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Comparing Beta-lLctamase Inhibition Activity of Phthalic Acid Derivatives

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Title
Structure-Activity Relationship of Inhibition of Bacterial Beta-Lactamase by Phthalic Acid Derivatives Using Colorimetric Assay

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Abstract
As a Cell and Molecular major with a Pre-Pharmacy emphasis, medicine has been an interest of mine. The major cause of increasing bacterial resistance to beta-lactam antibiotics is due to the expression of the enzyme beta-lactamase in bacteria. This leads to the inactivation of the antibiotics and preventing cell death. The currently known inhibitors of beta-lactamase are clavulanic acid, sulbactam, and tazobactam. These are the only inhibitors that have reached clinical importance. Phthalic acids derivatives have been identified as potential inhibitors. Phthalic acid, Terephthalic acid, and 1,2-Phenylenediacetic acid were tested for beta-lactamase inhibition at various concentrations to determine the IC50 values. The data showed that terephthalic acid was the most inhibitory of beta-lactamase. The IC50 for terephthalic acid was not fully determined, however the smallest concentration of terephthalic acid tested,1.5 mM, yielded a beta-lactamase activity of 34%, in other words an 66% inhibition. The IC50 values for phthalic acid and 1,2-Phenylenediacetic acid were 8.0 +/- 0.6 mM and 2.8 +/- 0.8 mM, respectively. Phthalic acid and 1,2-Phenylenediacetic acid are both similar in structure and inhibition of beta lactamase. Terephthalic is the most different with its carboxyl groups spread out the furthest. This could point towards the distance of the carboxyl groups having a greater impact on inhibition of beta-lactamase. Although all three phthalic acid derivatives showed the ability to inhibit b-lactamase, it is unlikely that these molecules would make it to the clinical level due to the high concentration of each molecule needed to inhibit beta-lactamase activity. Clinical drugs used are in the concentration of nanomolar while this experiment showed that millimolar concentrations were necessary for inhibition. In the future, I hope another student continues this research and takes a further look into the effect of the distance of the carboxyl group has on the inhibition of beta-lactamase activity.

Introduction

Beta-lactam antibiotics work by blocking cell wall synthesis of the bacteria and are a common drug used to treat bacterial infections. A major problem developing today in the world is the increase in bacteria being resistant to beta-lactam antibiotics. (1,4) The major cause of increasing bacterial resistance to beta-lactam antibiotics is due to the expression of the enzyme beta-lactamase in bacteria (1,4). Beta-lactamase is able to hydrolyzes beta-lactam antibiotics in bacteria that contain the beta-lactamase coding gene(1). This leads to the inactivating of the antibiotics and preventing cell death. This problem has provided a challenge in finding beta-lactamase inhibitors so these beta-lactam antibiotics can continue to kill bacterial infections.

There are a few inhibitors that have been found already for beta-lactamase. The currently know inhibitors are clavulanic acid, sulbactam, and tazobactam(2). Each of these are the only three inhibitors that have reached clinical importance(2). However, some other potential inhibitors of beta-lactamase have been identified, including phthalic acid derivatives.

A research study performed by Yukiko Hiraiwa, Akihiro Morinaka, Takayoshi Fukushima, and Toshiaki Kudo has given evidence to this. In their study, they set out to find Metallo-b-lactamase inhibitory activity of phthalic acid derivatives and found that the two carboxyl groups of phthalic acid derivative compounds might interact with zinc in the active site of IMP-1, which

could then inhibit the beta-lactamase produced. They then fixed the two carboxyl groups and investigated the effect of substitution of the phenyl ring on metallo-beta-lactamase inhibitory activity.(3) In the experiment 4-butyl-3-methylphthalic acid was recognized as a metallo-beta-lactamase inhibitor. The structure–activity relationship study of substituted phthalic acids afforded 3-phenylphthalic acid derivatives as potent metallo-beta-lactamase inhibitors.(3)

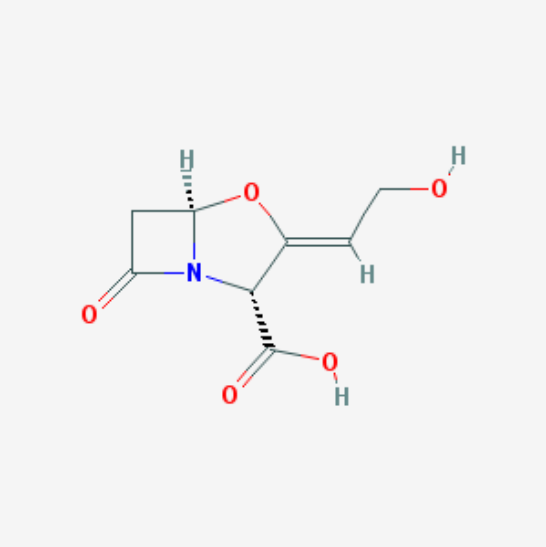
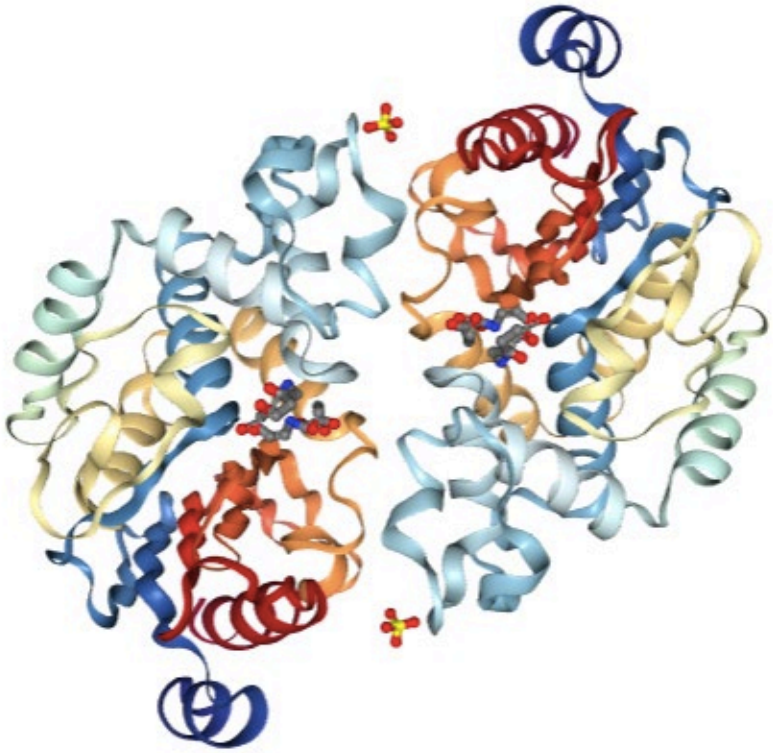
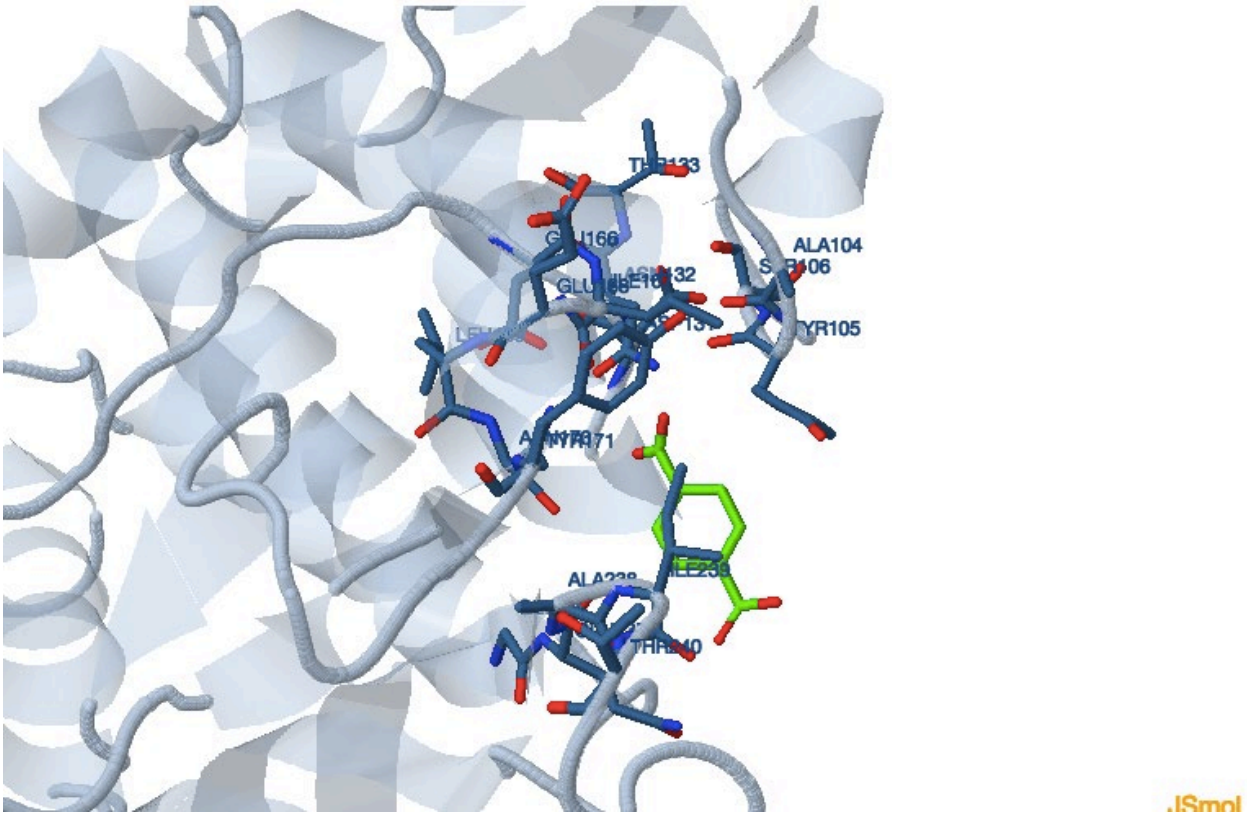


