PI: **smith, James**	Title: Lead Compound Discovery from Engineered Analogs of Occidiofungin	
Received: 09/03/2016	FOA: PA16-303	Council: 01/2017
Competition ID: FORMS-D	FOA Title: PHS 2016-02 OMNIBUS SOLICITATION OF THE NIH FOR SMALL BUSINESS TECHNOLOGY TRANSFER GRANT APPLICATIONS (PARENT STTR [R41/R42])	
1 R41 AI131792-01	Dual:	Accession Number: 3966533
IPF: 10038489	Organization: SANO CHEMICALS, INC	
Former Number:	Department: Microbiology	
IRG/SRG: ZRG1 BCMB-G (10)B	AIDS: N	Expedited: N
Subtotal Direct Costs (excludes consortium F&A) Year 1: [redacted]	Animals: N Humans: N Clinical Trial: N Current HS Code: 10 HESC: N	New Investigator: N Early Stage Investigator: N
Senior/Key Personnel:	*Organization:*	*Role Category:*
James Smith Ph.D	Sano Chemicals	PD/PI

OMB Number: 4040-0001
Expiration Date: 06/30/2016

APPLICATION FOR FEDERAL ASSISTANCE
SF 424 (R&R)

3. DATE RECEIVED BY STATE	**State Application Identifier**

1. TYPE OF SUBMISSION*

❍ Pre-application ● Application ❍ Changed/Corrected Application

4.a. Federal Identifier

b. Agency Routing Number

2. DATE SUBMITTED

Application Identifier
occidiofungin2016

c. Previous Grants.gov Tracking Number

5. APPLICANT INFORMATION

Organizational DUNS*: 078514829

Legal Name*: Sano Chemicals
Department: Microbiology
Division: Antibiotics
Street1*: [redacted]
Street2:
City*: [redacted]
County: [redacted]
State*: [redacted]
Province:
Country*: [redacted]
ZIP / Postal Code*: [redacted]

Person to be contacted on matters involving this application

Prefix: Dr. First Name*: James Middle Name: Leif Last Name*: Smith Suffix:

Position/Title: Chief Executive Officer
Street1*: 1000 San Jacinto Ln
Street2:
City*: [redacted]
County: [redacted]
State*: [redacted]
Province:
Country*: [redacted]
ZIP / Postal Code*: [redacted]
Phone Number*: [redacted] Fax Number: [redacted] Email: [redacted]

6. EMPLOYER IDENTIFICATION NUMBER *(EIN) or (TIN)** [redacted]

7. TYPE OF APPLICANT* R: Small Business

Other (Specify):
Small Business Organization Type ❍ Women Owned ❍ Socially and Economically Disadvantaged

8. TYPE OF APPLICATION*

● New ❍ Resubmission
❍ Renewal ❍ Continuation ❍ Revision

If Revision, mark appropriate box(es).

❍ A. Increase Award ❍ B. Decrease Award ❍ C. Increase Duration
❍ D. Decrease Duration ❍ E. Other *(specify)* :

Is this application being submitted to other agencies?* ❍Yes ●No What other Agencies?

9. NAME OF FEDERAL AGENCY*
National Institutes of Health

10. CATALOG OF FEDERAL DOMESTIC ASSISTANCE NUMBER
TITLE:

11. DESCRIPTIVE TITLE OF APPLICANT'S PROJECT*
Lead Compound Discovery from Engineered Analogs of Occidiofungin

12. PROPOSED PROJECT

Start Date* Ending Date*
04/01/2017 03/31/2018

13. CONGRESSIONAL DISTRICTS OF APPLICANT
TX-017

SF 424 (R&R) APPLICATION FOR FEDERAL ASSISTANCE

14. PROJECT DIRECTOR/PRINCIPAL INVESTIGATOR CONTACT INFORMATION

Prefix: Dr. First Name*: James Middle Name: Leif Last Name*: Smith Suffix: Ph.D

Position/Title: Chief Executive Officer
Organization Name*: Sano Chemicals
Department: Microbiology
Division: Antibiotics
Street1*: [redacted]
Street2:
City*: [redacted]
County: [redacted]
State*: [redacted]
Province:
Country*: [redacted]
ZIP / Postal Code*: [redacted]
Phone Number*: [redacted] Fax Number: [redacted] Email*: [redacted]

15. ESTIMATED PROJECT FUNDING

a. Total Federal Funds Requested*	$[redacted]
b. Total Non-Federal Funds*	$0.00
c. Total Federal & Non-Federal Funds*	$[redacted]
d. Estimated Program Income*	$0.00

16.IS APPLICATION SUBJECT TO REVIEW BY STATE EXECUTIVE ORDER 12372 PROCESS?*

a. YES ○ THIS PREAPPLICATION/APPLICATION WAS MADE AVAILABLE TO THE STATE EXECUTIVE ORDER 12372 PROCESS FOR REVIEW ON:

DATE:

b. NO ● PROGRAM IS NOT COVERED BY E.O. 12372; OR

○ PROGRAM HAS NOT BEEN SELECTED BY STATE FOR REVIEW

17. By signing this application, I certify (1) to the statements contained in the list of certifications* and (2) that the statements herein are true, complete and accurate to the best of my knowledge. I also provide the required assurances * and agree to comply with any resulting terms if I accept an award. I am aware that any false, fictitious, or fraudulent statements or claims may subject me to criminal, civil, or administrative penalties. (U.S. Code, Title 18, Section 1001)

● I agree*

** The list of certifications and assurances, or an Internet site where you may obtain this list, is contained in the announcement or agency specific instructions.*

18. SFLLL or OTHER EXPLANATORY DOCUMENTATION File Name:

19. AUTHORIZED REPRESENTATIVE

Prefix: Dr. First Name*: James Middle Name: Leif Last Name*: Smith Suffix:

Position/Title*: Chief Executive Officer
Organization Name*: Sano Chemicals
Department: Microbiology
Division: Antibiotics
Street1*: [redacted]
Street2:
City*: [redacted]
County: [redacted]
State*: [redacted]
Province:
Country*: [redacted]
ZIP / Postal Code*: [redacted]
Phone Number*: [redacted] Fax Number: [redacted] Email*: [redacted]

Signature of Authorized Representative*
James Smith

Date Signed*
09/03/2016

20. PRE-APPLICATION File Name:

21. COVER LETTER ATTACHMENT File Name:1235-CoverLetter.pdf

424 R&R and PHS-398 Specific Table Of Contents

OMB Number: 4040-0010
Expiration Date: 06/30/2016

Project/Performance Site Location(s)

Project/Performance Site Primary Location

❍ I am submitting an application as an individual, and not on behalf of a company, state, local or tribal government, academia, or other type of organization.

Organization Name: Sano Chemicals
Duns Number: [redacted]
Street1*: [redacted]
Street2:
City*: [redacted]
County: [redacted]
State*: [redacted]
Province:
Country*: [redacted]
Zip / Postal Code*: [redacted]
Project/Performance Site Congressional District*: TX-017

Project/Performance Site Location 1

❍ I am submitting an application as an individual, and not on behalf of a company, state, local or tribal government, academia, or other type of organization.

Organization Name: Texas A&M University
DUNS Number: [redacted]
Street1*: [redacted]
Street2: [redacted]
City*: [redacted]
County: [redacted]
State*: [redacted]
Province:
Country*: [redacted]
Zip / Postal Code*: [redacted]
Project/Performance Site Congressional District*: TX-017

File Name

Additional Location(s)

Funding Opportunity Number: PA-16-303. Received Date: 2016-09-03T22:03:46.000-04:00

OMB Number: 4040-0001
Expiration Date: 06/30/2016

RESEARCH & RELATED Other Project Information

1. Are Human Subjects Involved?* ❍ **Yes** ● **No**

1.a. If YES to Human Subjects

Is the Project Exempt from Federal regulations? ❍ Yes ❍ No

If YES, check appropriate exemption number: _ 1 _ 2 _ 3 _ 4 _ 5 _ 6

If NO, is the IRB review Pending? ❍ **Yes** ❍ **No**

IRB Approval Date:

Human Subject Assurance Number

2. Are Vertebrate Animals Used?* ❍ **Yes** ● **No**

2.a. If YES to Vertebrate Animals

Is the IACUC review Pending? ❍ Yes ❍ No

IACUC Approval Date:

Animal Welfare Assurance Number

3. Is proprietary/privileged information included in the application?* ❍ **Yes** ● **No**

4.a. Does this project have an actual or potential impact - positive or negative - on the environment?* ❍ **Yes** ● **No**

4.b. If yes, please explain:

4.c. If this project has an actual or potential impact on the environment, has an exemption been authorized or an environmental assessment (EA) or environmental impact statement (EIS) been performed? ❍ Yes ❍ No

4.d. If yes, please explain:

5. Is the research performance site designated, or eligible to be designated, as a historic place?* ❍ **Yes** ● **No**

5.a. If yes, please explain:

6. Does this project involve activities outside the United States or partnership with international collaborators?* ❍ **Yes** ● **No**

6.a. If yes, identify countries:

6.b. Optional Explanation:

	Filename
7. Project Summary/Abstract*	1236-projectsummary.pdf
8. Project Narrative*	1237-ProjectNarrative.pdf
9. Bibliography & References Cited	1238-References.pdf
10.Facilities & Other Resources	1239-FacilitiesAndOtherResources.pdf
11.Equipment	1240-Equipment.pdf
12. Other Attachments	1241-SBC_000701469.pdf

Funding Opportunity Number: PA-16-303. Received Date: 2016-09-03T22:03:46.000-04:00

Project Summary

Occidiofungin is a cyclic nonribosomally synthesized antifungal peptide with submicromolar fungicidal activity against a broad spectrum of fungi. Occidiofungin is produced by the Gram-negative bacterium *Burkholderia contaminans*. From our structural characterization studies, occidiofungin was determined to have a unique chemical composition. Our studies have also revealed that occidiofungin has a novel mechanism of action. Occidiofungin is a potent antifungal against fluconazole and caspofungin-resistant *Candida albicans*. Occidiofungin triggers cell death by inducing apoptosis in yeast cells. Occidiofungin has minimal toxicity in mice dosed with 2 mg/kg in a 28 day toxicity study. Furthermore, occidiofungin was shown to reduce *Candida glabrata* load in the kidneys of infected mice. All these studies point to the need to further the preclinical development of this novel compound. A major need for furthering investigational studies on this unique antifungal compound is the identification of a lead molecule for preclinical testing. The goal of this application is to screen a library of natural and semi-synthetic analogs of occidiofungin for bioactivity testing. The compound that has the best properties will be used in the required preclinical tests that are compulsory before our initial meeting with the FDA. This work is necessary for furthering additional studies aimed at developing the antifungal compound as a new therapeutic.

Project Narrative

The proposal addresses the need for the development of a novel broad spectrum antifungal for the treatment of systemic fungal infections. We will isolate natural analogs and produce semi-synthetic analogs of the antifungal occidiofungin. This works is essential for identifying a lead compound for preclinical studies that are needed for filing an Investigational New Drug (IND) application.

