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Total Synthesis and Biological Evaluation of Siderophores: Key to Develop Iron Chelators and Tackle Antibiotic Delivery to Gram-Negative Bacteria

Background

Siderophores are molecules used by bacteria to sequester iron from their environment by chelation[1]. These iron-siderophore chelates can bypass selective porin-mediated entry and easily enter Gram-negative bacterial cells via active transporters[2]. Ability to chelate iron makes siderophores excellent candidates for iron chelation therapy[3,4] and to be used as metalloenzyme inhibitors[5,6]. Also, due to the ability of siderophores to bypass the porin-mediated transport, antibiotic-siderophore conjugates (sideromycins) have proven to be a successful way of delivering antibiotics to Gram-negative bacteria[7,8,9,10]. Furthermore, some of the siderophores are reported to possess cytotoxic properties, mainly due to their inhibitory action on different metalloprotenases.[1] Hence, they have the potential of being developed into anticancer agents or potentiating agents as well.

Specific Objectives of Research

1. Synthesize catenulobactin B, talarazine A and their analogs
2. Biological evaluation of the synthesized molecules
3. Synthesis of sideromycins with synthesized siderophores

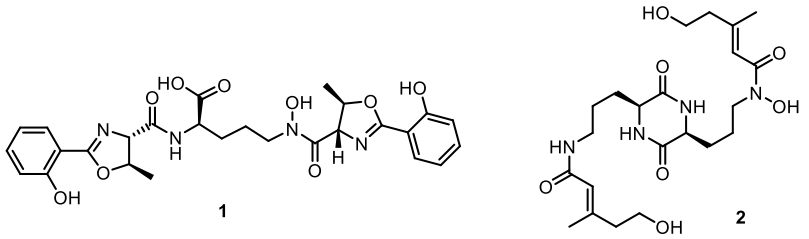


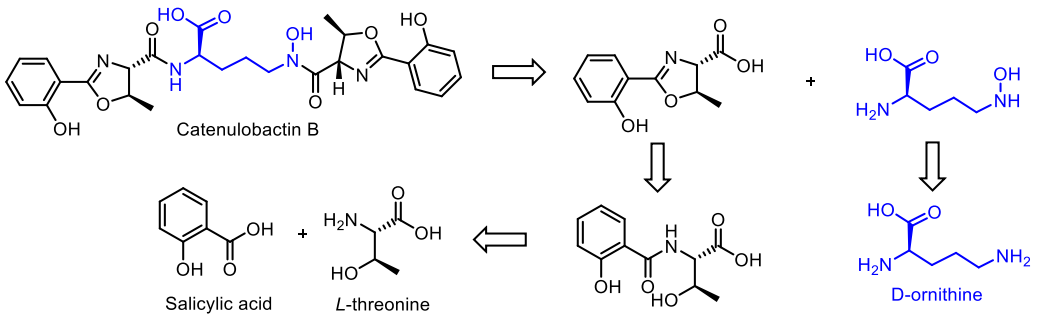
Figure 1: Structure of catenulobactin B (**1**)[11] and talarazine A (**2**)[12]

My research will focus on synthesis of naturally occurring siderophores, and analogs to explore their metal-chelation ability, bacterial-penetration ability, cytotoxicity, and to incorporate them in developing sideromycins. Noting that this work will be conducted by myself and eager undergraduate researchers, the selection of natural products were limited to structures that are amenable for undergraduate research. However, careful planning of the synthetic routes and having excellent technical skills are necessary to accomplish their total synthesis. Hence, this will be an excellent platform for undergraduate researchers to develop synthetic skills, master in purification techniques (e.g., column chromatography, recrystallization, and HPLC) and become thorough in analytical techniques to characterize molecules (NMR, IR, GC/MS). Synthesis of the molecules will be done in a modular way, employing multiple students with a fragment with attainable complexity. Further analysis done on the synthesized molecules will help students to be

trained on UV/visible spectrometer, HPLC and possible biological studies. This research intends to train students on molecular docking studies as well. Knowledge and skills my students acquire under my supervision will assuredly benefit them to flourish in their future career.

