Preparation of *C*-Glycosides as Potential Antihyperglycemic Agents for the Treatment of Type II Diabetes

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Abstract:

Type II diabetes mellitus affects millions of people worldwide, and there is an urgent need for novel anti-hyperglycemic drugs to combat this disease. In the project "Preparation of C-Glycosides as Potential Anti-hyperglycemic Agents for the Treatment of Type II Diabetes" carried out in Dr. Jennifer Chaytor's laboratory, aryl-*C*-glycosides will be synthesized by undergraduate student researchers via standard cross coupling and carbohydrate chemistry. These compounds will then be evaluated in enzymatic assays as potential antihyperglycemic agents. Their structures were designed based upon known antihyperglycemic agents which have therapeutic potential for the treatment of type II diabetes mellitus. The target compounds have a carbohydrate moiety linked to an aromatic portion via a short linker, and both the carbohydrate and aromatic portions can be varied to provide a small library of compounds.

Method for Recruiting Student Worker:

I am open to selecting a student worker from either the freshman-sophomore program or the junior-senior program. I view this program as an excellent opportunity to introduce students to independent, hands-on research and provide them with skills that they won't necessarily learn in the classroom (formulating a hypothesis, critical thinking, problem solving, organizing data, communicating results, etc.). I hope to provide a student with his/her first experience conducting independent undergraduate research.

I will mention this position to students in my CHEM 230 and 330 classes (generally sophomores and juniors, hence the two categories listed above), as well as students that have approached me to say that they are interesting in doing research in the Chemistry Department. I will recruit students by posting the job opening on the Cardinal Career Network.

Narrative:

Goals and Objectives:

This project will determine whether aryl-*C*-glycosides are effective antihyperglycemic agents due to their similar structure to known antihyperglycemic agent **4**. A small library of compounds will be synthesized and evaluated for antihyperglycemic activity using an alpha-glucosidase inhibition assay.

Goals and Objectives

The goals and objectives of this project are summarized as follows:

1) Optimize synthesis of C-glycosides using test compounds (acetophenone, iodobenzene, etc.)

2) Synthesize C-glycosides containing varying aromatic components to develop a small library of compounds

3) Purify and characterize all synthesized compounds

4) Test all prepared compounds for inhibition of the alpha-glucosidase enzyme using an alpha-glucosidase inhibition assay

Student Learning Objectives/Student Tasks

The undergraduate researcher will be expected to meet the following learning outcomes:

1) Conduct carbohydrate synthesis reactions under inert atmosphere (using appropriate techniques to keep all reagents water-free if necessary, using proper transfer procedures, conducting reactions under an argon atmosphere, etc.) to synthesize the desired compounds. The student will work towards the synthesis and purification of at least 4-5 compounds over the course of the year-long project.

2) Use appropriate purification and characterization methods (e.g. column chromatography, NMR spectroscopy, mass spectrometry, etc.) to confirm the synthesis/identity of each compound (including synthetic intermediates).

3) Carry out biological testing for inhibition of the alpha-glucosidase enzyme for their synthesized compounds (time permitting).

4) Maintain a proper lab notebook with all data recorded neatly and completely.

5) Communicate the results of their experiments through poster presentations.

In medicinal chemistry research, the careful and honest reporting of yields, characterization data, and procedural details is of the utmost importance. These skills along with those of keeping a proper lab notebook and organization/cleanliness in the laboratory will be taught to and expected of all students involved in this project.

Student researchers will be taught the necessary reactions, techniques, and procedures at the beginning of the project. They will be shown how to keep a proper lab notebook and the importance of maintaining a clean and organized work environment in the lab. The students will then conduct all experiments themselves under my supervision in order to practice the techniques and procedures they have learned.

I will ensure that the UGRP student meets the outcomes listed above by meeting with him/her weekly to assess their progress. During these meetings, I will monitor his/her lab notebook to ensure that it is up to date and discuss the results of their experiments that week. The student will purify and characterize each intermediate in the synthesis as well as their final target compounds. Once desired compounds are synthesized, the student will be taught the necessary cell-biological techniques to carry out the alpha-glucosidase inhibition assay. Finally, the student will present the results of their experiments to date through a poster presentation at the SE&T Symposium in April 2017 and the UGRP Symposium in April 2017. The student will also be encouraged to present at other conferences such as the local meeting of the American Chemical Society to be held in Fall 2016. This will allow the student researcher to learn how experimental results are communicated to both a scientific and non-scientific audience and will help to improve their communication and presentation skills.

