4-3-2019

Synthesis of An Ideal Inhibitor for the LpxC Enzyme

Taejun Lee
Andrews University, taejunl@andrews.edu

J. N. Andrews Honors Program

Andrews University

HONS 497

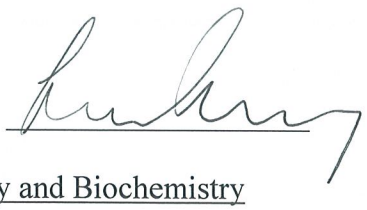
Honors Thesis

Synthesis of an ideal inhibitor for the LpxC enzyme

Taejun Lee

April 3, 2019

Advisor: Dr. Lisa Ahlberg, PhD

Primary Advisor Signature: 

Department: Chemistry and Biochemistry

Abstract

Due to the growing need of antibiotics in modern day medicine, this study attempts to apply a method of medicinal chemistry that would create a novel inhibitor for a lesser known target of gram negative bacteria, the LpxC enzyme. This study proposes that a synthetic plan which consists of combining components of different molecules would create a more effective inhibitor. These components include a binding group for the active site of the enzyme, a hydrophobic chain which has been shown to interact with an allosteric site on the LpxC enzyme, and the isoxazoline ring which interacts with the zinc ion. Recent developments have demonstrated that the inhibitor proposed may have had high inhibitory potential. Further, current research suggests that the current inhibitor proposed may also be ineffective against a specific method of bacterial resistance towards antibiotics.

Introduction

Recently, several Gram negative bacterial families have shown high resistance against most clinically available drugs (Neu 1992). An example to consider is *Enterococcus faecium*, which has been demonstrated to be resistant to ampicillin, gentamicin, and an antibiotic used only in desperate situations: vancomycin (Montecalvo et al. 1994). Further, this a problem of global proportions.

Bacteria that are resistant to antibiotics "are difficult, and sometimes impossible, to treat. In most cases, antibiotic-resistant infections require extended hospital stays, additional follow-up doctor visits, and costly and toxic alternatives. (CDC)" The WHO also illustrates this point by noting that bacterial infections such as pneumonia, tuberculosis, gonorrhea, and salmonellosis have become increasingly difficult to treat because the antibiotics used for them have become increasingly ineffective. Further, every year about 2 million people in America are infected with these drug-resistant bacteria and, of those, 23,000 people die. However, this problem extends globally as in the EU 25,000 deaths are caused by antibiotic resistance, and in Thailand, 38,000. Thus, this is an issue of global proportions, and is the problem that the current study seeks to address.

Our approach uses the standard methods of Medicinal Chemistry which begins with an analysis of structure activity relationships (SAR) within a biological system target such as proteins that are enzymes or receptors and then design a drug to interact with that system based on the binding features. The system analysis can itself be very time consuming, but this paper examines the literature for characteristics that can be applied to our drug design (Graham 2013).

In the past, studies have explored the effect of creating stronger antibiotics (Wright 2000). However, due to the increasing toxicity of these drugs, Michael Pirrung and colleagues

(2002) argue that the best way to combat high antibiotic resistance is not to create stronger antibiotics for existing targets, but to inhibit novel targets like the LpxC enzyme. Further, inhibition of LpxC has not only shown potential to kill the bacteria, but can enhance the inhibitory potential of other antibiotics (Lee et al 2018). This is because LpxC is an enzyme that is involved in the biosynthesis of Lipid A. Lipid A is a crucial building block of the bacterial outer-membrane, and LpxC is the enzyme that codes for the first intermediate in the synthesis of Lipid A. LpxC's role in the cell membrane is the reason that it can be an antibiotic, and, in some cases, potentiate other antibiotics. In the reaction catalyzed by LpxC, UDP-3-*O*-(*R*-3-hydroxymyristoyl)-*N*-acetylglucosamine is deacetylated, as shown in figure 1.

