 3 minutes. Glucagon-Like Peptide-1 (GLP-1) based therapies have been proposed recently, which include a GLP-1 mimetic as well as agents interfering with GLP-1 degradation. Agents that can prevent degradation of GLP-1 have therapeutic importance. Dipeptidyl Peptidase-IV (DPP-IV) and Neutral Endopeptidase (NEP) are the two enzymes, responsible for degradation of GLP-1 in vivo. Design of a dual ligand in the form of a single chemical entity that can modulate the two targets simultaneously will be advantageous in all aspects. This proposal works on design of a single chemical entity as a dual ligand which can simultaneoulsy inhibit two enzymes, DPP-IV and NEP, responsible for GLP-1 degradation in vivo. The designed molecule can thus raise the levels of GLP-1 thus showing the euglycemic effects. A few new chemical entities were designed by combining key pharmacophoric fetures, each of specific DPP-IV inhibitors and NEP inhibitors, reported in the literature. The desinged molecules were then subjected to docking into the protein structure of the two targetrs. Preliminary docking studies suggest that the newly designed molecules show better binding affinities compared to existing inhibitors. We intend to develop a synthetic method for the lead molecules designed and a series of analogs through parellel approach. The synthesized molecules will then be subjected to biological testing to test their DPP-IV and NEP inhibitory activity.

PERFORMANCE SITE(S) (organization, city, state)

The University of Mississippi, Oxford, MS
University of Albany, NY

Principal Investigator/Program Director (Last, First, Middle): Daga Pankaj R.

KEY PERSONNEL. See instructions. *Use continuation pages as needed* to provide the required information in the format shown below. Start with Principal Investigator(s). List all other key personnel in alphabetical order, last name first.

Name	eRA Commons User Name	Organization	Role on Project
Daga, Pankaj R.		University of Mississippi	Principal Investigator
Anderson, John		University of Albany	Postdoctoral Associate
Jones, Ronald		University of Mississippi	Postdoctoral Associate
Mathew, Andrew		University of Mississippi	Graduate Student
Li, Chenglong		University of Mississippi	Postdoctoral Associate
Welch, John		University of Albany	Collaborator

OTHER SIGNIFICANT CONTRIBUTORS

Name	Organization	Role on Project

Human Embryonic Stem Cells ☒ No ☐ Yes

If the proposed project involves human embryonic stem cells, list below the registration number of the specific cell line(s) from the following list: http://stemcells.nih.gov/registry/index.asp. *Use continuation pages as needed.*

If a specific line cannot be referenced at this time, include a statement that one from the Registry will be used.

Cell Line

The name of the principal investigator/program director must be provided at the top of each printed page and each continuation page.

RESEARCH GRANT

TABLE OF CONTENTS

Number of publications and manuscripts accepted for publication *(not to exceed 10)* _____

Other items (list):

Principal Investigator/Program Director (Last, First, Middle): Daga Pankaj R.

DETAILED BUDGET FOR INITIAL BUDGET PERIOD
DIRECT COSTS ONLY

FROM | THROUGH

PERSONNEL *(Applicant organization only)*		Months Devoted to Project				DOLLAR AMOUNT REQUESTED *(omit cents)*		
NAME	ROLE ON PROJECT	Cal. Mnths	Acad. Mnths	Summer Mnths	INST.BASE SALARY	SALARY REQUESTED	FRINGE BENEFITS	TOTAL
Dr. Daga Pankaj R	Principal Investigator	5.4	-	-	80,000	36,000	10,418	46,418
Jones, Ronald	PostDoctoral Associate	4.8	-	-	40,000	16,000	4,630	20,630
Li, Chenglong	PostDoctoral Associate	3.6	-	-	40,000	12,000	3,472	15,472
Mathew, Andrew	Graduate student	12	-	-	20,000	20,000	600	20,600
SUBTOTALS →						84,000	19,120	103,120

CONSULTANT COSTS		0
EQUIPMENT *(Itemize)*		0
SUPPLIES *(Itemize by category)* Chemicals including reagents and solvents (30,000) Expenses related to analytical techniques like NMR, mass, HPLC and IR (20,000) General glasswares (5,000) Software (30,000)		85,000
TRAVEL		5,000
PATIENT CARE COSTS	INPATIENT	0
	OUTPATIENT	0
ALTERATIONS AND RENOVATIONS *(Itemize by category)*		0
OTHER EXPENSES *(Itemize by category)* Tuition for graduate student (7,604)		7,604
CONSORTIUM/CONTRACTUAL COSTS	DIRECT COSTS	38,681
SUBTOTAL DIRECT COSTS FOR INITIAL BUDGET PERIOD *(Item 7a, Face Page)*		$ 239,405
CONSORTIUM/CONTRACTUAL COSTS	FACILITIES AND ADMINISTRATIVE COSTS	0
TOTAL DIRECT COSTS FOR INITIAL BUDGET PERIOD		$ 239,405

Principal Investigator/Program Director (Last, First, Middle): Daga Pankaj R.

DETAILED BUDGET FOR INITIAL BUDGET PERIOD
DIRECT COSTS ONLY

FROM | THROUGH

PERSONNEL *(Applicant organization only)*		Months Devoted to Project				DOLLAR AMOUNT REQUESTED *(omit cents)*		
NAME	ROLE ON PROJECT	Cal. Mnths	Acad. Mnths	Summer Mnths	INST.BASE SALARY	SALARY REQUESTED	FRINGE BENEFITS	TOTAL
	Principal Investigator							
Prof. John Welch	Collaborator	-	2.7	-	80,000	18,000	5,209	23,209
Dr. John Anderson	PostDoctoral Associate	3.6	-	-	40,000	12,000	3,472	15,472
SUBTOTALS →						30,000	8,681	38,681

CONSULTANT COSTS		
EQUIPMENT *(Itemize)*		
SUPPLIES *(Itemize by category)*		
TRAVEL		
PATIENT CARE COSTS	INPATIENT	
	OUTPATIENT	
ALTERATIONS AND RENOVATIONS *(Itemize by category)*		
OTHER EXPENSES *(Itemize by category)*		
CONSORTIUM/CONTRACTUAL COSTS	DIRECT COSTS	
SUBTOTAL DIRECT COSTS FOR INITIAL BUDGET PERIOD *(Item 7a, Face Page)*		$
CONSORTIUM/CONTRACTUAL COSTS	FACILITIES AND ADMINISTRATIVE COSTS	
TOTAL DIRECT COSTS FOR INITIAL BUDGET PERIOD		$ 38,681

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Principal Investigator/Program Director (Last, First, Middle): Daga Pankaj R.

BUDGET FOR ENTIRE PROPOSED PROJECT PERIOD
DIRECT COSTS ONLY

BUDGET CATEGORY TOTALS		INITIAL BUDGET PERIOD *(from Form Page 4)*	ADDITIONAL YEARS OF SUPPORT REQUESTED 2nd	3rd	4th	5th
PERSONNEL: *Salary and fringe benefits. Applicant organization only.*		103,120	106,214	109,401	0	0
CONSULTANT COSTS		0	0	0	0	0
EQUIPMENT		0	0	0	0	0
SUPPLIES		85,000	87,550	90,176	0	0
TRAVEL		5,000	5,150	5,304	0	0
PATIENT CARE COSTS	INPATIENT	0	0	0	0	0
	OUTPATIENT	0	0	0	0	0
ALTERATIONS AND RENOVATIONS		0	0	0	0	0
OTHER EXPENSES		7,604	7,833	8,068	0	0
CONSORTIUM/ CONTRACTUAL COSTS	DIRECT	38,681	39,842	41,037	0	0
SUBTOTAL DIRECT COSTS *(Sum = Item 8a, Face Page)*		239,405	246,589	253,986	0	0
CONSORTIUM/ CONTRACTUAL COSTS	F&A	0	0	0	0	0
TOTAL DIRECT COSTS		239,405	246,589	253,986	0	0

TOTAL DIRECT COSTS FOR ENTIRE PROPOSED PROJECT PERIOD **$** 739,980

JUSTIFICATION. Follow the budget justification instructions exactly. Use continuation pages as needed.

Pankaj R. Daga, Ph.D., Principal Investigator (effort = 45% through out the project tenure) will be responsible for the overall execution and coordination of the project. He will devote his experience on synthesis and molecular modeling to supervise all other personnel involved in the project.

Ronald Jones, Ph.D., Postdoctoral Associate (effort = 40% during the first year) with expertise in chemical synthesis will be responsible synthesizing the lead moleucles designed and initial lead optimization stage

Chenlong Li, Ph.D., Postdoctoral Associate (effort = 30%) has expertise in in-silico modeling of protein and small molecules. He will be responsible for in silico optimization of the ligand using protein-ligand docking studies. He will also be involved in designing of virtual combinatorial library in-silico and virtual screening of the library in the later stage of the project.

Andrew Mathew, MS., Graduate student (effort = 100%) will be exclusively involved in synthesis phase of the project under the direct supervision of Pankaj Daga and Ronald Jones.

Prof. John Welch, Ph.D., collaborator will devote his efforts only in academic months (effort = 30%) and will be responsible for the biological evaluation (in vivo and in vitro assays) of the synthesized compounds.

John Anderson, Ph.D., Postdoctoral Associate (effort = 30%) will work exclusively on the biological evaluation under the supervision of John Welch.

Principal Investigator/Program Director (Last, First, Middle): Daga Pankaj R.

BUDGET JUSTIFICATION PAGE MODULAR RESEARCH GRANT APPLICATION						
	Initial Period	**2nd**	**3rd**	**4th**	**5th**	**Sum Total (For Entire Project Period)**
DC less Consortium F&A	239,405 *(Item 7a, Face Page)*	246,589	253,586	0	0	739,980 *(Item 8a, Face Page)*
Consortium F&A	0	0	0	0	0	0
Total Direct Costs	0	0	0	0	0	$ 739,980

Personnel

Consortium

BIOGRAPHICAL SKETCH

Provide the following information for the key personnel and other significant contributors in the order listed on Form Page 2.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME	POSITION TITLE
Pankaj R. Daga	Assistant Professor
eRA COMMONS USER NAME	

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
M. G. V.'s College of Pharmacy, Nashik, University of Pune, INDIA	B. Pharm.	1997-2001	Pharmaceutical Sciences
National Institute of Pharmaceutical Education and Research (NIPER), Punjab, INDIA	M.S.(Pharm.)	2001-2002	Medicinal Chemistry
University of Mississippi	Ph.D.	2007	Medicinal Chemistry

A. Positions and Honors

Professional Experience

2002 – Jun 2003 Junior Scientist, Discovery Research, Dr. Reddy's Laboratories Ltd. (DRL), INDIA

Aug. 2003 – May 2007 Graduate Student/Research Assistant, University of Mississippi

Jun 2007 - Assistant Professor

Honors and Professional Membership

Jun. 2001– Dec. 2002 Research fellow at NIPER, Mohali, INDIA
Oct. 2006 Member of Phi Kappa Phi Honor Society.