Figure 1: Clavulanic acid

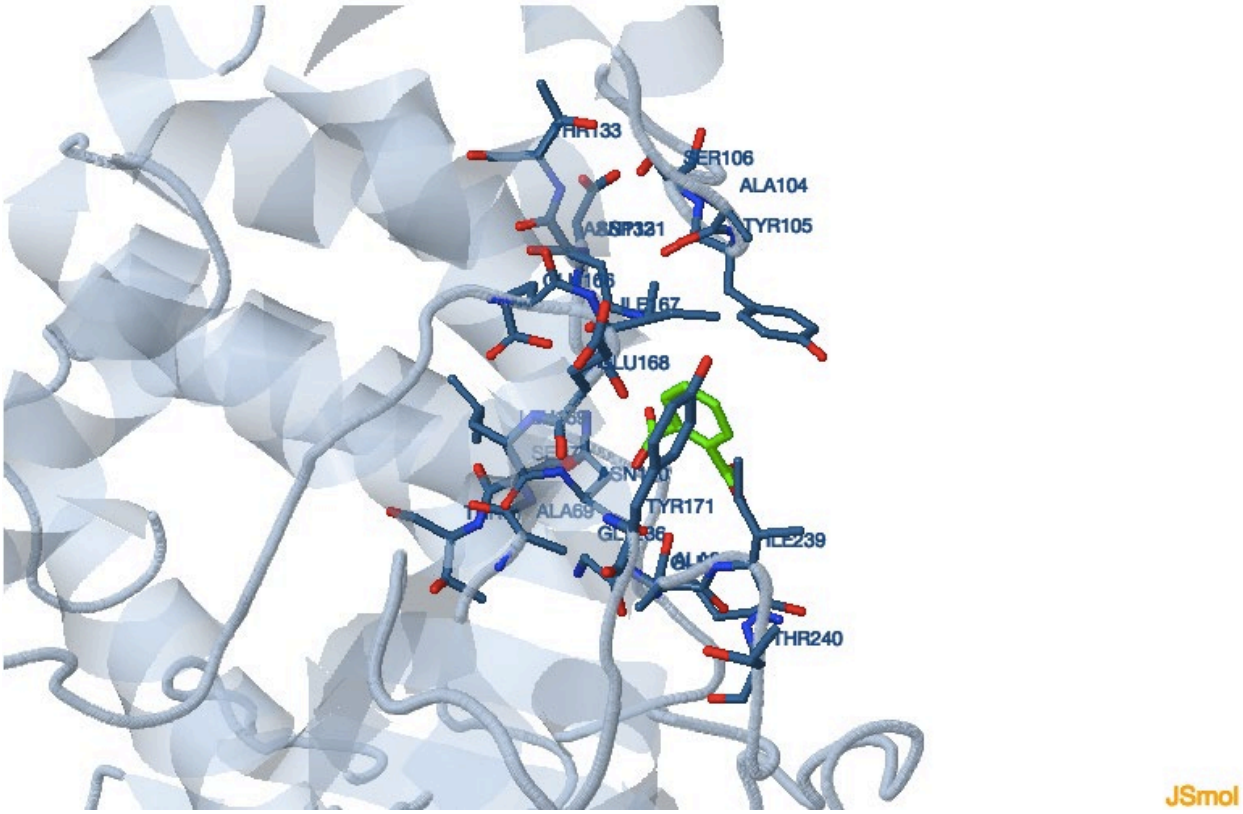


Inhibition of beta-lactamase by clavulanate. Trapped intermediates in cryo crystallographic studies. [5,6,7]

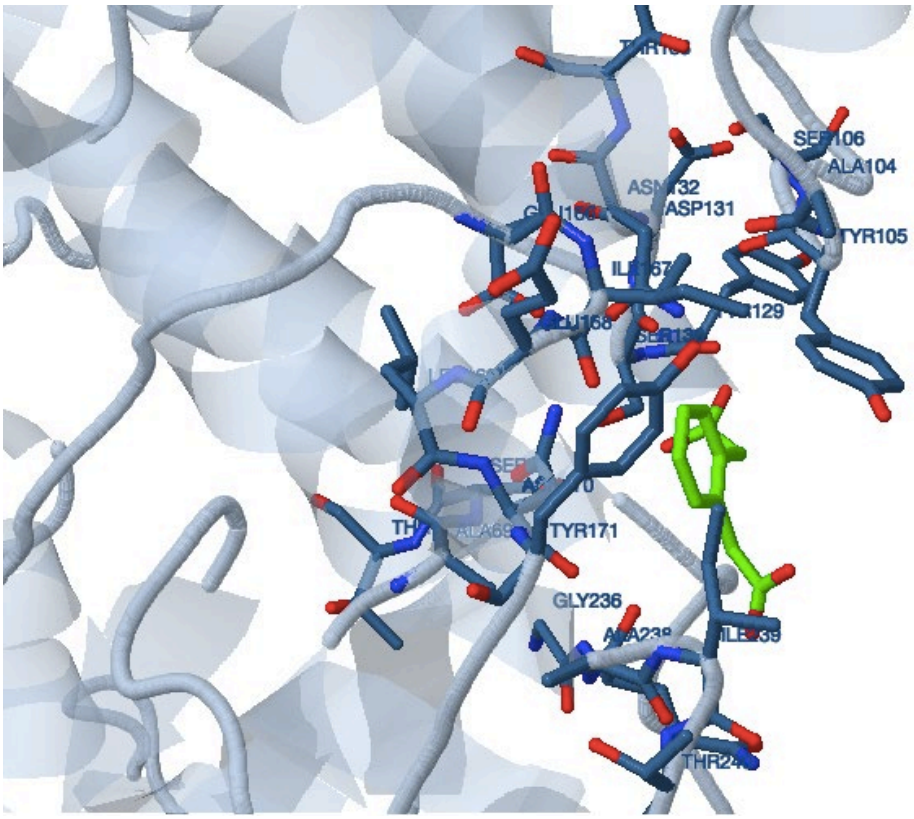
The docking simulation of Terephthalic acid at the active site of beta-lactamase (1BLC). The calculated KI was 2.1 mM [8].