Facilities and Other Resources –Sano Chemicals

Laboratory:
Sano Chemicals' laboratories are located approximately 9 miles from the campus of Texas A&M University. Sano Chemicals has fully equipped microbiology, molecular biology and chemistry laboratories occupying approximately 1,5000 square feet. The laboratories provide ample space for assorted large and small equipment and can accommodate up to 15 total investigators and supporting staff.

Clinical:
Not Applicable

Animal:
Not Applicable

Computer:
Sano Chemicals has high speed internet access. Sano Chemicals has a central server with over 15 work stations, all of which are protected by appropriate firewalls under the direction of a highly qualified administrative consultant.

Office:
Sano Chemicals' administrative offices are located in the Sano Chemicals facility, and occupy approximately 300 square feet. Sano Chemicals has a 350 square feet conference room used for regular meetings. The laboratories have eight computer working stations that can be used by the investigators.

Other Resources:
Sano Chemicals is located 9 miles from Texas A&M University and has access to state of the art research core facilities. Sano Chemicals has established relationship with The Research Valley Partnership, Texas Triangle Regional Center of Innovation and Commercialization, Texas A&M System Technology Commercialization, and the Center for New Ventures & Entrepreneurship at Texas A&M. These resources provide a rich environment for our company.

Facilities and Other Resources –Texas A&M University

Environment – Contribution to Success
Biology Department has 45 tenured and tenure-track faculty members. Six of the faculty members have well established careers in Microbiology. The Department of Biology promotes a supportive environment among faculty. Texas A&M University is a world class research and education institution. The university provides a rich environment promoting faculty interaction and collaboration. Texas A&M University supports seminars and social functions that enhance the research and educational environment, as well as provide and support outstanding facilities for cutting edge research.

Texas A&M University, Shared Facilities: (http://biophysics.tamu.edu/facilities.html)
X-ray Crystallography Laboratory and Center for Structural Biology
The X-ray Laboratory contains the newest X-ray diffraction instruments, high-speed computers and many graphics workstations. The Laboratory also provides training for macromolecular structural biology. Consistent with this goal, this laboratory organizes seminars, workshops, and always maintains an "open-door" policy for equipment use and training.

Biomolecular NMR Facilities
The Biomolecular NMR Laboratory is located in the Biochemistry Building and houses 500 MHz and 600 MHz Varian Inova NMR spectrometers. Both 500 and 600 MHz spectrometers are dedicated to performing all multidimensional, multinuclear NMR experiments required to solve the solution structures of proteins and nucleic acids (RNA and DNA), as well as probe the conformational dynamics and residue-specific energetics of biomolecules and macromolecular complexes. In addition, the NMR Laboratory in the Department of Chemistry contains three 500 MHz Varian spectrometers, one 400 MHz instrument, and several low-field instruments.

Each of the NMR facilities is overseen by a full-time NMR laboratory manager who is available to assist and train students in modern NMR techniques.

Microscopy and Imaging Center
The Microscopy and Imaging Center provides training and support services for current and emerging technologies involving microscopy and related imaging, sample preparation, in situ analysis and digital image processing. Several electron and light microscopes are available for use, including: a JEOL JEM-2010 high-resolution transmission electron microscope, a JEOL JSM-6400 scanning electron microscope, an ESEM E-3 environmental SEM, and a Zeiss Axiophot light microscope equipped for bright field, phase contrast, fluorescence, and Normarski differential interference contrast microscopy. An increasing number of instruments is being interfaced to the latest data collection and analysis/processing hardware and software.

Laboratory for Biological Mass Spectrometry (LBMS)
This laboratory provides modern mass spectrometry capabilities to researchers focused on the identification and characterization of biomolecules. The research activities of the LBMS include fundamental ion chemistry and developmental mass spectrometry, as well as mass spectrometry applications. Research in this laboratory provides the underpinning for developing new methods and mass spectrometry instrumentation, and these developments are tested and evaluated by collaborations with biological researchers in advance of commercialization.

Fluorescence Spectroscopy
The extensive fluorescence capabilities available at TAMU include ten photon-counting spectrofluorimeters, many using laser excitation, for steady-state or time-resolved measurements, in addition to a femtosecond mode-locked titanium-sapphire laser and inverted microscope suitable for fluorescence correlation spectroscopy and other fluorescence measurements. These instruments use either single- or multi-photon excitation to measure, among many other things, rates of macromolecular tumbling, distances between 30 and 100Å by fluorescence resonance energy transfer, the rates of reactions associated with individual molecules, and the kinetics and thermodynamics of macromolecular associations or receptor-ligand bindings.

Laboratory for Molecular Simulation
This facility brings molecular modeling and computational chemistry closer to the experimental scientist by offering advanced training and assistance to those who already use these tools in their research, and beginning training to those who have not yet adopted them. For this purpose, the LMS offers various workshops on topics such as UNIX operating systems, as well as the theoretical background and practical applications of molecular modeling. Advanced modeling software is available to use mathematical methods to calculate the properties of individual molecules, solids, and liquids.

Paramagnetic Resonance Facility
Paramagnetic probes can provide windows of observation into the structure and dynamics of biological systems. The Electron Paramagnetic Resonance (EPR) facility has Bruker X-band EPR capabilities with variable temperature control down to liquid helium (4.2¡ K) temperatures. A specialty ENDOR (electron nuclear double resonance) instrument operating at Q-band and pumped helium (1.8¡ K) temperatures also is available. Associated with these techniques is a fast freeze-quench system that allows trapping of potential intermediates in catalytic pathways.

ITC and DSC Microcalorimetry
Isothermal titration calorimetry (ITC) and Differential Scanning Calorimetry (DSC) are thermodynamic techniques for directly measuring the heat absorbed or released during molecular reactions. Our MicroCal MCS-ITC, VP-ITC, and VP-DSC instruments allow ultrasensitive characterizations of protein and nucleic acid unfolding, protein-ligand binding, protein-nucleic acid interactions, and metal ion binding to metalloproteins.

Analytical Ultracentrifugation (AUC)
AUC is an essential tool in determining molecular size in complex biological solutions. This can be used to provide information about a wide range of systems, from transcriptional activation complexes to the oligomerization state of biologically active proteins. We currently have a Beckman Optima XL-A centrifuge equipped with absorption optics.

Protein Chemistry Laboratory
The Protein Chemistry Laboratory provides instrumentation and skilled professional staff for the purification and microanalysis of proteins and peptides. In addition to teaching state-of-the-art protein chemical techniques to students, staff, and faculty, the laboratory offers a variety of analytical and consulting services, including the development of new approaches and protocols for advanced technology in protein characterization and analysis.

Institutional Commitment

Dr. James L. Smith has a 9 mo tenure–track position. Teaching obligations are 1.5 classes per year. He will teach a General Microbiology class in the Fall and team-teach a graduate level course on Antimicrobial Agents in the Spring semester. He is expected to devote 50% of his effort to research. His start up package provided him ample resources to begin his research program, but he is expected to maintain his research program through extramural funding. The department has a mentoring program for assistant and associate professors. Dr. Smith has two well-established professors, Richard Gomer and Ry Young, as his department mentors. Biology Department has a seminar series that brings in investigators that will enrich Dr. Smith's exposure to cutting edge research. The department is committed to maintaining Dr. Smith's international reputation.

Facilities:

Laboratory
Dr. Smith, laboratory is ~1000 square feet. His lab is equipped and approved for BSL-2 laboratory work and is equipped with the necessary molecular biology, protein expression, and protein purification tools necessary to conduct the proposal.

Office:
Dr. Smith has ~250 square feet of office space. Administrative and secretarial support staff is provided for faculty within the department.

Computer
Dell Desktop and Toshiba laptop computers with current operating systems and office application software.

Department of Biological Science Resources
There are shared instrument facilities on each floor of the Department of Biological Science buildings BSBE and BSBW that include centrifuges, -80° C freezers, cold rooms, deionized water, gel documentation, nanodrop spectrophotometers, and autoclaves. The department has the following support staff and facilities: Computing, Departmental Stockroom, Gene Technologies Laboratory, Instrumentation Shop, Microscopy and Imaging Center, and a Safety Office.

Sano Chemicals

Equipment located in the Sano Chemicals R&D facility that will be used for the project:

- biosafety cabinet (Class II)
- ultralow freezer (Nuaire)
- -20 freezer
- Oven
- environmental shaker (New Brunswick)
- 3 New Brunswick Celligen Bioreactor (5 L)
- UV/Vis Spectrophotometer (Jenway)
- pH meter (Denver)
- electrophoresis power supplies
- agarose and polacrylamide electrophoresis apparatus (BioRad)
- UVP Dual Intensity Transilluminator
- (2) polymerase chain reaction icycler (BioRad and MJ Research)
- microtiter plate reader (BioRad)
- refrigerated water bath (Haake)
- (2) 2L water baths (VWR)
- vortexes
- teflon vacuum pump
- microbiological Incubators 180 Liter
- rocker and a variable rotator
- heated stir plates
- sonifier-Energy sonicator
- (2) refrigerated centrifuges with assorted rotors (Beckman)
- speedvac (Jouan)
- lyophilizer (Virtis)
- autoclave Pelton and Crane (Magnaclave)
- cold room
- 5ft Chemical Hood (One Point Solution)
- analytical balance (120/0.0001 grams)
- digital balances
- benchtop variable speed centrifuges (Asteria and Eppendorf)
- 4 Dell computers.
- LCMS System, Thermo Scientific TSQ Quantum Access with HESI Probe with Agilent Front End with Binary Pump and variable wavelength detector

Investigators Equipment

Texas A&M University - Dr. Smith

The equipment located in Dr. Smith's laboratory includes:

- Bio-Rad cell and mini trans blot apparatus
- Bio-Rad wide and mini sub-cell GT system for DNA electrophoresis and two power supplies for protein electrophoresis, westerns, and DNA electrophoresis
- Two BioRad iCycler with the gradient upgrades
- BioRad HPLC DuoFlow F10 series with a Quadtec UV detection
- Carter 4/6 Peristaltic pump
- Virtis Benchtop Lyophilizer ZL
- Buchi Rotovap
- Speedvac
- Benchtop shaker incubator
- Sorval RC-5B superspeed refrigerated floor centrifuge
- Bio-Rad GelDoc XR+ with ImageLab software
- 5ft Labconco Biosafety cabinet,

- 5ft Chemical Hood
- Three Eppendorf benchtop centrifuges
- VWR Oven Gravity Medium
- Bio-Rad SmartSpec Plus Spectrophotometer
- Bio-Rad Gene Pulser XCell
- Bio-Rad xMark Microplate Absorbance Spectrophotometer
- Metler Toledo pH meter
- Oasis balance and analytical balance
- Two 2L water baths
- 15 L water Bath
- rocker and a variable rotator
- Two Genie vortexes
- Teflon vacuum pump
- Two Corning heater and stirrers
- Benchtop shaker incubator
- Sonifier-Energy sonicator
- -20 freezer
- 45 cuft Chromatography refrigerator
- Sanyo V.I.P. Series Space Saving Ultra-Low Freezer
- Two Percival Biological Incubator Model I-36NL
- Isotemp 5 cuft incubator
- Thermo Mega Pure Glass Still
- 3 Dell Computers.