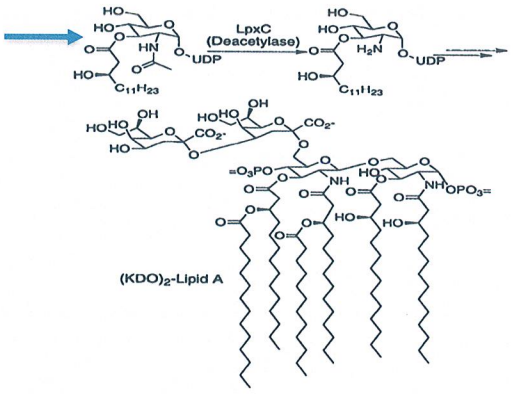


*Figure 1: Figure 1 shows the deacetylation of UDP-3-*O*-(R-3-hydroxymyristoyl)-N-acetylglucosamine by the LpxC enzyme. The arrow shows the substrate for the LpxC enzyme UDP-3-*O*-(R-3-hydroxymyristoyl)-N-acetylglucosamine.* From Pirrung, M. et al (2002). *Figure 1: Biosynthesis and structure of Lipid A.* https://pubs.acs.org/doi/pdf/10.1021/jm020183v

For LpxC, Pirrung and colleagues (2002) suggested that an inhibitor with an isoxazoline scaffold and a preferential binding group would have high inhibitory activity. The isoxazoline moiety also demonstrates structural similarities to histidine (Fig 2) which has been shown to interact with the zinc ion within the active site of LpxC. Enzyme histidine generally surrounds

the zinc ion, and is a ligand for the ion. These histidine allow zinc to associate with a ligand, while allowing the zinc to retain all its positive charge, which then potentiates its binding potential towards inhibitors (McCall 2000). In consequence, because the isoxazoline has structural similarities to histidine we are hoping that the isoxazoline will help keep the electrostatic field neutral, and limit binding of the zinc ion strictly to the binding group. Thus, because isoxazoline has shown success inhibiting gram negative bacteria in vitro and shares structural similarities with histidine, which has been shown to interact with the LpxC active site, isoxazoline was chosen as a starting point for our novel antibiotic. The current study seeks to use the past literature to build a potential antibiotic around isoxazoline for LpxC.

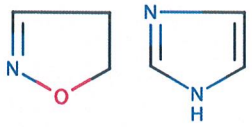


Figure 2: Figure 2 demonstrates the structural similarities between the histidine side chain (right) and isoxazoline (left). They may also serve similar functions as nitrogen and oxygen are polar H-bond acceptors.

Methods

To create a potential antibiotic with the highest inhibitory potential, the current study sought to identify the active site of the LpxC, and any other allosteric sites of LpxC. Then, we design a novel compound with these binding sites in mind and then create a synthetic plan using these qualities.

Results

Previously, Pirrung and colleagues (2002) have identified an inhibitor of LpxC with an oxazoline scaffold and high inhibitory potential. However, the researchers found that the oxazoline scaffold within the inhibitor was physiologically unstable (Pirrung et al. 2002). Thus,

Pirrung and others (2002) first replaced the original oxazoline scaffold with a more physiologically stable isoxazoline scaffold and then demonstrated that the novel compound possessed similar inhibitory potential as the initial compound with the oxazoline scaffold (Figure 3). Additionally, the researchers also described the affinity of various binding groups for the LpxC enzyme, concluding that the following binding groups had the highest affinity for LpxC: hydroxamate, phosphonate, and thioacetoxyacetyl (Pirrung et al. 2002). Finally, Pirrung and colleagues (2002) inform our work by demonstrating that a zinc ion catalyzes substrate binding to the LpxC enzyme. The Pirrung work suggests that a novel compound with an isoxazoline ring and a hydroxamate, phosphonate, or a thioacetoxyacetyl binding group would best inhibit the LpxC enzyme. It also tells us that a zinc ion catalyzes most interactions between LpxC and the substrate. This is further substantiated by a study done by Clayton et al. who found the crystal structure of LpxC and isolated the active site of the enzyme (Figure 4). Through the crystal structure, it can be seen how the zinc within the active site and the allosteric site interact with the substrate.