Synthesis of Siderophores

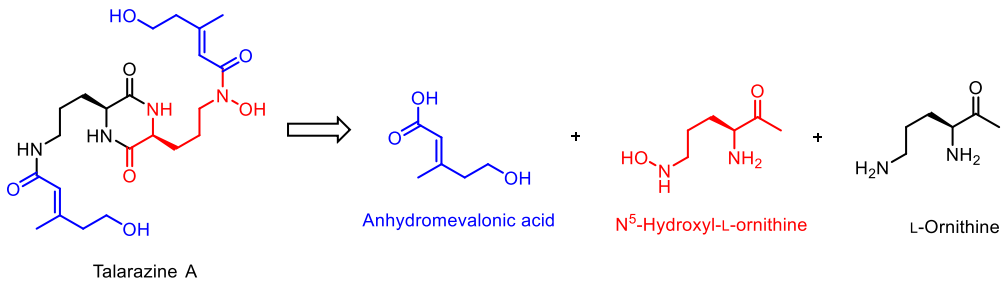
Catenulobactin B



Scheme 1: Retrosynthetic analysis of catenulobactin B

Catenulobactin B was isolated by Abe and co-workers from *Catenuloplanes* sp. RD067331 in 2018. It has demonstrated iron chelating ability and moderate cytotoxicity against P388 murine leukemia cells.[11] Synthesis of catenulobactin B can be performed in a modular approach where the oxazoline fragment (colored in black) and the hydroxamate fragment (colored in blue) can be handled by different students before the final coupling. The oxazoline fragment will be synthesized by coupling commercially available *L*-threonine and salicylic acid followed by a cyclization[13] whereas the hydroxamate fragment will be accessed via a sequence of reaction starting with commercially available *D*-ornithine.[14] Analogs of catenulobactin B will be synthesized altering stereocenters of the molecules and substituents. Since this molecule possesses moderate antileukemic activity[11], its potential to be developed into an antileukemic drug, a carrier to deliver antileukemic drugs to target cells or a potentiating agent will be evaluated. Also, potential of developing novel sideromycins using this siderophore will be explored (discussed later).

Talarazine A



Scheme 2: Retrosynthetic analysis of talarazine A

Isolation of talarazine A from *Talaromyces* sp. CMB-W045 was reported in 2017 by Capon and co-workers. It was reported as a non-cytotoxic iron chelator.[12] Synthesis of talarazine A also will be performed in a modular approach, involving multiple students. N^5-hydroxyl ornithine will be

synthesized from *L*-ornithine in one module[14], anhydromevalonic acid will be synthesized from 4-hydroxy-2-butanone[15] in another module and all components will be coupled together in a separate module. Analogs of talarazine A also will be synthesized altering stereocenters of the molecules and substituents. Novel sideromycins will be synthesized using talarazine A as well. Since this molecule has no reported cytotoxicity[12], it can be an ideal siderophore to develop novel sideromycins.

Synthesis of both the siderophores and their analogs heavily involve many important reactions commonly used in natural product synthesis such as peptide coupling chemistry, protection/ deprotection reactions, rational designing of synthesis routes and careful selection of reaction conditions to retain stereochemistry. This chemistry will expose students to a variety of important concepts in organic synthesis which they can use in their future career to solve much more complex research problems. Students will also be trained on proper safety protocols and being a "good laboratory citizen".

Biological Evaluation of the Synthesized Molecules

Several siderophores are capable of binding with other metal ions in addition to iron.[1] Therefore, it is important to evaluate the ability of the synthesized molecules to chelate iron and other metals as well. Siderophore-metal chelation will be evaluated *in vitro* using UV-visible spectrometry. Molecules with selective iron chelation ability will be good candidates to be further investigated for their suitability in iron-chelation therapy or metalloenzyme inhibitors involving iron.

