



## 1R41AI131792-01 SMITH, JAMES

**RESUME AND SUMMARY OF DISCUSSION:** Funds are requested to develop a new class of broad spectrum antifungal agents based on a cyclic nonribosomally synthesized peptide, occidiofungin, for the treatment of systemic fungal infections. An entirely novel mechanism of action of occidiofungin speaks to a high level of conceptual innovation and makes the proposed work very promising. The proposed experimental strategy is based on abundant and highly encouraging preliminary results; the work is well planned and full feasibility will likely be established at the end of Phase I. The investigators are highly qualified to perform these studies and the environment is adequate for the stated goals. Overall, very high enthusiasm was expressed for this scientifically solid and potentially impactful antifungal drug discovery proposal.

**DESCRIPTION (provided by applicant):** 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.

**PUBLIC HEALTH RELEVANCE:** 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 work is essential for identifying a lead compound for preclinical studies that are needed for filing an Investigational New Drug (IND) application.

### CRITIQUE 1:

Significance: 2  
Investigator(s): 1  
Innovation: 2  
Approach: 4  
Environment: 1

**Overall Impact:** The applicant proposes to continue efforts to characterize the antifungal properties of the cyclic peptide occidiofungins. Aim 1, which involves a combination of biosynthetic and synthetic approaches to prepare occidiofungins, addresses the current, major limitation regarding moving occidiofungins forward for additional preclinical assessment, that is, the ability to access a highly pure occidiofungin to replace the mixtures that have heretofore been used. Aim 2 involves routine bioactivity assessment of the analogs, including in vitro activity (MIC and time-kill assays) and in vitro toxicity. There are many strengths to this application. The major strength of the proposal is the significance: the published and preliminary data suggest occidiofungins have very promising fungicidal activity with a distinct mechanism of action compared to known antifungals. Additionally, the occidiofungins do not

appear have acute toxicity to mice at 20 mg/kg. A second strength of the application is the proposed multi-pronged approaches to access and prepare pure occidiofungin, which increases that likelihood that new analogs will be realized. At the end of the project, the identity of the active ingredient will be known and 2 complementary platforms will be in place for preparing pure occidiofungins and analogs, will be important for downstream experiments for identifying the target and further SAR studies when the target is in hand. There are a few minor weaknesses in the approach, specifically related to yields from the producing strain, under-developed semisynthesis and solid phase synthesis particularly with respect to the targeted structural modifications that will be pursued. Nonetheless, the productivity and experience of the applicant directly related to occidiofungins suggests the results will have a sustained influence in antifungal drug discovery.

## 1. Significance:

### Strengths

- New antifungals with new modes of action and/or structures are desperately needed since relatively few options are clinically available.
- Occidiofungins have broad spectrum and potent antifungal activity, including against *Candida* species and *Cryptococcus*.
- Occidiofungin, a cyclic peptide, has a distinct structure relative to the other FDA-approved cyclic peptide antifungal, echinocandins.
- Scientific premise is well justified in the application and within the applicants publications on the topics.

### Weaknesses

- The lack of knowledge regarding the mechanism of action or having any results with a purified compound makes the analog generation project somewhat of a fishing expedition at this stage.

## 2. Investigator(s):

### Strengths

- The PI has led the way in the isolation and characterization of occidiofungins, which have been documented in several high quality publications.

### Weaknesses

- None stated.

## 3. Innovation:

### Strengths

- Occidiofungins potentially represent an entirely new class of antifungal antibiotics.
- A genetic system in the producing strain has been demonstrated.

### Weaknesses

- The screening is routine (although needed).

## 4. Approach:

### Strengths

- Synergistic biosynthetic (genetic inactivation) and synthetic approaches are utilized to generate analogs.
- The utility of thioesterases (and potentially OcfN) to catalyze lactonization/lactamization has been demonstrated in other systems and is a powerful approach to access cyclized molecules. The premise for this approach is strongly supported by literature precedent and economic considerations.

#### **Weaknesses**

- Genetic mutations will likely decrease the occidiofungin yields significantly as previously noted for the *ocfC* mutant. The applicant has also published the *ocfN* mutant and has an *ocfD*-TE domain mutant in-hand. Occidiofungin titres were never discussed or referenced within the proposal.
- Semisynthesis by chemical modification of the amine of DABA is only very briefly mentioned. What is the reaction condition? What are the target modifiers? How many analogs will be prepared and will the production levels be sufficient to support this endeavor? Another problem with this approach is that it will be presumably be performed with the mixture of occidiofungins, hence will give a mixture of products.
- The solid phase synthesis will potentially be more challenging than anticipated due to the unusual amino acid precursors (for example,  $\beta$ -hydroxy-Tyr and the fatty acid-like NAA2 component) that will ultimately be needed to synthesize the natural product.