B. Selected peer-reviewed publications (in chronological order)

- Singh S. K., Saibaba V., Ravikumar V., Rudrawar S. V., **Daga P.** , Rao C. S., Akhila V., Hegde P., Rao Y. K.. Synthesis and Biological Evaluation of 2,3-Diarylpyrazines and Quinoxalines as Selective COX-2 Inhibitors. *Bioorg. Med. Chem.* **2004**, *12* (8), 1881-1893.
- Thaimattam R., **Daga P.**, Razzak S. A., Banerjee R., Iqbal J. 3D-QSAR CoMFA, CoMSIA Studies on Substitued Ureas as Raf-1 Kinase Inhibitors and its Confirmation with Structure-Based Studies. *Bioorg Med Chem.* **2004**, *12* (24), 6415-6425.
- Thaimattam R., **Daga P. R.**, Banerjee R., Iqbal J. 3D-QSAR studies on c-Src kinase inhibitors and docking analyses of a potent dual kinase inhibitor of c-Src and c-Abl kinases. *Bioorg Med Chem.* **2005**, *13* (15), 4704-4712.

- Singh S. K., Saibaba V., Rao K. S., Reddy P. G., **Daga P. R.**, Rajjak S. A., Misra P., Rao Y. K. Synthesis and SAR/3D-QSAR Studies on the COX-2 Inhibitors Activity of 1,5-Diarylpyrazoles to Validate the Modified Pharmacophore. *Euro. J. Med. Chem.* **2005**, *40* (10), 977-990.
- Patel D. S., **Daga P.**, Bharatam P. V., Dongare R. K., Gadre S. R. Molecular Electrostatic Potential (MESP) studies on the anti-hyperglycemic agents — 2,5-dihydroxyquinones. *Ind. J. Chem. Sect. A*, **2006**, *45A* (1), 13-20.
- **Daga P. R.**, Doerksen R. J. Stereoelectronic Properties of Spiroquinazolinones in Differential PDE7 Inhibitory Activity. *J. Comp. Chem.***2007** (Resubmitted after revision)

RESOURCES

FACILITIES: Specify the facilities to be used for the conduct of the proposed research. Indicate the performance sites and describe capacities, pertinent capabilities, relative proximity, and extent of availability to the project. If research involving Select Agent(s) will occur at any performance site(s), the biocontainment resources available at each site should be described. Under "Other," identify support services such as machine shop, electronics shop, and specify the extent to which they will be available to the project. Use continuation pages if necessary.

Laboratory:

The Medicinal Chemistry Labs are located in the Faser Hall. The Department of Medicinal Chemistry has received funding through the Centre for Disease Control and Prevention for creation of the Laboratory for Applied Drug Design and Synthesis (LADDS).All of the laboratories involved in this project are well equipped.

Clinical:

NA

Animal:

C57BL/6N mice

Computer:

The principal investigator of the project has a Macintosh workstation and a WindowsXP laptop in his office. 3 Windows XP Dell workstations, 2 Dell Linux computers, and 1 SGI Octane2 workstations are located in the PIs lab.

Office:

Office equipments include a fully functional photo copier, FAX machines, computers, laptop, color printers and scanners.

Other:

The National Center for Natural Products Research (NCNPR) has a science library and a central animal facility.

MAJOR EQUIPMENT: List the most important equipment items already available for this project, noting the location and pertinent capabilities of each.

Listed and described in detail in next few pages.

RESOURCES

FACILITIES: Specify the facilities to be used for the conduct of the proposed research. Indicate the performance sites and describe capacities, pertinent capabilities, relative proximity, and extent of availability to the project. Under "Other," identify support services such as machine shop, electronics shop, and specify the extent to which they will be available to the project. Use continuation pages if necessary.

LABORATORY: 1. THE LABORATORY FOR APPLIED DRUG DESIGN AND SYNTHESIS, DEPARTMENT OF MEDICINAL CHEMISTRY, THE UNIVERSITY OF MISSISSIPPI

1. School of Pharmacy:

Founded in 1908, the School of Pharmacy offers the Doctor of Pharmacy as the entry-level practice degree and is divided into six departments. All departments, with the exception of pharmacy practice, offer graduate degrees in their respective field. The School of Pharmacy is the sole occupant of Faser Hall, a four-story building completed in 1969, housing the teaching and research activities of the faculty and students. The fourth floor of Faser Hall, which houses the majority of The Department of Medicinal Chemistry laboratory and office space, has been fully renovated in 2005. Plans are underway for renovation of the third floor of Faser Hall.

2. Department of Medicinal Chemistry, School of Pharmacy

Currently, six faculty members make up the Department of Medicinal Chemistry, with expertise and interests in the areas of organic synthesis, rational drug design, molecular modeling, enzymology, isolation of new drug targets, lead optimization, combinatorial and parallel synthesis, purification and analysis, biotechnology, and opioid research. The Department currently has on-going research projects in the therapeutic emphasis areas of: emerging infectious diseases, cancer chemotherapy, immune modulation, diseases of the CNS, cardiovascular disease, female and male endocrine disorders, prevention and treatment of drug abuse.

2.1. The Laboratory for Applied Drug Design and Synthesis

The Department of Medicinal Chemistry, through a multi-year grant from The Centers for Disease Control and Prevention, has created and implemented The Laboratory for Applied Drug Design and Synthesis (LADDS). LADDS consists of a multi-million dollar array of state-of-the-art instrumentation that provides the infrastructure required to support the mission of LADDS.

"The mission of LADDS is to achieve in parallel the multiple goals of teaching rational drug design and synthesis while carrying out the actual research endeavour of identifying novel antiinfective agents."

The focus of LADDS initially involved the chemotherapy of drug-resistant malaria. Other infectious organisms, particularly those with emerging resistance to conventional drug therapies, are now targeted in parallel projects. The core of LADDS instrumentation is the contrasting fields of computer aided-drug design (CADD), and combinatorial and/or parallel chemistry (CPC). The rational area of CADD is integrated with the more random seeming library synthesis by first constructing rational drug templates whose ultimate pharmaceutical properties are optimized by parallel synthesis of analogs. In collaboration with academic or industrial partners, biochemical or biological assays of these libraries then provides the necessary information for iterative rounds of design and synthesis until a unique agent with desirable properties can be identified. Finally, LADDS faculty are committed to the ultimate commercialization of the best of these new drugs and work with the Office of Research to ensure that intellectual property is generated before publications are obtained

Core instrumentation has been acquired to streamline medicinal chemistry projects in a contemporary fashion, allowing for the preparation of libraries of compounds, ranging from tens to thousands of compounds, in a short time frame. Graduate students in our program receive extensive training using state-of-the-art instrumentation, and learn how to integrate the aspects of design, construction, analysis, purification, and data management of chemical libraries.

2.2. Instrumentation at Department of Medicinal Chemistry (University of Mississippi)

2.2.1. NMR Facilities (4 High Field FT-NMR): The School of Pharmacy is equipped with two suites of NMR-spectrometers. Each instrument is specifically tailored to provide inverse proton or inverse carbon detection. The NMR systems use SGI platforms and are networked to provide data transfer between instruments. NMR suite 1, located in Faser Hall, houses2 Bruker 400 MHz Avance NMR spectometers. NMR suite 2, located in the Natural Product Center, houses two Bruker Avance NMR spectrometers (500 and 600 MHz).

2.2.2. Molecular Modeling Facilities Department of Medicinal chemistry has two suits of computer aided drug design, to b used for research as well as teaching efforts. Suite 1 Consists of six Silicon Graphics Workstations (four INDYs, Four Indigo2 Impacts, and a Challenge), a MicroVAX II and AT&T mini-mainframe computers. Suite 2 contains (6) SGI Octane2 with 360 MHz MIPS R12000A processors, 256MB of memory, a 9GB Ultra SCSI disk, 32MB of graphics memory and 21" monitors. Various Software packages available for the research as well as teaching include:

- **Tripos Inc.**: SYBYL/Base, CONCORD, Biopolymer, GeneFold, Composer, ProTable, FlexX, CScore, Flexidock, CoMFA, CoMSIA.
- **Accelrys Inc.:** InsightII, Builder, Discover, Homology, Affinity, Modeler, LUDI, Biopolymer, Profiles-3D, Cerius2-QSAR, ADME, Catalyst.
- GOLD from **CCDC, UK**
- PC-based Spartan available for PC-based small molecular modeling from **Wavefunction Inc**. are also available at disposal.

2.2.3. Parallel Synthesis-Combinatorial Chemistry

- Argonaut® Trident Combinatorial System: Equipped with Trident Processing Station, Trident Workstation, Autosampler (Gilson 223), 4 Reaction Cassettes, and Agitation-Thermal Unit for temperature control from –40 to 150 oC. The Trident Library Synthesizer runs up to 192 reactions in parallel. The Trident software controls the temperature, agitation, reagent/solvent deliveries and product collection. The Trident Workstation uses a manual interface to deliver reagents, while still maintaining inert conditions, and performs parallel resin washing and sample collection. The Trident Processing Station is a multi-purpose liquid handler with a special interface to the Trident Reaction Cassette, used to perform liquid, extraction's, solid-phase extraction's, reverse filtration, dry solid loading to open vessels, sampling of reaction vessel contents to vials, reformatting (e.g. from tubes to microplates), delivery of reagent/solvent to Reaction Cassette, draining/washing of resin in Reaction Cassette, transfer of reaction vessel contents to vials, transfer of tube/vial contents to reaction vessels, and addition of solvent to external vials.
- Six (6) Argonaut Quest 210 Parallel Organic Synthesizers: Equipped with 20 reaction vessels, automated solvent wash module, solid phase extraction rack, and hydrogenation module. The Quest 210 handles both solid-phase and solution-phase chemistry. These systems are ideal for the synthesis of small focused libraries on solid-support, chemistry development, exploratory reactions, and the resynthesis of active compounds. This instrument handles up to 20 simultaneous reactions in either 5 ml or 10 ml reaction vessels.
- Four (4) Argonaut FirstMate Benchop Modules: These are the scaled-down versions of the Quest systems, and are used routinely for larger scale solution chemistry.

2.2.4. HPLC-Mass Spectrometry

LC-MS Systems.

(1) Waters Alliance HT LC/MS System consists of: Waters 2790 Separations Module, the Waters ZQ™ Mass Detector (Waters 996 Photodiode Array (PDA) Detector, and FractionLynx™ software. (2) Waters Alliance LC/MS Systems, consists of Waters ZQ Mass Detector, Photodiode Array Detector, and Alliance HPLC system.

HPLC Systems

Five (5) Waters Alliance HPLC Systems: (UV, Diode Array, Evaporative Light Scattering detectors).

Four (4) Waters DeltaPrep 4000 series HPLC Systems: Equipped with preparative capability – from hundreds of milligrams to grams per run, a PrepLC Controller, solvent delivery unit, an integral injector panel, a Rheodyne 7725I injector, and Waters 996 Photodiode Array or Waters 2487 Dual Wavelength Absorbance Detector.

High Resolution Q-TOF (Quadropole-Time-of-Flight)

Micromass Q-TOF micro Hybrid Quadrupole/Orthogonal High Resolution Time of Flight MS with Micromass capillary HPLC. ESI and APCI (positive and negative ion modes) including LockSpray source (right) and NanoFlow CapLC source (left). Biochemical analysis sofware suites include ProteinLynx, BioLynx, and MetaboLynx. Routine instrument for high-resolution analysis of small molecules or proteins. Nanoflow CapLC accessory performs microflow capabilities at nL/min flow rates.

