SOAR Research Proposal – Summer 2018

DNA Binding Rates and Cytotoxicity of Rhodium Compounds

Faculty: Shari U. Dunham, Ph.D., Associate Professor of Chemistry and Anastasia Thévenin, Ph.D., Assistant Professor of Biology
Student: Miles Lizak (post-baccalaureate student)[1]
Project Start Date: May 21, 2018[2]
Length of Project: 10 weeks

Description of the project
DNA Binding Rates and Cytotoxicity of Rhodium Compounds

Transition metal elements (those in the short and wide center of the periodic table) are underexplored for their potential use in pharmaceuticals. Although most drugs are primarily "organic" in nature (meaning they are made up of carbon, hydrogen, oxygen, nitrogen, with perhaps the occasional sulfur, phosphorous or halide atom), one very successful cancer drug has a platinum atom at its core. This platinum-based drug, commonly known as cisplatin and referred to as the "penicillin of cancer drugs"[3], has been used in the clinic for over 35 years and for decades has inspired the search for other transition-metal based drugs.

Small complexes containing the transition metal **rhodium** were early identified as having anticancer activity similar to that of cisplatin.[4] Since cisplatin is generally believed to kill cells due to its ability to (1) access and damage cellular DNA, (2) change the structure and stability of that DNA, and (3) ultimately interfere with normal DNA processing that can lead to programmed cell death, research in Dr. Shari Dunham's laboratory has focused on the DNA interactions of novel rhodium complexes. These rhodium complexes have two rhodium atoms at their core and are surrounded by four bridging groups. Previous work in her laboratory has shown that, like cisplatin, a series of rhodium (Rh) complexes can bind to double-stranded DNA and that minor changes in the bridging groups of these rhodium complexes can greatly affect the rate at which they damage DNA.[5,6]

Recent work in the laboratory of Dr. Stephen Dunham (Austin Mates '18 and Inderjit Sandhu '18) has resulted in the synthesis, characterization, and purification of several new rhodium compounds

[1] Miles has already earned a BA in Creative Writing from Fairleigh Dickinson University in 2014. During the summer of 2016 he matriculated in the post-baccalaureate program at Moravian College to start his path toward a Biochemistry major. He has taken 3 courses each semester since and has been a full time student at Moravian College (3 or more course units) during the fall of 2016, the fall of 2017, and this spring of 2018.
[2] SOAR officially starts on May 30 but Miles is a commuter and can start early for training on cell culture techniques.
[3] S. Trzaska, "Top Pharmaceuticals: Cisplatin", *Chemical & Engineering News*, https://pubs.acs.org/cen/coverstory/83/8325/8325cisplatin.html, Accessed March 5, 2018.
[4] B.L. Rosenberg, L. Van Camp, and T. Krigas, "Inhibition of Cell Division in *Escherichia coli* by Dlectrolysis Products from a Platinum Electrode", *Nature* 205, pp 698-699 (1965).
[5] S.U. Dunham, A.E. Burr, S. Mikulski, H.T. Chifotides and K.R. Dunbar, "Covalent Binding and Interstrand Cross-Linking of Duplex DNA by Dirhodium(II,II) Carboxylate Compounds", *Biochemistry* 44(3), pp 996-1003 (2005).
[6] S.U. Dunham, T.S. Remaley, B.S. Moore, D.L. Evans and S.U. Dunham, "Isolation, Characterization, and DNA Binding Kinetics of Three Dirhodium(II,II) Carboxyamidate Complexes: $Rh_2(\mu\text{-}L)(HNOCCF_3)_3$ where L= $[OOCCH_3]^-$, $[OOCCF_3]^-$, $[HNOCCF_3]^-$", *Inorganic Chemistry*, **50**, pp 3458-3463 (2011).

that differ systematically in the bridging groups that surround the rhodium centers. Work to determine the DNA-binding rates of a series of these new rhodium compounds has begun in the Dunham laboratories with a SOAR summer ’17 project by Lauren Caronia, independent studies in Spring ’17 and Fall ‘17 by Ana Bustamante, and most recently with Winter term and Spring ’18 independent studies by Miles Lizak. With the recent arrival of Dr. Anastasia Thévenin in the Department of Biological Sciences, the expertise and facilities to explore the affect of these compounds on mammalian cells is now also present at Moravian College. The goals for this summer work are (1) to determine the DNA-binding rates for the remaining two rhodium complexes in this series, (2) to compare the DNA-binding rates for all of the compounds in the series, and then (3) to evaluate the extent to which these compounds are toxic to both cancerous and non-cancerous cells. Ultimately, our intent is to compare the DNA-binding rates and cytotoxicity of each rhodium compound in the series, and to use this information along with their DNA unwinding angles and their extent of DNA interstrand crosslink formation to identify the most likely potential candidates for new chemotherapy drugs.

