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Project Title: Preparation of *C*-Glycosides as Potential Anti-hyperglycemic Agents for the Treatment of Type II Diabetes Mellitus

Field of Study: Bioorganic Chemistry, Organic Synthesis

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 this project conducted in Dr. Jennifer Chaytor's laboratory at SVSU, aryl-*C*-glycosides will be synthesized by undergraduate students via standard cross coupling reactions and carbohydrate chemistry. These compounds will then be evaluated in enzymatic assays as potential anti-hyperglycemic agents. Their structures were designed based upon known anti-hyperglycemic 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. Active compounds can be used in structure-activity relationship studies to provide information about the mechanism of action of anti-hyperglycemic drugs.

Submission to the Internal Review Board: No (not applicable)

Start date of project: January 01, 2016 End date of project: December 31, 2018

Preparation of *C*-Glycosides as Potential Anti-hyperglycemic Agents for the Treatment of Type II Diabetes Mellitus

1. Description of proposed project and activities

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 and often combination therapies are required for optimal outcomes.[2] Three main categories of anti-hyperglycemic drugs are currently used.[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 anti-hyperglycemic lead compounds are shown in Figure 1. *O*-Glycosides such as phlorizin (**1**)[3] suffer from metabolic instability, whereas *C*-glycosides such as dapaglifozin (**2**)[4] and compound **3**[5] have shown promise as selective SGLT2 inhibitors that are resistant to degradation. Furthermore, compound **4** was demonstrated to be an inhibitor of the alpha-glucosidase enzyme, but has not been evaluated for SGLT2 activity.[6]

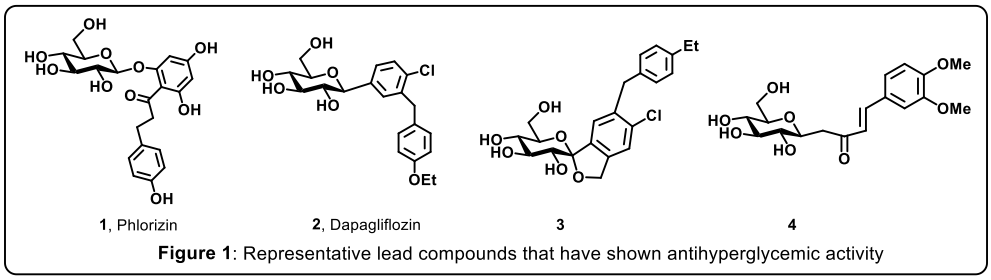

Figure 1: Representative lead compounds that have shown antihyperglycemic activity

The aim of this project is to prepare *C*-glycosides (**5** – **7**, Figure 2) that are structurally based upon lead compounds (**1** – **4**) that have shown potential as anti-hyperglycemic agents. These compounds retain the conjugated system of compound **4** while shortening the linker between the sugar and aryl portions of the molecule. It is hypothesized that shortening the linker may result in compounds that display activity against both the alpha-glucosidase enzyme as well as inhibition of SGLT2, possibly leading to "dual-action" anti-hyperglycemic drugs. *C*-Glycosides have recently shown promise as therapeutic agents for the treatment of diabetes, and dapaglifozin **2** is currently on the market as the diabetes drug Farxiga™.[4] *C*-Glycosides have been shown to be more stable than their *O*-glycoside counterparts while retaining similar conformations. If active compounds are identified, these compounds can be utilized in

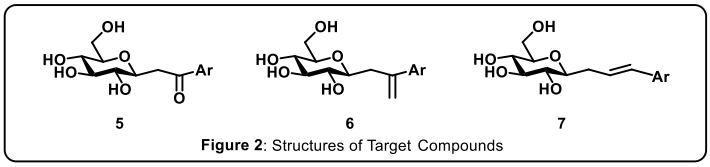

Figure 2: Structures of Target Compounds

structure-activity relationship studies for the treatment of type II diabetes. These studies could potentially provide information about the mechanism of action of gluconeogenesis inhibitors, which is currently not well understood.

Compounds **5 – 7** can be readily synthesized via known chemistry[7,8] and are easily amenable to structure-activity relationship studies as the aromatic component (Figure 2, Ar = aromatic) can be easily varied. 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-based enzymatic essay that uses para-nitrophenyl alpha-D-glucopyransoside as the substrate.[9] In addition, their ability to promote liver cell glucose utilization may be assessed in an established glucose oxidase enzymatic assay and a cell viability UV-based assay.[10] Activity results will guide the design of second generation compounds. Compounds that show promising biological activity can subsequently be modified at the carbohydrate portion, where the effect of the nature of substituents and stereochemistry at each position will be evaluated. It has previously been shown that small structural changes can have a drastic effect on biological activity of glycosides.[11,12] As the program is developed, synthesized compounds can be evaluated for other modes of anti-hyperglycemic activity, such as SGLT2 inhibition. Collaborations with researchers skilled in these assays in animal models will be explored. To date, we have explored the synthesis of compounds with the general structure **5** through a Faculty Research Award. We have very recently optimized this synthetic procedure to work for glucose (instead of mannose as was previously reported) and are preparing a small library of compounds in this structural category. We are now beginning to focus on compounds with the general structure **7**, while category **6** will be explored in subsequent years of this program.