The docking simulation of phthalic acid at the active site of beta-lactamase (1BLC). The calculated KI was 2.2 mM [8]

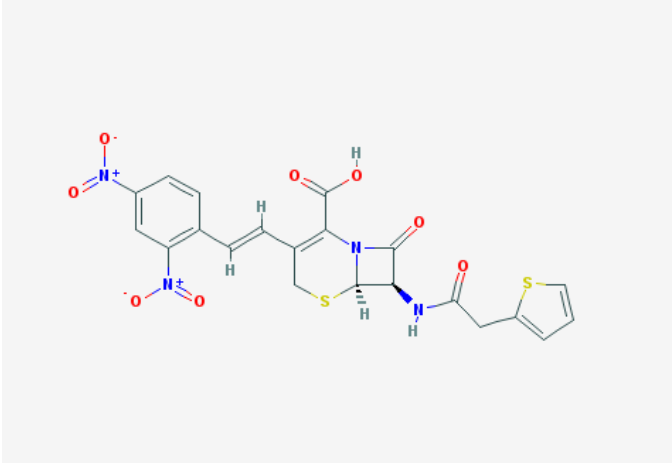


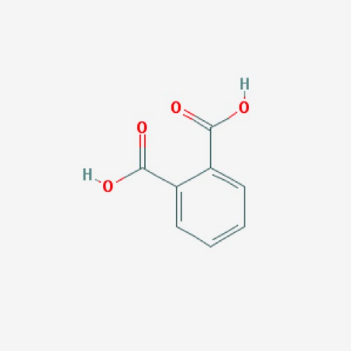
The docking simulation of 1,2-Phenylenediacetic acid at the active site of beta-lactamase (1BLC). The calculated KI was 1.4 mM. [8]

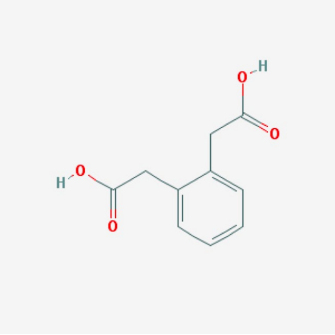


My proposal is to use the beta-Lactamase enzyme activity to find the beta-lactamase inhibiting ability for phthalic acid, 1,2-phenylenediacetic acid, and terephthalic acid.

Beta-lactamase enzyme activity was assayed colorimetrically at 490 nm using the Sigma Aldrich Beta-Lactamase Screening Kit (MAK222). The activity was measured by monitoring a colored product by hydrolyzing the lactam ring of a substrate, nitrocefin. The structure of nitrocefin shown below.







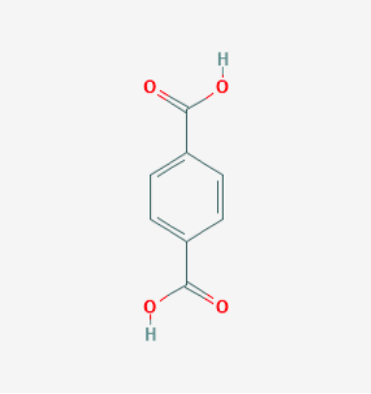


Figure 2: Phthalic Acid Figure 3: 1,2-Phenylenediacetic Acid Figure 4: Terephthalic Acid

Materials and Methods

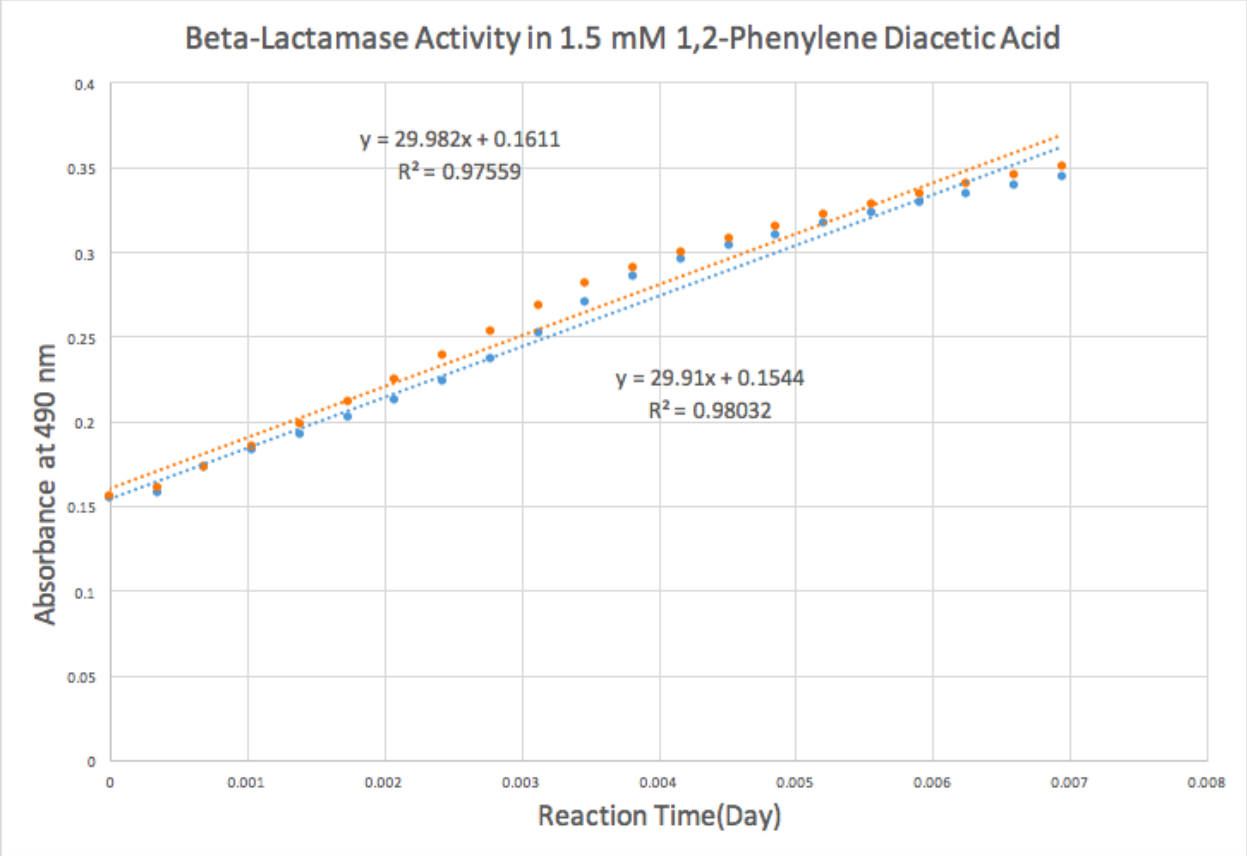
Phthalic acid, terephthalic acid, 1,2-phenylenediacetic acid, the Beta-Lactamase Screening Kit (MAK222) including beta-lactamase, beta-lactamase assay buffer and clavulanic acid, and nitrocefin were purchased from Sigma Aldrich. Concentrations of 500mM of Phthalic acid, terephthalic acid, and 1,2-phenylenediacetic acid were made using the the drugs and DMSO. From the 500mM concentration, concentrations of 1.5mM, 2.5mM, 5mM, 25mM, and 50mM were made for each drug. 30ul of enzymatic reaction mix consisting of 29uL b-Lactamase Assay Buffer and 1uL Nitrocefin was added to 20 uL of each drug. The drug mixtures were then incubated at 25 °C for 10 minutes. A blank sample without inhibitor was ran to establish a 100% activity level. Clavulanic acid was also used as a known inhibitor of beta-lactamase activity to have a control to compare results to. 30 uL enzyme was added to initiate the reaction. The absorbance at 490nm was measured using a SpectraMax M5 microplate reader from Molecular Devices in kinetic mode for 10 minutes, taking the absorbance reading every 30 seconds. The data was then used to see beta-lactamase activity over time for each concentration to establish an activity curve.