Departmental Resources

All ultralow freezers are on a central freezer alarm system, 2 autoclaves on same floor as my lab are checked weekly for proper maintenance, DNA sequencing facility, and laboratory grade water (deionized and distilled). The Microscopy Center is located within the Department of Biological Sciences. It is fully equipped for conventional transmission and scanning electron microscopy and confocal laser scanning microscopy.

Texas A&M Protein Chemistry Lab

The LSBI was established in 2001 and is a core facility for major instrumentation. Equipment at the LSBI is available for all TAMU faculty, and it is located less than a 10 minute walk from the Biological Science Building BSBE. The facility is equipped with protein sequencing and mass spectrometry resources.

Biomolecular NMR Facilities

The Biomolecular NMR Laboratory is located in the Biochemistry Building and houses 500 MHz and 600 MHz NMR spectrometers. Both 500 and 600 MHz spectrometers are dedicated to performing all multidimensional, multinuclear NMR experiments required to solve the solution structures of proteins and nucleic acids (RNA and DNA), as well as probe the conformational dynamics and residue-specific energetics of biomolecules and macromolecular complexes. In addition, the NMR Laboratory in the Department of Chemistry contains three 500 MHz spectrometers. Each of the NMR facilities is overseen by a full-time NMR laboratory manager who is available to assist and train students in modern NMR techniques.

U.S. Small Business Administration

SBIR.gov SBC Registration

Company Information

SBC Control ID	SBC_000701469				
Company Name	Sano Chemicals Inc				
Address	606 Coachlight CT				
City	College Station	**State**	TX	**Zip**	77845
TIN/EIN	[redacted]	**DUNS**	[redacted]		
Company URL	sanochemicals.com				
Number of Employees	6				
Is this SBC majority-owned by multiple venture capital operating companies, hedge funds, or private equity firms?	No				
What percentage (%) of the SBC is majority-owned by multiple venture capital operating companies, hedge funds, or private equity firms?	0%				

OMB Number: 4040-0001
Expiration Date: 06/30/2016

RESEARCH & RELATED Senior/Key Person Profile (Expanded)

PROFILE - Project Director/Principal Investigator

Prefix: Dr. First Name*: James Middle Name Leif Last Name*: Smith Suffix: Ph.D

Position/Title*:	Chief Executive Officer
Organization Name*:	Sano Chemicals
Department:	Microbiology
Division:	Antibiotics
Street1*:	[redacted]
Street2:	
City*:	[redacted]
County:	
State*:	
Province:	
Country*:	[redacted]
Zip / Postal Code*:	

Phone Number*: [redacted] Fax Number: [redacted]

E-Mail*: [redacted]

Credential, e.g., agency login: [redacted]

Project Role*: PD/PI Other Project Role Category:

Degree Type: PhD Degree Year: 2002

Attach Biographical Sketch*: File Name: 1247-SmithBiosketch.pdf

Attach Current & Pending Support: File Name:

Funding Opportunity Number: PA-16-303 . Received Date: 2016-09-03T22:03:46.000-04:00

OMB No. 0925-0001 and 0925-0002 (Rev. 10/15 Approved Through 10/31/2018)

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FIVE PAGES.**

NAME: James Leif Smith

eRA COMMONS USER NAME (credential, e.g., agency login): [redacted]

POSITION TITLE: Chief Executive Officer and Associate Professor

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
University of Florida, Gainesville, FL	Ph.D.	08/2002	College of Medicine's Multidisciplinary Program, Microbial Genetics and Protein Chemistry
University of Florida, Gainesville, FL	MBA	08/2002	Finance and Competitive Strategy
Oragenics Inc., Alachua, FL	Postdoctoral	05/2006	Antimicrobial Development

A. Personal Statement

Infectious diseases are still the second leading cause of death worldwide. Septicemia alone accounts for nearly 30,000 deaths per year in the United States. I am committed to finding avenues to minimize the suffering of those who desperately need alternatives to conventional therapy. My strength in this area of research lies in my cross disciplinary background, which encompasses bacterial cultivation, bacterial genetics, process biochemistry, and biophysical applications such as NMR and Mass Spectrometry. As a postdoctoral fellow at Oragenics Inc, I gained considerable amount of experience under the guidance of the distinguished microbiology professor Dr. Jeffrey D. Hillman. Dr. Jeffrey Hillman founded Oragenics to commercialize the fruits of 25 years of research at Harvard and the University of Florida. It was here that I expanded my knowledge of Microbiology and learned several molecular, proteomic, and animal handling techniques. I have developed several scientific programs while at Oragenics, one of which was the development of novel antibiotics. Since leaving Oragenics in 2006, I have successfully built my own research program around isolating, identifying, and characterizing novel antimicrobials. Our group laid the ground work, i.e. production, purification and the structural activity determination of mutacin 1140, for the future studies aimed at understanding the compounds activity. Synthesis of novel structural variants of mutacin 1140 with enhanced bioactivities have reinvigorate the development of the compound for treating Gram-positive bacterial infections. My work over the last seven years on a novel antifungal compound named occidiofungin has been equally as fulfilling. The project has a good potential for development of a new drug for the treatment of serious fungal infections. I am eager to put my knowledge and experience toward the therapeutic development of occidiofungin.

B. Positions and Honors

Positions and Employment

August 1996 – August 2002

Graduate Research Assistant, College of Medicine, University of Florida

September 2002 – May 2006

Postdoctoral/Project Leader, Antimicrobial Division, Oragenics, Inc.

August 2006 – June 2010

Assistant Professor, Department of Biological Sciences and Department of Basic Sciences at the College of Veterinary Medicine, Mississippi State University

July 2010 – August 2013

Assistant Professor, Department of Biological Sciences, Texas A&M University

March 2012 - present

Chief Executive Officer, Sano Chemicals

August 2013 – present

Associate Professor, Department of Biological Sciences, Texas A&M University

Other Experience

February 1999 – March 2004,

Director and Treasurer, The Able Trust, 106 East College Avenue, Suite 820, Tallahassee, FL 32301 Served on the Finance Committee (Chairman), Executive Committee, and the By-laws Committee

October 2009 – October 2010

Guest-Editor, Current Pharmaceutical Biotechnology , Special topics issue will be centered on the importance of antimicrobial research for promoting our understanding of microbial physiology.

Honors

December, 2007

National High Magnetic Field Laboratory, Winter 2007-2008 issue of *Mag Lab Reports*., Science Starts Here, Featured as an Upcoming Scientist.

March, 2009

Smith J. L., Dahal, N., Wilson-Stanford S., Application number US PPA *61/273,403;* Small Peptide Induction Technology

April, 2009

Hillman J. D., Orugunty R. S., and Smith J. L., Application number US Utility Patent *7,521,529*; Synthesis of differentially protected orthogonal lanthionines

May, 2009,

Invited Speaker, Finnish Biosciences Conference, Helsinki, Finland

May, 2009

Lu S, Smith J. L., Austin F., and Gu Ganyu, Application number US PPA 61/217,026; Occidiofungin, a Unique Glycopeptide Produced by a Strain of Burkholderia contaminans

October, 2009

Smith J. L., and Orugunty R. S., Application number US Utility *12/589,115*.Functional Enhancement of Antimicrobials

November 2009,

Charles C. Randall Lectureship Award of the South Central Branch of the American Society for Microbiology

July 2010

Lu S, Smith J. L., Austin F., and Gu G., US Utility Patent 20110136729; WO 2010/138173; Occidiofungin, a Unique Glycopeptide Produced by a Strain of Burkholderia contaminans

September 2012

Smith J.L., Wilson-Stanford S., Hillman J.D., US PPA 61/603,661; Variants of the Lantibiotic MU1140 and Other Lantibiotics with Improved Pharmacological Properties and Structural Features

September 2012

Smith J.L., Wilson-Stanford S., Hillman J.D., US PPA 61/603,693; Improved Replacement Therapy for Dental Caries

September 2013

Editorial Board Member for Applied and Environmental Microbiology

C. Contribution to Science

1. The study of antimicrobials may lead to life-saving therapies and help ensure a sustainable food supply. This in itself is enough to motivate my research efforts. However, there is more to antimicrobial research. This work also leads to a better understanding of microbial physiology and the complexity of the processes that microorganisms undergo to maintain a sustainable niche. I have focused my research efforts towards enabling a better understanding of peptide-based antibiotics, particularly on lantibiotics and a novel antifungal peptide named occidiofungin. Novel peptide-based antimicrobials are of great interest to my group. Whether ribosomally or non-ribosomally synthesized, peptide-based antimicrobial compounds often contain complex structures and biosynthetic systems. The discovery of unique structures, and determination of their roles in antimicrobial function, is a fascinating area of research. We have characterized the structure of the Gram-positive active antibiotic mutacin 1140 and the broad spectrum antifungal occidiofungin. The identification and bioactivity characterization of these antibiotics has been the basis for the development of these natural products for the treatment of serious bacterial and fungal diseases.

a. Gu G, **Smith L**, Liu A, Lu SE. Genetic and biochemical map for the biosynthesis of occidiofungin, an antifungal produced by Burkholderia contaminans strain MS14. Appl Environ Microbiol. 2011 Sep;77(17):6189-98. doi: 10.1128/AEM.00377-11. Epub 2011 Jul 8. PubMed PMID: 21742901; PubMed Central PMCID: PMC3165383.
b. Lu SE, Novak J, Austin FW, Gu G, Ellis D, Kirk M, Wilson-Stanford S, Tonelli M, **Smith L**. Occidiofungin, a unique antifungal glycopeptide produced by a strain of Burkholderia contaminans. Biochemistry. 2009 Sep 8;48(35):8312-21. doi: 10.1021/bi900814c. PubMed PMID: 19673482; PubMed Central PMCID: PMC2771368.
c. **Smith L**, Zachariah C, Thirumoorthy R, Rocca J, Novák J, Hillman JD, Edison AS. Structure and dynamics of the lantibiotic mutacin 1140. Biochemistry. 2003 Sep 9;42(35):10372-84. PubMed PMID: 12950164.
d. **Smith L**, Novák J, Rocca J, McClung S, Hillman JD, Edison AS. Covalent structure of mutacin 1140 and a novel method for the rapid identification of lantibiotics. Eur J Biochem. 2000 Dec;267(23):6810-6. PubMed PMID: 11082191.

