Streptococcus Pyogenes Sortase A Inhibition Using Fluorinated Chalcones

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Abstract

Antibiotic drugs have been widely used in an effort to fight diseases caused by different bacteria strains, such as Streptococcus pyogenes. Some of these bacteria have over time adapted to become resistant to these antibiotic drugs. We chose to focus on the inhibition effects of different structured chalcones on Streptococcus pyogenes, specifically the enzyme Sortase A and its ability to anchor proteins to its cell wall. We focused on 5 different fluorinated chalcones and used High-Performance Liquid Chromatography (HPLC) to determine each chalcone's inhibition activity with the Sortase A enzyme. Compiling our trials with other teams' trail runs, we found inhibition rates from chalcone vials #148, 109, 92, 27, and 113 to be 57%, 53%, 75%, 25%,67% respectively. Further research could be done looking into the inhibition effects of different fluorinated chalcones based on position in the structure.

Introduction (Research Question & Background)

Since antibiotic resistance has become a worldwide issue in regard to common bacterial infections [1] such as Streptococcus pyogenes, we conducted an experiment which evaluates the effect of fluorinated chalones on Sortase A enzyme inhibition. Sortase A enzyme is responsible for anchoring bacterial proteins to the cell wall of another organism [2]. Some proteins have virulent factors that contribute to the ability of pathogenic organisms to cause illness. The ability to inhibit this enzyme and block this pathway, could be a new effective way of fighting bacterial infections without antibiotics [2].

We found previous claims that fluorinated organic compounds have natural anti-bacterial properties [3]. This claim has been tested in several previous studies. Based on previous research [1,3,4,5], our experimental question was "Do fluorinated chalcone derivatives inhibit the Sortase A enzymatic activity of the S. pyogenes organism? If these chalcones do show the ability to inhibit Sortase A, which isomer structures are most effective?". In order to test this hypothesis, we evaluated the effectiveness of different fluorinated chalcones for Sortase A inhibition.

Methods

Materials & Instrumentation

- DMSO Chalcone 27, 92, 109, 113, 148 Stock and Working Solution (100 mM, 10 mM)
- DMSO Dimethyl Sulfoxide (Kulshan 120D)
- Solution A Sortase A Enzyme (prepared by WWU collaborators)
- Solution B Peptide Substrate (AnaSpec-III)
- N-ethylmaleimide Quenching Solution (5mM, prepared by Tommaso A. Vannelli)
- Micropipettes: P100-1000 (Rainin), P20-200 (Gilson), P2-20 (Biorad), P0.2-2 (Fisherbrand)
- Waters 2690 HPLC Separations Module

Chalcones derived from WWU Organic Chemistry Students. Stock solutions prepared from those compounds by our group, and previous WCC research groups.

Procedure

Thin Layer Chromatography (TLC) Analysis:

Chalcones diluted in methanol and spotted on TLC paper → Solution was allowed to elute in a methylene chloride and hexane elution → Solvent purity and composition were verified using UV illumination

Inhibition Assays:

Solution A and Chalcone DMSO Working Solution incubate for 30 minutes at room temperature → Add solution B and incubate for 30 minutes at 37° → Quench solution with NEM solution, then submit for HPLC analysis

Procedure adapted for WCC by Tommaso A. Vannelli from a CHEM356 lab developed by Profs. Amanda Murphy and John Antos, no further modifications were made.

Data & Results

Molecular Structure of Tested Chalcone Derivatives

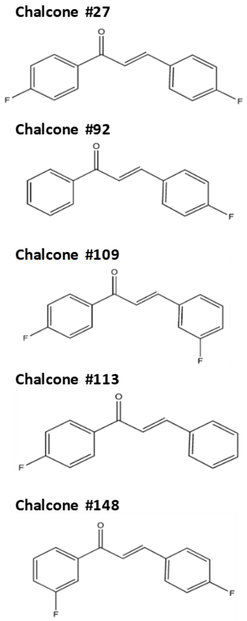


Figure 1 - Monofluorinated (#92, #113), and Difluorinated (#27, #109, #148) chalcone derivatives used for the experiment.

