Discovering Antibiotic Activity of Anthranilic Acid Derivatives

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Introduction

We have all seen it in the news. Antibiotic resistance is increasing [1]. In fact, the World Health Organization has said that antibiotic resistance is becoming a very serious threat to global health [2]. With bacteria and fungi being able to readily adapt and fend off the drugs that were designed to kill them, new medicines will need to be made in order to protect us. A common household antibiotic drug that is used is penicillin. Bacteria has a guardian – an enzyme that works hard to build up the bacteria's outer defense. Penicillin inhibits this enzyme with a weapon called the β-Lactam ring structure [3] (**Figure One**). This weapon disables the enzyme and weakens the protective peptidoglycan outer layer of the bacteria. However, over time, bacteria have mutated to become heavily resistant to the β-Lactam ring [4]. New antibiotics with varied structures are needed to treat this new form of resistant bacteria.

Research Question

Our research project will focus on the synthesis and characterization of a different breed of antibiotics – anthranilic acid derivatives (**Figure Two**). Discovered at GVSU, we have a lead compound that has antibiotic activity while not having a β-Lactam ring. This chemical is called 2-amino-5-bromobenzoic acid and has a common name called anthranilic acid [5]. With this research project, we will be attaching different atoms and structures onto this lead compound via organic synthesis. These novel structures will then be biologically tested to see if we can discover something with more activity than the lead compound.

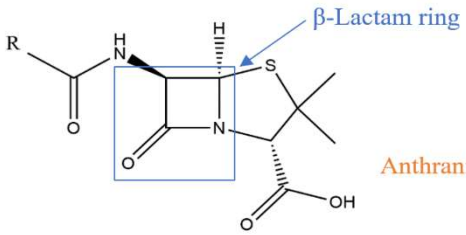

Figure One: Penicillin

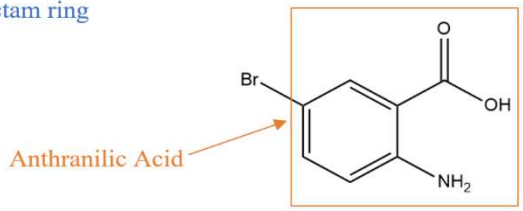

Figure Two: 2-amino-5-bromobenzoic acid

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[1] "About Antibiotic Resistance." *About Antibiotic Resistance*, Center for Disease Control and Prevention, www.cdc.gov/drugresistance/about.html.

[2] World Health Organization. Antimicrobial resistance: global report on surveillance. World Health Organization, 2014.

[3] Roderik Morgan Ph.D.; William Schroeder Ph.D.; Robert Smart Ph.D; and Linda Chamberlain Ph.D. Executive Director Technology Commercialization 270C DeVos Center Grand Valley State Unviersity grand Rapids MI 49504 616-331-7377 Carboxylic Amides as Antimicrobial Agents. pp.1. GVSU.

[4] Sauvage, Eric & Powell, Ailsa & Heilemann, Jason & Josephine, Helen & Charlier, Paulette & Davies, Christopher & Pratt, R.F.. (2008). Crystal Structures of Complexes of Bacterial DD-Peptidases with Peptidoglycan-mimetic Ligands: The Substrate Specificity Puzzle. Journal of molecular biology. 381. 383-93. 10.1016/j.jmb.2008.06.012.

[5] Sun, S., Selmer, M., & Andersson, D. I. (2014). Resistance to β-lactam antibiotics conferred by point mutations in penicillin-binding proteins PBP3, PBP4 and PBP6 in salmonella enterica. PLoS One, 9(5) doi:http://dx.doi.org/10.1371/journal.pone.0097202

Methods

The specific goals of this project are to (1) Chemically synthesize anthranilic acid derivatives, (2) Purify and characterize these new derivatives, and (3) have them tested for antibiotic activity. During this project, the synthesized chemicals will be characterized using modern spectroscopic methods including: ^{1}H-NMR, ^{12}C-NMR, thin layer chromatography, melting point, and infrared spectroscopy . Then, these chemicals will be tested for antibiotic properties against several strains of bacteria. Staphylococcus aureus (**Figure Three**) – which causes staph infections – will be one of the bacteria that the compounds will be tested against [6].

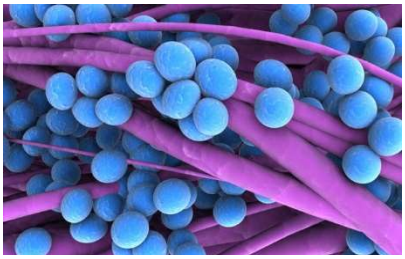

Figure Three: Staphylococcus aureus

One of the products synthesized was 4,5-dibromo-2-(undec-10-enamido)benzoic acid. The overall synthesis was a combination of 2-amino-4,5-dibromobenzoic acid and 10-undecenoylchloride in a solution of K_2CO_3 and 1,2-dimethoxyethane. Nucleophilic acyl substitution drives this reaction. The amine nitrogen of 2-amino-4,5-dibromobenzoic acid attacks the carbonyl of the alkenoyl chloride in the solution of K_2CO_3 and 1,2-dimethoxyethane. The chloride is the leaving group. A sample reaction can be seen below (**Figure Four**).