2. Gaining an understanding of the coordinated function of the enzymes leading to their production is equally as interesting, because of the potential for manipulating these enzymes for synthesizing and engineering unique compounds. Mutacin 1140 is post-translationally modified and we have furthered the understanding of the coordinated interaction of the PTM enzyme function during the synthesis of mutacin 1140. Furthermore, we have furthered our understanding of the biosynthesis of the non ribosomally synthesized antifungal occidiofungin. These studies provide a basis for manipulating the biosynthetic pathway for producing novel analogs of these compounds.

a. Escano J, Stauffer B, Brennan J, Bullock M, **Smith L**. Biosynthesis and Transport of the Lantibiotic Mutacin 1140 Produced by Streptococcus mutans. J Bacteriol. 2015 Apr 1;197(7):1173-84. doi: 10.1128/JB.02531-14. Epub 2015 Jan 20. PubMed PMID: 25605307.
b. Escano J, Stauffer B, Brennan J, Bullock M, **Smith L**. The leader peptide of mutacin 1140 has distinct structural components compared to related class I lantibiotics. Microbiologyopen. 2014 Dec;3(6):961-72. doi: 10.1002/mbo3.222. Epub 2014 Nov 17. PubMed PMID: 25400246; PubMed Central PMCID: PMC4263518.
c. Chen S, Wilson-Stanford S, Cromwell W, Hillman JD, Guerrero A, Allen CA, Sorg JA, **Smith L**. Site-directed mutations in the lanthipeptide mutacin 1140. Appl Environ Microbiol. 2013 Jul;79(13):4015-23. doi: 10.1128/AEM.00704-13. Epub 2013 Apr 19. PubMed PMID: 23603688; PubMed Central PMCID: PMC3697549.
d. Ravichandran A, Gu G, Escano J, Lu SE, **Smith L**. The presence of two cyclase thioesterases expands the conformational freedom of the cyclic Peptide occidiofungin. J Nat Prod. 2013 Feb 22;76(2):150-6. doi: 10.1021/np3005503. Epub 2013 Feb 8. PubMed PMID: 23394257; PubMed Central PMCID: PMC4142711.

3. During my time at Oragenics Inc., I learned the methods used to optimize the production and purification of antimicrobial compounds. I continued some of these studies when I first began as an assistant professor. I have developed methods to extract the antibiotic mutacin 1140 from complex media and enabled the antibiotics production in minimal media. These methods have been translated over to Oragenics Inc, which is developing mutacin 1140 for the treatment of *Clostridium difficile* infections.

a. Dahal N., Chaney N., Ellis D, Lu S., **Smith L**. Optimization of the Production of the Lantibiotic Mutacin 1140 in Minimal Media. Process Biochem. 2010 Jul 1;45(7):1187-1191. PubMed PMID: 20711515; PubMed Central PMCID: PMC2918913.
b. Chaney N, Wilson-Stanford S, Kastrantas J, Dahal N, **Smith L**. Rapid method for extracting the antibiotic mutacin 1140 from complex fermentation medium yeast extract. Can J Microbiol. 2009 Nov;55(11):1261-6. doi: 10.1139/w09-091. PubMed PMID: 19940934.

Complete List of Published Work in MyBibliography:
http://www.ncbi.nlm.nih.gov/sites/myncbi/10WFtnmKHuo55/bibliography/47767237/public/?sort=date&direction=ascending

D. Research Support

Current Research Support

Agency: NIH-NIAID
Award 1R41AI122441-01A1
Principal Investigator: James L. Smith
Duration: 04/01/2016 – 3/31/2018
Proposal Title: Optimization of the Production and Isolation of the Novel Antifungal Occidiofungin

Completed Research Support

[redacted]

OMB Number: 4040-0001
Expiration Date: 06/30/2016

RESEARCH & RELATED BUDGET - SECTION A & B, Budget Period 1

ORGANIZATIONAL DUNS*: [redacted]

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: Sano Chemicals

Start Date*: 04-01-2017 **End Date*:** 03-31-2018 **Budget Period: 1**

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1 .	Dr.	James	Leif	Smith	Ph.D	PD/PI	[redacted]	0.60			[redacted]	[redacted]	[redacted]
2 .	Dr.	Akshaya		Ravichandran		Research Scientist	[redacted]	12.00			[redacted]	[redacted]	[redacted]

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name:

Total Senior/Key Person [redacted]

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
	Total Number Other Personnel					**Total Other Personnel**	
						Total Salary, Wages and Fringe Benefits (A+B)	[redacted]

RESEARCH & RELATED Budget {A-B} (Funds Requested)

Funding Opportunity Number: PA-16-303 . Received Date: 2016-09-03T22:03:46.000-04:00

RESEARCH & RELATED BUDGET - SECTION C, D, & E, Budget Period 1

ORGANIZATIONAL DUNS*: [redacted]

Budget Type*: ● Project ❍ Subaward/Consortium

Organization: Sano Chemicals

Start Date*: 04-01-2017 **End Date*:** 03-31-2018 **Budget Period: 1**

C. Equipment Description	
List items and dollar amount for each item exceeding $5,000	
Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	
Total Equipment	
Additional Equipment: File Name:	

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	0.00
2. Foreign Travel Costs	0.00
Total Travel Cost	**0.00**

E. Participant/Trainee Support Costs		**Funds Requested ($)***
1. Tuition/Fees/Health Insurance		0.00
2. Stipends		0.00
3. Travel		0.00
4. Subsistence		0.00
5. Other:		
Number of Participants/Trainees	**Total Participant Trainee Support Costs**	**0.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

Tracking Number: GRANT12241216 Funding Opportunity Number: PA-16-303 . Received Date: 2016-09-03T22:03:46.000-04:00

RESEARCH & RELATED BUDGET - SECTIONS F-K, Budget Period 1

ORGANIZATIONAL DUNS*: [redacted]

Budget Type*: ● Project ❍ Subaward/Consortium

Organization: Sano Chemicals

Start Date*: 04-01-2017 **End Date*:** 03-31-2018 **Budget Period: 1**

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	[redacted]
2. Publication Costs	0.00
3. Consultant Services	0.00
4. ADP/Computer Services	0.00
5. Subawards/Consortium/Contractual Costs	[redacted]
6. Equipment or Facility Rental/User Fees	0.00
7. Alterations and Renovations	0.00
Total Other Direct Costs	[redacted]

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	[redacted]

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1 . MTDC	40.00	[redacted]	[redacted]
		Total Indirect Costs	[redacted]

Cognizant Federal Agency
(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	[redacted]

J. Fee	Funds Requested ($)*

K. Budget Justification*	File Name: 1234-BudgetJustificationSano.pdf
	(Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

Budget Justification - Sano Chemicals

Statement of Work
Sano Chemicals will perform the work involved in the synthesis of the semi-synthetic analogs of occidiofungin. The bioactivity of these analogs will be evaluated at Texas A&M university.

Salaries and Wages
Principal Investigator: Dr. Smith (5% Effort, 0.6 cal month Effort for year 1). Dr. Smith will be actively involved in the day to day activities of the project and will ensure successful completion by providing his experience. Furthermore, Dr. Smith will be responsible for the overall administration and coordination of the project.

Research Scientist: Akshaya Ravichandran (100% Effort, 12 cal month Effort for year 1) Dr. Ravichandran will have to commit 100% of her time on this project, which is a sufficient time commitment for the successful completion of the project. Akshaya has over 6 years of experience working with occidiofungin. Her experience will be invaluable towards the completion of the genetic modification studies outlined in the proposal. She will also work on engineering the semi-synthetic analogs of occidiofungin.

Equipment
No additional equipment needs for this project.

Materials and Supplies
The cost of materials and supplies is based on previous experience with the fermentation supplies, molecular biology supplies, and expected analytical fees. Molecular reagents and bacterial cultivation supplies will be needed to produce occidiofungin analogs, validate, and characterize the products. Commodity funds are requested for laboratory supplies including single-use disposable plastic ware such as centrifuge tubes, micropipetter tips, pipettes and culture plates; reagents and chemicals required for bacterial culture; personal protective equipment such as gloves, safety glasses, and masks; and other incidental supplies.

Travel:
None Requested

Indirect Cost Rate
Indirect costs are computed at the rate of 40% of total modified total direct costs.

RESEARCH & RELATED BUDGET - Cumulative Budget

	Totals ($)	
Section A, Senior/Key Person		[redacted]
Section B, Other Personnel		
Total Number Other Personnel		
Total Salary, Wages and Fringe Benefits (A+B)		[redacted]
Section C, Equipment		
Section D, Travel		0.00
1. Domestic	0.00	
2. Foreign	0.00	
Section E, Participant/Trainee Support Costs		0.00
1. Tuition/Fees/Health Insurance	0.00	
2. Stipends	0.00	
3. Travel	0.00	
4. Subsistence	0.00	
5. Other		
6. Number of Participants/Trainees		
Section F, Other Direct Costs		[redacted]
1. Materials and Supplies	[redacted]	
2. Publication Costs	0.00	
3. Consultant Services	0.00	
4. ADP/Computer Services	0.00	
5. Subawards/Consortium/Contractual Costs	[redacted]	
6. Equipment or Facility Rental/User Fees	0.00	
7. Alterations and Renovations	0.00	
8. Other 1		
9. Other 2		
10. Other 3		
Section G, Direct Costs (A thru F)		[redacted]
Section H, Indirect Costs		[redacted]
Section I, Total Direct and Indirect Costs (G + H)		[redacted]
Section J, Fee		

Funding Opportunity Number: PA-16-303 . Received Date: 2016-09-03T22:03:46.000-04:00

OMB Number: 4040-0001
Expiration Date: 06/30/2016

RESEARCH & RELATED BUDGET - SECTION A & B, Budget Period 1

ORGANIZATIONAL DUNS*: [redacted]

Budget Type*: ❍ Project ● Subaward/Consortium

Enter name of Organization: Texas A&M University

Start Date*: 04-01-2017 **End Date*:** 03-31-2018 **Budget Period: 1**

A. Senior/Key Person

Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1 . Dr.	James		Smith		PD/PI	[redacted]	0.60			[redacted]	[redacted]	[redacted]

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name:

Total Senior/Key Person [redacted]

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
	Post Doctoral Associates						
1	Graduate Students	6.00			[redacted]	[redacted]	[redacted]
	Undergraduate Students						
	Secretarial/Clerical						
1	**Total Number Other Personnel**					**Total Other Personnel**	[redacted]
						Total Salary, Wages and Fringe Benefits (A+B)	[redacted]

RESEARCH & RELATED Budget {A-B} (Funds Requested)

Funding Opportunity Number: PA-16-303 . Received Date: 2016-09-03T22:03:46.000-04:00

RESEARCH & RELATED BUDGET - SECTION C, D, & E, Budget Period 1

ORGANIZATIONAL DUNS*: [redacted]

Budget Type*: ❍ Project ● Subaward/Consortium

Organization: Texas A&M University

Start Date*: 04-01-2017 **End Date*:** 03-31-2018 **Budget Period: 1**

C. Equipment Description

List items and dollar amount for each item exceeding $5,000

Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	
Total Equipment	

Additional Equipment: File Name:

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	
2. Foreign Travel Costs	
Total Travel Cost	

E. Participant/Trainee Support Costs		**Funds Requested ($)***
1. Tuition/Fees/Health Insurance		[redacted]
2. Stipends		
3. Travel		
4. Subsistence		
5. Other:		
Number of Participants/Trainees	**Total Participant Trainee Support Costs**	[redacted]

RESEARCH & RELATED Budget {C-E} (Funds Requested)

Funding Opportunity Number: PA-16-303 . Received Date: 2016-09-03T22:03:46.000-04:00

RESEARCH & RELATED BUDGET - SECTIONS F-K, Budget Period 1

ORGANIZATIONAL DUNS*: 0202718260000
Budget Type*: ❍ Project ● Subaward/Consortium
Organization: Texas A&M University

Start Date*: 04-01-2017 **End Date*:** 03-31-2018 **Budget Period: 1**

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	[redacted]
2. Publication Costs	0.00
3. Consultant Services	0.00
4. ADP/Computer Services	0.00
5. Subawards/Consortium/Contractual Costs	0.00
6. Equipment or Facility Rental/User Fees	[redacted]
7. Alterations and Renovations	0.00
Total Other Direct Costs	[redacted]

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	[redacted]

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1 . MTDC	[redacted]	[redacted]	[redacted]
		Total Indirect Costs	[redacted]

Cognizant Federal Agency (Agency Name, POC Name, and POC Phone Number) Arif Karim HHS 2147673261

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	[redacted]

J. Fee	Funds Requested ($)*

K. Budget Justification* File Name: 1242-BudgetJustificationAM.pdf
(Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

Budget Justification-Texas A&M

STATEMENT OF WORK

Texas A&M will perform the work involved in characterizing the bioactivity of each of the natural or synthetic analogs of occidiofungin. The generation of some of the amine and the synthetic solid phase peptide synthesis analogs of occidiofungin will be done at Texas A&M University.

