Inhibition of Sortase A Using Bridged Chalcones

Where's My Stipend?

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Abstract

Streptococcus pyogenes (S. pyogenes) is a common bacteria treated with antibiotics, but the rise of antibiotic resistance calls for alternative approaches. In this study, we investigated the inhibition of *Sortase A* (SrtA), a key enzyme to the bacteria's infection process, using chalcones with a methylenedioxy substituent. Inhibition rates were measured via High-Performance Liquid Chromatography (HPLC). Chalcones #95, #81, and #134 exhibited percent inhibitions of 12% (±5.6), 13% (±2.6), and 9% (±2.5), respectively, which were significantly lower than the 40% (±2.5) inhibition observed with the unsubstituted chalcone. These findings suggest that the methylenedioxy group may have negatively impacted SrtA inhibition. Further research could explore repositioning the methylenedioxy group or testing alternative structural modifications to improve inhibition efficiency.

Introduction

Streptococcus pyogenes (S. pyogenes) is the bacterium responsible for strep throat, scarlet fever, pneumonia, and various other infectious diseases. Since the 1940s, *S. pyogenes* has developed increasing resistance to antibiotic treatment [1]. The use of antibiotics typically kills most of the bacteria, but some- particularly those with resistant traits- survive and pass these traits on to future generations. Over time, this reduces the effectiveness of antibiotics as the population of resistant bacteria becomes dominant.

A promising approach to addressing this issue is inhibiting Sortase A (SrtA), an enzyme in the bacterium that facilitates biofilm formation and anchoring to human cells [6]. By inhibiting this function, the immune system has a better chance of attacking and eliminating the bacteria, which may reduce the potential for resistance [3]. Therefore, SrtA inhibition could serve as an anti-infective strategy and an alternative to traditional antibiotics [2].

In this project, we explored how chalcones with a methylenedioxy substituent inhibit SrtA (Figure 2). Chalcones with a methylenedioxy bridge have not been extensively studied for their effects on SrtA inhibition. Our research aimed to answer the question: Do chalcones with a methylenedioxy substituent (Figure 2) inhibit SrtA more effectively than unsubstituted chalcones (Figure 1)?

Methods

Materials & Instrumentation

The Following materials were provided by Whatcom Community College: Dimethyl Sulfoxide (DMSO), Solution B (peptide substrate), 5mM n-ethylmaleimide (NEM), 2mL HPLC testing vials, a Waters 2690 Separations Module equipped with an in-line degasser, thermostatted 96-sample carousel autosampler, column compartment heater, Waters 996 Photodiode Array Detector controlled by OpenLab ChemStation CDS (Agilent). We also had our solution A enzyme provided by Professor John Antos and Carly Finerty at Western Washington University. The following material were made by Western Washington University students and used without further purification 4'-methoxy, 3,4-methylenedioxy chalcone, 4'-methyl-3,4,-methylenedioxy chalcone , 4'-chloro-3,4-methylenedioxy chalcone, unsubstituted chalcone.

Reactions were analyzed by HPLC on a on a Hypersil GOLD C18 column (50mm x 4.6mmID, 5 mm, Thermo Scientific) protected with a Hypersil GOLD C18 (10x4mmID, 5mm) drop-in guard column and maintained at 25 °C. The substrate and product peaks were resolved using a linear gradient of the mobile phase combination (A) water with 0.1 v:v% formic acid and (B) acetonitrile with 0.1 v:v% formic acid with a flow rate of 2.0 mL/min. SrtA substrate and product peak areas were determined at 360 nm.