2.2.5 Additional Instrumentation

- **Polarimeter**. Rudolph Autopol IV, a six-wavelength polarimeter equipped with stainless steel jacketed cells, for measuring optical rotations. Volumes accomodated range from 1.0 mL to 0.05 mL
- **Fourier Transform Infrared Spectrophotometer.** Bruker Vector33 FTIR, with NIR and MID FTIR capabilities, equipped with a fiber optics accessory for remote sensing, and an HYPERION IRScope with ATR capabilities for solid-phase analysis of chemically modified resins.
- **UV-VIS Spectrophotometer.**Hewlett-Packard 8453 Diode-Array Biochemical Analysis Spectrophotometer equipped with 7-cell thermostatted multicell transport accessory and dedicated water circulator. Biochemical Analysis software suite allows for kinetic analysis and real-time monitoring.
- **Elemental Analysis.** Perkin-Elmer CHN/SO Series II Analyzer
  Elemenatal analyzer equipped with a 60 sample carosel for performing high-throughput elemenatal analyses.

3. National Center for Natural Product Research

NCNPR, a $27.5 million Center is a research facility engaged in the discovery of both pharmaceutical and agrichemical state-of-art technologies based on natural resources like plants, animals and microbes. NCNPR was fully functional since 1995 and was authorized by congress in 1988. It is housed in a building named Thad Cochran natural products center. Currently, NCNPR holds 120 researchers working on diverse disciplines like molecular biology, biochemistry, microbiology, pharmacognosy, pharmacology, toxicology, medicinal chemistry, botany, pharmaceutics, plant physiology, analytical chemistry etc. Ample natural products are studied as successful candidate to treat diseases like AIDS, malaria, fungal infections, tuberculosis and other tropical diseases. Also, measures to control fungal diseases in crops and algae growth in catfish are special areas of research. NCNPR began its collaboration with USDA in 1996 and with U.S Food and Drug Administration in 2001. Also, American herbal pharmacopoeia holds research collaboration with NCNPR with an objective to develop quality standards for botanical dietary supplements. NCNPR was approved by the U.S. Department of Agriculture in 1989. About 115,000 gross square feet of space was provided under phase I for constructing laboratories, offices, conference space, classrooms, purchase of major instrument and computer facilities, maintenance of a Science Library and an animal care facility. Due to the spectacular research progress a phase II 120,000 gross square feet addition to the NCNPR is underway.

- Repository of more than 18,000 natural product specimens, extracts, and pure compounds
- NCNPR screening database including more than 30,000 samples
- Specialized natural products isolation and purification laboratories
- Screening laboratories for determining biological activity of natural products
- Central instrumentation laboratory including nuclear magnetic resonance and mass spectrometers
- Medicinal chemistry synthesis laboratories
- Natural products analytical laboratories utilizing a broad spectrum of analytical techniques, including gas and liquid chromatography, densitometry, and capillary electrophoresis
- Molecular modeling laboratory where sophisticated computer-based techniques reveal molecular structure properties of drugs and their targets, leading to new drug design
- Production greenhouses, demonstration garden beds, shade houses, and field plots
- Science library of more than 69,000 volumes in house, with electronic access to thousands more journals, databases, and books

4. Mississippi Center for Supercomputing Research (MCSR)

MCSR is funded by the state of Mississippi and it offers high-performances computing facilities to all the Mississippi universities. It currently provides two supercomputers and a Linux cluster for the use of various research groups within university of Mississippi. Super computers include Redwood which is a 224-CPU SGI Altix 3700 model and Sweetgum which is a 128-CPU SGI Origin 2800. Redwood has 64 Itanium2 900 MHz processors and 160 Itanium2 1.3 GHz processors with 1GB memory on each. The SGI Altix 3700 supercluster helps researchers to take advantage of multiple processors to run parallel calculations that have particularly high performance codes and complex calculations especially when dealing with biological problems. Sweetgum includes 128 CPUs with 64 gigabyte of memory. The Beowulf Linux cluster includes Mimosa which is a 253-

node Intel cluster. Several programs are available in these hardware for research purpose. Some of them include:

- **Programming**. Fortran, C/C++, Java, etc.,
- **Molecular Modeling**. Amber, Gaussian 98/03 on mimosa/sweetgum, Gaussian 03 on redwood, NWChem, GAMESS, CPMD, GROMACS
- **Math/sci/viz** PV-WAVE, IMSL, GSL, Math/Scientific Libraries, Abaqus on sweetgum, Abaqus on redwood
- **Math/Stats** MATLAB, Abaqus on redwood, SPSS example part I, SPSS example part II, Mathematica.

A. SPECIFIC AIMS

Diabetes Mellitus was reported to be the sixth leading cause of death in U.S. in 2002. It can lead to serious complications such as heart diseases and stroke, hypertension, blindness, kidney and nervous system diseases and many others. A number of oral anti-diabetic agents along with insulin preparations are available for the treatment of Diabetes. Various side-effects associated with these agents in addition to patient compliance limit the use of these agents. Glucagon-Like Peptide-1 (GLP-1) based therapies have been proposed recently, which include a GLP-1 mimetic as well as agents interfering with GLP-1 degradation. GLP-1 is degraded *in vivo* by two enzymes dipeptidyl peptidase IV (DPP-IV) and Neutral Endopeptidase (NEP). Inhibiting these two enzymes leads to increase in serum GLP-1 level which ultimately exerts antidiabetic activity via various processes. Design of a dual ligand in the form of a single chemical entity that can modulate the two targets simultaneously will be advantageous in all terms. The overall objective of my research proposal is to design and synthesize small molecule entities to simultaneously inhibit DPP-IV and NEP. Such a goal can be achieved by combining pharmacophoric features of the two classes of inhibitors to design a dual inhibitor. The aims presented herein represent a novel approach for the treatment of Type 2 Diabetes Mellitus.

To accomplish this objective, the following specific aims will be executed:

1. To combine specific pharmacophore elements to design lead dual ligands taking into consideration the key components of both the selective DPP-IV and NEP inhibitors
2. To carry out synthesis of the lead molecules and to perform *in vitro* biological evaluation of synthesized compounds for DPP-IV and NEP inhibition
3. To carry out synthesis of various analogs of lead compounds to find the structure-activity relationship and to perform *in vitro* biological evaluation of newly synthesized compounds for DPP-IV and NEP inhibition
4. To perform *in vivo* biological testing of few best compounds, based on *in vitro* inhibition

B. BACKGROUND AND SIGNIFICANCE

B.1. Introduction

Diabetes Mellitus (DM) is a major debilitating disease that is caused by abnormal glucose homeostasis and is commonly associated with obesity. Approximately 5% of the world's population is suffering from this disorder.[1] DM is a global problem, with a projected prevalence from approximately 171 million people in 2000 to 336 million individuals worldwide by 2030.[2] It can lead to serious complications such as heart diseases, stroke, hypertension, blindness, kidney and nervous system diseases and many others. Total cost of diabetes in United States, in 2002, was reported to be $132 billion.

Two major types of diabetes Type 1 and Type 2, T1DM and T2DM, respectively, are reported. T1DM develops as a result of pancreatic β cells being destroyed by the immune system thus altering the insulin secretion. Continuing loss of function of islet β cells is held to be a major determinant along with development of resistance to insulin for the development of T2DM. T2DM is increasingly becoming a worldwide epidemic. It accounts for about ≥ 90 percent of the cases diagnosed.[1]

B.2. Existing Pharmacotherapy of Diabetes Mellitus

As mentioned earlier, in T1DM, insulin secretion is altered because of destruction of β cells; insulin is the only treatment of choice for T1DM. However, a more preventative approach is used to treat T2DM namely diet and exercise. Oral medications such as sulphonylureas, biguanides, PPAR regulators and others are prescribed in these cases. *Sulphonylureas* like Tolbutamide (**1,** Figure 1) and glyclazide (**2**) have been a popular choice as first-line therapy for the treatment of T2DM for nearly 50 years. These agents lower the blood glucose level primarily by stimulating insulin secretion from β cells of pancreatic islet.[3] Various first and second generation sulphonylureas are available in the market showing short to intermediate to long onset of action. *α-Glucosidase Inhibitors* provide an alternative means of therapy to reduce hyperglycemia. Acarbose (**3**) was introduced in the early 1990's, which was followed by two additional agents, miglitol and voglibose. These agents inhibit activity of α-glucosidase enzymes preventing them from cleaving disaccharide and oligosaccharide substrates into monosaccharide prior to absorption.[4]

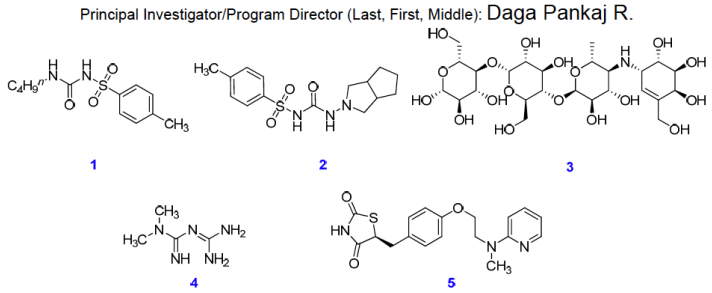


Figure 1. Available Therapeutic agents for the treatment of T2DM.

Biguanides such as Metformin (**4**) and Phenformin improve insulin sensitivity at the cellular level. Metformin is widely used as the initial drug of choice for the treatment of T2DM because of its safety and cost.[5] Agents, from *thiazolidinedione* or *glitazone* class, like rosiglitazone (**5**) and pioglitazone are some of the latest therapeutic agents causing improvement in whole-body insulin sensitivity. These effects result from stimulation of a nuclear receptor peroxisome proliferaor-activator receptor-γ (PPARγ).[6] In recent years, the incretin hormone glucagon-like peptide 1 (GLP-1) has been the subject of intense research efforts related to the treatment of type 2 diabetes.[7] These approaches will be discussed in detail below.

B.3. Adverse Effects Associated with Current Therapy

Hypoglycaemia is the most common and potentially most serious adverse effect of sulphonylurea therapy.[8] Apart from these two major side-effects other adverse events like sensitivity reactions, fever, jaundice and blood dyscriasis are also observed. Gastrointestinal disturbances caused by α-glucosidase inhibitors in addition to high cost have substantially limited their use.[8] Phenformin was withdrawn from the market in many countries because of high incidence of lactic acidosis. Despite having some gastrointestinal adverse effects, metformin is widely regarded as the drug of choice for most patients with type 2 diabetes, as it does not promote weight gain and has beneficial effects on several cardiovascular risk factors.[5] Though very effective in reducing the blood glucose levels, glitazones can cause fluid retention with increase plasma volume, a reduced haemarocrit and a decrease in hemoglobin concentration. Troglitazone, the first glitazone launched in the market, was withdrawn in 2000 because of cases of idiosyncratic hepatotoxicity resulting in fatalities. Recently pioglitazone and rosiglitazone are shown to increase cardiovascular risk in T2DM patients[9] and found to be associated with more fractures in upper arm, hand or foot, in female patients.

B.4. GLP-1 Based Therapies for Diabetes Mellitus

Glucose-dependent insulin secretion is promoted by incretin hormones, predominantly Gastro-Insulinotropic peptide (GIP) and glucagon-like peptide-1 (GLP-1). GLP-1 possesses a number of properties that make it a potentially ideal antidiabetic target. GLP-1 is released from intestinal L-cells in response to food intake and has been shown to affect the pancreas, gastro-intestinal tract and brain. After release, GLP-1 stimulates meal-induced insulin secretion, and helps in insulin biosynthesis. It is also found to increase pancreatic β cell mass, by stimulating β cell proliferation and differentiation and reducing the β cell apoptosis. In the GI tract, it is found to decrease gastric motility and secretions.[10] Central administration of GLP-1 antagonist Exendin (9-39) was found to increase food intake, which suggests that GLP-1 may exert satiating effects, centrally.