PERSONNEL

James Leif Smith, PhD, Principal Investigator (5% Effort, 0,6 cal month Effort; year 1); He will be actively involved in the day to day activities of the project and will ensure successful completion by providing his experience. He will oversee the efforts of his research scientist on the project and will be actively involved in training and day to day trouble shooting that is necessary for successful completion of the experimental aims. Furthermore, Dr. Smith will be responsible for the overall administration and coordination of the project.

Mengxin Geng, Graduate Research Assistant, She (50% effort, 6 mo; year 1), will be working under the supervision of Dr. Smith. An effort of 50% is the maximum reportable effort for a graduate research assistant given their educational training. Mengxin is knowledgeable of techniques used isolating occidiofungin and analogs of occidiofungin. Her efforts, along with Dr. Smith, will be aimed at furthering the understanding of the bioactivity of each analogs and making the decision as to which analog has the best properties to move forward with preclinical development.

MATERIALS AND SUPPLIES

Costs for disposable supplies, chemicals, media, and chromatography supplies are based prior experience from past practices. Additional costs are being budgeted for peptide synthesis.

EQUIPMENT

No equipment is being requested

TRAVEL

No travel funds are being requested.

FEDERALLY NEGOTIATED INDIRECT COST RATE

Texas A&M recovers under Modified Total Direct Cost basis with an indirect cost recovery of 48.5%.

RESEARCH & RELATED BUDGET - Cumulative Budget

	Totals ($)	
Section A, Senior/Key Person		[redacted]
Section B, Other Personnel		[redacted]
Total Number Other Personnel	1	
Total Salary, Wages and Fringe Benefits (A+B)		[redacted]
Section C, Equipment		
Section D, Travel		
1. Domestic		
2. Foreign		
Section E, Participant/Trainee Support Costs		[redacted]
1. Tuition/Fees/Health Insurance	[redacted]	
2. Stipends		
3. Travel		
4. Subsistence		
5. Other		
6. Number of Participants/Trainees		
Section F, Other Direct Costs		[redacted]
1. Materials and Supplies	[redacted]	
2. Publication Costs	0.00	
3. Consultant Services	0.00	
4. ADP/Computer Services	0.00	
5. Subawards/Consortium/Contractual Costs	0.00	
6. Equipment or Facility Rental/User Fees	[redacted]	
7. Alterations and Renovations	0.00	
8. Other 1		
9. Other 2		
10. Other 3		
Section G, Direct Costs (A thru F)		[redacted]
Section H, Indirect Costs		[redacted]
Section I, Total Direct and Indirect Costs (G + H)		[redacted]
Section J, Fee		

Funding Opportunity Number: PA-16-303 . Received Date: 2016-09-03T22:03:46.000-04:00

Total Direct Costs less Consortium F&A

NIH policy (NOT-OD-05-004) allows applicants to exclude consortium/contractual F&A costs when determining if an application falls at or beneath any applicable direct cost limit. When a direct cost limit is specified in an FOA, the following table can be used to determine if your application falls within that limit.

Category	Budget Period 1	Budget Period 2	Budget Period 3	Budget Period 4	Budget Period 5	TOTALS
Total Direct Costs less Consortium F&A	[redacted]	0	0	0	0	[redacted]

OMB Number: 4040-0001
Expiration date: 06/30/2016

SBIR/STTR Information

Program Type (select only one)*

❍ SBIR ● STTR ❍ Both (See agency-specific instructions to determine whether a particular agency allows a single submission for both SBIR and STTR)

SBIR/STTR Type (select only one)*

● Phase I ❍ Phase II ❍ Fast-Track (See agency-specific instructions to determine whether a particular agency participates in Fast-Track)

Questions 1-7 must be completed by all SBIR and STTR Applicants:

1a. Do you certify that at the time of award your organization will meet the eligibility criteria for a small business as defined in the funding opportunity announcement?* ● Yes ❍ No

1b. Anticipated Number of personnel to be employed at your organization at the time of award.* 5

2. Does this application include subcontracts with Federal laboratories or any other Federal Government agencies?* ❍ Yes ● No

If yes, insert the names of the Federal laboratories/agencies:*

3. Are you located in a HUBZone? To find out if your business is in a HUBZone, use the mapping utility provided by the Small Business Administration at its web site: http://www.sba.gov * ❍ Yes ● No

4. Will all research and development on the project be performed in its entirety in the United States?* ● Yes ❍ No

If no, provide an explanation in an attached file. Explanation:*

5. Has the applicant and/or Program Director/Principal Investigator submitted proposals for essentially equivalent work under other Federal program solicitations or received other Federal awards for essentially equivalent work?* ❍ Yes ● No

If yes, insert the names of the other Federal agencies:*

6. Disclosure Permission Statement: If this application does not result in an award, is the Government permitted to disclose the title of your proposed project, and the name, address, telephone number and e-mail address of the official signing for the applicant organization, to organizations that may be interested in contacting you for further information (e.g., possible collaborations, investment)?* ● Yes ❍ No

7. Commercialization Plan: If you are submitting a Phase II or Phase I/Phase II Fast-Track Application, include a Commercialization Plan in accordance with the agency announcement and/or agency-specific instructions.*

Attach File:*

OMB Number: 4040-0001
Expiration date: 06/30/2016

SBIR/STTR Information

SBIR-Specific Questions:

Questions 8 and 9 apply only to SBIR applications. If you are submitting ONLY an STTR application, leave questions 8 and 9 blank and proceed to question 10.

8. Have you received SBIR Phase II awards from the Federal Government? If yes, provide a company commercialization history in accordance with agency-specific instructions using this attachment.* ❍ Yes ❍ No

Attach File:*

9. Will the Project Director/Principal Investigator have his/her primary employment with the small business at the time of award?* ❍ Yes ❍ No

STTR-Specific Questions:

Questions 10 and 11 apply only to STTR applications. If you are submitting ONLY an SBIR application, leave questions 10 and 11 blank.

10. Please indicate whether the answer to BOTH of the following questions is TRUE:* ● Yes ❍ No

(1) Does the Project Director/Principal Investigator have a formal appointment or commitment either with the small business directly (as an employee or a contractor) OR as an employee of the Research Institution, which in turn has made a commitment to the small business through the STTR application process; AND

(2) Will the Project Director/Principal Investigator devote at least 10% effort to the proposed project?

11. In the joint research and development proposed in this project, does the small business perform at least 40% of the work and the research institution named in the application perform at least 30% of the work?* ● Yes ❍ No

Funding Opportunity Number: PA-16-303. Received Date: 2016-09-03T22:03:46.000-04:00

PHS 398 Cover Page Supplement

OMB Number: 0925-0001

Expiration Date: 10/31/2018

1. Human Subjects Section

Clinical Trial? ❍ Yes ● No

*Agency-Defined Phase III Clinical Trial? ❍ Yes ❍ No

2. Vertebrate Animals Section

Are vertebrate animals euthanized? ❍ Yes ● No

If "Yes" to euthanasia

Is the method consistent with American Veterinary Medical Association (AVMA) guidelines?

❍ Yes ❍ No

If "No" to AVMA guidelines, describe method and proved scientific justification

3. *Program Income Section

*Is program income anticipated during the periods for which the grant support is requested?

❍ Yes ● No

If you checked "yes" above (indicating that program income is anticipated), then use the format below to reflect the amount and source(s). Otherwise, leave this section blank.

*Budget Period	*Anticipated Amount ($)	*Source(s)

PHS 398 Cover Page Supplement

4. Human Embryonic Stem Cells Section

*Does the proposed project involve human embryonic stem cells? ❍ Yes ● No

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://grants.nih.gov/stem_cells/registry/current.htm. Or, if a specific stem cell line cannot be referenced at this time, please check the box indicating that one from the registry will be used:

Specific stem cell line cannot be referenced at this time. One from the registry will be used.

Cell Line(s) (Example: 0004):

5. Inventions and Patents Section (RENEWAL)

*Inventions and Patents: ❍ Yes ❍ No

If the answer is "Yes" then please answer the following:

*Previously Reported: ❍ Yes ❍ No

6. Change of Investigator / Change of Institution Section

❑ Change of Project Director / Principal Investigator

Name of former Project Director / Principal Investigator

Prefix:

*First Name:

Middle Name:

*Last Name:

Suffix:

❑ Change of Grantee Institution

*Name of former institution:

Tracking Number: GRANT12241216 Funding Opportunity Number: PA-16-303 . Received Date: 2016-09-03T22:03:46.000-04:00

PHS 398 Research Plan

OMB Number: 0925-0001
Expiration Date: 10/31/2018

Introduction	
1. Introduction to Application (Resubmission and Revision)	
Research Plan Section	
2. Specific Aims	1243-specificaims.pdf
3. Research Strategy*	1244-researchstrategy.pdf
4. Progress Report Publication List	
Human Subjects Section	
5. Protection of Human Subjects	
6. Data Safety Monitoring Plan	
7. Inclusion of Women and Minorities	
8. Inclusion of Children	
Other Research Plan Section	
9. Vertebrate Animals	
10. Select Agent Research	
11. Multiple PD/PI Leadership Plan	
12. Consortium/Contractual Arrangements	
13. Letters of Support	1245-departmentletter.pdf
14. Resource Sharing Plan(s)	1246-ResourceSharingPlan.pdf
15. Authentication of Key Biological and/or Chemical Resources	
Appendix	
16. Appendix	

Tracking Number: GRANT12241216
Funding Opportunity Number: PA-16-303. Received Date: 2016-09-03T22:03:46.000-04:00

RESEARCH PLAN

Specific Aims

Occidiofungins, isolated from Burkholderia *contaminans* MS14, are a newly discovered class of antifungals. From our structural characterization studies, occidiofungin was determined to have a unique chemical composition. These studies revealed four main analogs, occidiofungins A-D, and the presence of two distinct diastereomers. All analogs are composed of eight amino acids and a novel C18 fatty amino acid (NAA) containing a xylose sugar, and a 2,4- diaminobutyric acid (DABA). The structural analogs differ by an addition of oxygen to asparagine 1 (Asn1) forming a β-hydroxy asparagine 1 (BHN1) and by the addition of chlorine to β-hydroxy tyrosine 4 (BHY) forming 3-chloro β-hydroxy tyrosine 4 (chloro-BHY). So far, a mixture of occidiofungin A-D analogs show promise for developing a novel therapeutic option for treating life threatening fungal infection. There is a ***critical need*** to isolate and characterize the bioactivity and toxicity of each of these natural analogs, as well as semi-synthetically produced analogs. The potential to develop new clinically useful approaches to mitigate human susceptibility to infections caused by fungal pathogens like *Candida albicans* will likely remain limited, unless we further understand the therapeutic potential for each of these compounds.