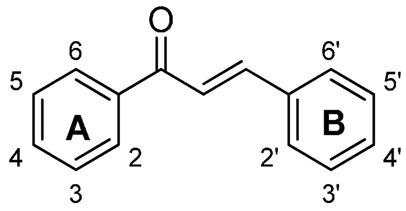


Figure 2 - Labeled basic chalcone structure and numbering system

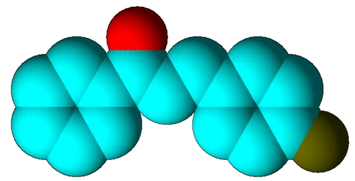
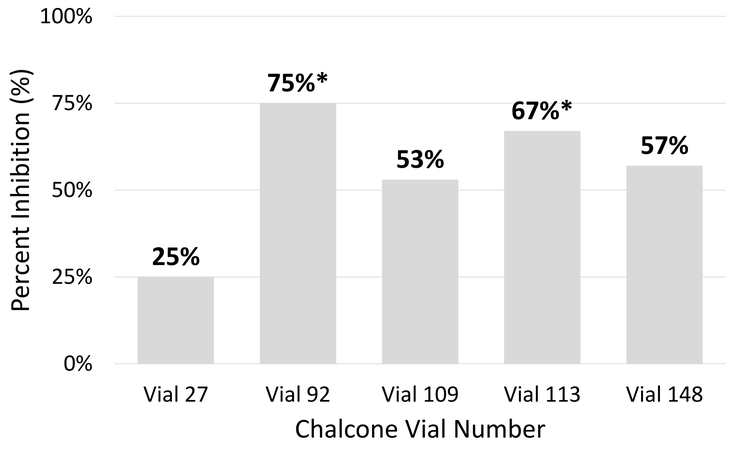


Figure. 3 - ChemSketch diagram showing the polarity of chalcone derivative #92. The fluorine atom shown in green is the most electronegative region of the molecule.

Chalcone Derivative Inhibition Percentages by Vial Number



Graph 1. *Vials #92 and #113 show the largest percent inhibitions. Both these chalcones are monofluorinated with fluorine bonded in the B4' position (#92) or the A4 position (#113). It's important to note that the inhibition data for Vial #113 is based on only one trial, so the reliability and validity of these results are uncertain. Furthermore, the 75% inhibition value for Vial #92 is using the WWU substrate, however, our latest data using the AnaSpec-III substrate shows inhibition values of 28%.*

Discussion

After completing all our HPLC assays, we found that some fluorinated chalcones have a higher average percent inhibition, which seems to be correlated with the fluorine position in the molecule. Chalcone derivative #92 showed the highest average percent inhibition at 75%. This molecule has a single fluorine atom in the 4' position of the B ring (4'B). Chalcone derivative #113 has a similar result to #92, the average percent inhibition being 67%. This molecule also has a single fluorine atom, in the 4A position. Chalcones #109 and #148 showed average inhibition percentages of 53% and 57% respectively. These chalcones have 2 fluorine atoms. #109 has fluorine atoms in the 4A the 3'B positions. Chalcone #148 is the exact opposite arrangement with fluorine atoms in the 3A and 4'B positions. Chalcone #27 significantly underperformed compared to the other chalcone derivatives. This chalcone has 2 fluorine atoms across from each other in the 4A and 4'B positions and had an average percentage inhibition of only 25%. (Please refer to Figure 2 for the numbering system.)

Regarding our experimental question, our data shows that fluorinated chalcones do inhibit the Sortase A enzyme. The data shows a correlation between mono-fluorinated chalcones and a greater ability to inhibit the Sortase A. Chalcone derivative #92 was the most effective. The second most effective chalcone derivative was #113, which is also a mono-fluorinated chalcone. Based on this data, we can see that the addition of a fluorine atom in the 4 or 4' position on either carbon ring structure yielded the best results. Chalcones #92 and #113 both have the highly electronegative fluorine atom on one pole. This gives the molecule the capacity to attract other molecules. If the goal was to attract the Sortase A molecules and keep them from attaching to cell walls, this could be a plausible explanation for the high percent inhibition from these two chalcones.

Though we were able to generate a plausible explanation for the trends in our data, we also need to acknowledge our faults in conducting this experiment, since they may have affected our data. We had time and material restrictions, so we used other groups' chalcone solutions to conduct our experiment. We also borrowed data from other groups and had inconsistent data points across the board. Since we were unsure how the other groups prepared their solutions, we know this could have caused significant errors in the data. Overall, if we were to repeat this experiment, we would aim for greater consistency in preparing solutions and an even number of trials for each chalcone. We found that having one person run the assays for a specific chalcone vial, decreased the standard deviation. This is because the technique stays more consistent when it is just one person pipetting, compared to having multiple people running assays with different pipetting techniques. However, this can potentially lead to hidden errors if one's technique is poor but hidden due to the standard deviation. We recommend ensuring one's micro-pipetting technique is satisfactory prior to running assays—that way the error is mitigated when running assays individually or collaboratively. For future studies, we suggest an evaluation of mono-fluorinated chalcones with fluorine atoms in different positions on the molecule. We think this could further the research in this field and lead to significant discoveries in inhibiting the effects of antibiotic-resistant bacteria.

Acknowledgments

All our resource material used was provided to us by Whatcom Community College. Professor John Antos at Western Washington University developed the outline procedure which we used for our project starting point. Our professor, Tommaso A. Vannelli, Ph.D., assisted us in guidance on narrowing down our hypothesis and questions regarding the research. The stockroom staff at Whatcom Community College (Mark Price and Bethany Tegt) also assisted us in making sure we had materials provided during lab.

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