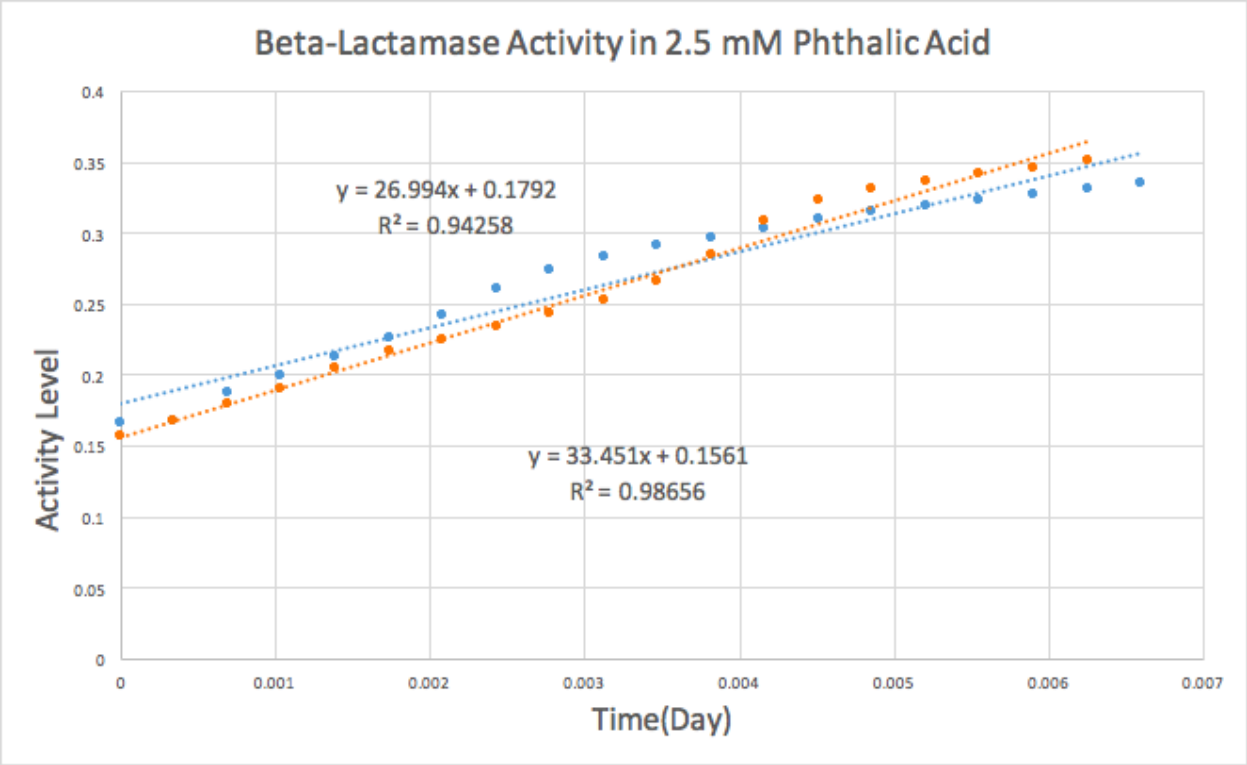
1. Using phthalic acid and DMSO, make a 500mM concentration in order to make additional concentration strengths to be tested. Add 249.21mg of phthalic acid with 3mL of DMSO.
2. Make concentrations from the 500mM concentration of 1.5mM, 2.5mM, 5mM, 25mM 50mM
3. To make 1.5mM solution, add 3ul of the 500mM stock solution, 750ul of beta lactamase assay buffer, and 247ul DMSO. Pipet 20ul into the first two wells of row A in the microplate.
4. To make 2.5mM solution, add 5ul of the 500mM stock solution, 750ul of beta lactamase assay buffer, and 245ul DMSO. Pipet 20ul into the first two wells of row B in the microplate.
5. To make 5mM solution, add 10ul of the 500mM stock solution, 750ul of beta lactamase assay buffer, and 240ul DMSO. Pipet 20ul into the first two wells of row C in the microplate.
6. To make 15mM solution, add 30ul of the 500mM stock solution, 750ul of beta lactamase assay buffer, and 220ul DMSO. Pipet 20ul into the first two wells of row D in the microplate.
7. To make 25mM solution, add 50ul of the 500mM stock solution, 750ul of beta lactamase assay buffer, and 200ul DMSO. Pipet 20ul into the first two wells of row E in the microplate.

8. To make 50mM solution, add 100ul of the 500mM stock solution, 750ul of beta lactamase assay buffer, and 150ul DMSO.
9. Set up Inhibition Reaction Mixes consisting of 48uL of b-Lactamase Assay buffer and 2ul of b-Lactamase. 50ul of the Inhibition Reaction Mix is required for each well.
10. Add 50ul of the appropriate Inhibition Reaction Mix to each well. Mix well by pipetting.
11. Incubate the plate at 25 °C for 10 minutes. Protect the plate from light during the incubation
12. Set up an Enzymatic Reaction Mix consisting of 29uL b-Lactamase Assay Buffer and 1uL Nitrocefin. 30uL of the Enzymatic Reaction Mix is required for each well.
13. Add 30 uL of the Enzymatic Reaction Mix to each reaction well. Mix well using a pipette.
14. Measure the absorbance (ABS, A490) in a microplate reader in kinetic mode for 10–30 minutes. Take absorbance readings every minute

15. Using terephthalic acid and b-Lactamase Assay, make a 500mM concentration in order to make additional concentration strengths to be tested. Add 249.195mg with 3 mL of b-Lactamase Assay.
16. Repeat steps 2-14
17. Using homophthalic acid and b-Lactamase Assay, make a 500mM concentration in order to make additional concentration strengths to be tested. Add 180.16mg of homophthalic acid with 3mL of b-Lactamase Assay.
18. Repeat steps 2-14.
19. Using 1,2 Phenylenediacetic acid and b-Lactamase Assay, make a 500mM concentration in order to make additional concentration strengths to be tested. Add 291.27mg of 1,2 Phenylenediacetic acid with 3mL b-Lactamase Assay.
20. Repeat steps 2-14.
21. Run a blank sample consisting of 50uL and 30uL Enzymatic Reaction Mix to establish a 100% activity level can be established.