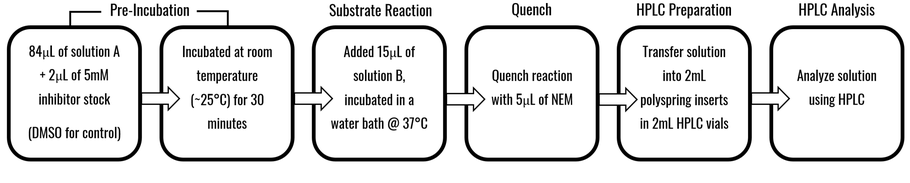


Figure 1. A flow chart of our steps when running assays

Data & Results

Chalcone Structures

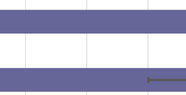
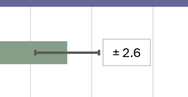
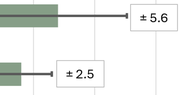
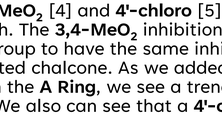
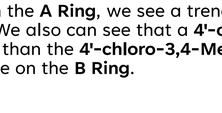
Name	Chalcone Structure	% Inhibition/SD/RSD
Unsubstituted Chalcone		40% (±2.5 SD) 6% RSD
3,4-methylenedioxy (#84)		40% (±1.0 SD) (RSD not provided)
4'-chloro (#15)		25% (±5.1 SD) (RSD not provided)
4'-methoxy,3,4-methylenedioxy (#81,83)		13% (±2.6 SD) 19% RSD
4'-methyl-3,4-methylenedioxy (#95,100)		12% (±5.6 SD) 47% RSD
4'-chloro-3,4-methylenedioxy (#134)		9% (±2.5 SD) 28% RSD

Table 1. Each chalcone has a structure name that indicates where on the A and B rings the substituents are placed. In the chalcone library, they are also organized by vial number, as indicated in parenthesis in the name column. The SD in each result is the standard deviation accounting for the variance between team members samples. The RSD is relative standard deviation which is what precent the standard deviation is of the percent inhibition.

Figure 2. Chalcones **3,4-MeO$_2$** [4] and **4'-chloro** [5] data was provided by previous student research. The **3,4-MeO$_2$** inhibition was observed by this other student research group to have the same inhibition percent as we tested for our unsubstituted chalcone. As we added additional substituents to this same structure on the **A Ring**, we see a trend of inhibition percentage decreasing. We also can see that a **4'-chloro** chalcone has a higher percent inhibition than the **4'-chloro-3,4-MeO$_2$** chalcone that had the added **MeO$_2$** structure on the **B Ring**.

Discussion

Overall, chalcones with a 3,4-methylenedioxy bridge showed moderate to poor inhibition of Sortase A (SrtA) in Streptococcus pyogenes. Despite the large error, we can confidently say that substituting a methyl or methoxy on the 4' position of bridged chalcones significantly negatively affects inhibition compared to unsubstituted and unbridged chalcone, with a chlorine substitution in the same position appearing to inhibit even worse, albeit slightly. This is in contrast to the work of Sebastian Dales' team in Spring quarter of 2024 which shows 3,4-methylenedioxy chalcone with an inhibition rate comparable to our unsubstituted chalcone (~40%). On the other hand, Shelby Fisher's team from that same quarter showed that a 4'-chloro chalcone with no bridge had higher inhibition (~25%) than our results with a bridge (~9%). This seems to suggest that the 3,4-methylenedioxy bridge alone did not have a significant negative effect on inhibition, but the bridge in combination with other substitutions on the 4' position did negatively affect SrtA inhibition.

Despite this, there are plenty of avenues left to explore when it comes to bridged chalcones. First of all, other substitutions on the 4' position could still potentially work, especially fluorine due to its high electronegativity. Substituting positions other than 4' could also work, such as adding a 3'-nitro. While adjusting the position of the methylenedioxy bridge on the B-ring is another potential route, these compounds do not appear in the library and would have to be synthesized or special ordered. Another potential route of interest would be exploring the inhibition of chalcones with a bridge on the A-ring instead of the B-ring. More broadly, analyzing percent inhibition of chalcones across bacterial species could provide some additional insight into their overall effects on SrtA inhibition. Finally, we would advise future groups interested in chalcones with a 3,4-methylenedioxy bridge to avoid or be cautious when choosing chalcones with a bromine on the 2 position as we observed that some of them (2-bromo-4'-chloro-3,4-methylenedioxy and 2-bromo-4,5-methylenedioxy-3'-nitro chalcones) exhibited unacceptably poor solubility in DMSO, methanol, and butanol. These compounds in particular may not be ideal for further research.

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