Our ***long-term goal*** is to develop an alternative treatment option for serious fungal infections, in which currently available antifungals are failing too many people. Our ***objective in this application*** is to characterize the naturally produced and semi-synthetically made analogs of occidiofungin. Our ***central hypothesis*** is that one of these analogs has a superior set of qualities for preclinical development and that these analogs need to be evaluated to identify a lead compound. This hypothesis was based on our preliminary data showing a difference in the spectrum of activity for some of these structural analogs. The ***rationale*** for the proposed research is that a lead compound needs to be identified in order to ensure success with the required preclinical studies before we have our pre-IND meeting with the FDA. We propose the following aims:

Objective: Develop a novel therapeutic approach to treat life-threatening fungal infections. The occidiofungin analogs in this application will be screened based on their bioactivity in the presence of serum and toxicity against a mouse cell line. A lead drug candidate for therapeutic development will be identified from this study.

Specific Aim 1: Synthesize, engineer, and isolate structural analogs of occidiofungin.

Given our structural characterization and understanding of occidiofungin biosynthesis, we are able to engineer the synthesis of natural analogs of occidiofungin. The disruption and overexpression of gene products in the biosynthetic pathway will provide means to produce a homogenous culture of analogs of interest. Furthermore, we propose to screen semi-synthetic analogs by modifying an available amine in the molecule. Lastly, we propose to evaluate the possibility of producing occidiofungins by solid phase peptide synthesis. These studies are aimed to expand our understanding of the bioactivity of the naturally occurring analogs, while simultaneously evaluating the utility of chemically synthesizing novel analogs.

Specific Aim 2: Characterize the bioactivity of each structural analogs.

Microorganisms do not normally expend additional energy unless there is a good reason. It is likely that each naturally produced analog has a distinct set of bioactivities and toxicity. Fundamentally, we hope to understand the structure activity relationships (SAR) of the different structural elements found within occidiofungins A-D and the semi-synthetic analogs. Towards this aim, the bioactivity of each occidiofungin analog shall be determined using several methods to evaluate the spectrum of activity, serum binding, and time-kill kinetics. Occidiofungin analogs shall also be tested for differences in their *in vitro* toxicity profile using a rat hepatoma (H4IIE) cell line. These studies will expand our understanding of the biological activity and toxicity of each occidiofungin analog.

At the completion of these studies, it is our ***expectation*** that we will have identified a lead compound of occidiofungin that has the best attributes for preclinical testing. These results are expected to have an important ***positive impact*** because current antifungals have limitations in use and are failing a significant population of susceptible patients. Occidiofungin is rapidly fungicidal, which may improve the therapeutic outcome for these patients. We are well equipped with the knowledge and experience to successfully complete this proposal.

Research Strategy

(A) Significance

Antifungals are very important for the treatment of fungal infections particularly in immunocompromised and organ transplant patients (1-11). There is an absolute need for an alternative antifungal treatment option in the clinic. Hence, the development of newer class of antifungals with different mechanism of action and antifungal spectrum is desperately needed. Occidiofungin is a unique natural product (Figure 1) that has demonstrated attributes that can potentially fill this need. We are currently working to optimize the production of occidiofungin in *B. contaminans* or heterologous system. In connection to these studies, we would like to propose further studies aimed at engineering the production of natural and semi-synthetic analogs of occidiofungin. *A major obstacle is the separation of natural occidiofungin variants.* It is possible to move forward with the clinical development of a mixture of defined occidiofungin analogs. However, it would be better to isolate each of these analogs and determine differences in bioactivity and toxicity. We propose to evaluate these activities by improving our isolation methods, but we are also proposing to engineer the biosynthetic pathway to produce the analogs of interest. This system will not only lead to the isolation of the desired product, but also a novel system for the production of a defined analog of occidiofungin. This information can be integrated into our current production and optimization studies. *Following the completion of the proposed studies in this application, identification of the desired occidiofungin analog/s will be done along with an engineered system to promote the production of the desired analog/s.*

Global market for antifungal therapeutics (in humans) was 11.8 billion dollars in 2013 and is expected to be 13.9 billion by 2018 (1). The U.S. antifungal market in 2013 was 4.9 billion dollars and expected to grow to 5.5 billion dollars by 2018 (1). More than 60% of the antifungal market is for the treatment of life threatening infections, which is also the proposed application for occidiofungin. Fluconazole, belonging to the azole class of antifungals, has had over one billion dollars in annual sales, despite limitations in its uses. Our current findings suggest that occidiofungin is structurally (Figure 1) and functionally a unique antifungal (12, 13); it has a superior spectrum of activity and lower toxicity than the drugs currently used to treat serious fungal infections. Advantages of occidiofungin over currently used antifungals arise from its fungicidal activity and novel mechanism of action (14, 15) in conjunction with the minimal toxicity observed in an animal studies (16). Composition and methods of use patent for occidiofungin has been filed in the U.S (US 2011/0136729). Patent was filed on May 25, 2010. PCT National Phase Applications have been filed in November 2011 for Japan, European Patent Office, China, and South Korea. These filing will help secure worldwide protection. We do not anticipate any problems with the issue of our patents.

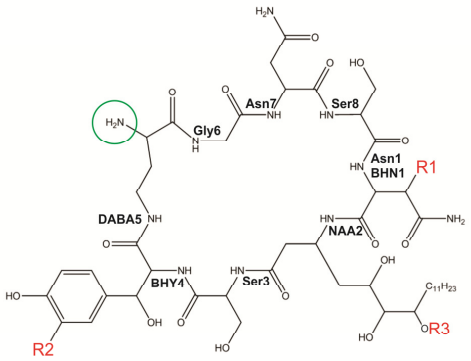

Figure 1. Covalent Structure of occidiofungin. R1=H or OH; R2=H or Cl; R3=H or Xylose. Green circle designates region for site directed modification.

(B) Innovation

Current antifungals that are used to treat systemic infections only target membrane and cell wall integrity. Azoles and echinocandins are fungistatic and moderately fungicidal (10, 11, 17, 18), respectively. This often poses a problem for successful treatment, given that individuals with serious fungal infections are usually severely immunocompromised. Current antifungal treatments lead to abnormal liver function test and have limitations with respect to their spectra of activity and toxicities. Occidiofungin is fungicidal with a unique mechanism of action as it induces apoptosis in yeast cells (14, 15). Current mouse studies with occidiofungin shows no organ specific toxicity (16). *The proposed research is innovative, in our opinion, as we will identify a lead occidiofungin based compound for developing a novel therapeutic to alleviate the healthcare burden associated with fungal infections.* Engineering the biosynthetic pathway or developing a semi-synthetic route for producing pure analogs of occidiofungin will promote its development for treating serious fungal infections.

Currently, we are working with a natural mixture of the major occidiofungin products that are not easily separable. Following successful completion of Aim I, we shall have purified fraction of all eight naturally occurring analogs of occidiofungin for future studies. Each of these bacterial strains, engineered to produce a

defined analog of occidiofungin, can be utilized to produce the desired analog for drug development studies. We also propose to evaluate a semi-synthetic production approach for occidiofungin analogs. Identification of a lead compound is an important consideration that needs to be made before we start our new investigational drug studies (IND). It is our aim to put forward the best occidiofungin analog for preclinical studies. Successful completion of this study will provide a means to identify the lead compound that is most likely to succeed during clinical development.

(C) Approach

Introduction:

There is a substantial need for an alternative drug treatment for serious fungal infections. Limitations of one or more of the currently used antifungal drugs include high recurrence of infection, high mortality rates, poor oral bioavailability, limitations in spectrum of activity, and liver toxicity. Mechanism of action studies with occidiofungin support our assertion that the novel molecule has a unique fungal target (15). All three major thresholds for developing a novel antifungal for clinical use have been met by the antifungal occidiofungin.

<u>First threshold is to have a unique chemical composition.</u>

• Occidiofungin is a hybrid product of NRPS-PKS whose complete chemical composition has been determined. It is a 1200 Da cyclic glyco-lipopeptide (Figure 1) (12, 13).

<u>Second threshold is having a similar or better spectrum of activity as compared to amphotericin B and fluconazole.</u>

- Minimum inhibitory concentrations (MICs) of occidiofungin against *Candida* species are between 0.5 and 1.0 µg/mL (14).
- Occidiofungin retains its *in vitro* potency against *Candida* in the presence of 5% and 50% human serum with a minimum lethal concentration (MLC) of 2 and 4 µg/mL, respectively (14).
- Time-kill experiments revealed that occidiofungin is rapidly fungicidal against *Candida albicans(14)*.
- Post-antifungal experiments, where occidiofungin was washed away from the media, still exhibited potent antifungal activity against *Candida*. These experiments suggest that occidiofungin has a strong interaction with its target (14).
- *Cryptococcus neoformans* is insensitive to echinocandins, but is susceptible to occidiofungin at sub-micromolar concentrations. Antifungal activity of occidiofungin has been demonstrated against a wide array of fungi (13, 14).
- Mechanism of action studies suggests that an alternate target exists for occidiofungin other than targeting ergosterol production, binding to ergosterol or inhibiting the 1,3-β-glucan synthase enzyme, which are the current therapeutic targets for treating fungal infections (14, 15).