Roles and Responsibilities

- Anastasia Thévenin will train Miles on cell culture protocols (cell media preparation, passaging, freezing and thawing of cells). Miles will work with two cell lines: HeLa – a cervical cancer cell line and MDCKs (Madin-Darby Canine Kidney cells) – a non-cancerous epithelial cell line. In later weeks (see timeline), Anastasia Thévenin will work with Miles on establishing cell treatment conditions and testing cell viability using an established calorimetric assay (MTT - that measures ability of intact cells to break down a metabolite, leading to an observable color change).
- Shari Dunham will be available regularly to train Miles on procedures needed to prepare solutions for DNA-binding work (rhodium complex stock solutions, DNA stock solutions, reaction buffer solutions), to carry out the Rh-DNA kinetic studies (time point collection and processing in centrifiltration devices), to analyze the kinetic data (atomic and molecular spectroscopy methods), and to appropriately dispose of all materials used in the laboratory.
- Shari Dunham will also oversee Miles’ data analysis in Excel and visual representations of results in Microsoft Word or PowerPoint.
- The project directors will work with Miles to make plans to identify appropriate experiments and appropriate contact faculty for those occasions when either project director will be out of town.
- In addition to attending and occasionally presenting at weekly SOAR program meetings, Miles will prepare and present at approximately biweekly meetings (~15-30 min) to the project directors.
- Miles will maintain a research laboratory notebook that will include regular and complete entries and have an updated table of contents at the beginning. Entries will be dated, clearly written and organized, and made at least daily with details of ideas for experiments, planning of experiments, clear reference to location and organization of electronic data for each experiment, and a summary of results from each experiment. Shari Dunham will look at Miles’ notebook periodically and provide informal feedback throughout the summer. The notebook will be submitted to Shari Dunham upon completion of the Summer Research.

- Throughout the summer, Miles will prepare a summary figure for each set of experiments (with a detailed figure caption!) to clearly illustrate the results. These summary figures will be submitted electronically to the project directors before completion of the Summer Research.
- Miles will be encouraged to present the results of his summer work in either a poster or talk format at the Landmark Conference for Science Research near the end of the summer (location and date TBD). If Miles does not continue this research into the 2018-2019 academic year, he will be required to submit a draft of a research report on his work before the first day of classes in the fall. If Miles does continue this research in some form during the 2017-2018 academic year, a final report/poster will instead be due by the end of the experience and in time for the Annual Student Scholarship and Creative Endeavors Day in spring of 2019.

Project Timetable

- ☐ Weeks 1-2: Cell culture training in Thévenin lab on two different cell lines prior to any treatments with rhodium compounds; cell viability will be initially tested using an MTT assay – a calorimetric cytotoxicity assay that leads to a color change from yellow to purple in viable cells.

- ☐ Weeks 2-3: Propose conditions for a 7- to 10-day kinetic study of DNA-binding of one of the new rhodium complexes, prepare solutions for the study, carry out the binding reaction and collection of reaction timepoints, and analyze all timepoints to determine %Rh bound to DNA as a function of time.

- ☐ Weeks 4-5: Prepare a figure of %Rh bound vs time for the kinetic reaction run in Weeks 2&3, present the figure and these results to Dr. Shari Dunham, and propose any additional kinetic studies needed for this rhodium complex. Plan and carry out a cytotoxicity study using up to three rhodium complexes on one or more cell lines using the established MTT assay protocol from weeks 1&2.

- ☐ Weeks 6-7: Prepare a visual representation of cytotoxicity results from the experiments performed in Weeks 4&5, present the figure and these results to the project directors, and propose any additional cytotoxicity studies needed for these rhodium complexes. Plan and carry out a 7- to 10-day kinetic study of DNA-binding of the remaining new rhodium complexes in the series.

- ☐ Weeks 8-9: Prepare a figure of %Rh bound vs time for the kinetic reactions run in Weeks 6&7, present the figure and these results to Dr. Shari Dunham, and propose any additional kinetic studies needed for these rhodium complexes. Plan and carry out a cytotoxicity study using up to three rhodium complexes on one or more cell lines.

- ☐ Week 10: Perform any final replicate measurements, prepare final tables and figures for presentation, complete lab clean up and appropriate cataloging of samples and reactions.

Summary of benefits

Student engagement in discipline-appropriate scholarly research. Miles has already been working in Dr. Shari Dunham's research laboratory for two 0.5 unit independent studies this academic year.