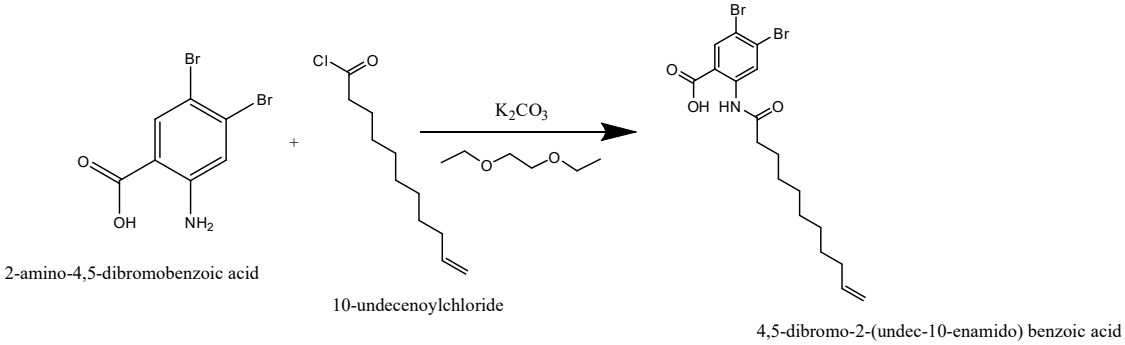

Figure Four: Nucleophilic acyl substitution reaction

[6] What Causes Staph Infections, and How Can You Avoid Them? 2019, Cleveland Clinic, Cleveland. health.clevelandclinic.org/staph-infections-what-are-they-and-when-should-you-worry/.

Results

These new derivatives of anthranilic acid will help establish antibiotic structure activity relationships. We will modify the alkyl chain of the lead structure as well as different atoms attached to the base of the molecule to find a relationship between the structures and their antibiotic activity.

This research project includes data collection via ^{1}H-NMR, ^{12}C-NMR, thin layer chromatography, melting point, and infrared spectroscopy to determine purity for biological testing. An example of how this will be tested for biological activity, can be seen in area 13 (**Figure Five**) – treated with 5-chloro-2-(undec-10-enamido) benzoic acid – where the inability of Staphylococcus aureus to grow into that area shows that this chemical was active against the bacteria [7]. An example of how this will be characterized is shown below (**Figure Six**). More data results are pending return to the lab.

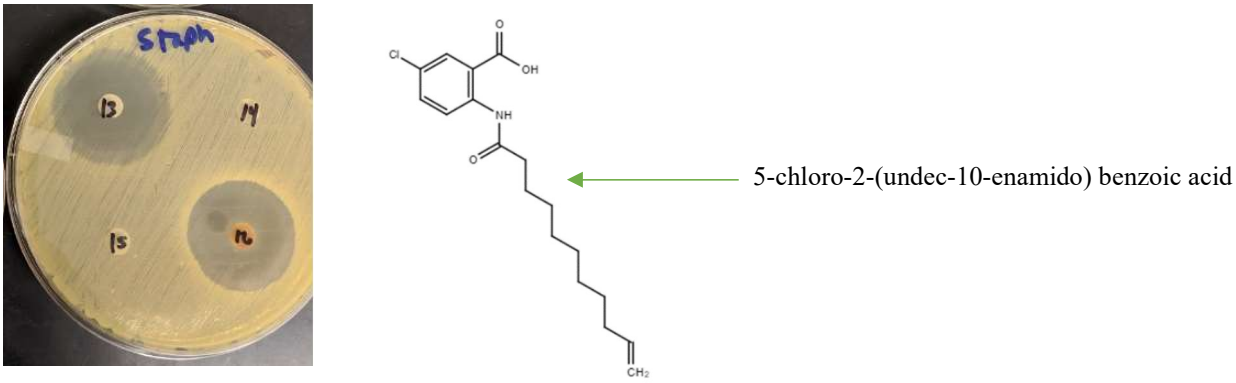

Figure Five: 5-chloro-2-(undec-10-enamido) benzoic acid against a Staphylococcus aureus colony [7]

3,5-dibromo-2-(undec-10-enamido) benzoic acid		38.9	186-207 and 183-202	0.94	-MP and IR shows some impurities.

Figure Six: Characterized for 3,5-dibromo-2-(undec-10-enamido)benzoic acid

[8] Witucki, Laurie. "Activity for compounds, with compound list." Received by the author

Discussion of Results

The synthesis of 4,5-dibromo-2-(undec-10-enamido) benzoic acid had a yield of 38.9%. The overall synthesis was a combination of 2-amino-4,5-dibromobenzoic acid and 10-undecenoylchloride in a solution of K_2CO_3 and 1,2-dimethoxyethane. The melting points upon recrystallization were 186-207°C and 183-202°C. These melting points suggest impurities. A TLC plate was ran using 75% ethyl acetate, 25% hexanes, and 2% acetic acid as the mobile phase. The TLC ran for about 10 minutes and notably, the reactants had a color change to yellow. The Rf value in the substrate lane was 0.94. The Rf values in the product lane were 0.94 and 0.80. The Rf values in the co lane were 0.94 and 0.80. This specific chemical is not considered pure until an NMR is taken and analyzed. Data from other chemicals are currently unavailable pending return to the lab.

Future Work

Overall, the chemistry synthesis worked well, but there is still much analysis and purification that needs to happen. Research in the future will include an experiment to test different reagents in an attempt to increase yield. Additional research will include brominating of the alkenes to see how this change will impact the structure activity relationship with bacteria. After purification and further analysis of the data, these chemicals will be able to undergo microbial testing with different kinds of bacteria and find the relationship between the structure and the activity with the bacteria.