Intravenous administration of GLP-1 is shown to normalize blood glucose levels in T2DM patients.[11] However, peptidic nature and short half-life of the GLP-1 makes it unavailable for oral administration. Recently, Knudsen and coworkers have discovered a series of small molecules known as ago-allosteric modulators selective for the human GLP-1 receptor.[12] These molecules are still in the initial stages of discovery. An indirect way is to develop degradation resistant GLP-1 analog or inhibit the degradation of GLP-1. Exendin-4 (**Byetta®**), a close analog of GLP-1, was approved for the treatment of T2DM in 2005.[13] Various long-acting GLP-1 mimetics have already reached different phases of clinical trials. Structure-activity relationship studies of analogs of GLP-1 have shown that residues in the C-terminal region are important for receptor binding while N-terminal dipeptide is essential for receptor activation.[14] *Dipeptidyl Peptidase IV* (DPP-IV) is responsible for cleavage of the two N-terminal amino acids of active GLP-1 to yield inactive GLP-1.[15] Fewer studies have been carried out to study other enzymes involved in GLP-1 degradation. *In vitro*, *Neutral Endopeptidase* (NEP) was also

reported to degrade members of glucagon/secretin/glucose-dependent insulinotropic polypeptide family, including GLP-1.[16, 17]

B.6. Dipeptidyl Peptidase IV (DPP-IV).

DPP-IV (*aka* CD26), is ubiquitously distributed serine protease. It cleaves GLP-1(7-36) to GLP-1(9-36) by selectively cleaving two N-terminal amino acid residues, which are important for receptor binding.[15] Numerous evidences suggest that DPP-IV may represent a novel target for the treatment of type 2 diabetes. DPP-IV knockout mice were healthy, showed increased active GLP-1 levels, improved glucose tolerance, and were resistant to body weight gain on a high-fat diet. More importantly, several human clinical trials indicate that small-molecule DPP-IV inhibitors are well-tolerated, lower blood glucose and/or HbA_{1c} levels, and increase glucose tolerance. **Januvia®** (**6**, Sitagliptin) was approved for the treatment of type 2 diabetes in the United States on October 2006.[18] DPP-IV inhibitors are unlikely to cause serious hypoglycemia for actions of GLP-1 are strictly glucose-dependent.

Extensive research efforts have resulted in a number of potent DPP-IV inhibitors. Apart from Sitagliptin, various DPP-IV inhibitors like Vildagliptin (**7**)[19] Saxagliptin (**8**)[20] are in different phases of human clinical trials for the treatment of diabetic patients. A few of the representative structures are shown in Figure 2.

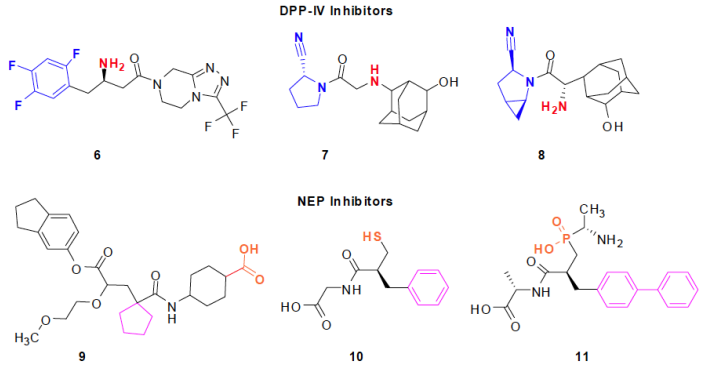


Figure 2: Known DPP-IV and NEP inhibitors. Pharmacophoric features are colored in red and blue for DPP-IV inhibitors; magenta and orange for NEP inhibitors.

B.7. Neutral Endopeptidase (NEP).

NEP, *aka* neprilysin, is a widespread membrane zinc-metallopeptidase with broad substrate specificity. Although mainly present in the brush-border membranes of the kidney, it is also found in diverse tissues like lymph nodes, small intestine and CNS.[21] NEP is known to be involved in inactivating opioid peptides in the CNS, atrial natriuretic peptide, vasoactive intestinal peptide and many other peptides mainly by cleaving at hydrophobic residues.[22] NEP is also shown to be involved in degradation of β-amyloid peptides, thus showing a role in Alzheimer's disease.[23] *In vitro,* NEP can degrade members of the glucagon/secretin/glucose-dependent insulinotropic polypeptide (GIP) family of peptides, including GLP-1. Plamboeck and coworkers have reported influence of candoxatril (**9**), a NEP selective inhibitor, on metabolic stability and antihyperglycaemic /insulinotropic effects of GLP-1.[24] Many reports are available showing importance of NEP along with ACE for the treatment of hypertension. Various specific NEP inhibitors are reported in the literature, a few of which are shown in Figure 2.

C. Scientific Rationale

According to studies reported by Plamboeck and coworkers NEP is responsible for degradation of 50% of GLP-1 in plasma. When candoxatril was co-administered with a DPP-IV inhibitor, GLP-1 degradation was completely prevented, and metabolic stability of GLP-1 was improved, resulting in a six-fold increase in its plasma half-life.[25]

Available evidence suggests that metformin is more effective as a monotherapy than either sitagliptin or the related DPP-IV inhibitor, vildagliptin.[18] The study carried out by Plamboeck and coworkers may provide the probable reason for this report. The design of a dual ligand in the form of a single chemical entity that can

modulate multiple targets simultaneously would be advantageous and prove to be an effective way of treatment. Conclusively a dual ligand targeting both DPP-IV and NEP would provide more effective therapy than monotherapy with only DPP-IV.

C.1. Pharmacophoric Features of DPP-IV Inhibitors.

Analysis of known small molecule inhibitors and available protein-ligand co-crystal structures has revealed important features necessary for binding. A substituted five or six-membered ring structure (shown in blue, Figure 2) fits well into S1 pocket of protease while the ammonium group (shown in red, Figure 2) interacts via a hydrogen bonding network with two carboxylic acid residues of glutamate side chain as well as the hydroxyl group of tyrosine residue present in the active site.[26]

C.2. Pharmacophoric Features of NEP Inhibitors.

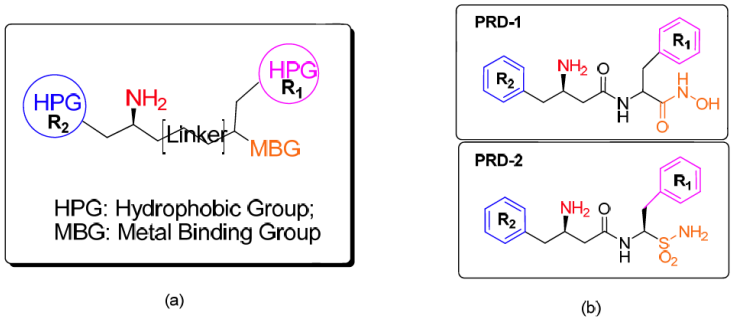
Crystallographic analysis of available protein-ligand co-crystal structures reveals S1' pocket of the protease being very hydrophobic and able to accommodate a hydrophobic group including substituted five or six-membered and biphenyl group (shown in magenta, Figure 2). The amino-acid side chains of two Histidine residues and one glutamate residue are involved in zinc ligation, together with chelating group (shown in orange, Figure 2) in the ligand.[27]

Combination of pharmacophoric features to design multiple ligands design is becoming a popular approach, to target more than one enzyme simultaneously. The 'merged' form of designed multiple ligand gives rise to smaller and simpler molecules. To combine the pharmacophores, the key structural elements that occur in both selective ligands are overlapped. Taking this fact into consideration, this proposal puts forward the design of a single chemical entity as a dual ligand, which can inhibit DPP-IV and NEP simultaneously for the treatment of T2DM patients. Recently, Oefner and coworkers reported first small molecule that can serve as dual DPP-IV and NEP inhibitor.[28] This study provides a proof-of-concept for this proposal as well as demonstrates the importance of the current research proposal.

D. PRELIMINARY STUDIES.

D.1. Development of Lead

The lead compound was designed by combining key pharmacophore features of both selective Dipeptidyl Peptidase-IV and Neutral Endopeptidase inhibitors. The general structure of the lead compounds is shown in Scheme 1. The lead molecules, represented in Scheme 3, namely PRD-1 and PRD-2, were designed so as to include the structural elements of both pharmacophore. These examples represent the integrated form of multiple ligand in which the pharmacophore features from different ligands are overlapped.



Scheme 1. (a) General structure of the proposed lead molecule (b) Representative structures of proposed lead molecules

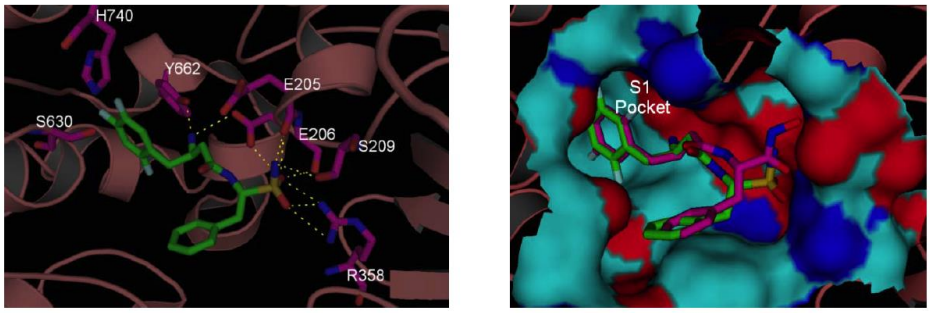
D.2. Docking Studies

For years, docking studies have been used to study the probable binding mode and binding affinity of designed molecules. The knowledge revealed by docking tools allows for possible modifications in lead structure, which can in turn improve the binding affinity and biological activity of the analogs. Myriads of protein crystal

structures of DPP-IV and NEP enzymes are reported in literature, which can be used to study the binding mode of proposed lead molecules and probable modifications.

Protein crystal structures for DPP-IV and NEP were downloaded from protein data bank. Depending upon careful analysis of these protein structures and co-crystallized ligand, structures, each for DPP-IV and NEP, few molecules were designed and subjected to docking studies. Docking analysis were performed using docking software GOLD, which is genetic algorithm based tool to carry out docking.

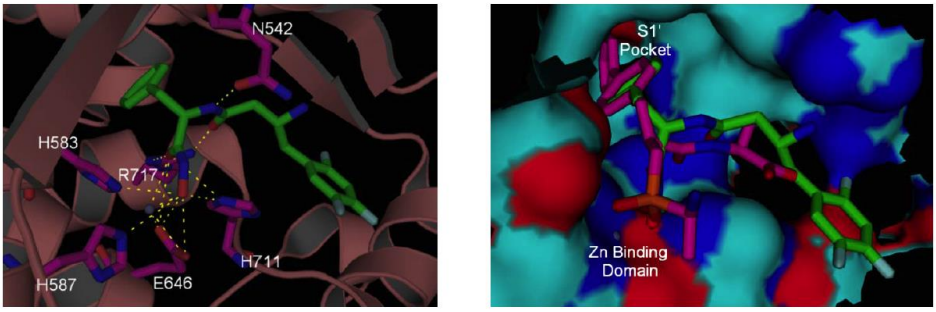
Docking into DPP-IV active site. Docking analysis suggested that PRD-1 and PRD-2 should bind to DPP-IV more effectively than sitagliptin (**6**). Introduction of polar chelating group in the molecule resulted in extra hydrogen bonding partner to Arg358 and Ser209, thus increasing the binding affinity of these molecules. Figure 3a shows docked conformation of PRD-2 (shown in green) into the active site of DPP-IV while (b) shows overlay of PRD-1 and PRD-2. PRD-1 binds in the similar way as that of PRD-2. As expected, the hydrophobic group corresponding to R2 enters the S1 pocket of active site while R1 group lies facing the solvent.