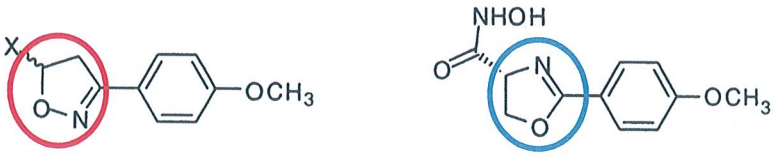


Figure 3: Figure 3 compares the structure of the oxazoline (structure circled in blue) and the isoxazoline (structure circled in red). From Pirrung, M. et al (2002). *Table 1: Enzymatic and Antibacterial Activity of the Various Inhibitors.* https://pubs.acs.org/doi/pdf/10.1021/jm020183v

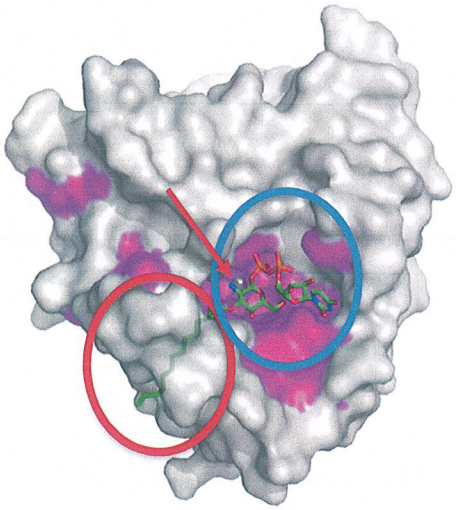


Figure 4: Figure 4 shows the crystal structure of LpxC bound to the substrate. Circled in red shows how a hydrophobic substrate interacts with the enzyme. Circled in blue shows how the active site of the enzyme interacts with the substrate. The arrow is pointing to the zinc ion within the active site.) From Clayton, G. et al (2002). *Figure 2: Overall structure of E. coli LpxC.* doi: 10.1074/jbc.M113.513028.

In their review of zinc metalloenzymes, McCall, et al (2000) revealed some binding characteristics of a catalytic zinc ion in a typical enzyme. Firstly, the researchers suggested that a catalytic zinc ion within a generic enzyme has been commonly correlated with the histidine amino acid residue of the enzyme's substrate (McCall et al. 2000). McCall and others also found that zinc tends to bind within an enzyme in a tetrahedral geometry. This is further substantiated by Lee et al. who bound a compound with a moiety like isoxazoline (oxazolidone) to the active site of the LpxC enzyme (Figure 5). Further, McCall and others (2002) identified that the catalytic zinc ion preferentially binds to a sulfur-containing binding group of a substrate. Thus, to the current study, McCall and others (2002) provide support for a isoxazoline ring because of its

structural similarity to histidine and suggest a possible interaction between the isoxazoline ring and the zinc ion. Further, this paper also substantiates a relationship between a sulfur-containing binding group and the zinc ion, providing the thioacetoxyacetyl as the binding group in our current compound.