<u>The third threshold is having a similar or better toxicity profile compared to amphotericin B and fluconazole.</u>

- Female B6C3F1 mice were used to assess the acute toxicological effects of occidiofungin. Mice were given occidiofungin up to 20 mg/kg body weight (single dose) or 2mg/kg body weight daily for 5 days by intraperitoneal injection. All mice survived these dosages. Variables evaluated included body weight, selected organ weights, clinical chemistry, clinical and anatomic pathology, and behavioral phenomena. Key effects included a transient irritation at the site of injection, and body weight reductions. There was a significant increase in the percentage of neutrophils in the blood and a decrease in thymus weight relative to body weight after a single dose, which is characteristic of a non-specific

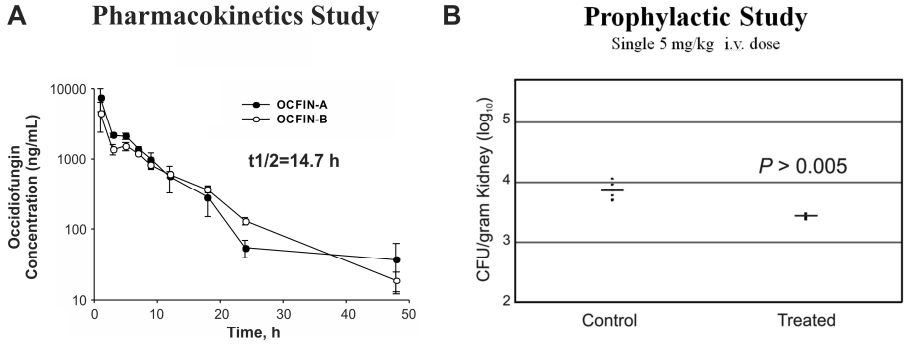

Figure 2. Animal Studies A. Pharmacokinetics of occidiofungin following a 2.5 mg/kg i.v. administration. B. Single prophylactic dose of occidiofungin one hour prior to the i.v. administration of *Candida glabrata* at 10^6 CFU.

stress response. Adaptation seems to be rapid, because these effects were not observed after 5 daily doses. Histological sections did not exhibit organ specific toxicity in liver, lung, thymus, and kidney (16).

- Occidiofungin has a long half-life following i.v. administration and has been shown to reduce mortality and fungal load in the kidneys of female BALB/C mice (Figure 2A and 2B).

Justification:

Occidiofungin, along with very few antifungal compounds, has met these three criteria, and therefore holds promise as a potential antifungal therapeutic agent. The current need towards clinical development is identifying the lead compound of occidiofungin for preclinical studies. The aims described in our application will enable our group to produce a defined analog of occidiofungin that can be further evaluated for differences in bioactivity and toxicity. This information is crucial as it will improve the success rate for developing occidiofungin as a new antifungal therapeutic.

Objective: Produce structurally defined analogs of occidiofungin to identify a lead compound for preclinical studies.

Specific Aim 1: Synthesize, engineer, and isolate structural analogs of occidiofungin.

Preliminary Data for the Biosynthesis of Occidiofungin (12, 19-21):

Sequencing of the *ocf* gene cluster and the detailed structural characterization of occidiofungin provided an excellent opportunity to model the biosynthesis of occidiofungin (Figure 3). The *ocf* gene cluster encodes all the gene products required for the synthesis of occidiofungin (12). The order in which the modules function, so that they are in agreement with the structure are as follows: OcfJ-OcfI-OcfH-OcfF-OcfE-OcfD. The amino acid sequence of the cyclic peptide is Asn/β-hydroxyasparagine (BHN1)-novel amino acid(NAA2)-Ser3-β-hydroxytyrosine (BHY4)-Diaminobutyric acid (DABA5)-Gly6-Asn7-Ser8. The *ocfG* gene encodes a homolog of an enzyme, StaM of the A47934 cluster, known to be involved in the formation of BHY(12). It is likely that the enzyme is responsible for the β-hydroxylations of Tyr4 and Asn1. Given the presence of Asn and BHN at position 1, it is also possible that the hydroxylation of Asn by ocfG is inefficient, leading to a mixture of Asn and BHN residues at position 1. The C-terminal region of OcfD, is predicted to have a TE domain, which is important for the condensation reaction of Ser8 to Asn/BHN1, which terminates synthesis and forms the cyclic peptide. The *ocfN* gene also codes for a thioesterase that is involved in the cyclization of the peptide. The *ocfK* gene codes for a homolog of nonheme iron dioxygenases that can catalyze chlorinations(12). It is likely that this enzyme catalyzes the 3-chloro addition that is observed on BHY4 (12).

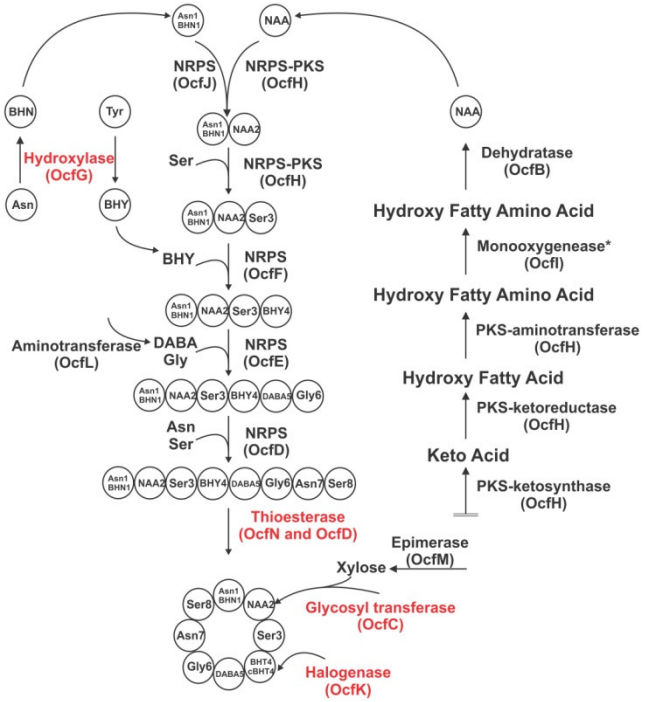

Figure 3. Biosynthesis of occidiofungin. Enzymes that will be modulated are colored red.

There are more than eight different structural analogs of occidiofungin. Only some of the analogs have been successfully separated by chromatography. To date, the spectrum of activity, mechanism of action, and toxicity studies have been done using a mixture of four structural analogs (A-D). The structural analogs of occidiofungin are known to result from the presence or lack of a: β-hydroxyl group on asparagine 1 (occidiofungins A and B), chlorine on BHY4 (occidiofungins C and D), or xylose on NAA2 (Figure 1). In addition, there are two diastereomers formed by two distinct cyclase thioesterases, OcfN and OcfD (21). Given the risk and cost associated with the development of new antimicrobial compounds, it is crucial to identify a specific analog or a well characterized combination of structural analogs that have the best antifungal properties for the treatment of systemic fungal infections. The production of said analogs or combination must be well regulated and reproducible towards scalability of the process for large scale production.

We plan to isolate the products of each natural analog of occidiofungin via genetic modification and chromatography. We have previously shown that the biosynthetic pathway for occidiofungin is amenable to genetic modification enabling the directed synthesis of different structural analogs of occidiofungin (12, 21, 22). The disruption and over-expression of specified gene products in the biosynthetic pathway provides a specific bacterial strain that will produce a well characterized structural analogs of occidiofungin. Engineering of a strain that can produce a specific occidiofungin analog would also provide a pure starting material for determining activity differences, thus, promoting lead compound identification. In addition, we propose a semi-synthetic route by modifying a free amine in occidiofungin and by utilizing the OcfN thioesterase to cyclize a synthesized peptide. This approach may lead to new analogs of occidiofungin that may have different antifungal spectrum and or improved pharmacokinetic, pharmacodynamic properties. The combination of site directed modification and solid phase peptide synthesis (SPPS), as proposed in this application, will expand the structural repertoire of compounds that may have improved bioactivities.

Research Strategy:

Manipulating the producing organism to make each structural analog of occidiofungin is a practical approach as long as it is used in conjunction with the characterization of the structural analogs that can be isolated via chromatographic separation. We have the experience and molecular tools to produce the stereoisomer analogs and the individual β-hydroxylated and chlorinated analogs proposed below (12, 21, 22).

Production of the stereoisomers formed by the thioesterase OcfD and OcfN. We predicted that the thioesterase domain of OcfD and the thioesterase coded by ocfN were both required for cyclization of each distinct diastereomer. A nonpolar mutation was constructed in the open reading frame of wild-type ocfN by the insertion of a kanamycin resistance gene, nptII. The *B. contaminans* MS14 mutant (ocfN::nptII) produced only one of the diastereomers. NMR analysis revealed the loss of the stereoisomers formed by OcfN (5). The bioactivity of the OcfD diastereomer products was compared to the bioactivity of the purified wild-type occidiofungin analogs A-D containing both diastereomers. There was a two-fold decrease in activity for the OcfD diastereomer product against Candida spp. An observed two-fold decrease in activity for these analogs, given that they make up approximately half of the wild-type occidiofungin analogs, suggests that the activity of the OcfN diastereomer is four-fold more active against Candida spp. This data does support our hypothesis that the bacterium is making different analogs of occidiofungin to increase its spectrum of activity (5). Recently, the production of the OcfN diastereomer has also been accomplished following the same mutagenesis procedure described for MS14 mutant (ocfN::nptII). We are in the process of purifying enough of the MS14 mutant (ocfD::nptII) product for bioactivity and NMR studies.

Production of the occidiofungin analogs lacking xylose (ΔXyl). The *B. contaminans* MS14 mutant (ocfC::nptII) produces an analog that no longer has the xylose sugar attached to the novel amino acid (NAA2) (8). The bioactivity of the analogs lacking the xylose, which we call occidiofungin A-D(ΔXyl), has a similar bioactivity against Candida spp as the analogs containing the xylose (8). Therefore, the presence of the xylose sugar is not essential for antifungal activity. This is good news as we plan to synthesize new analogs by SPPS. We need to characterize occidiofungin A-D(ΔXyl) according to the experiments outlined in specific aim 2. We do see an increase in neutrophils in our animal studies following administration of occidiofungin containing the xylose sugar. We can also alleviate lethargy observed in mice at higher doses (5-10mg/mL) by administering the antihistamine cetirizine. Given that xylose is not normally present in animal systems, it may be responsible for triggering an innate immune response and the occidiofungin A-D(ΔXyl) analogs may minimize these observed effects.

Production of the occidiofungin analogs AB or CD. Before embarking on the bioengineering of *B. contaminans* MS14 to produce individual analogs of occidiofungins A-D, we need to characterize the bioactivity differences of occidiofungins A-B and occidiofungins C-D. We have recently been able to separate these structural analogs by chromatography. Depending on the outcome of the spectrum of activity and toxicity studies using these fractions, we will determine the most beneficial structural analog to bioengineer. Production of mixed analogs A-B and C-D will be carried out by knocking out the halogenase ocfK or by overexpressing ocfK. The practical reason for this approach is that it should lead to a differential increase in the amount of the A-B product or C-D product expressed by the bacterium. The vector pMLS7 was constructed to overexpress genes in the Burkholderia cells (9) and successfully used by my group (1, 5, 8). To promote the halogenase activity, 0.1-1mM CaCl2 will also be supplemented into the media. This has been shown to be successful in

other halogenated natural products (10-12). Once we show an increase in chlorination within our system, we can use this system to determine the halogenase's specificity to other halides such as bromide and iodide.