The proposed work will generate a small library of *C*-glycosides of varying structure that will be evaluated for anti-hyperglycemic activity. If successful, the benefits of this research are that active compounds can be used in structure-activity relationship studies that can potentially shed light on the mechanism of action of these inhibitors. In addition, the mechanism of action of gluconeogenesis inhibitors such as metformin is currently poorly understood.[2] A greater understanding of this mechanism will aid in the design of new inhibitors and subsequently new diabetes therapies.

Goals and Objectives

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

1) Optimize synthesis of *C*-glycosides in all three categories (compounds with general structures **5** – **7**) using test compounds (acetophenone, iodobenzene, etc.).

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

3) Synthesize compound **4** as a control for biological testing.

4) Purify and characterize all synthesized compounds.

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

References:

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(10) Zou, C., Shen, Z. *J. Pharmacol. Toxicol. Methods*, **2007**, *56*, 58-62.
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2. Description of benefits to faculty, community, and Saginaw Valley State University

Medicinal chemistry research is essential in order to identify novel drugs and drug targets against a variety of diseases. Furthermore, carbohydrates are involved in many biological processes and are the basis of several drug structures. The development of a medicinal chemistry and carbohydrate chemistry research laboratory at SVSU has the potential to identify novel drug targets that will open the doors to further research in this area. This is a direct benefit to both SVSU and myself as a **faculty** member. The undergraduate research program that I have established at SVSU has built on my graduate and post-doctoral research and has already provided many opportunities for both myself and my undergraduate research students to present at local and national conferences. The proposed project will provide many more opportunities for presentations and also publications in this area, which will also allow us to showcase undergraduate research at SVSU.

In addition to the above mentioned benefits to SVSU, there are many benefits to **SVSU students** as well. When working in my laboratory, undergraduate students 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 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 scientific 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.

My research students have and will continue to strongly benefit from this project as it involves aspects of synthetic organic chemistry, medicinal chemistry, structure characterization, analytical chemistry, and biological techniques. This interdisciplinary program will not only provide students with hands-on laboratory experience, but will also expose them to problem solving skills that are often necessary in a

laboratory setting. This project provides opportunities for student presentations at regional and national chemistry conferences, and may culminate in their co-authorship on a peer-reviewed journal article.

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] Anti-hyperglycemic 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 anti-hyperglycemic drug targets as they have potential to aid more patients to control their blood sugar levels. Therefore, this project has direct potential benefits to the local **community** as well as the wider population.

3. Description of the professional or academic work for publication or presentation

The results of this project will be presented at institutional, regional and national chemistry conferences. There is potential for undergraduate students to publish the results of their experiments in journals such as the Journal of Undergraduate Research. Furthermore, if active compounds are identified, the results will be submitted to a reputable scientific journal (such as Bioorganic and Medicinal Chemistry Letters) for publication. I have strong publication (9 publications and 1 patent) and presentation (18 presentations) records to date, and this will surely continue throughout the duration of this project.

4. Description of teaching, research, and service activities at SVSU

Teaching

My teaching load consists largely of teaching our Organic Chemistry curriculum (lectures and labs) at SVSU. I also assist with General Chemistry and Biochemistry as needed. The chemistry described in this project is an extension of many of the reactions that we discuss in my classes. It is very exciting for students to realize that the chemistry that they learn in the classroom is actually done in research laboratories, which makes the concepts much less abstract and theoretical. The students also remember reactions much better when they can perform them in the laboratory, and unfortunately we cannot cover all reactions in the Organic Chemistry Laboratory curriculum. I am beginning to incorporate a biomolecules section into my Organic II course, and the carbohydrate aspect of this project fits very nicely into the curriculum. Interesting results from my laboratory often make their way into my lectures, and discussion of my research in my classes helps to excite students about chemistry in general as well as undergraduate research.

Research

I have developed an active research program at SVSU. To date, I have supervised 10 undergraduate students in research and three high school students from local high schools. In the past three years, these students have delivered 13 presentations at conferences, including two at national meetings of the American Chemical Society. I have presented at four conferences since joining SVSU in 2012. I was incredibly excited to be invited to give an oral presentation at the national meeting of the American Chemical Society on my undergraduate research at SVSU, thus allowing me to showcase this project and

the work of our undergraduate students. My grant application record has been very successful since joining SVSU. I have received two external grants, one from the American Chemical Society and one from the Michigan Space Grant Consortium. Additionally, I have been awarded nine internal grants at SVSU, including several Undergraduate Research Program awards, Faculty Research Awards, a grant from the Dow Science and Sustainability Education Center, and the Herbert H. and Grace A. Dow Professor Award. These awards have a combined total of over $50,000 in financial support for my research and teaching programs. Finally, I currently have nine publications in peer-reviewed scientific journals. The most recent was published earlier this year (2015) in the Journal of Agricultural and Food Chemistry, and an additional five have been published since 2010. The attached CV highlights some of my recent research accomplishments.