Results


Beta-Lactamase Activity in 1.5 mM 1,2-Phenylene Diacetic Acid
y = 29.982x + 0.1611
R² = 0.97559
y = 29.91x + 0.1544
R² = 0.98032
Absorbance at 490 nm
Reaction Time(Day)
0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
0
0.001
0.002
0.003
0.004
0.005
0.006
0.007
0.008



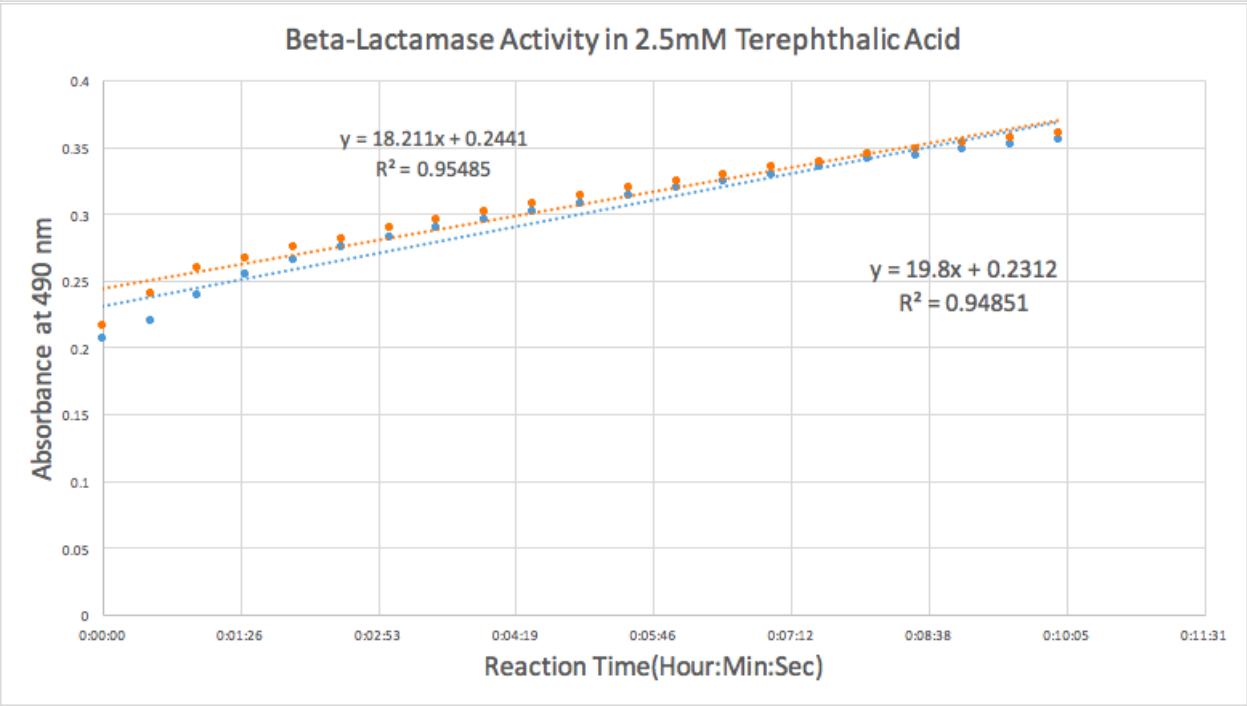


Figure 5: Beta-Lactamase activity graphs for terephthalic acid, phthalic acid, and 1,2-phenylene diacetic acid. The two lines represent the two wells ran for each inhibitor.

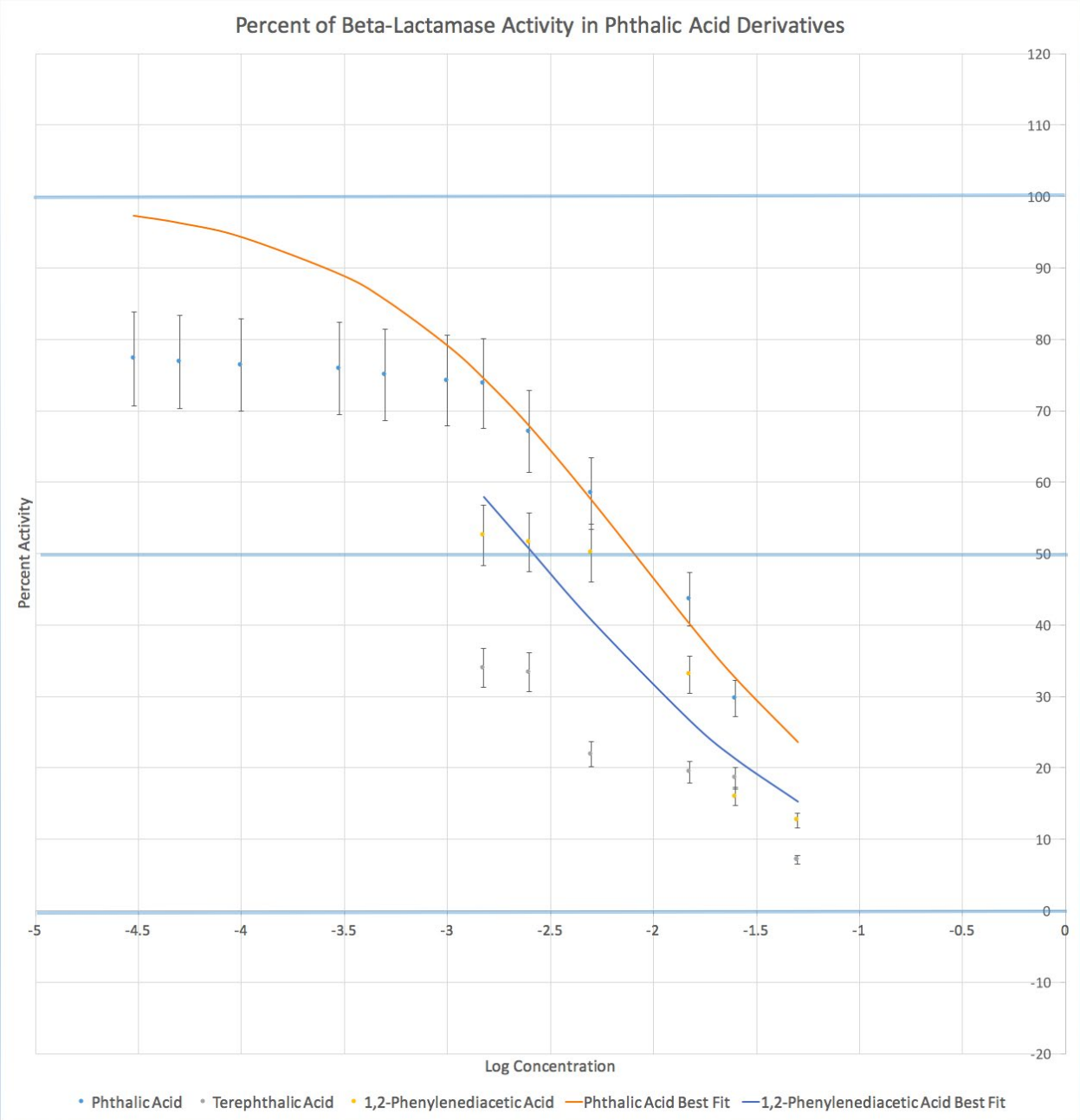
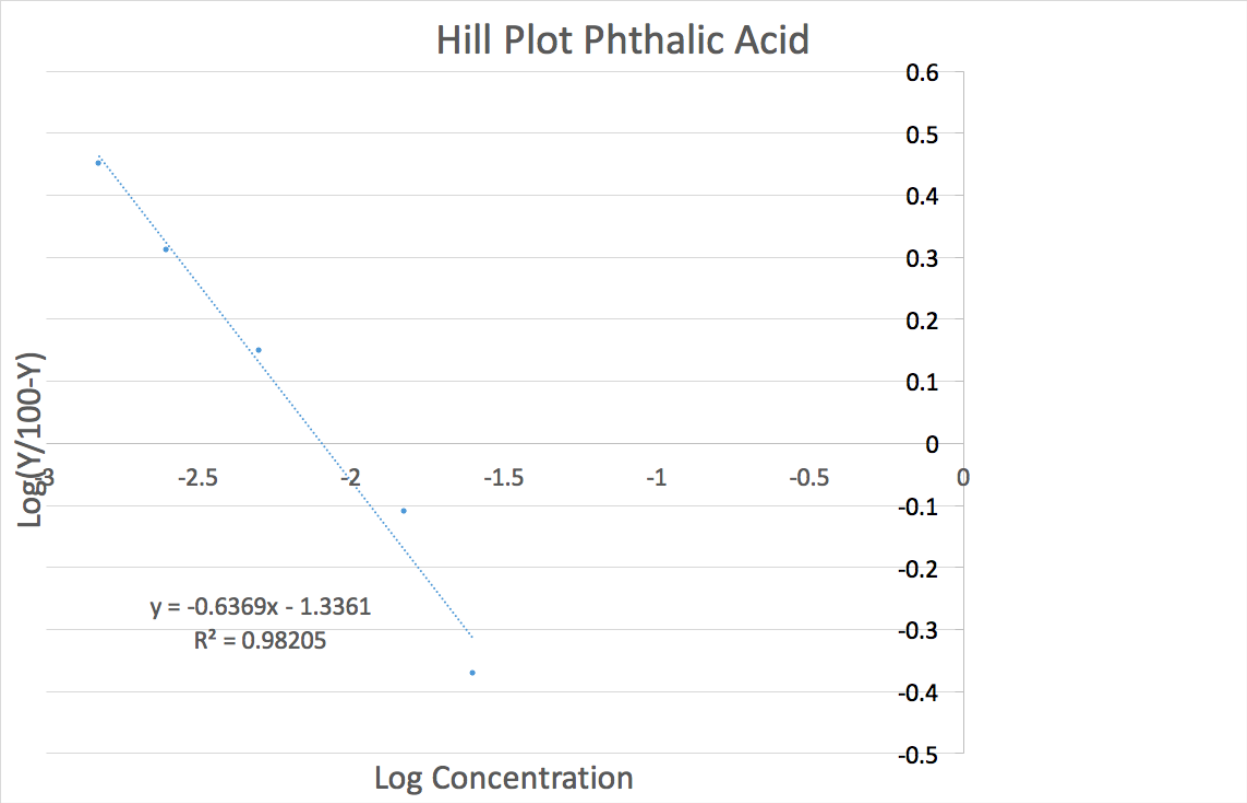