(a) (b)

Figure 3. (a) Binding mode of lead PRD-2 in the active site of DPP-IV. Hydrogen bonding interactions are shown in yellow dotted lines. (b) Overlay of PRD-1 (magenta) and PRD-2 (green) in the binding pocket of DPP-IV.

Docking into Active site of NEP. Docking of PRD-1 and PRD-2 into the active site of NEP revealed probable binding conformation of the leads. The R_2 group of the molecule fits well in the S1' pocket of NEP active site. S1' pocket of NEP is very hydrophobic and can accommodate larger hydrophobic groups including biphenyl and indole ring system. The binding mode of PRD-2 (green) into the active site of NEP is shown in Figure 4 (a).



(a) (b)

Figure 4. (a) Binding mode of lead PRD-1 in the active site of NEP. Hydrogen bonding interactions are shown in yellow dotted lines. (b) Overlay of existing ligand (magenta) and PRD-2 (green) in the binding pocket of NEP.

Similar to DPP-IV binding, these molecules show extra hydrogen bonding interactions. As expected, the R1 hydrophobic group fits well into the hydrophobic S1' pocket of active site while R2 hydrophobic group is facing

the solvent. An overlay of PRD-2 (green) with the existing ligand (magenta) in the active site is shown in Figure 4b.

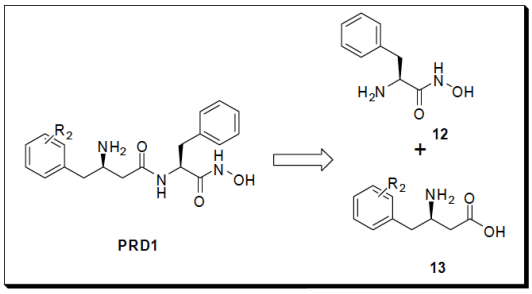
Preliminary docking studies suggest that the designed molecules can serve as a very potent lead in the development of a dual inhibitor. Furthermore, extensive docking studies can also be used to design more effective inhibitors of the series.

E. CHEMISTRY AND METHODS

Synthesis of PRD-1 and PRD-2 will be carried out using solution phase chemistry. It will be followed by synthesis of library of compounds through parallel or combinatorial approach.

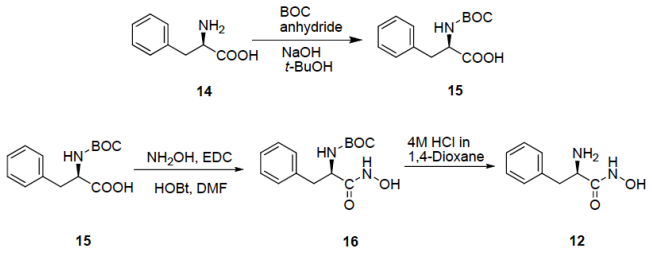
E.1. Outline of proposed chemical approach for PRD-1

The retro-synthetic scheme for the PRD-1 is represented in Scheme 1. The molecule will be synthesized by combining substituted hydroxamic acid derivatives and substituted β-phenyl-alanine derivatives as two starting mate-rials, which can be synthesized from the general methods cited in the literature or purchased depending upon cost and availability. Precautions will be taken while deciding the substitution pattern of the phenyl rings to avoid metabolism.

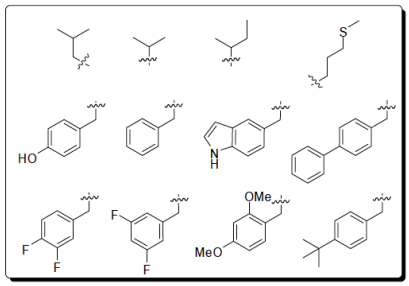


Scheme 1: Retro-synthetic scheme of PRD-1

The synthesis of hydroxamic acid derivatives can be accomplished in two steps. First, the free amine group **14** will be protected using t-butoxy carbonyl (Boc) anhydride in presence of sodium hydroxide and t-butyl alcohol. Resultant Boc-protected amino acid **15** will then be converted into hydroxamic acid **13** through treatment with EDC and hydroxylamine hydrocholoride in presence of N-Hydroxybenzotriazole (HOBt) and DMF,[29] followed by Boc-deprotection of **16**, as shown in Scheme 2.



Scheme 2: Synthesis of hydroxamic acid derivatives.

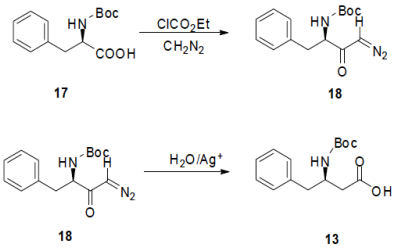


Scheme 3: List of diverse side-chains of aromatic amino acid at position R_2

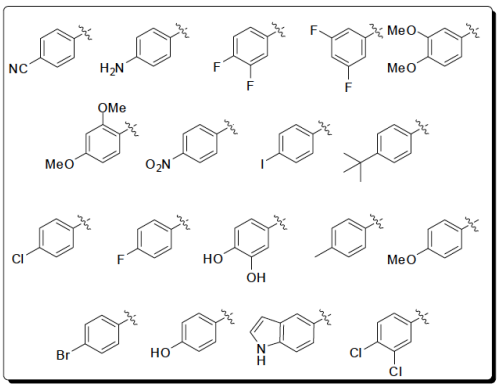
Preliminary studies show that the hydrophobic group corresponding to phenyl ring (R_2) in **12** fits well in the hydrophobic pocket of NEP. This pocket can accommodate a wide variety of hydrophobic groups including the side-chain of other hydrophobic amino acids like Val, Leu, Met and Ile. Various substituted or unsubstituted amino acid derivatives will be used in the synthesis of intermediate **12** of a series of PRD1, a few of them are shown in Scheme 3.

Homologation of substituted phenylalanine will be carried out using Arndt-Eistert Homologation as shown in Scheme 4. Boc-protected phenylalanine, **17** will be converted into acid chloride, with the help of thionyl chloride or ethyl-choloroformate, which will then further be treated with diazomethane to yield diazoketone,

18.[30] Diazoketones undergo Wolf-rearrangement in presence of catalytic amounts of silver salt and water to yield corresponding Boc protected β-amino acid, **13**.[31]

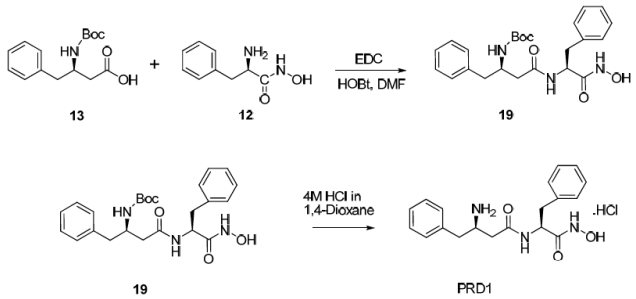


Scheme 4: Arndt-Eistert Homologation of α-amino acid derivatives



Scheme 5: List of diverse substituted phenylalanine rings for position R_2

According to preliminary studies discussed above, the S1 pocket in DPP-IV can accommodate various substituted phenyl ring as well as five-membered ring. Few of the phenylalanine derivatives which will be considered for synthesis of intermediate **13** are shown in Scheme 5.

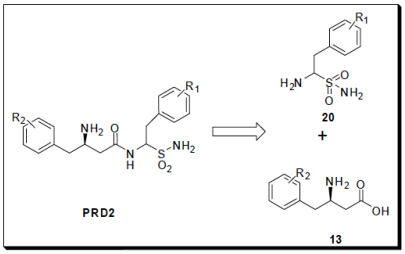


Scheme 6: Peptide coupling using EDC.

Intermediate **12** and **13** will then be coupled using standard peptide coupling procedures, as shown in Scheme 6.[32] Coupling will be carried out using EDC, in presence of HOBt and DMF, to yield intermediate **19**.[33] This intermediate will then be Boc-deprotected using 4M hydrochloric acid in 1,4-dioxane.

E.2. Outline of proposed chemical approach for PRD-2

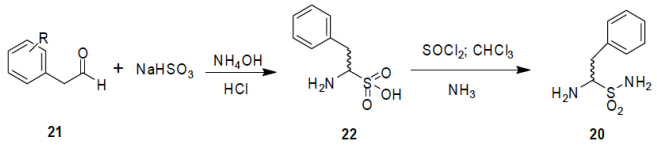
Retro-synthetic scheme for the PRD-2 is represented in Scheme 7. The molecule will be synthesized by combining substituted α-amino-sulfonamide derivatives, and substituted β-phenylalanine derivatives as two starting materials, which can be synthesized from the general methods cited in the literature.



Scheme 7: Retro-synthetic scheme of PRD-2

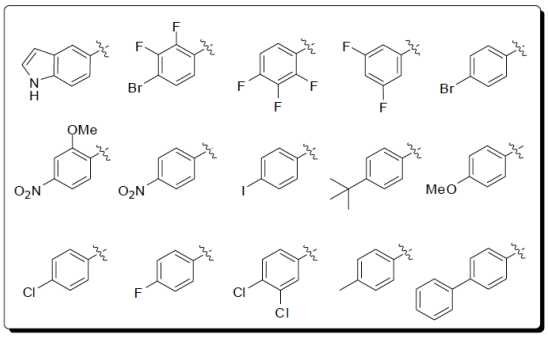
The synthesis of α-amino-sulfonamide derivatives will be accomplished in two steps as shown in Scheme 8. Reaction of substituted phenyl acetaldehyde, **21** with sodium bisulphite will yield α-amino-sulfonic acid, **22**,[34]

which will further be treated with ammonia in presence of thionyl chloride and chloroform, to get intermediate **20**. At this stage, we do not plan to carry out the chiral resolution of **20**, although computational studies show preference for the *R* isomer.



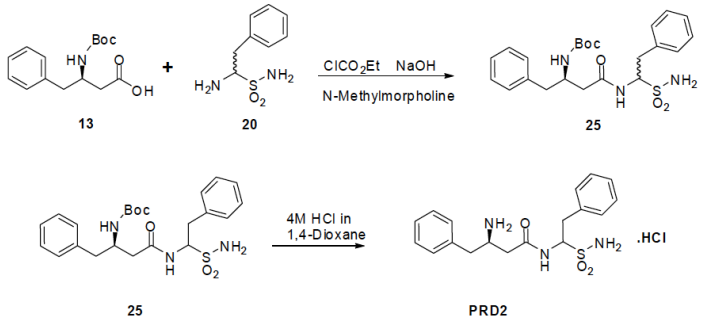
Scheme 8. Synthesis of sulfonamide derivatives.

A number of substituted phenyl acetaldehyde derivatives will be considered for synthesis of this imtermediate. A few of them are shown in Scheme 9.