Background:

Type II diabetes mellitus is a significant health problem affecting hundreds of millions of people worldwide.[1] Increased blood glucose levels, known as hyperglycemia, is a characteristic of type II diabetes that leads to retinopathy, nephropathy, and neuropathy, among other diseases.[1] Although lifestyle factors such as diet and exercise play a vital role in diabetes therapy, most patients also require drug therapy to combat hyperglycemia.[2] In addition, individualized treatment is a necessity

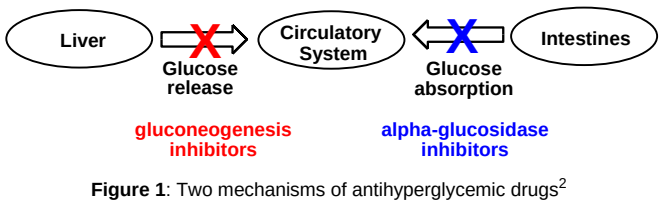

Figure 1: Two mechanisms of antihyperglycemic drugs[2]

and often combination therapies are required for optimal outcomes.[2] Two mechanisms of antihyperglycemic drugs are shown in Figure 1.[2] Gluconeogenesis inhibitors block the synthesis of new glucose in the liver, thereby reducing the amount of glucose that is released into the circulatory system. These compounds tend to promote liver cell glucose utilization and increase skeletal cell glucose uptake, but the mechanism by which these processes occur remains unclear.[2] Alpha-glucosidase inhibitors also reduce blood glucose levels by preventing glucose absorption from the intestines. More recently, inhibitors of the sodium-glucose co-transporter 2 (SGLT2) have been identified as insulin-independent potential targets for the treatment of hyperglycemia. SGLT2 is responsible for the majority of renal glucose readsorption, and inhibition results in an increase in glucose excretion in the urine. Some antihyperglycemic lead compounds are shown in Figure 2. *O*-Glycosides such as phlorizin (**1**)[3] suffer from metabolic instability, whereas *C*-glycosides such as dapaglifozin (**2**)[4] and **3**[5] have shown promise as selective SGLT2 inhibitors that are resistant to degradation. Furthermore, **4** was demonstrated to be an inhibitor of the alpha-glucosidase enzyme, but has not been evaluated for SGLT2 activity.[6]

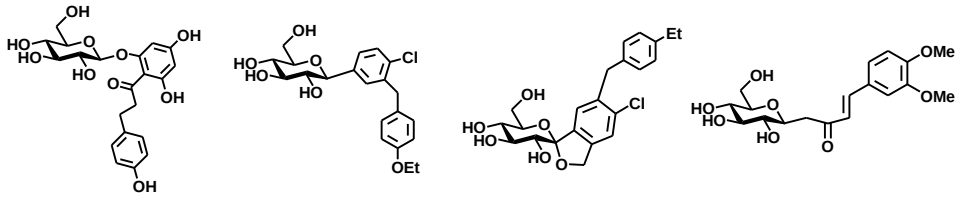

Figure 2: Representative lead compounds that have shown antihyperglycemic activity

Methodology

The aim of this project is to prepare *C*-glycosides (**5** – **7**, Figure 3) that are structurally based upon lead compounds (**1** – **4**) that have shown potential as antihyperglycemic agents. These compounds retain the conjugated system of compound **4** while shortening the linker between the sugar and aryl portions of the molecule. *C*-Glycosides have recently shown promise as therapeutic agents for the treatment of diabetes, and dapaglifozin **2** is currently in phase III clinical trials as a diabetes drug.[4] *C*-Glycosides have been shown to be more stable than their *O*-glycoside counterparts while retaining similar conformations.

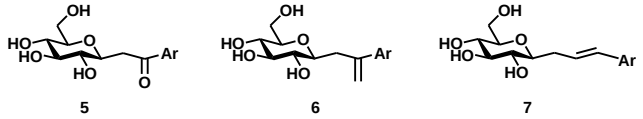

Figure 3: Structures of Target Compounds

Compounds **5 – 7** can be readily synthesized via known chemistry and are easily amenable to structure-activity relationship studies as the aromatic component (Figure 2, Ar = aromatic) can be easily varied. Retrosyntheses of the key steps in the formation of compounds **5 – 7** are shown in Figure 4. Compound **5** can be prepared by the reaction of an appropriately protected thioglycoside (**8**) with an aryl silyl ether (**9**).[7] Suzuki coupling of vinyl triflates (**10**) with arylboronic acid (**11**) gives access to compound **6**, while Heck coupling of protected allyl glucose (**12**) with an aryl iodide (**13**) furnishes compound **7**.[8] In all cases the glucose moiety can be prepared in three or fewer steps and the aryl component is readily varied to provide a small library of compounds. Various aromatic coupling partners can be arrived at through well-established metal-catalyzed cross-coupling approaches. The final compounds' ability to inhibit alpha-glucosidase will be evaluated in an UV-