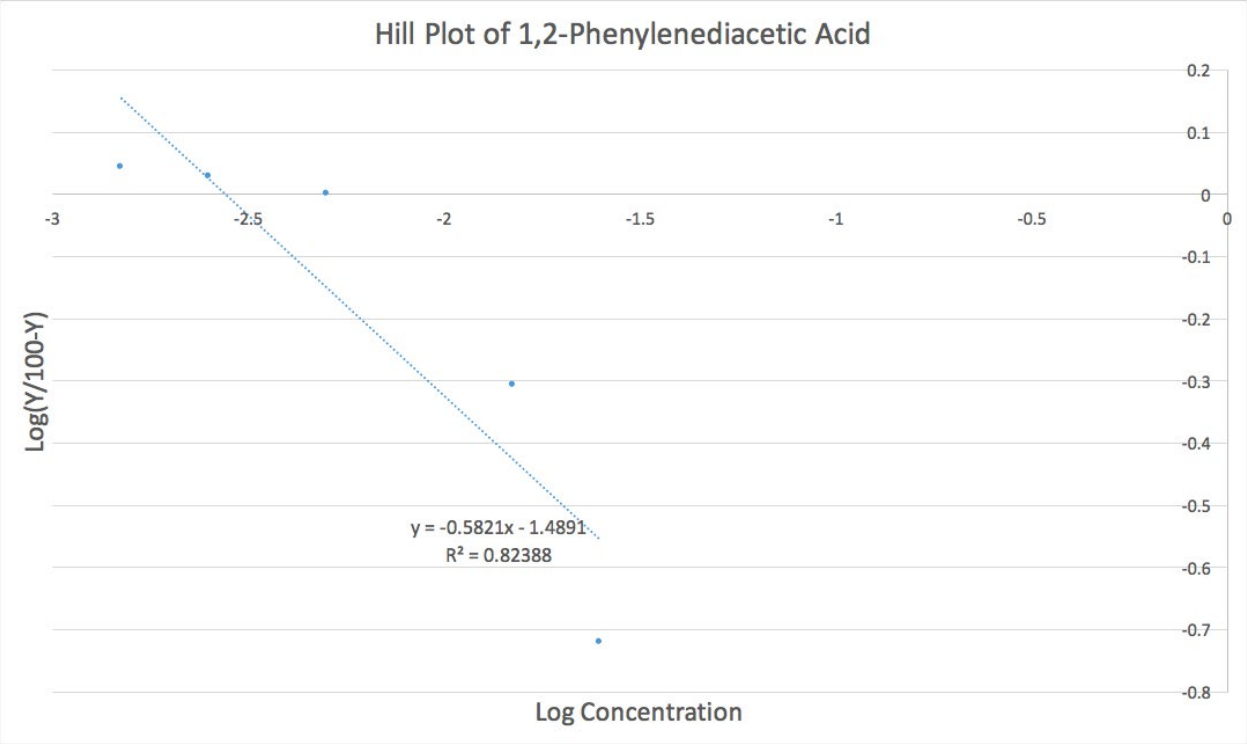
Figure 6: The beta-lactamase activity is shown at each concentration level of the three inhibitors tested. The error bar are individually set to the error for each data point gathered.

The data analyse consisted of graphing the increase in absorbance at 490 nm as a function of reaction time for each concentration of the inhibitor and getting an average slope for the two trials. The average slope of activity was then divided by that of activity without an inhibitor to determine the percent activity for the varying concentrations. The percent activity was then graphed against common log of the inhibitor concentration. These values were regraphed to construct the Hill plot to determine the Hill coefficient and at what concentration each molecule inhibited b-lactamase 50%(IC 50). The Hill plot was constructed by plotting Log (Y/(1-Y)) as a function of Log X, where Y is the percent activity and X is the molar concentration of the inhibitor. The Hill coefficient and IC50 were determined from the slope and the x-intercept of the Hill plot. The best fit curve was obtained using the following equation:

$$f(x) = Max - \frac{Max - Min}{1 + (\frac{X}{I50})^{\ Hill}}$$

Where Max is the maximum activity, Min is the minimum activity, X is the molar concentration of the inhibitor, Hill is the Hill coefficient from the Hill plot.





Discussion

The purpose of this study was to determine if phthalic acid, terephthalic acid, and 1,2-phenylenediacetic acid could inhibit beta-lactamase activity and then compare them against one another by finding the IC50 for each inhibitor. In the research study referenced in the intro, it was inferred that the carboxyl groups of 4-butyl-3-methylphthalic acid were the major factor that lead it to being an inhibitor [3]. Due to this information, it was predicted that 1,2-phenylenediacetic acid would be the best inhibitor due to its large functional groups and their close placement to one another on the molecule. It was determined that these inhibitors were not very successful inhibitors of beta-lactamase. The results seem to be confirmed with the docking simulation. The simulation shows that the inhibitors chosen don't specifically bond to the active site, making it a non-successful inhibitor.

The prediction that 1,2-phenylenediacetic acid would be the best inhibitor was not supported by the data gathered from this experiment. Looking at the graph in Figure 6, it is clear that all of the molecules inhibited the beta-lactamase activity. The data showed that actually terephthalic acid was the most inhibitory of beta-lactamase. The IC50 for terephthalic acid was not able to be determined, however the smallest concentration of terephthalic acid tested,1.5 micromolar, yielded a b-lactamase activity of 34%, in other words an inhibition rate of 66%. The IC 50 values found for phthalic acid and 1,2-phenylenediacetic acid was 8.0 +/- 0.6 mM and 2.8 +/- 0.8 mM,

respectively. This shows that 1,2-phenylenediacetic acid was slightly better inhibitor than phthalic acid. Phthalic acid and 1,2-phenylenediacetic acid are both similar in structure and inhibition of beta-lactamase. Terephthalic is the most different in structure with its carboxyl groups spread out the furthest. This could point towards the distance of the carboxyl groups having a greater impact on inhibition of beta-lactamase. Even though these molecules showed the ability to inhibit b-lactamase activity, it is unlikely that these molecules would make it to the clinical level due to the large concentration of each molecule needed to inhibit b-lactamase activity. Clinical drugs used are in nanomolar concentration while this experiment showed that micromolar concentrations were necessary for inhibition.
One challenge that arose during this experiment was the limited source of beta-lactamase buffer. The protocol required a lot of it and only 25mL was able to be purchased due to high costs. As seen in Fig.4, phthalic acid has more data points than 1,2-phenylenediacetic acid and terephthalic acid. This was due to phthalic acid being used first to determine what concentrations would produce 50% inhibition on b-lactamase. Once determining a set of concentrations that provided a thorough range of inhibition, those concentrations were also used for terephthalic acid and 1,2-phenylenediacetic acid. Another challenge that arose was that during the pipetting of the enzyme reaction mix into the wells, air bubbles would form. It was difficult to get rid of all the air bubbles and they may have impacted the data obtained. The original protocol also called for the potential inhibitors to be dissolved into beta-lactamase buffer but due to the insolubility of the inhibitors, DMSO had to be used instead. The activity versus time graph was also a challenge to deal with due to the graph leveling off fast and being non-linear. This occured when low concentrations of the inhibitors were used and introduced lare errors when the activity of beta-lactamase was high.