#### **5. Environment:**

##### **Strengths**

- The facilities are excellent.

##### **Weaknesses**

- None stated.

##### **Protections for Human Subjects:**

Not Applicable (No Human Subjects)

##### **Vertebrate Animals:**

Not Applicable (No Vertebrate Animals)

##### **Biohazards:**

Not Applicable (No Biohazards)

##### **Authentication of Key Biological and/or Chemical Resources:**

Acceptable

##### **Budget and Period of Support:**

Recommended budget modifications or possible overlap identified:

- Possible overlap with 1R41AI122441-01A1 for "optimization of the production and isolation of the Novel antifungal occidiofungin".

## CRITIQUE 2:

Significance: 1  
Investigator(s): 2  
Innovation: 2  
Approach: 2  
Environment: 2

**Overall Impact:** Occidiofungin is a new cyclic nonribosomally synthesized antifungal peptide that kills a broad spectrum of fungi with submicromolar fungicidal activity. Occidiofungin has a novel mode of action and is a potent antifungal against fluconazole and caspofungin-resistant *Candida albicans*. This compound does not achieve fungus killing via inhibition of ergosterol production, binding to ergosterol or inhibiting the 1,3- $\beta$ -glucan synthase enzyme, which are the current therapeutic targets for treating fungal infections. Thus it has a different mode of killing and worthy of further investigation. The PIs have applied for IP rights to this molecule and analogs thereof. Encouragingly, occidiofungin has minimal toxicity in mice dosed with 2 mg/kg in a 28 day toxicity study and at this dose, it was shown to reduce *Candida glabrata* load in the kidneys of infected mice. A limitation of occidiofungin however is that it is currently difficult to obtain pure analogs and the biological works have used mixtures of stereoisomers. Based on the favorable efficacy, alternative mode of action and safety profile of occidiofungin, the PIs aim to screen a library of natural and semi-synthetic analogs of occidiofungin (obtained via biosynthesis and chemical derivatizations) for bioactivity testing. Enthusiasm for this proposal is very high, although slightly tempered due to the difficulty of making the compounds, because occidiofungin could provide a solution to the incidence of drug-resistant agents.

### 1. Significance:

#### Strengths

- Occidiofungin has a novel mode of action and is a potent antifungal against fluconazole and caspofungin-resistant *Candida albicans*.

#### Weaknesses

- It appears that it is difficult to obtain pure stereoisomer of occidiofungin and the difficulty in separating the components of the mixture or making large quantities of this compound could ultimately hamper commercial development of this compound.

### 2. Investigator(s):

#### Strengths

- The PIs have appropriate skills for this project. Importantly they have already provided some of the fundamental insights into occidiofungin activity (several publications) and they have filed a Composition and Methods of Use patent for occidiofungin (US 2011/0136729).

#### Weaknesses

- None stated.

### 3. Innovation:

#### Strengths

- This compound does not achieve fungus killing via inhibition of ergosterol production, binding to ergosterol or inhibiting the 1,3- $\beta$ -glucan synthase enzyme, which are the current therapeutic targets for treating fungal infections.

**Weaknesses**

- None stated.

**4. Approach:**

**Strengths**

- Genetic modification and chromatographic separation could lead to pure stereoisomers of occidiofungin.
- The xylose moiety is not necessary for activity so the use of a mutant strain to make analogs without the xylose unit could be a fruitful endeavor.
- The PI's plan to identify which analogs have optimal activity before expending resources to engineer *B. contaminans* MS14 strains. This is a rigorous strategy that will ensure that only the required strains are made.

**Weaknesses**

- None stated.

**5. Environment:**

**Strengths**

- The environment is appropriate for proposed work.

**Weaknesses**

- None stated.

**Protections for Human Subjects:**

Not Applicable (No Human Subjects)

**Vertebrate Animals:**

Not Applicable (No Vertebrate Animals)

**Biohazards:**

Not Applicable (No Biohazards)

**Authentication of Key Biological and/or Chemical Resources:**

Acceptable

**Budget and Period of Support:**

Recommend as Requested.