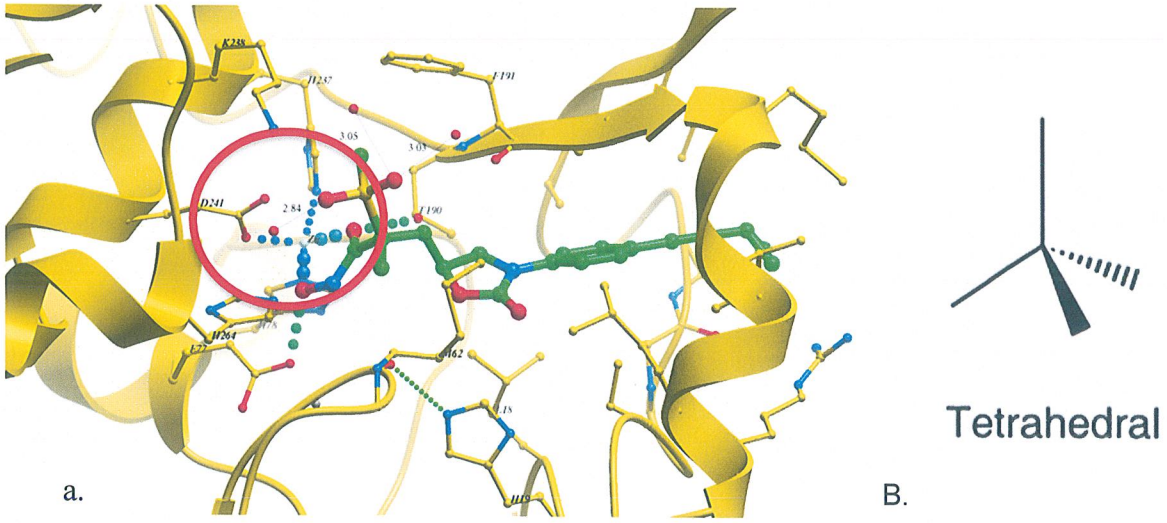


Figure 5: 5a) Circled in red is the binding of the binding group to the zinc ion, and how it adopts a tetrahedral geometry. From Lee, P.S. et al. *Figure 3. Cocrystal oxazolidinone 13f with P. aeruginosa LpxC enzyme (PDB code 6mae).*

https://pubs.acs.org/doi/pdf/10.1021/acs.jmedchem.8b01287.

5b) 5b shows a scheme of tetrahedral geometry. From McCall, K. et al (2000). *Scheme 1.*

https://academic.oup.com/jn/article/130/5/1437S/4686409

McClerren and others (2005) then demonstrated another characteristic of zinc ion binding within LpxC. In their study, McClerren and others (2005) found that a large, bulky, di-benzene ring molecule named CHIR-090 had inhibited the zinc ion within the LpxC enzyme. This is further substantiated by the structure of the LpxC substrate and an LpxC inhibitor, both of which have large bulky scaffolds (Figure 4,5). In consequence, a relationship between a large,

bulky molecule and inhibition of LpxC was suggested, and thereby we incorporated this feature into our design.

Whittington and colleagues (2003) performed an experiment where they determined that an interaction between LpxC and its substrate occurs independent of LpxC's catalytic zinc ion. The researchers discovered that LpxC contained a unique fold within its structure that leads to the catalytic zinc ion within the enzyme (Whittington et al. 2003). Further they found that this fold in LpxC has characteristics that allow only a long, hydrophobic, "fat-like" molecule to interact with it (Whittington et al. 2003). Whittington and colleagues (2003) provides the current inhibitor with another point of interaction with the enzyme separate from the catalytic zinc ion, and thus suggests that a long, hydrophobic carbon chain should be incorporated into the novel compound. This is consistent with what we see in the crystal structure of LpxC (Figure 4, 5).

In the study conducted by Kurth and colleagues (1990) a novel method of synthesizing isoxazolines was demonstrated. In their study, Kurth and others (1990) describe a method to synthesize polyether antibiotic sub structural units. However, in the description of their synthesis of these units, it was found that an isoxazoline intermediate was used to create this product (Kurth et al 1990). They report that an isoxazoline ring can be created by a mixture of two chemicals: triphenylacetonitrile oxide and an excess of 1,5-hexadiene (Kurth et al. 1990).

In conclusion, the current study aims to synthesize a novel compound that contains a large scaffold which includes an isoxazoline ring (synthesized using the method outlined by Kurth et al. 1990), a fatty-acid like hydrophobic chain, and a thioacetytoxyacetyl binding group (synthesized using the method outlined by Pirrung et al. 2002).

Synthetic Plan

The synthesis of the novel compound with the characteristics listed above (further referred to as TL1) will occur in three major phases. One half of the molecule will be created (further referred to as TL2) containing an isoxazoline ring, a benzene ring and it will also contain the thioacetoxyacetyl group (further referred to as TL4). Then the second half of the molecule (TL3) containing the hydrophobic segment destined for the hydrophobic channel within LpxC will be synthesized (Figure 6). After the synthesis of the individual halves, they will be combined to create the compound TL1 using the reaction by Anderson et al.