Conclusion

According to the results gathered from this experiment, phthalic acid, 1,2-phenylenediacetic acid, and terephthalic acid are all inhibitors or b-lactamase activity. While it was predicted that 1,2-phenylenediacetic acid would have the best inhibition, it was actually terephthalic acid with the best rate of inhibition. IC 50 values found for phthalic acid and 1,2-phenylenediacetic acid was 8.0 +/- 0.6 mM and 2.8 +/- 0.8 mM, respectively. respectively. Even though these molecules showed the ability to inhibit beta-lactamase activity, it is unlikely that these molecules would make it to the clinical level due to the large amount of each molecule needed to inhibit beta-lactamase activity. Clinical drugs used are in the concentration of nano molar while this experiment showed that micro molar concentrations were necessary for inhibition. In the future, I hope other students continues this research and take a further look into the effect of the distance of the carboxyl groups has on the inhibition of beta-lactamase activity.

Acknowledgements

A large thank you goes to Winona State University for supporting and funding this research with the Winona State Student Research Grant. Another thank you goes out to the WInona State Chemistry department for letting me use the laboratory, equipment, and chemicals necessary to perform this experiment.

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ABSTRACT

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MATERIALS and METHODOLOGY

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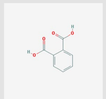


Figure 2: Phthalic Acid

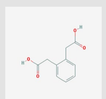


Figure 3: 1,2-Phenylenediacetic Acid

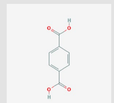
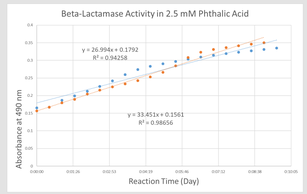
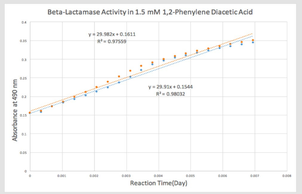


Figure 4: Terephthalic Acid

RESULTS





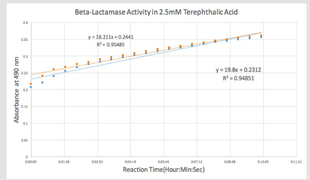
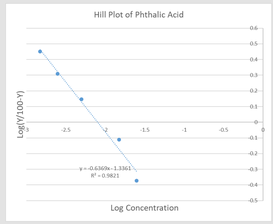
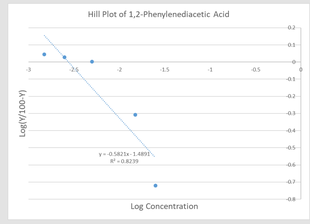


Figure 5: Beta-lactamase activity graphs for phthalic acid, 1,2-phenylenediacetic acid, and terephthalic acid. The two lines represent the two trials for each inhibitor.

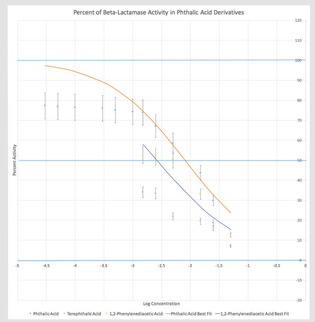
The data analysis consisted of graphing the increase in absorbance at 490 nm as a function of reaction time for each concentration of the inhibitor and getting an average slope for the two trials. The average slope of activity was then divided by that of activity without an inhibitor to determine the percent activity for the varying concentrations. The percent activity was then graphed against common log of the inhibitor concentration. These values were then used to construct the Hill plot to determine the Hill coefficient and the concentration of each molecule at 50% beta-lactamase activity (IC50). The Hill plot was constructed by plotting Log (Y/(100-Y)) as a function of Log X, where Y is the percent activity and X is the molar concentration of the inhibitor.





The Hill coefficient and IC50 were determined from the slope and the antilog of the x-intercept of the Hill plot.

Figure 6: Percent activity versus log concentration
The best fit curve was obtained using the following equation:



$$f(x) = Max - \frac{Max - Min}{1 + (\frac{X}{I50})^{\ Hill}}$$

where Max is the maximum activity, Min is the minimum activity, X is the molar concentration of the inhibitor, and Hill is the Hill coefficient from the Hill plot. The percent beta-lactamase activity is shown at each concentration level of the three inhibitors tested. The error bar are individually set to the error for each data point gathered.

ACKNOLEDGMENTS

A large thank you goes to Winona State University for supporting and funding this research with the Winona State Student Research Grant. Another thank you goes out to the Winona State University Chemistry department for letting me use the laboratory, equipment, and chemicals necessary to perform this experiment.

DISCUSSION

The purpose of this study was to determine if phthalic acid, terephthalic acid, and 1,2-phenylenediacetic acid could inhibit beta-lactamase activity and then compare them against one another by finding the IC50 for each inhibitor. In the research study referenced in the intro, it was inferred that the carboxyl groups of 4-butyl-3-methylphthalic acid were the major factor that lead it to being an inhibitor (3). Due to this information, it was predicted that 1,2-phenylenediacetic acid would be the best inhibitor due to its longer and more flexible functional groups and their close placement to one another on the molecule. It was determined that these inhibitors were not very successful inhibitors of beta-lactamase. The results seem to be confirmed with the docking simulation. The simulation shows that the inhibitors chosen don't specifically bond to the active site, making them nonspecific inhibitors.

The prediction that 1,2-phenylenediacetic acid would be the best inhibitor was not supported by the data gathered from this experiment. Looking at the graph in Figure 6, it is clear that all of the molecules inhibited the beta-lactamase activity. The data showed that actually terephthalic acid was the most inhibitory of beta-lactamase. The IC50 for terephthalic acid could not be determined, however the smallest concentration of terephthalic acid tested, 1.5 mM, yielded a beta-lactamase activity of 34%, in other words an inhibition rate of 66%. The IC 50 values found for phthalic acid and 1,2-phenylenediacetic acid was 8.0 ± 0.6 mM and 2.8 ± 0.8 mM, respectively. This shows that 1,2-phenylenediacetic acid was slightly better inhibitor than phthalic acid. Phthalic acid and 1,2-phenylenediacetic acid are both similar in structure and inhibition of beta-lactamase. Terephthalic is the most different in structure with its carboxyl groups spread out the furthest. This could point towards the distance of the carboxyl groups having a greater impact on inhibition of beta-lactamase. Even though these molecules showed the ability to inhibit b-lactamase activity, it is unlikely that these molecules would make it to the clinical level due to the large concentration of each molecule needed to inhibit beta-lactamase activity. Clinical drugs used are in the concentration of nanomolar while this experiment showed that millimolar concentrations were necessary for inhibition.