Scheme 9. List of few substituted phenyl acetaldehyde derivatives for at position R_1

Synthesis of Boc-protected β-amino acids **13** will be carried out in the similar way as described above. Treatment of **13** in presence of ethyl choloroformate and N-methylmorpholine with **20** (dissolved in sodium hydroxide) is expected to yield Boc-protected PRD-2, **23**,[35] which upon deprotection using 4M hydrochloric acid in 1,4-dioxane will yield hydrochloride salt of **PRD-2**.



Scheme 10. Coupling of β-amino acid with α-amino-sulfonamide with subsequent deprotection.

F. BIOLOGICAL EVALUATION

The synthesized molecules will be tested for their biological activity using various reported bioassays. This various tests to be performed include oral glucose tolerance test, measurement of plasma active GLP-1 levels

and *in vitro* testing of newly synthesized compounds for DPP-IV and NEP inhibitory activity. The various bioassay procedures for these tasks are already available in the literature. Standard procedures will be followed unless otherwise required.

F.1. Oral Glucose Tolerance Test in Lean Mice.[36] Mice (n = 7/group) will be randomly assigned to treatment groups and fasted overnight (~18-21 h). Baseline (t = -60 min) blood glucose concentration will be determined by glucometer from tail nick blood. Animals will then be treated orally with vehicle (0.25% methylcellulose, 5 mL/kg) or test compound (3, 1, 0.3, and 0.1 mg/kg; 5 mL/kg). Blood glucose concentration will be measured 1 h after treatment (t = 0 min) and mice will then be orally challenged with dextrose (5 g/kg, 10 mL/kg). One group of vehicle-treated mice will be challenged with water as a negative control. Blood glucose levels will be determined from tail bleeds taken 20, 40, 60, and 120 min after dextrose challenge. The blood glucose excursion profile from t = 0 to t = 120 min will be used to integrate an area under the curve (AUC) for each treatment. Percent inhibition values for each treatment will be generated from the AUC data normalized to the water-challenged controls.

F.2. Measurement of Plasma Active (intact) GLP-1. Measurement of intact GLP-1 in the plasma will be carried out by using RadioImmuneAssay (RIA) or Enzyme-Linked ImmunoSorbent Assay (ELISA). General procedures for the assays are mentioned below.

RIA. C-terminal GLP-1 (total) immunoreactivity will be measured using standards of synthetic GLP-1(7–36)amide (= proglucagon 78–107amide) and antiserum no. 89390. The assay cross-reacts with less than 0.01% with C-terminally truncated fragments and 83% with GLP-1(9–36)amide and has a detection limit of 1 pM. The N-terminal GLP-1 assay, measuring the concentration of intact GLP-1, will be measured using a two-site sandwich immunoassay based on two monoclonal antibodies: the near C-terminally directed GLP1F5 as catching antibody and the strictly N-terminal Mab26.1 as detecting antibody. The assay shows less than 0.1% cross-reactivity with N-terminally truncated or extended forms of GLP-1, GLP-1(1–37), GLP-1(8–37), or GLP-1(9–37).[37]

ELISA. Alternatively, Plasma intact GLP-1 will be measured using a 96-well ELISA kit for active hormone, purchased from Linco Research Inc (St. Charles, MO, cat # EGLP-35K). The assay has a detection limit of 2 pM and is selective for active GLP-1 (GLP-1[7-36] amide and GLP-1[7-37]). The DPP-IV inhibitor valine thiazolidide (100 µM) will be added to plasma aliquots for active GLP-1 measurements to prevent degradation of the hormone.[36]

F.3. Measurement of Plasma DPP-IV Activity.

Rationale. General procedure to measure plasma DPP-IV activity includes continuous fluorometric assay with the chromogenic substrates Gly-Pro-AMC or Gly-Pro-*p*-nitroanilide, which are cleaved by DPP-IV to release 7-amino-4-methylcoumarin (AMC) or p-nitroaniline (*p*NA) leaving group, respectively (Figure 5). AMC and *p*NA emit fluorescence which can be measured at appropriate wavelength. The emission is then correlated to the activity of the compounds.

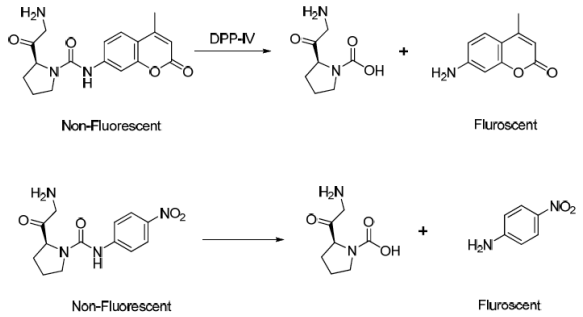


Figure 5: Schematic representation of reaction catalyzed by DPP-IV to generate fluorescent substrate

Bioassay using AMC. A typical reaction contains 50% plasma, 50 µM Gly-Pro-AMC, and buffer (100 mM HEPES, pH 7.5, 0.1 mg/mL BSA) in a total reaction volume of 50 to 70 µL. Liberation of AMC will be monitored continuously in a 96-well plate fluorometer, using an excitation wavelength of 360 nm and an emission wavelength of 460 nm. Under these conditions, approximately 5 µM AMC is produced in 5 min at 37° C. The assay exhibits linear rates only for about 5 min due to the rapid substrate depletion. Therefore, it is important to preincubate all assay components to the assay temperature prior to the assay.

Bioassay using pNA.[38] Test compounds will be dissolved and diluted in DMSO (final concentration DMSO during assay 5% v/v). The initial evaluation of compounds will be carried out at 1 mM, or in case of solubility limits, the highest concentration possible. If vi/vo (velocity in the presence of inhibitor/velocity in the presence of control) is < 0.5, an IC_{50} value will be determined experimentally using at least 6 different concentrations of inhibitor.

F.4. NEP 24.11 activity determination.

Rationale. NEP bioassay involves a two-stage enzymatic reaction (Figure 6), where NEP 24.11 cleaves the substrate (suc-Ala-Ala-Leu-4-nitroanilide) on the amino side of leucine (reaction 1). The Leu-4-nitroanilide formed is subsequently degraded by aminopeptidase M to Leucine and 4-nitroaniline (reaction 2), resulting in an increase in absorbance.[39]

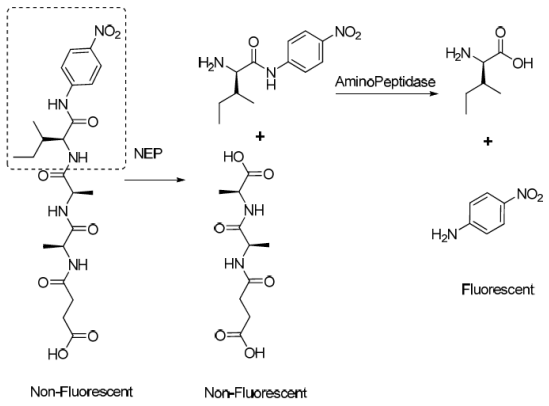


Figure 6: Schematic representation of reaction catalyzed by NEP and aminopeptidase to generate fluorescent substrate

Procedure. Briefly, plasma will be diluted ninefold in 0.9% NaCl, and 50 µl will be added to a quartz cuvette (1-cm path), followed by 20 µl of aminopeptidase M containing 4 µg of enzyme protein (0.096 U, Sigma). The reaction will be initiated by addition of 280 µl of substrate (0.262 µmol, diluted in 0.05 mol/l Tris buffer, pH 7.5; Bachem). Samples will be incubated at 26°C for 90 min, and the increase in absorbance (405 nm) will be recorded in a spectrophotometer every 15 min. However, possible contamination of commercially available aminopeptidase M preparations with NEP 24.11 can't be ruled out, a control incubation (50 µl of 0.9% NaCl, 20 µl of aminopeptidase M, and 280 µl of substrate) will be made. As a positive control, porcine kidney NEP 24.11 (Calbiochem) will be added in place of plasma. The specificity of the reaction for NEP 24.11 will be tested by the addition of the active NEP 24.11 inhibitor candoxatrilat (3.5 µmol/l final dilution).[25]

Time Phased Objectives of the Proposal

During the *first year*, the post-doctoral associate Dr. Ronald Jones will synthesize the proposed compounds using solution phase chemistry. Dr. Jones will be accompanied by graduate student Andrew Mathew. Prof. John Welch from University of Albany will carry out the biological testing of the synthesized compounds. His post-doctoral associate Dr. John Anderson and graduate student Chris Wells will screen the compounds at the end of first year. Meanwhile, Dr. Ronald Jones and Andrew Mathew will initiate synthesis of starting materials and other reagents for combinatorial synthesis.

Second year – As the project continues with biological evaluation of newly synthesized molecules, post doctoral associate Dr. Chenglong Li will help in structure- based optimization of existing ligands to design new molecules. At the same time the combinatorial synthesis of the proposed derivatives will be carried out by Dr. Ronald Jones and Andrew Mathew.

Third year – At the beginning of third year, Dr. Chenglong Li will assist in designing of virtual combinatorial library of the analogs with combinations of various hydrophobic and metal chelating groups. The virtual library will then be subjected to docking to get best possible dual ligands. Dr. Ronald Jones and Andrew Mathew will carry out synthesis of newly designed molecules. Biological testing of the derivatives synthesized will be carried out.

G. HUMAN SUBJECTS

Not Applicable

H. VERTEBRATE ANIMAL USE

1. Description of animal use: All animals will be housed, treated and cared in accordance to NIH guidelines for humane treatment of laboratory animals and animal welfare act that is accredited by the American association for accreditation of laboratory animal care. Specific description of animals as required are as follows:

   Species: laboratory mouse (*Mus musculus*)

   Strain: C57BL/6N mouse model

   Sex: male

   Age: 7-12 weeks old

   Weight: 22-25g

2. Justification of animal use: Mouse models are very common to evaluate new drug candidates in research areas like infectious diseases, immunology, toxicology, cancer pharmacology, behavioral studies. Hence, mouse models will be used in this project and also liver damage due to malaria could be studied in detail once the mouse model is sacrificed on the fourth day after drug treatment. ICR strain is preferred because in this project's assay system, genetic variability is not desired. Swiss-Webster strain is also an outbred like ICR.

3. Veterinary care: A well managed facility with strict hygienic conditions will be provided for the outbred strains by well trained personnel and animal caretakers under the supervision of qualified veterinarian. Also, the institutional animal care and use committee (IACUC) will monitor the animal facility regularly to make sure the operations are in accordance to PHS policy.

4. Procedures for pain reduction: Xylazine (with ketamine) 10mg/kg will be given intramuscularly for pain reduction. This amount will be adequate to prevent any suffering of the hamsters.

5. Euthanasia: This will be performed by the trained personnel and this method will be consistent with the recommendations of the panel on euthanasia of the American Veterinary Medical Association.