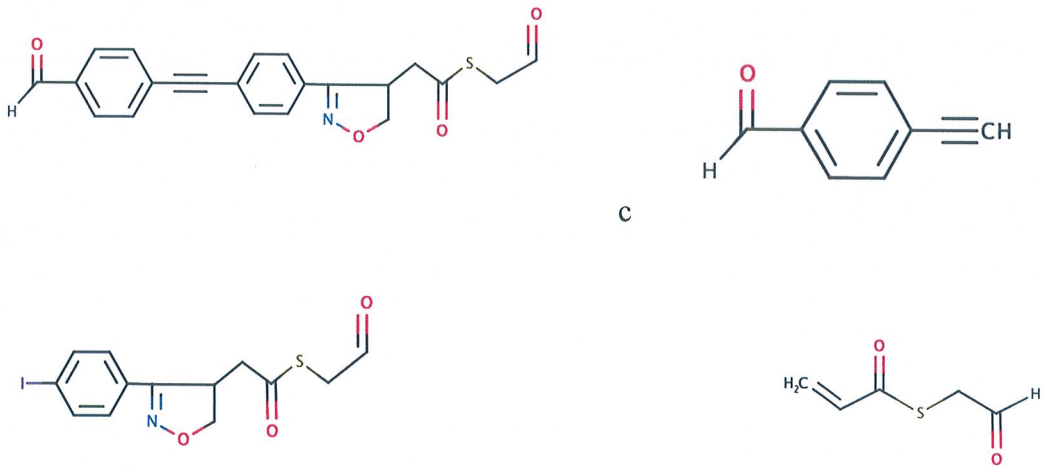


Figure 6: Figure 6a shows the structure of TL1, 6b shows the structure of TL2, 6c shows the structure of TL3 and 6d shows the structure of TL4

Phase 1: Synthesis of TL2. To synthesize TL2 two main steps are required. First the TL4 will be synthesized, then it will be combined with the scaffold to create TL2. TL4will be synthesized using the method proposed by Pirrung and colleagues (figure 7,8), and TL2 will be synthesized by using the method suggested by Kurth and colleagues (figure 9).

Figure 7: Figure 7 shows the first step of binding group synthesis

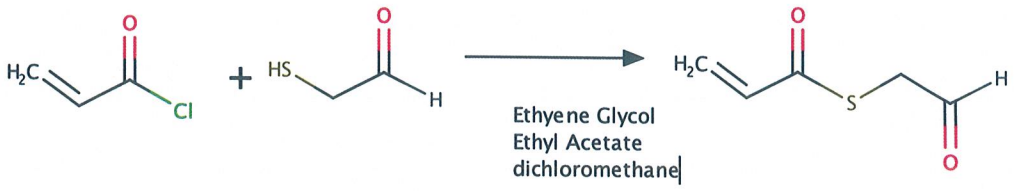


Figure 8: Figure 8 shows the second step of TL4 synthesis

Figure 9: Figure 9 shows the synthesis of TL2 in the solvent, benzene

Phase 2: Synthesis of TL3. To synthesize TL3, three steps will be required. First, the aldehyde within chlorobenzaldehyde must be transformed in a reversible reaction to an acetal. this is because the aldehyde must be protected from the potential side reactions that are possible when synthesizing TL3 (Figure 10) . Transformation of the aldehyde to an acetal will be done using the method developed by Dr. Lisa Ahlberg (L. Ahlberg, personal communication). Then,

using the method suggested by Cheung and colleagues (Cheung, Ren, Hu 2014), the protected chlorobenzaldehyde will be used to synthesize TL3. After TL3 is created, the chlorobenzaldehyde will be transformed back into the aldehyde using the method found by Gregg and others (Gregg, Golden, Quinn 2007).

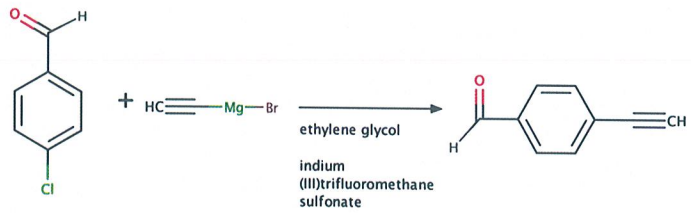


Figure 10: Figure 10 shows the synthesis of TL3

Phase 3: Synthesis of TL1. Based on a method used by Anderson and colleagues, TL2 and TL3 will be combined in a reaction to form TL1(Figure 11) .