Service

In addition to a heavy teaching load and developing an active research program, I have been involved in serving the Chemistry department, SVSU, and the community. As I value effective written and oral communication skills, I am a member of the University Writing Committee and have been involved in National Day of Writing events. Through this committee I have also selected recipients of the Innovative Writing in Teaching (IWIT) award and the Braun writing award. In an attempt to recruit and retain students, I have served in many recruitment events (Fall Open House, Admissions Fair, Cardinal College Days, etc.). To help recruit K-12 students, I have assisted with the Regional Science Bowl and Tuscola Academic Games for three years each. In terms of retaining current students, I use my role as Advisor of the Chemistry Club to keep students engaged and excited about Chemistry. In an effort to maintain ethical standards at SVSU, I have served as a Faculty Representative of the Student Conduct Board since 2013. Finally, I have served on five Faculty Search Committees since 2012 for the Chemistry and Biology departments. In serving the community, I have been active with the local Midland Chapter of the American Chemical Society, served on University of Michigan – Flint's Curriculum Advisory Committee, and served as a Faculty Mentor for local K-12 teachers through the Dow Corning Foundation/SVSU STEM Community Partnership and the Dow Science and Sustainability Education Center.

5. Budget:

Year 1: 2016

Reagents/equipment/consumables for carbohydrate synthesis (chemicals, solvents, syringes, pipets, dry ice, glassware, etc.) and cell biology consumables (pipets, 96 well plates, etc.).	$5,307.47
Student compensation (wages and FICA): Spring/Summer 2016 semester: 204 hours x $9/hour = $1,836; Fall 2016 semester 104 hours x $9/hour = $936. Total wages $2,772. FICA @ 7.65%= $212.06 for a total of $2,984.06.	$2,984.06
Summer salary @6% of base = $3,517.32 plus 19.65% fringe benefits (FICA @7.65% and Retirement @12%) = $691.15. Total Summer salary and fringe benefits= $4,208.47.	$4,208.47
Year 1 Total	**$12,500.00**

Year 2: 2017

Reagents/equipment/consumables for carbohydrate synthesis (chemicals, solvents, syringes, pipets, dry ice, glassware, etc.) and cell biology consumables (pipets, 96 well plates, etc.).	$3,113.85
One 2.4 credit lab for Fall 2017 term (adjunct replacement) = $700 x 2.4 cr = $1,680.00 plus FICA @ 7.65%= $128.52 for a total of $1808.52.	$1,808.52
Student compensation (wages and FICA): Spring/Summer 2017 semester: 204 hours x $9/hour = $1,836; Fall 2017 semester 104 hours x $9/hour = $936; Winter 2017 semester 104 hours x $9/hour=$936. Total wages $3,708. FICA @ 7.65%= $283.66 for a total of $3,991.66.	$3,991.66
Summer salary @5% of base = $2,997.05 plus 19.65% fringe benefits (FICA @7.65% and Retirement @12%) = $588.92. Total Summer salary and fringe benefits= $3,585.97.	$3,585.97
Year 2 Total	**$12,500.00**

Year 3: 2018

Reagents/equipment/consumables for carbohydrate synthesis (chemicals, solvents, syringes, pipets, dry ice, glassware, etc.) and cell biology consumables (pipets, 96 well plates, etc.).	$2,439.26
One 2.4 credit lab for Winter 2018 and Fall 2018 terms (adjunct replacement) = $700 x 4.8 cr = $3,360 plus FICA @ 7.65%= $257.04 for a total of $3,617.04.	$3,617.04
Student compensation (wages and FICA): Spring/Summer 2018 semester: 180 hours x $10/hour = $1,800; Winter 2018 semester 95 hours x $10/hour = $950. Total wages $2,750. FICA @ 7.65%= $210.38 for a total of $2,960.38.	$2,960.38
Summer salary @4.75% of base = $2,911.26 plus 19.65% fringe benefits (FICA @7.65% and Retirement @12%) = $572.06. Total Summer salary and fringe benefits= $3,483.32.	$3,483.32
Year 3 Total	**$12,500.00**

In addition to the student salaries requested, other students can conduct research for credit or for research experience during the regular academic year and the spring/summer semesters. Additional student salary support will be sought through the SVSU Undergraduate Research Program. Funds are requested for chemical reagents and equipment, organic synthesis consumables (NMR and synthesis solvents, syringes, needles, etc.), and cell biology consumables (pipets, 96-well plates, cell growth media, etc.). Finally, funds are requested to cover payment for an adjunct instructor to cover one of my lab sections in the second year and two sections in year 3 in order to have more time to commit to this research project. Additional funds to cover small summer salaries are requested so that I can avoid some spring/summer teaching in order to have more time to focus on this research project.