LITERATURE CITED:

1. American Diabetes Association. Economic Costs of Diabetes in the U.S. in 2002. *Diabetes Care* **2003,** *26*, 917-932.
2. Wild, S.; Roglic, G.; Green, A.; Sicree, R.; King, H. Global Prevalence of Diabetes: Estimates for the year 2000 and projections for 2030. *Diabetes Care* **2004,** *27*, 1047-1053.
3. Ashcroft, F. M.; Gribble, F. M. ATP-sensitive K+ channels and insulin secretion. Their role in health and disease. *Diabetologia* **1999,** *42*, 903-919.
4. Lebovitz, H. E. Oral antidiabetic agents. The emergence of alpha-glucosidase inhibitors. *Drugs* **1992,** *44 Suppl 3*, 21-8.
5. Kirpichnikov, D.; McFarlane, S. I.; Sowers, J. R. Metformin: an update. *Annals Int. Med.* **2002,** *137*, 25-33.
6. Day, C. Thiazolidinediones: a new class of antidiabetic drugs. *Diabetic Med.* **1999,** *16*, 179-192.
7. Knudsen, L. B. Glucagon-like Peptide-1: The Basis of a New Class of Treatment for Type 2 Diabetes. *J. Med. Chem.* **2004,** *47*, 4128-4134.
8. Krentz, A. J.; Ferner, R. E.; Bailey, C. J. Comparative tolerability profiles of oral antidiabetic agents. *Drug Saf.* **1994,** *11*, 223-41.
9. Donnelly, R. Effect of pioglitazone on the drivers of cardiovascular risk in type 2 diabetes. *Int. J. Clin. Pract.* **2007,** *61*, 1160-1169.
10. Nauck, M. A.; Niedereichholz, U.; Ettler, R.; Holst, J. J.; Orskov, C.; Ritzel, R.; Schmiegel, W. H. Glucagon-like peptide 1 inhibition of gastric emptying outweighs its insulinotropic effects in healthy humans. *Am J Physiol Endocrinol Metab* **1997,** *273*, E981-988.
11. Rachman, J.; Barrow, B. A.; Levy, J. C.; Turner, R. C. Near-normalisation of diurnal glucose concentrations by continuous administration of glucagon-like peptide-1 (GLP-1) in subjects with NIDDM. *Diabetologia* **1997,** *40*, 205-211.
12. Knudsen, L. B.; Kiel, D.; Teng, M.; Behrens, C.; Bhumralkar, D.; Kodra, J. T.; Holst, J. J.; Jeppesen, C. B.; Johnson, M. D.; de Jong, J. C.; Jorgensen, A. S.; Kercher, T.; Kostrowicki, J.; Madsen, P.; Olesen, P. H.; Petersen, J. S.; Poulsen, F.; Sidelmann, U. G.; Sturis, J.; Truesdale, L.; May, J.; Lau, J. Small-molecule agonists for the glucagon-like peptide 1 receptor. *Proc. Nat. Acad. Sci.* **2007,** *104*, 937-942.
13. Keating, G. M. Exenatide. *Drugs* **2005,** *65*, 1681-1692.
14. Adelhorst, K.; Hedegaard, B. B.; Knudsen, L. B.; Kirk, O. Structure-activity studies of glucagon-like peptide-1. *J. Biol. Chem.* **1994,** *269*, 6275-6278.
15. Kieffer, T. J.; McIntosh, C. H.; Pederson, R. A. Degradation of glucose-dependent insulinotropic polypeptide and truncated glucagon-like peptide 1 in vitro and in vivo by dipeptidyl peptidase IV. *Endocrinology* **1995,** *136*, 3585-3596.
16. Hupe-Sodmann, K.; McGregor, G. P.; Bridenbaugh, R.; Goke, R.; Goke, B.; Thole, H.; Zimmermann, B.; Voigt, K. Characterisation of the processing by human neutral endopeptidase 24.11 of GLP-1(7-36) amide and comparison of the substrate specificity of the enzyme for other glucagon-like peptides. *Reg. Pep.* **1995,** *58*, 149-156.
17. Hupe-Sodmann, K.; Goke, R.; Goke, B.; Thole, H. H.; Zimmermann, B.; Voigt, K.; McGregor, G. P. Endoproteolysis of Glucagon-like Peptide (GLP)-1(7-36) amide by Ectopeptidases in RINm5F Cells. *Peptides* **1997,** *18*, 625-632.
18. Drucker, D.; Easley, C.; Kirkpatrick, P. Sitagliptin. *Nat Rev Drug Discov* **2007,** *6*, 109-110.
19. Villhauer, E. B.; Brinkman, J. A.; Naderi, G. B.; Burkey, B. F.; Dunning, B. E.; Prasad, K.; Mangold, B. L.; Russell, M. E.; Hughes, T. E. 1-[[(3-Hydroxy-1-adamantyl)amino]acetyl]-2-cyano-*S*-pyrrolidine: A Potent, Selective, and Orally Bioavailable Dipeptidyl Peptidase IV Inhibitor with Antihyperglycemic Properties. *J. Med. Chem.* **2003,** *46*, 2774-2789.
20. Augeri, D. J.; Robl, J. A.; Betebenner, D. A.; Magnin, D. R.; Khanna, A.; Robertson, J. G.; Wang, A.; Simpkins, L. M.; Taunk, P.; Huang, Q.; Han, S. P.; Abboa-Offei, B.; Cap, M.; Xin, L.; Tao, L.; Tozzo, E.; Welzel, G. E.; Egan, D. M.; Marcinkeviciene, J.; Chang, S. Y.; Biller, S. A.; Kirby, M. S.; Parker, R. A.; Hamann, L. G. Discovery and Preclinical Profile of Saxagliptin (BMS-477118): A Highly Potent, Long-

Acting, Orally Active Dipeptidyl Peptidase IV Inhibitor for the Treatment of Type 2 Diabetes. *J. Med. Chem.* **2005,** *48*, 5025-5037.

21. Turner, A. J.; Isaac, R. E.; Coates, D. The neprilysin (NEP) family of zinc metalloendopeptidases: Genomics and function. *BioEssays* **2001,** *23*, 261-269.
22. Rogues, B. P.; Beaumont, A. Neutral endopeptidase-24.11 inhibitors: from analgesics to antihypertensives? *Trends Pharmacol. Sci.* **1990,** *11*, 245-249.
23. Yasojima, K.; McGeer, E. G.; McGeer, P. L. Relationship between beta amyloid peptide generating molecules and neprilysin in Alzheimer disease and normal brain. *Brain Research* **2001,** *919*, 115-121.
24. Plamboeck, A.; Holst, J.; Carr, R.; Deacon, C. Neutral Endopeptidase 24.11 and Dipeptidyl Peptidase IV are Both Involved in Regulating the Metabolic Stability of Glucagon-like Peptide-1 in vivo. In *Dipeptidyl Aminopeptidases in Health and Disease*, 2004; pp 303-312.
25. Plamboeck, A.; Holst, J.; Carr, R.; Deacon, C. Neutral endopeptidase 24.11 and dipeptidyl peptidase IV are both mediators of the degradation of glucagon-like peptide 1 in the anaesthetised pig. *Diabetologia* **2005,** *48*, 1882-1890.
26. Hunziker, D.; Hennig, M.; Peters, J.-U. Inhibitors of Dipeptidyl Peptidase IV - Recent Advances and Structural Views. *Curr. Top. Med. Chem.* **2005,** *5*, 1623-1637.
27. Oefner, C.; Roques, B. P.; Fournie-Zaluski, M.-C.; Dale, G. E. Structural analysis of neprilysin with various specific and potent inhibitors. *Acta Cryst. Sect. D* **2004,** *60*, 392-396.
28. Oefner, C.; Pierau, S.; Schulz, H.; Dale, G. E. Structural studies of a bifunctional inhibitor of neprilysin and DPP-IV. *Acta Crys. Sect. D* **2007,** *63*, 975-981.
29. Smith, H. K.; Beckett, R. P.; Clements, J. M.; Doel, S.; East, S. P.; Launchbury, S. B.; Pratt, L. M.; Spavold, Z. M.; Thomas, W.; Todd, R. S.; Whittaker, M. Structure-activity relationships of the peptide deformylase inhibitor BB-3497: modification of the metal binding group. *Bioorg. Med. Chem. Lett.* **2002,** *12*, 3595-3599.
30. Podlech, J. Preparation of enantiopure β-amino acids by homologation of α-amino acids. In *Enantioselective Synthesis of β-Amino Acids*, 2nd ed.; Juaristi, E.; Soloshonok, V. A., Eds. 2005; pp 93-106.
31. Kirmse, W. 100 years of the Wolff rearrangement. *Eur. J. Org. Chem.* **2002**, 2193-2256.
32. Sheehan, J. C.; Hess, G. P. A New Method of Forming Peptide Bonds. *J. Am. Chem. Soc.* **1955,** *77*, 1067-1068.
33. Rebek, J.; Feitler, D. Improved method for the study of reaction intermediates. Mechanism of peptide synthesis mediated by carbodiimides. *J. Am. Chem. Soc.* **1973,** *95*, 4052-4053.
34. Neelakantan, L.; Hartung, W. H. α-Aminoalkanesulfonic acids. *J. Org. Chem.* **1959,** *24*, 1943-8.
35. Shiba, T.; Miyoshi, K.; Kusumoto, S. Synthesis of alanyl-1-aminoethanesulfonic acid. *Bull. Chem. Soc. Jap.* **1977,** *50*, 254-257.
36. Kim, D.; Wang, L.; Beconi, M.; Eiermann, G. J.; Fisher, M. H.; He, H.; Hickey, G. J.; Kowalchick, J. E.; Leiting, B.; Lyons, K.; Marsilio, F.; McCann, M. E.; Patel, R. A.; Petrov, A.; Scapin, G.; Patel, S. B.; Roy, R. S.; Wu, J. K.; Wyvratt, M. J.; Zhang, B. B.; Zhu, L.; Thornberry, N. A.; Weber, A. E. (2*R*)-4-Oxo-4-[3-(Trifluoromethyl)-5,6-dihydro[1,2,4]triazolo[4,3-*a*]pyrazin- 7(8*H*)-yl]-1-(2,4,5-trifluorophenyl)butan-2-amine: A Potent, Orally Active Dipeptidyl Peptidase IV Inhibitor for the Treatment of Type 2 Diabetes. *J. Med. Chem.* **2005,** *48*, 141-151.
37. Vilsboll, T.; Krarup, T.; Sonne, J.; Madsbad, S.; Volund, A.; Juul, A. G.; Holst, J. J. Incretin Secretion in Relation to Meal Size and Body Weight in Healthy Subjects and People with Type 1 and Type 2 Diabetes Mellitus. *J Clin Endocrinol Metab* **2003,** *88*, 2706-2713.
38. Wang, A.; Huang, Y.; Taunk, P.; Magnin, D. R.; Ghosh, K.; Robertson, J. G. Application of robotics to steady state enzyme kinetics: analysis of tight-binding inhibitors of dipeptidyl peptidase IV. *Anal. Biochem.* **2003,** *321*, 157-166.
39. Indig, F. E.; Ben-Meir, D.; Spungin, A.; Blumberg, S. Investigation of neutral endopeptidases (EC 3.4.24.11) and of neutral proteinases (EC 3.4.24.4) using a new sensitive two-stage enzymatic reaction. *FEBS Letters* **1989,** *255*, 237-240.

Principal Investigator/Program Director (last, First, Middle): Daga Pankaj R.

CHECKLIST

TYPE OF APPLICATION *(Check all that apply.)*

☒ NEW application. *(This application is being submitted to the PHS for the first time.)*

☐ REVISION/RESUBMISSION of application number:
(This application replaces a prior unfunded version of a new, competing continuation/renewal, or supplemental/revision application.)

☐ COMPETING CONTINUATION/RENEWAL of grant number:
(This application is to extend a funded grant beyond its current project period.)

INVENTIONS AND PATENTS *(Competing continuation/renewal appl. only)*
☐ No ☐ Previously reported
☐ Yes. If "Yes," ☐ Not previously reported

☐ SUPPLEMENT/REVISION to grant number:
(This application is for additional funds to supplement a currently funded grant.)