Figure 11: Figure 11 shows the combination of TL2 and TL3 to form the final novel compound

Discussion

Due to the reliability of most of the reactions used within this synthetic plan, this reaction has a high probability of success. The two novel reactions, the synthesis of the isoxazoline, and the combination of TL2 and TL3, however do present some issues. Isoxazoline synthesis has proven to be highly unpredictable, as the number of substrates attached to each reagent, as well as the agent used to catalyze the reaction need to be considered, and a slight change in either could lead to low yields of the isoxazoline. However, the substrate originally used, and the

current study share structural similarities, and thus we predict that it will work. The second novel reaction, which is the synthesis of TL2 and TL3, is based on a reaction to create CHIR-090, which is very similar in general shape, and in fact the bond we intend to create, is the same bond as the one in CHIR-090 (Fig 8). Thus, we predict a high success rate with this reaction as well.

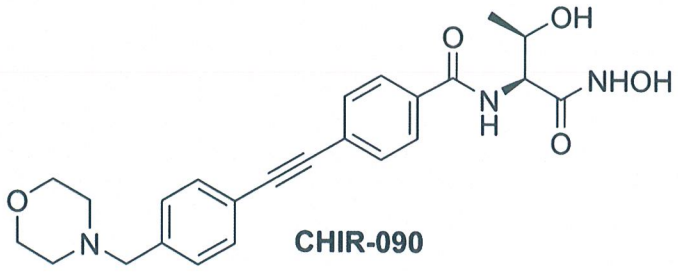


Figure 8: Figure 8 shows CHIR-090 and its similarities to the model currently in study. From Tomaras A. et al (2014). Structures of LpxC inhibitors used in these studies. *Fig 1: Structures of LpxC inhibitors used in these studies.* https://mbio.asm.org/content/5/5/e01551-14#F1

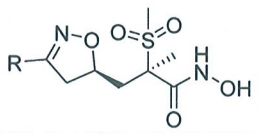
We not only predict that it will have high inhibitory potential, but we also have reason to believe that this molecule will be immune to common resistance mechanisms of bacteria for antibiotics. A common mechanism of bacteria that allows it resistance to antibiotics is through one of the following efflux pumps: *MexAB-OprM, MexCD-OprJ,* and *MexXY-OprM.* These pumps are all structural similar and have the same efflux function. The only difference is that different species of bacteria contain different species of the efflux pump. Further, this pump has very low specificity and extrudes a broad spectrum of antibiotics from the bacterial cell, rendering the antibiotic useless. Examples of antibiotics that are extruded from the cell are as follows: quinolones, macrolides, tetracyclines, lincomycin, chloramphenicol, meropenem, and S-4661, and most variants of penicillin. However, not all antibiotics are extruded from the bacterial cell. Some antibiotics remain within the cell; however, some seem to differ depending on the

species of the efflux pump. Firstly, there are general antibiotics that do not get extruded by any of the species: polymyxin B or imipenem. But there are also antibiotics that seem to not be extruded by only certain efflux pumps, these include: ceftazidime, carbenicillin, sulbenicillin, novobiocin, cefsulodin, and flomoxef (Masuda et al. 2000). When looking at the antibiotics that are not extruded from the cell, one motif was present throughout all antibiotics, they all had sulfur within the compound except for polymyxin B, which is typically salted with a sulfate ion, and novobiocin. Thus, it seems heavily correlated that the presence of sulfur influences the efflux pump which prevents the efflux pump from extruding the sulfur containing antibiotic. The novobiocin and polymyxin B are exceptions, however polymyxin B is typically salted with sulfur leaving novobiocin as a seemingly anomalous result. Another interesting trend is that none of the antibiotics that are extruded contain a sulfur group. Thus, while the mechanism is unclear, sulfur does seem to play a role in extrusion of an antibiotic from the bacteria. Thus, the use of sulfur within the current binding group alludes immunity from the adaptive mechanisms of bacteria to antibiotics.