He has started to see the differences between biochemistry research in the teaching laboratory (where the experimental variables are quite limited and the instructor often has a good sense of the experimental outcome) versus true biochemistry research on a novel project (where planning and problem-solving are critical and regular processes in which the instructor and the student researcher regularly take part). During winter term (Jan '18) Miles learned the methodologies necessary to measure DNA-binding rates of rhodium compounds, kept a detailed laboratory notebook, maintained organized Excel spreadsheets, and started to prepare visual representations of his binding curve data. During the spring semester he is applying these methodologies to measure the DNA-binding rates for 1 or 2 novel dirhodium compounds and he is preparing a poster to co-present at Scholarship Day with Ana Bustamante ('18). What the summer SOAR experience offers for Miles is (1) the opportunity to apply the methods he has learned to complete and compare the DNA-binding rates of an entire series of six rhodium compounds, (2) to learn a new methodology to see if these DNA-binding rates correlate with the ability of these compounds to kill mammalian cells, (3) and to be part of a larger research community where he will be presenting talks about his work and engaging in similar presentations by his peers. We believe that experience in all of these areas should help Miles confirm his desired path of graduate school in Chemistry/Biochemistry and make him an even more competitive applicant for graduate programs.

Impact on faculty, campus community, and discipline. As an illustration of how scientific questions are often answered by collaborative teams, this project brings together the expertise of three faculty from two different departments at Moravian College: Dr. Stephen Dunham (Chemistry) who designs, synthesizes and characterizes the novel rhodium complexes, Dr. Shari Dunham (Chemistry) who investigates their DNA interactions, and Dr. Anastasia Thévenin (Biology) who has the expertise to study their effect on mammalian cells. Because Miles has already begun work with Dr. Shari Dunham, there is great potential for him to contribute significantly to our ongoing study to determine if the speed of DNA interactions formed by a rhodium complex correlates with its ability to kill cancer cells. We are hopeful that his work will be part of a larger publication on the design, synthesis, DNA-interactions, and cytotoxicity of potential antitumor agents. In addition, Miles will be strongly encouraged to present the findings of his summer work at appropriate local and national conferences.

Proposed Expenses (beyond stipends)

$250 Centrifiltration devices for processing time points of DNA-binding reactions (Millipore #UFC903024, 30kDa MWCO, pack of 24 from Fisher Scientific). One new device is required for each DNA-binding reaction, and often 2 or more reactions are required to determine the DNA-binding rate of a single rhodium complex.

$100 MTT, 1g (from Calbiochem). This reagent is a required component of the assay that will count the number of viable cells following treatment by a rhodium compound.

$100 High glucose DMEM (4 bottles at $25 per 500 mL bottle from Fisher Scientific). This is the media that will be used to grow mammalian cells.

$450 Total laboratory expense request

DNA Binding Rates and Cytotoxicity of Rhodium Compounds

Miles Lizak, Biochemistry Major, expected graduation Dec 2018
Shari U. Dunham, Ph.D., Associate Professor of Chemistry and Anastasia Thévenin, Ph.D., Assistant Professor of Biology
On-campus housing is not needed

I was drawn to the study of biochemistry by an intense interest in the workings of physiology and pharmaceuticals. Once I finish my education, I hope to go on to work in a pharmaceutical research laboratory. Naturally, I was excited by Dr. Shari Dunham's research into novel dirhodium compounds which bind to DNA, since these compounds may have chemotherapeutic applications. When Dr. Dunham sat me down and explained her work to me last fall, I was hooked.

I began working in Dr. Dunham's research laboratory in January 2018. Throughout the winter term, I learned the techniques necessary for carrying out the rhodium compound/DNA reactions and analyzing the results. I have continued working with Dr. Dunham in the spring semester, moving on to analyze different reactions. I keep a carefully-compiled research notebook, which Dr. Dunham reviews on a regular basis. Thus far, the project has taught me valuable laboratory skills that I would not have learned in a classroom setting, as well as adaptability and problem-solving skills. I have the opportunity to work with advanced instruments, such as a graphite furnace atomic absorption spectrometer, and figure out ways of mathematically modeling and displaying my findings with the help of Microsoft Excel.

I am eager to continue this research both because I am fascinated by the subject and because I want to continue to contribute to what I feel is important work. Learning to assay the cytotoxicity of these compounds with Dr. Thévenin will allow me to help move the project forward and gain valuable experience in working with live cell cultures. My prior experience in Dr. Dunham's laboratory will have prepared me well for working on the project over the summer, since I have a good understanding of the project, the theories behind it, and the protocols involved.

A hands-on research experience like this one will help prepare me for a competitive graduate program. After I graduate from Moravian, I intend to go on to pursue a PhD in biochemistry, and spending the summer working with Dr. Dunham and Dr. Thévenin would be a wonderful opportunity to experience full-time research while learning and applying new laboratory techniques. A summer of research through SOAR would reflect well on my work ethic and willingness to learn, making me a stronger applicant for graduate-level research labs.

Thank you for your time and consideration.
Miles Lizak