☐ CHANGE of principal investigator/program director.
Name of former principal investigator/program director:

☐ CHANGE of Grantee Institution. Name of former institution:

☐ FOREIGN application ☐ Domestic Grant with foreign involvement List Country(ies) Involved:

1. PROGRAM INCOME ***(See instructions.)***
All applications must indicate whether program income is anticipated during the period(s) for which grant support is request. If program income is anticipated, use the format below to reflect the amount and source(s).

Budget Period	Anticipated Amount	Source(s)

2. ASSURANCES/CERTIFICATIONS ***(See instructions.)***
In signing the application Face Page, the authorized organizational representative agrees to comply with the following policies, assurances and/or certifications when applicable. Descriptions of individual assurances/certifications are provided in Part III. If unable to certify compliance, where applicable, provide an explanation and place it after this page.
•Human Subjects Research •Research Using Human Embryonic Stem Cells •Research on Transplantation of Human Fetal Tissue •Women and Minority Inclusion Policy •Inclusion of Children Policy •Vertebrate Animals• •Debarment and Suspension •Drug- Free Workplace *(applicable to new [Type 1] or revised/resubmission [Type 1] applications only)* •Lobbying •Non-Delinquency on Federal Debt •Research Misconduct •Civil Rights (Form HHS 441 or HHS 690) •Handicapped Individuals (Form HHS 641 or HHS 690) •Sex Discrimination (Form HHS 639-A or HHS 690) •Age Discrimination (Form HHS 680 or HHS 690) •Recombinant DNA Research, Including Human Gene Transfer Research •Financial Conflict of Interest •Smoke Free Workplace •Prohibited Research •Select Agent Research •PI Assurance

3. FACILITIES AND ADMINSTRATIVE COSTS (F&A)/ INDIRECT COSTS. See specific instructions.

☐ DHHS Agreement dated: ☐ No Facilities And Administrative Costs Requested.

☐ DHHS Agreement being negotiated with Regional Office.

☐ No DHHS Agreement, but rate established with Date

CALCULATION* *(The entire grant application, including the Checklist, will be reproduced and provided to peer reviewers as confidential information.)*

a. Initial budget period:	Amount of base $	239,405	x Rate applied	44.00	% = F&A costs	$	105,339
b. 02 year	Amount of base $	246,589	x Rate applied	44.00	% = F&A costs	$	108,500
c. 03 year	Amount of base $	253,986	x Rate applied	44.00	% = F&A costs	$	111,754
d. 04 year	Amount of base $		x Rate applied		% = F&A costs	$	
e. 05 year	Amount of base $		x Rate applied		% = F&A costs	$	
					TOTAL F&A Costs	$	325,593

*Check appropriate box(es):

☐ Salary and wages base ☒ Modified total direct cost base ☐ Other base *(Explain)*

☐ Off-site, other special rate, or more than one rate involved *(Explain)*

Explanation *(Attach separate sheet, if necessary.):*

Principal Investigator/Program Director (Last, First, Middle): Daga Rankaj R.

Place this form at the end of the signed original copy of the application.
Do not duplicate.

PERSONAL DATA ON PRINCIPAL INVESTIGATOR(S)/PROGRAM DIRECTOR(S)

The Public Health Service has a continuing commitment to monitor the operation of its review and award processes to detect—and deal appropriately with—any instances of real or apparent inequities with respect to age, sex, race, or ethnicity of the proposed principal investigator(s)/program director(s).

To provide the PHS with the information it needs for this important task, complete the form below and attach it to the signed original of the application after the Checklist. When multiple PIs/PDs are proposed, complete a form for each. **Do not attach copies of this form to the duplicated copies of the application.**

Upon receipt of the application by the PHS, this form will be separated from the application. This form will **not** be duplicated, and it will **not** be a part of the review process. Data will be confidential, and will be maintained in Privacy Act record system 09-25-0036, "Grants: IMPAC (Grant/Contract Information)." The PHS requests the last four digits of the Social Security Number for accurate identification, referral, and review of applications and for management of PHS grant programs. Although the provision of this portion of the Social Security Number is voluntary, providing this information may improve both the accuracy and speed of processing the application. Please be aware that no individual will be denied any right, benefit, or privilege provided by law because of refusal to disclose this section of the Social Security Number. The PHS requests the last four digits of the Social Security Number under Sections 301(a) and 487 of the PHS Acts as amended (42 U.S.C 241a and U.S.C. 288). All analyses conducted on the date of birth, gender, race and/or ethnic origin data will report aggregate statistical findings only and will not identify individuals. If you decline to provide this information, it will in no way affect consideration of your application. Your cooperation will be appreciated.

DATE OF BIRTH (*MM/DD/YY*)	06/11/80	SEX/GENDER
SOCIAL SECURITY NUMBER (last 4 digits only)	XXX-XX- 9649	☐ Female ☒ Male

ETHNICITY

1. Do you consider yourself to be Hispanic or Latino? (See definition below.) Select one.

Hispanic or Latino. A person of Mexican, Puerto Rican, Cuban, South or Central American, or other Spanish culture or origin, regardless of race. The term, "Spanish origin," can be used in addition to "Hispanic or Latino."

☐ **Hispanic or Latino**

☒ **Not Hispanic or Latino**

RACE

2. What race do you consider yourself to be? Select one or more of the following.

☐ ***American Indian or Alaska Native.*** A person having origins in any of the original peoples of North, Central, **or** South America, and who maintains tribal affiliation or community attachment.

☒ ***Asian.*** A person having origins in any of the original peoples of the Far East, Southeast Asia, or the Indian **subcontinent**, including, for example, Cambodia, China, India, Japan, Korea, Malaysia, Pakistan, the Philippine Islands, Thailand, and Vietnam. (Note: Individuals from the Philippine Islands have been recorded as Pacific Islanders in previous data collection strategies.)

☐ ***Black or African American.*** A person having origins in any of the black racial groups of Africa. Terms such as "Haitian" or "Negro" can be used in addition to "Black" or African American."

☐ ***Native Hawaiian or Other Pacific Islander.*** A person having origins in any of the original peoples of Hawaii, Guam, Samoa, or **other** Pacific Islands.

☐ ***White.*** A **person** having origins in any of the original peoples of Europe, the Middle East, or North Africa.

☐ Check here if you do not wish to provide some or all of the above information.

GRADUATE SCHOOL REQUEST FOR TRAVEL FUNDS FORM

Graduate Student Travel Form

The Graduate Student Travel Award Program makes available limited support for a graduate student who is first author on a research presentation and/or recognized through an award for original research/scholarship. The travel award will provide $100 for presentations at regional meetings and $200 for presentations at national meetings. State meetings generally are not eligible for support. Students should complete this form and submit it to the Senior Administrative Secretary at the Graduate School **at least ten days prior to date of trip.**

Name: _____ Date: _____

Department: _____ Travel Dates: _____

Destination: _____

Organization: _____

Title and type of presentation: (attach (1) abstract or description of award AND (2) notice of presentation acceptance)

_____

_____

_____

_____

Estimated Cost of trip: _____ Other sources of funding: _____

The Graduate School Travel award is dependent upon availability of funds and the Graduate School reserves the right to limit the amount and frequency of support for graduate students as necessary.

If you are requesting a travel advance or require prepaid registration, you must also submit a Travel Authorization Form which can be obtained from your department.

In order to obtain this award, a Travel Reimbursement Form should be submitted to all sponsoring parties (including the Graduate School) as soon as possible after returning from the trip.

I certify that the student meets the above Travel Award qualifications.
__ **Regional** __**National** Meeting

_____ _____

Department Chair Date

DO NOT WRITE BELOW THIS LINE-OFFICE USE ONLY

To Be Complete by Graduate School:

APPROVAL:		DATE:		COPIES TO:		DATE MAILED:	
$100 award	_____	_____		Chair	_____	_____	
$200 award	_____	_____		Student	_____	_____	

Posted _____ Date Posted _____ Posted By _____ Dbase# _____

EXAMPLE OF A TRAVEL REIMBURSEMENT FORM

The University of Mississippi
Travel Reimbursement Voucher

Employee			**Trip #**
e-mail			**S.S.N.**
Dept Name			**Personnel #**

A CONTACT

Contact Person:	**Meeting:** Time Date Began @ Ended @	Accompanied By:	**Phone #**
Purpose and Place of Trip:			

B DAILY TRAVEL EXPENSES

	Date								Totals
1	Actual Meal Expenses:								
	a. Breakfast								
	b. Lunch								
	c. Dinner								
2	Lodging*								
								Total Meals and Lodging	
3	Meal Tips (not to exceed 15%)								
4	Taxi*								
5	Parking, Tolls*								
6	Gasoline*								
7	Business Calls*								
8									
								Total Other	

C TRANSPORTATION

	Personal Car						Airfare*	Rental Car*	Bus/Train*
	C	Date	From	To	Mileage	Amount			
1									
2									
3									
4									
5									
6									
7									
8									
9									
10		Insert total dollar amount from Mileage Worksheet in this row							
					Totals				

D EXPENDITURES

	Registration Fees		Other Reimbursable Expenses					
			Date	Description	Amount	Date	Description	Amount
1	Conference Fee*							
2	Banquet Fee*							
3	Dues*							
4								
5								
	Total Fees					Total Other Expenses		

E

COMMENTS	H Total Expenses
	I Exchange Rate**

F APPROVAL

CERTIFICATION: SUBJECT TO ANY DIFFERENCES DETERMINED BY AUDIT, I CERTIFY THAT THE ABOVE AMOUNT CLAIMED FOR TRAVEL EXPENSES FOR THE PERIOD INDICATED IS TRUE AND ACCURATE AND THAT PAYMENT FOR ANY PART HAS NOT BEEN RECEIVED.

EMPLOYEE'S SIGNATURE (REQUIRED)	DATE	DEPARTMENT HEAD SIGNATURE (REQUIRED)	DATE	AUDITED BY	DATE

G CIAL

COST CENTER #	AMOUNT	APPROVAL	INTERNAL ORDER #	AMOUNT	APPROVAL	ADDRESS OR BANK CHANGES

EXAMPLE OF WASTE DISPOSAL FORM

THE UNIVERSITY OF MISSISSIPPI
DEPARTMENT OF HEALTH & SAFETY
(662) 915-5433

REQUEST FOR DISPOSAL OF HAZARDOUS CHEMICALS

Supervisor:	Department:
Building:	Room #:
Physical State:	Phone #:

IDENTIFICATION OF THE MATERIAL

Chemical Components	% or ppm	Quantity
TOTAL		

I hereby certify that the material listed on this form does NOT contain any pathogenic or infectious material, pesticides, PCB's or radioactive materials; and THAT THE IDENTIFICATION OF THE MATERIAL(S) LISTED ABOVE IS TRUE AND CORRECT TO THE BEST OF MY KNOWLEDGE. I TAKE FULL RESPONSIBILITY FOR ANY ERRORS OCCURRING HEREIN.

Signature of Supervisor Date

DATE RECEIVED DHS:	CONTROL #:

Form: DHS-4-web Rev. 03/07

THE UNIVERSITY OF MISSISSIPPI
DEPARTMENT OF BIOMOLECULAR SCIENCES

ACKNOWLEDGMENT OF GRADUATE STUDENT HANDBOOK

I have read the graduate student handbook and acknowledge my responsibilities as a graduate student and the policies and procedures at the University of Mississippi, Department of BioMolecular Sciences.

Employee Signature **Date**

Print Employee Name