**CRITIQUE 3:**

Significance: 1  
Investigator(s): 2  
Innovation: 1  
Approach: 3  
Environment: 4

**Overall Impact:** This is a proposal for the development of discoveries in Dr. Smith's laboratories of an antifungal complex of compounds (occidiofungins). Extensive preliminary work has resulted in: (1) Determination of the biosynthetic pathways involved; (2) The ability to manipulate the pathways through genetic engineering; (3) Preliminary demonstration of a spectrum of antifungal activity highly competitive with existing agents; (4) Preliminary SAR data; and (5) An acceptable toxicity profile. Based on the extensive preliminary work development of an occidiofungin for the treatment of human fungal infections would be highly significant. The PI is highly qualified to carry out the proposed work. The level of innovation is also quite high and the approach proposed is logical. While the environment is sufficient for Phase I additional personnel and facilities would probably be needed to proceed to a Phase II submission and, eventually, an IND filing.

### 1. Significance:

#### Strengths

- The identification of a family of antifungal agents possessing low toxicity while having fungicidal properties against clinically significant pathogens is of very high significance.
- The investigators have done a large amount of excellent preliminary work that demonstrates that the occidiofungins possess these properties.
- They have determined the biosynthetic pathways involved and have demonstrated their ability to manipulate the same so as to gather structure activity relationship (SAR) data on the various components of the occidiofungin complex.
- This suggests that they will be able to prepare pure components so as to be able to select one for clinical development. It will also enable them to obtain additional patent protection with extended market.

#### Weaknesses

- None stated.

### 2. Investigator(s):

#### Strengths

- Dr. Smith has done a great deal of work to put himself and coworkers in excellent position to advance this program to clinical trials.
- He has also picked up business experience in his relatively short career (MBA). The proposal reflects this in that he has laid out a highly logical path forward.

#### Weaknesses

- As the project progresses into IND preparation the company will need to add preclinical/clinical development personnel.

### 3. Innovation:

#### Strengths

- The preliminary work reported has been quite innovative.

**Weaknesses**

- None stated.

**4. Approach:**

**Strengths**

- Dr. Smith has laid out a highly logical plan for the exploration of the SARs for the occidiofungins and for the fine tuning of the fermentation processes so as to allow for production of a lead product in essentially pure state.

**Weaknesses**

- Dr. Smith is overly pessimistic regarding the need for high levels of improvements in separation methods. In the experience of the reviewer these are problems that should be resolvable. In the final analysis the compounds could be made by total synthesis.
- Development of a mixture of compounds would be very difficult from a regulatory viewpoint.

**5. Environment:**

**Strengths**

- The environment for the phase I program is adequate.

**Weaknesses**

- It would have helped had the PI supplied more information regarding the production methods, yields, and plans for fermentation scale up.
- A better description of the UT facilities should have been given.

**Protections for Human Subjects:**

Not Applicable (No Human Subjects)

**Vertebrate Animals:**

Not Applicable (No Vertebrate Animals)

**Biohazards:**

Not Applicable (No Biohazards)

**Authentication of Key Biological and/or Chemical Resources:**

Acceptable

**Budget and Period of Support:**

Recommend as Requested.

**THE FOLLOWING SECTIONS WERE PREPARED BY THE SCIENTIFIC REVIEW OFFICER TO SUMMARIZE THE OUTCOME OF DISCUSSIONS OF THE REVIEW COMMITTEE, OR REVIEWERS' WRITTEN CRITIQUES, ON THE FOLLOWING ISSUES:**



**COMMITTEE BUDGET RECOMMENDATIONS: The budget was recommended as requested.**

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Footnotes for 1 R41 AI131792-01; PI Name: smith, James

NIH has modified its policy regarding the receipt of resubmissions (amended applications). See Guide Notice NOT-OD-14-074 at <http://grants.nih.gov/grants/guide/notice-files/NOT-OD-14-074.html>. The impact/priority score is calculated after discussion of an application by averaging the overall scores (1-9) given by all voting reviewers on the committee and multiplying by 10. The criterion scores are submitted prior to the meeting by the individual reviewers assigned to an application, and are not discussed specifically at the review meeting or calculated into the overall impact score. Some applications also receive a percentile ranking. For details on the review process, see [http://grants.nih.gov/grants/peer\\_review\\_process.htm#scoring](http://grants.nih.gov/grants/peer_review_process.htm#scoring).

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