One challenge that arose during this experiment was the limited source of beta-lactamase buffer. The protocol required a lot of it and only 25mL was able to be purchased due to high costs. As seen in Fig.6, phthalic acid has more data points than 1,2-phenylenediacetic acid and terephthalic acid. This was due to phthalic acid being used first to determine what concentrations would produce 50% inhibition on beta-lactamase. Once determining a set of concentrations that provided a thorough range of inhibition, those concentrations were also used for terephthalic acid and 1,2-phenylenediacetic acid. Another challenge that arose was that during the pipetting of the enzyme reaction mix into the wells, air bubbles would form. It was difficult to get rid of all the air bubbles and they may have impacted the data obtained. The original protocol also called for the potential inhibitors to be dissolved into beta-lactamase buffer but due to the insolubility of the inhibitors, DMSO had to be used instead. The activity versus time graph was also a challenge to deal with due to the graph leveling off fast and being non-linear. This occurred when low concentrations of the inhibitors were used and introduced large errors when the activity of beta-lactamase was high.

CONCLUSION

According to the results gathered from this experiment, phthalic acid, 1,2-phenylenediacetic acid, and terephthalic acid are all inhibitors or b-lactamase activity. While it was predicted that 1,2-phenylenediacetic acid would have the best inhibition, it was actually terephthalic acid with the best rate of inhibition. Even though these molecules showed the ability to inhibit beta-lactamase activity, it is unlikely that these molecules would make it to the clinical level due to the large amount of each molecule needed to inhibit beta-lactamase activity. Clinical drugs used are in the concentration of nanomolar while this experiment showed that millimolar concentrations were necessary for inhibition. In the future, I hope other students continues this research and take a further look into the effect of the distance of the carboxyl groups has on the inhibition of beta-lactamase activity.

INTRODUCTION

Beta-lactam antibiotics work by blocking cell wall synthesis of the bacteria and are a common drug used to treat bacterial infections. A major problem developing today in the world is the increase in bacteria being resistant to beta-lactam antibiotics(1,4). The major cause of increasing bacterial resistance to beta-lactam antibiotics is due to the expression of the enzyme beta-lactamase in bacteria (1,4). Beta-lactamase is able to hydrolyzes beta-lactam antibiotics in bacteria that contain the beta-lactamase coding gene(1). This leads to the inactivating of the antibiotics and preventing cell death. This problem has provided a challenge in finding beta-lactamase inhibitors so these beta-lactam antibiotics can continue to kill bacterial infections.

There are a few inhibitors that have been found already for beta-lactamase. The currently know inhibitors are clavulanic acid, sulbactam, and tazobactam(2). These are the only three inhibitors that have reached clinical importance(2). However, some other potential inhibitors of beta-lactamase have been identified, including phthalic acid derivatives.

A research study performed by Yukiko Hiraiwa, Akihiro Morinaka, Takayoshi Fukushima, and Toshiaki Kudo has given evidence to this. In their study, they set out to find metallo-beta-lactamase inhibitory activity of phthalic acid derivatives and found that the two carboxyl groups of phthalic acid derivative compounds might interact with zinc in the active site of the enzyme, which could then inhibit the beta-lactamase produced (3). They then fixed the two carboxyl groups and investigated the effect of substitution of the phenyl ring on metallo-beta-lactamase inhibitory activity(3). In the experiment 4-butyl-3-methylphthalic acid was recognized as a metallo-beta-lactamase inhibitor. The structure–activity relationship study of substituted phthalic acids afforded 3-phenylphthalic acid derivatives as potent metallo-beta-lactamase inhibitors(3).

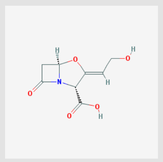
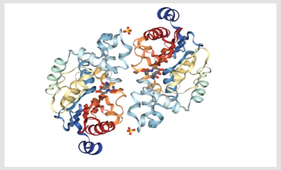


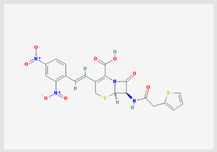
Figure 1: Clavulanic acid



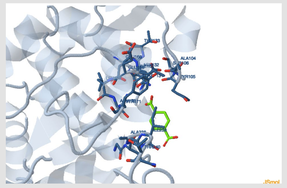
Inhibition of beta-lactamase by clavulanate. Trapped intermediates in cryo crystallographic studies (5,6,7).

My proposal is to use the beta-lactamase enzyme activity to find the inhibiting ability for phthalic acid, 1,2-phenylenediacetic acid, and terephthalic acid.

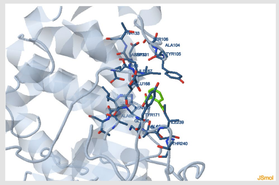
Beta-lactamase enzyme activity was assayed colorimetrically at 490 nm using the Sigma Aldrich Beta-Lactamase Screening Kit (MAK222). The activity was measured by monitoring a colored product by hydrolyzing the lactam ring of a substrate, nitrocefin. The structure of nitrocefin is shown below.



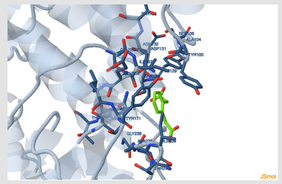
Docking simulation of Terephthalic acid at the active site of beta-lactamase (1BLC). The calculated KI was 2.1 mM (8).



Docking simulation of phthalic acid at the active site of beta-lactamase (1BLC). The calculated KI was 2.2 mM (8).



Docking simulation of 1,2-Phenylenediacetic acid at the active site of beta-lactamase (1BLC). The calculated KI was 1.4 mM (8).



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WINONA STATE UNIVERSITY
UNDERGRADUATE STUDENT RESEARCH & CREATIVE PROJECTS FINAL REPORT

Electronically submit complete final report ten (10) days following completion of project to Grants & Sponsored Projects (grants@winona.edu).
Hover over fill-able fields for additional guideline and completion information.

Student Name: Curtis Felton

Student Email: cfelton13@winona.edu

Student Major: Cell and Molecular Biology

Faculty Sponsor: Myoung Lee

Faculty Sponsor Email: mlee@winona.edu

Title of Project: Structure-Activity Relationship of Inhibition of Bacterial Beta-Lactamase by Phthalic Acid Derivatives Using Colorimetric Assay

Project Abstract:

As a Cell and Molecular major with a Pre-Pharmacy emphasis, medicine has been an interest of mine. The major cause of increasing bacterial resistance to beta-lactam antibiotics is due to the expression of the enzyme beta-lactamase in bacteria. This leads to the inactivation of the antibiotics and preventing cell death. The currently known inhibitors of beta-lactamase are clavulanic acid, sulbactam, and tazobactam. These are the only inhibitors that have reached clinical importance. Phthalic acids derivatives have been identified as potential inhibitors. Phthalic acid, Terephthalic acid, and 1,2-Phenylenediacetic acid were tested for beta-lactamase inhibition at various concentrations to determine the IC50 values. The data showed that terephthalic acid was the most inhibitory of beta-lactamase. The IC50 for terephthalic acid was not fully determined, however the smallest concentration of terephthalic acid tested,1.5 mM, yielded a beta-lactamase activity of 34%, in other words an 66% inhibition. The IC50 values for phthalic acid and 1,2-Phenylenediacetic acid were 8.0 +/- 0.6 mM and 2.8 +/- 0.8 mM, respectively. Phthalic acid and 1,2-Phenylenediacetic acid are both similar in structure and inhibition of beta lactamase. Terephthalic is the most different with its carboxyl groups spread out the furthest. This could point towards the distance of the carboxyl groups having a greater impact on inhibition of beta-lactamase. Although all three phthalic acid derivatives showed the ability to inhibit b-lactamase, it is unlikely that these molecules would make it to the clinical level due to the high concentration of each molecule needed to inhibit beta-lactamase activity. Clinical drugs used are in the concentration of nanomolar while this experiment showed that millimolar concentrations were necessary for inhibition. In the future, I hope another student continues this research and takes a further look into the effect of the distance of the carboxyl group has on the inhibition of beta-lactamase activity.

The student-authored final report **MUST** include each of the following (check boxes to verify inclusion of each component):

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☑ A copy of the project end product, appropriate to the standards of the discipline

Applicant Signature: Curtis Felton

Date: 11/28/2018

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