Production of synthetic analogs of occidiofungin. The free amino group on the DABA residue provides a region in which we can synthesize additional structural analogs (Figure 1). We have prepared alkyne NHS ester with > 90% yield for microscopy imaging studies using azide derivatized fluorescent dyes. In these studies, we have determined that occidiofungin is intracellularly localized. We will investigate additional structural modifications within this region that may positively impact bioactivity. We have recently finished the Marphey's derivitization studies on occidiofungin to determine the chirality of each amino acid. Based on this data, and supported by the knowledge of known epimerase domains within the NRPS enzymes, the stereochemistry of the individual amino acids are: (L)Asn1-(L)NAA2-(L)Ser3-(D)BHY4-(D)DABA5-Gly6-(L)Asn7-(D)Ser8. Given the predicted increase in bioactivity of the OcfN cyclase thioesterase product (5), we will use this enzyme for the last cyclization step for our SPPS peptides with a C-terminal N-acetylcysteamine (SNAC) group. This approach will enable us to substitute amino acids and will provide a detailed understanding of the "structure activity relationship" of this molecule. We have expressed and purified the OcfN cyclase thioesterase (Figure 4). The SPPS peptides will be purchased from a commercial peptide supplier (Peptide Elephants). The first peptide will contain a β-homo alanine at the NAA2 position to test the activity of the OcfN cyclase. This substitution will maintain the peptide backbone structure for optimizing the enzymatic cyclase activity. We believe that NAA2 is important for penetrating the fungal plasma membrane. Synthesis of NAA2 is simplified with the knowledge that the xylose is not needed for activity (22). Other functional groups will be explored that may have more desirable attributes while still promoting the peptide's entry into the cell. We will need to synthesize some of the desired β-amino acids for NAA2 position, but many of the Fmoc and Boc labeled β-amino acids are available through Sigma Aldrich.

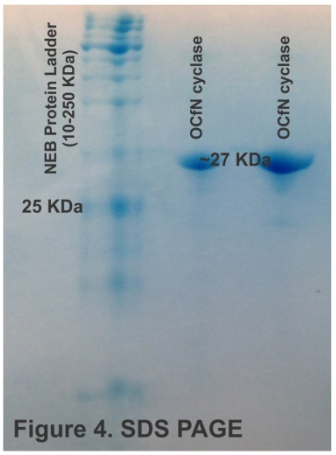

Figure 4. SDS PAGE

Potential Problems and Alternative Approaches: One potential problem that can occur is that the insertion of nptII in the ocfD thioesterase domain, may interfere with the function of other modules within the protein, resulting in a loss of occidiofungin biosynthesis. An alternative approach is to use the suicide vector pJQ200mp18 with a Kmr cassette insertion in the plasmid region of the gentamicin resistance gene (gtmR) (pJQ200mp18:Kmr). The 800 bp fragment described above will be amplified with flanking BamHI restriction sites and cloned into pGEM-T Easy Vector System I. The conserved serine in the GXSXG active site motif of the thioesterase will be replaced by an alanine. Primers with 5' overlapping regions will be used to change the serine codon to an alanine by circle PCR mutagenesis. The PCR product will be introduced into *E. coli* and the bacterium will form the plasmid using the overlapping 5' regions introduced by the primers. The desired plasmid will be isolated from *E. coli* grown on LB-Amp plates for selection. If circle PCR doesn't work, two-step PCR will be used to clone the mutated product. Plasmids will be sequenced to confirm the mutation. The product will be digested with BamHI and cloned into pJQ200mp18:Kmr. Integration of the plasmid into the *ocfD* gene will be selected for using kanamycin. The sacB/sucrose counterselection system will be used to identify the revertants (loss of plasmid). Half of these revertants should contain the mutation. PCR and sequencing will be used to identify the mutation introduced into *B. contaminans* MS14. If mutating the active site of the thioesterase does not yield the desired product, another approach will be used to make the Asn1 analogs. Disruption of the hydroxylase gene, ocfG, should prevent the synthesis of both BHN and BHY. This mutation may prevent the biosynthesis of occidiofungin, in the event that BHY is required for biosynthesis. The activity can presumably be restored by supplementing BHY into the media as has been reported for the production of balhimycin (23).

Expected outcomes. We will have isolated natural structural analogs of occidiofungin upon completion of Aim 1. The outlined approaches will provide a unique basis for not only testing the bioactivity of each analog, but provide a means to specifically produce each compound. The compounds isolated in this study will be evaluated for its bioactivity and toxicity for identifying new therapeutic agents for the treatment of invasive fungal infections in patients who are intolerant or not responding to other therapies.

Specific Aim 2: Characterize the bioactivity of each structural analog.

We have the necessary experience to characterize the bioactivity of the occidiofungin analogs (12, 14, 15, 19-21).

Research Strategy:

In vitro susceptibility testing. MICs for Aspergillus, Penicillium, Cryptococcus, Microsporum, Pythium, Trichophyton, and Candida spp. will be determined by using Clinical Laboratory Standards Institute (CLSI) method M27-A3 and M38-A2. MIC endpoints for occidiofungin are then determined by visual inspection and will be based on the wells that have no visible growth (an optically clear well) after 24 and 48 hours of incubation. Susceptibility testing will be done in duplicates. DMSO containing no antifungal agent will be used as a negative control and Amphotericin B as a positive control.

Determine the effects of protein binding on the activity of occidiofungin. The most direct approach to determine the effect of protein binding on the antifungal activity of occidiofungin is to test the compound in the presence of serum. Susceptibility testing, as described above, will be performed in duplicate according to the CLSI M27-A3 method in RPMI or YPD growth medium in the presence of 5% and 50% (vol/vol) human sera (Sigma Aldrich, St. Louis, MO). This will provide information as to the amount of unbound antifungal compound that is present in blood. This information is also important for future formulations for efficacy studies in an animal model. Given the turbidity of 50% serum, 50µL of cell suspension in each well of the microtiter well will be plated to determine the minimum lethal concentration (MLC).

Determine the kinetics of activity and the post antifungal effects on Candida spp. These experiments provide important information about the activity of the occidiofungin analogs. These studies will determine the rate of fungicidal activity. The post antifungal effect (PAFE) experiments will determine the relative effect occidiofungin has on cells exposed for a short time period. Time-kill assays are a measurement of CFUs following the addition of the antifungal to the yeast. We will observe the kinetics over a 24 hour period following addition of a suspension of *C. albicans* or *C. glabrata* to a culture tube containing varying concentrations of occidiofungin. The suspension is prepared from colonies that are removed from a fresh (24 hour old) YPD plate and suspended in sterile water to an $O.D._{530}$ of 0.13. The suspension is then diluted ten-fold by adding 1mL to 9mL of RPMI 1640 containing the desired concentration of the antifungal compound and incubated at 35°C with agitation. At time 0, 2, 4, 8, 24 hours, 100µL of cell suspension is drawn from the culture and serially diluted to obtain the CFUs. PAFE experiments are performed in a similar manner. Following a 1-hour exposure to occidiofungin, the tubes are spun down at 5000 rpm for ten minutes, washed three times, and resuspended in fresh, warm RPMI 1640 media. CFU are measured at 2, 4, 8, and 24 hours. These experiments will be conducted on all newly isolated analogs of occidiofungin. Amphotericin B will be used as a control for comparison between data sets.

In vitro toxicity screen. Cytotoxicity will be evaluated by monitoring membrane integrity and mitochondrial function. Cellular ATP assay, MTT and Glutathione S-transferase assays will be performed as previously reported by our group (16), using a rat hepatoma (H4IIE) cell line. Amphotericin B and rotenone will be used as a control.

Potential Problems and Alternative Approaches: We have expertise with the assays outlined in this aim. They are widely used in the antimicrobial research community. There may need to be some modifications to these protocols as problems arise that do not conform to these assays. For instance, several strains of *S. cerevisiae* in my lab will not grow in RPMI 1640 used for MICs and time-kill assays and YPD broth has to be used instead. We expect similar situations to arise as we perform our spectrum of activity screening and will make modifications to protocols as needed.

Expected outcomes. *Aim 2* will enable us to determine the importance of these structural modifications relative to their spectrum of activity, time-kill kinetics and toxicity. These results are important in that they will enable our group to identify a lead analog with the most promising attributes for successfully developing a new antifungal therapeutic.

Timeline: These aims will identify lead occidiofungin analog/s, which is necessary before initiating our pre-IND meeting with the FDA. The completion of this study will coincide with the completion of our optimized production and purification studies and provide us with the knowledge needed to move occidiofungin into clinical studies. Experiment outline in Aim 1 are expected to take approximately one year, while the studies in Aim 2 can be done as the analogs become available.

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COLLEGE OF SCIENCE

Department of Biology

TEXAS A&M UNIVERSITY

30 August 2016

To Whom It May Concern:

This letter is to confirm that Dr. James L. Smith is a full-time, tenured faculty member in the Department of Biology at Texas A&M University where he holds the rank of Associate Professor. Dr. Smith's current research involves drug development and structure-activity relationship studies of antimicrobial compounds. His excellent past productivity indicates that he will be successful in achieving his research goals. He has the department's full support for his application titled, "Lead Compound Discovery from Engineered Analogs of Occidiofungin".

Sincerely,

Thomas D. McKnight
Professor and Head of Biology

Butler Hall 100
3258 TAMU
College Station, Texas 77843-3258

Tel. 979.845.7747 Fax. 979.845.2891
www.bio.tamu.edu

Resource Sharing Plan

Data Sharing Plan

Nature of the materials to be shared includes, but is not limited to, experimental techniques, information about experimental materials, methods employed for data interpretation, raw data and our conclusions drawn from the data. Given the nature of the study, intellectual property decisions will need to be made prior to the release of information. However, there are no complicating factors that would prevent the investigator from sharing information after the filing of a provisional application. Data and materials will be shared in the following manners:

A. Attendance at Scientific Meetings

Local

Members of the PI laboratory will present data acquired from this proposal to the local research community within the state of Texas. Opportunities exist for personnel to present their research accomplishments in poster format at regional Symposiums held on Texas A&M campus. These events are open to the entire University community. Investigators will disseminate research findings by giving research seminars at local universities including Rice and University of Texas.

Regional

Research results obtained with the support of this grant will be the basis of poster and oral presentations to be made at yearly regional meetings. Personnel will be encouraged to attend these events. These regional meetings are excellent opportunities for networking and the exchange of ideas and research materials. Example meeting includes the Texas Branch American Society for Microbiology.

National/International

To reach large numbers of the scientific community, data derived during the execution of this proposal will be presented at national scientific meetings. Example meetings include those held by the American Society for Cell Biology, American Society for Biochemistry and Molecular Biology, and the American Society for Microbiology Candida and IACACC Meeting.

These meetings are well attended and a large number of international labs participate providing an opportunity for our research findings to reach a broader audience.

B. Peer-reviewed Publications

Data acquired during this project will be written up for publication in appropriate peer-reviewed journals. All results will be published in a timely manner.