Cytotoxicity of all the synthesized molecules will be evaluated in collaboration with Dr. William Ranahan at ORU and Dr. Ryan Rafferty at Kansas State University. As catenulobactin B has reported antileukemic activity[11], a special emphasis will be given to leukemic cell lines in evaluating this molecule and its analogs for cytotoxicity. If the molecules synthesized penetrate cancer cells preferentially, they can be used as therapeutic delivery agents even if they do not possess cytotoxicity. Therefore, cellular penetration studies also will be done on each cell line being tested. For this, cellular incubation will be done at the Rafferty Lab and cell lysates will be sent to my lab where students will use HPLC to analyze the samples.

Synthesis of Sideromycins with Synthesized Siderophores

Figure 2: Design of a sideromycin

As antibiotic resistance emerges worldwide and threatens to make bacterial infections a leading cause of death in the future,[16] it demands the discovery of new ways to combat this problem. When the most difficult-to-treat and antibiotic resistant bacteria are considered, the majority are Gram-negative bacteria.[17] Due to the complexity of their cell wall with an additional outer membrane, it is extremely difficult for drug molecules to penetrate Gram-negative bacteria.[17] Therefore, many antibiotics that are active against Gram-positive bacteria are inactive against Gram-negative bacteria. One of the elegant ways to overcome this problem is to synthesize sideromycins (Figure 1), conjugating siderophores to antibiotics. Sideromycins have proven activity against

Gram-negative bacteria, penetrating the outer membrane, irrespective of having larger molecular weights.[7,8,9,10]

Siderophores synthesized in my lab will be used to design novel sideromycins, conjugating them with available antibiotics. In this component of research, a range of Gram-positive active antibiotics with little or no activity against Gram-negative bacteria will be selected to conjugate to siderophores. Molecular docking studies will be performed between the binding target of the antibiotic molecule and the proposed siderophores to estimate the ability of each conjugate to retain the same binding energy as the unconjugated antibiotic. The best candidates will be synthesized in lab. This process will introduce students to various software and resources commonly used in molecular docking studies and visualizing molecules. It will be immensely helpful for them not only in obtaining first-hand experience in using these resources but also in getting a taste of rational designing of drugs in medicinal chemistry.

Upon accomplishing the synthesis of sideromycins, their ability to penetrate Gram-negative bacteria and their minimal inhibitory concentrations will be evaluated in collaboration with Dr. Joel Gaikwad at ORU. Bacterial incubation for testing molecular penetration will be done under Dr. Gaikwad's supervision and the cell lysates will be analyzed by my students using HPLC. These studies will reveal how effective our sideromycins are in penetrating Gram-negative bacteria and inhibiting them.

My research will be a good learning environment for students who wish to go to graduate school or industry as they will be well trained on essential synthetic and analytical skills and they will be ready to thrive in the next stage of their lives with confidence. Throughout research, students will be encouraged to think rationally in solving the problems they encounter. This research will be a good fit for undergraduates, as the procedures do not involve working long hours at a stretch, so that it will be practical for them to find research hours that will fit into their already busy schedule. Also, it will not involve handling toxic material and running dangerous reactions. Students whom I mentor will continually be motivated to expose themselves to new research and new chemistry as I will be encouraging them to pursue Summer REU and SUROP opportunities to further their education.

Material involved in research are relatively affordable and all the equipment required are available in the department. All the software and other resources used in performing molecular docking studies are freely available and do not add any cost to research. In the future, I wish to explore funding opportunities with external funding agencies. Knowing that "unless the Lord builds the house, those who build it labor in vain", I will be prayerfully striving to make the best use of available resources at ORU and trust on God's provision and guidance to train students with a bold vision who are excellent in their field for the glory of God. The research proposed here will train multiple students over many years as it progresses and leads us in different directions. I will be taking my initial steps to open the doors of my lab to train students during Summer 2021 with support from the Biology and Chemistry Department faculty and staff, particularly Dr. William Ranahan and Dr. Joel Gaikwad with whom I will be collaborating in my research.

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