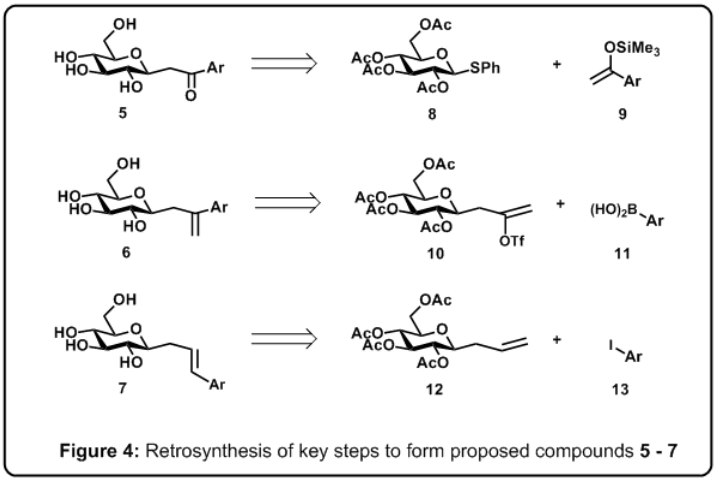

Figure 4: Retrosynthesis of key steps to form proposed compounds **5 - 7**

based enzymatic essay that uses para-nitrophenyl alpha-D-glucopyransoside as the substrate.[9,] To date, we have explored the synthesis of compounds with the general structures **5** and **7**. We are currently optimizing this synthetic procedure to work for glucose (instead of mannose as was previously reported) and then will be preparing a small library of compounds.

The desired compounds will be synthesized using known procedures[7,11] by the undergraduate student researcher and evaluated for alpha-glucosidase activity using an established procedure.[8] The purified compounds will be characterized using Nuclear Magnetic Resonance and Mass Spectrometry. As of July 2017, at least 10 compounds should be available, providing a small compound library of potential anti-hyperglycemic agents.

Significance and Impact:

Type II diabetes is an ever-growing problem in our society which will affect an estimated 380 million people by 2025.[1] In addition, diabetes and its associated health problems are a drastic strain on the economy due to both direct medical costs and secondary outcomes such as loss of work and disabilities.[1] Antihyperglycemic drug therapy is often required in order to gain control over glycemic levels, and these drugs are often used in combination to create individualized treatment plans. Therefore, there is an urgent need for new antihyperglycemic drug targets as they have potential to aid more patients to control their blood sugar levels.

During this research project, undergraduate students will have an opportunity to see a research project through from start to finish. The medicinal application of the project tends to excite students from different disciplines and allows them to see the benefits of interdisciplinary research. Students will learn techniques of air-sensitive chemical synthesis as well as purification of synthesized compounds. Furthermore, they will learn how to conduct biological assays and cell biological techniques. Most importantly, students will be exposed to the research method (developing a hypothesis, conducting experiments and making observations, and revising hypothesis accordingly), lab record keeping, and communication of experimental results, which are skills that are needed in all careers in the sciences.

Timeline:

The undergraduate researcher will be asked to prepare 2-3 aryl glycosides (target compounds) per semester, thus synthesizing approximately 4-6 target compounds by April 2017. Through this exercise they will learn aspects of carbohydrate chemistry and appropriate use of protecting groups to prepare the glycoside component, and they will subsequently prepare the required aromatic component and couple the two to give the desired product. Final compounds will be evaluated for activity using the alpha-glucosidase inhibition assay described above through comparison to compound **4** and other known standards. By August 2016, one target compound should be made and each reaction in the synthesis will have been optimized. By December 2016, 3-4 additional targets should be prepared. Finally, by April 2017, the biological testing of the prepared compounds should be completed and additional compounds will be synthesized if time permits.

Progress Evaluation

Synthesized compounds will be evaluated at each step using Nuclear Magnetic Resonance Spectroscopy and Mass Spectrometry and compared to known compounds to confirm their structure. Each synthetic step should take 1-2 weeks to complete. Progress will be evaluated weekly through regular meetings with the student to ensure that he/she is on track to synthesize the desired compounds within the predicted timeframe. Once synthesized, compounds will be evaluated for activity through comparison to known anti-hyperglycemic compounds (positive controls).

The results of this project will be presented at regional and national chemistry conferences. If active compounds are identified, the results will be submitted to a reputable scientific journal (such as Bioorganic and Medicinal Chemistry Letters) for publication.

References:

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Budget:

Student Salary: $4000.00

One student for Spring/Summer 2016 ($2000), Fall 2016 ($1000) and Winter 2017 ($1000)

Number of Students: 1

Total: $4000.00

Previous Funding:

Equipment/supplies for this project have previously been funded by the SVSU Faculty Research Grant. Additionally, a salary for one student was funded through the UGRP program from May 2015– April 2016.

Other Funding/Explanation:

I am requesting $4000.00 in financial support from the SVSU UGRP for this project. I have already received funding from SVSU's Ruth and Ted Braun Fellowship program for this project. The funds that have been received will cover reagents, equipment, and supplies for this project, as well as a salary for one student research assistant. Of the requested funds, $4000 will be used to pay one student researcher at a rate of $9/hour to assist with this project. Having a student salary to fund a second undergraduate researcher on this project will be essential to its successful completion. In order to receive payment, the student will need to work in the lab for an average of 12 hours per week during Spring/Summer (2-3 days per week) and 7 hours per week during the academic year and will need to complete at least one reaction (including purification/characterization) every two weeks.

Please note that as I will receive a small faculty summer salary through the Braun Fellowship, I am not requesting a faculty salary through the UGRP despite supervising the student worker throughout the spring/summer semesters.

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