

Proposal for Senior Honors Thesis

HONS 497 Senior Honors Thesis Credits 2 (2 minimum required)

Directions: Please return signed proposal to the Honors Office **at least one week prior to your scheduled meeting with the Honors Council (or by Oct. 1 if you are a senior)**. This proposal must be accepted by Honors Council the semester before presentation. (2-3 pages)

Student's Name: Nicole R. Van Allen

Primary Advisor: David Nowack, Ph.D

Secondary Advisor: Desmond Murray, Ph.D

Thesis Title: Inhibition of Bacterial Carbohydrate Metabolizing Enzymes by Novel Chalcones

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Expected date of Graduation: May 2009

I. Goals and brief description

My goals in conducting this research project are to synthesize and isolate novel chalcones, and establish enzyme assay protocols for β -galactosidase and α -glucosidase. Finally, I want to test the novel chalcones that will be synthesized at varying concentrations in the enzyme assays. The result of these tests will determine whether any inhibition occurred as a result of the presence of the chalcone.

II. Methodology

First I will attempt to synthesize and isolate novel chalcones. I am working with Dr. Desmond Murray on this part of the project. The majority of the chalcones, being organic in nature, previously synthesized by Dr. Murray have been insoluble in water. This is a problem because the enzyme activity must occur in water. However, I am seeking to add specific functional groups onto the chalcone that will enhance its solubility in water.

The enzyme assay protocols are methods used in the laboratory for the study of enzyme activity. It is crucial to have enzyme assays in order to study enzyme kinetics and enzyme inhibition. By establishing the enzyme assay protocols, I will have precise environmental requirements and procedures necessary to study the activities of both β -galactosidase and α -glucosidase.

Two enzyme activities will be evaluated; the activities of both β -galactosidase and α -glucosidase. These enzymes use commercially prepared reactants (substrates). The enzyme acts on these colorless substrates to produce highly colored products. The intensity of the colored products is directly related to the amount of activity of the enzyme. I will observe the varying intensity of light in the enzyme mixture of both enzyme and chalcone by using a UV/VIS spectrophotometer. This will tell me the varying intensities of light, which is directly related to the enzymatic activity of the enzyme. The data that I collect using the UV/VIS spectrophotometer will be analyzed using a Lineweaver-Burk plot. This plot will demonstrate the enzymatic inhibition, if any, observed.

III. Relevance and Uniqueness

I am seeking to synthesize and isolate a very unique type of chalcone. The chalcone that I am attempting to synthesize and study is one that has not been studied or researched in the laboratory with the use of

enzymes and enzymatic activity. The possible effects that these “new” chalcones have on the carbohydrate-metabolizing enzymes (β -galactosidase and α -glucosidase) may indicate their possible use as drugs.

It has been shown in previous research that enzyme inhibitors are important tools; they have potential use as drugs by modulating cellular functions. Sulfa drugs are an early and common class of drugs that were used as antibacterials. There is structural similarity between sulfa drugs and chalcones, this fact prompted researchers to look at chalcones as a possible drug (enzyme inhibitor). The result of this research has indicated that chalcones do exhibit some degree of inhibition on enzymes.

β -galactosidase and α -glucosidase both serve as carbohydrate-metabolizing enzymes. Both of the enzymes (β -galactosidase and α -glucosidase) play key roles in the processing of glycoproteins and glycolipids, which are directly related to obesity, diabetes, and other metabolic diseases. Bacteria, both harmful and benign in their growth processes, also utilize these enzymes.

IV. Annotated Bibliography

Heightman, T.; Vasella, A *Angew. Chem. Int. Ed* **1999**, 38, 750

Glycosidase inhibitors have aroused interest as therapeutic agents as the glycosidase recognition processes improve. They have possibilities in treatment of diabetes, HIV, infections and as antifungal agents. This article explains the importance of both shape and charge of the glycosidase inhibitor. It seems to be a combination of both of these factors that leads to proper binding to the substrate.

Kato, A; et al. *J. Med. Chem* **2005**, 48, 2036

Blocking or modifying glycosidases, as apparent in previous articles, hold great interest from a therapeutic standpoint. This research report involves the usage of various sugars as glycosidase inhibitors. Since I am focusing on chalcones, this article is less desirable than others. However it provides important information pertaining to my goal in studying glycosidases etc.

Mohrig, J. R., et al. *Experimental Organic Chemistry A Balanced Approach: Macroscale and Microscale*. New York, NY: W.H. Freedman and Company, 1998

This text provided procedural information for the extraction of the chalcone from the aqueous solution. It also provides experimental information for organic chemistry that may prove useful throughout the chalcone synthesis process.

Moreno-Vargas, A; et al. *Bioorganic & Medicinal Chemistry Letters* **2002**, 12, 2335

Presented the use of carboxylic acid derivatives as beta-galactosidase inhibitors, which is also related to my work and synthesis of a water-soluble chalcone. These enzymatic inhibitors can also be used to modulate cellular functions and are potential drugs in new therapeutic strategies.

Also demonstrates that mimics of glycosyl cation intermediates (liberated during the enzyme-catalyzed hydrolytic processes) prove to be some of the most potent glycosidase inhibitors (polyhydroxypiperidines).

Seo, W et al. *Bioorganic & Medical Chemistry Letters* **2005**, 15, 5514

This article seems to be the most closely related article to chalcone synthesis and use as an enzymatic inhibitor. Since glycosidases are directly responsible for the processing and synthesis of complex carbohydrates this provides a wide variety of use in biological testing etc. Inhibitors of glycosidases can be used to modulate cellular functions; in some cases such as the control of oligosaccharide metabolism linked to cellular dysfunction they can be used as potential drugs.

V. Progress to Date

As of January 24, 2008, I have experimented with synthesizing a water-soluble chalcone and have successfully done so. I have come up with a method to isolate the desired chalcone, and have successfully isolated the chalcone. I formed about 45% of the expected yield; however, I lost significant amounts of product while trying to find a method to isolate the chalcone out of the aqueous solvent. In addition, I have identified the enzyme assays for β -galactosidase and α -glucosidase and they have been ordered. I was trained in the operation of the UV/VIS spectrophotometer.

This student's performance in his/her major field is acceptable and I understand the plans to graduate with honors.

Dept Chair (signature) _____

I have read and support this proposal: Primary Advisor (signature) _____

I have read and support this proposal: Secondary Advisers (signature) _____

If human subjects or if live vertebrate animals are involved, evidence of approval from the Human Subjects Review Board or an Animal Use Committee is needed through the campus scholarly research offices (Ext. 6360).