Limitations: The first limitation to this study is the high chance of error. Because so many experiments are performed, and the experiments are additive, there is a high chance of error. The possibility of experimental error remains in any chemical reaction. However, repeatedly in this experiment, products from one chemical reaction are used as the reagents for the next. Thus, we see that the consequences for each mistake are higher as they would require us to restart the whole reaction, rather than just a small portion. While measures like purification and checking for the product intermittently using NMR, may lower the chance of error, it is difficult to lower error to a conservative percentage.

The final limitation is the price, because of the extensive nature of the reaction as well as the price of some of the materials, this reaction is expensive. Some materials like palladium, while they are included in this reaction in catalytic amounts, are still costly. Further, because of the number of reactions that must be run, as well as the reactions that may have to be rerun, supplies may be quickly siphoned, and inevitably cost more money.

Final notes- The original plan was to slightly alter the sulfur binding group of the molecule provided by Pirrung and others. There was moderate success in doing this as results were obtained, and NMR spectroscopy taken. However, in the search for isoxazoline synthesis much more information about the LpxC enzyme was recovered, and thus the aim of the research changed. We then used the current methods to synthetic plan presented. Another note is that in 2018, a year after research for the current study completed, a paper by Lee et al (2018) was found with a very similar molecule to the current molecule and it proved to have high inhibitory potential. Further, compound 25d has an MIC (minimum inhibitory concentration) as low as 0.5 micrograms/mL and as high as 8 micrograms/mL in different species of gram negative bacteria. This effectiveness is comparable to the effectiveness of most clinically available antibiotics (Yamazhan T. et al 2005). Thus, these results of Lee et al (2018) confers validity to my synthetic plan as there seems to be a high structural similarity between the novel compound and compound 25d, referenced in Table 2 of the study done by Lee et al (2018) (Fig. 9).



25a-e

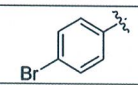
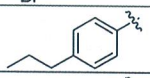
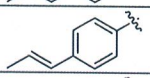
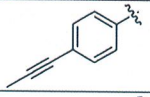
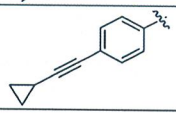
	R	*PA* Kd (nM)	MIC (μg/mL)[a]			logD[b]
			PA	*EC*	*KP*	
CHIR-090 (2)	-	0.48	2	0.125	0.5	
25a		2.6	4	32	>64	1.2
25b		0.2	1	4	16	1.7
25c		0.3	1	2	16	2.0
25d		0.44	0.5	1	8	1.4
25e		0.17	1	0.125	0.5	2.2

Figure 12: Figure 12 shows multiple inhibitors created by Lee et al (2018). Figure 9 also shows compound 25d which has structural similarities to the novel compound mentioned in this study. From Lee P. et al. (2018) Application of Virtual Screening to the Identification of New LpxC Inhibitor Chemotypes, Oxazolidinone and Isoxazoline. *Table 2: Isoxazoline SAR.* https://pubs.acs.org/doi/pdf/10.1021/acs.jmedchem.8b01287

Conclusion

In conclusion, this synthetic plan has promise due to the reliability of the reactions within the plan, and it further has inhibitory potential, as later confirmed by Lee et al (2018). Further, the novel molecule has potential to be immune to adaptive mechanisms by the bacteria towards most antibiotics. Thus, future study could explore an actual synthesis of the molecule as well as

optimization of the synthesis. Future studies could also test the immunity to bacteria's adaptive mechanisms for antibiotics.

Experimental

TL4 synthesis- First, acrylic acid (0.0045 mols, 0.3243 g) will be stirred together with thionyl chloride (15 mL, 0.207 mols) in the solvent dichloromethane (50 mL), and the solution will be stirred for two hours. Then the solvent will be removed, and the product will be added with diazomethane (0.76 g, 0.018 mols) in the solvent ether (25 mL) (Fig. 7) The solution will then be left to sit for an hour and then the solvent will be encouraged to evaporate using a steady stream of air. After being left to sit, the solution will then be recrystallized (which improves purity) before the next step. Following the purification, the product (0.0004 mols or 0.1 g) will be dissolved in tetrahydrofuran solvent (7 mL) and then left to sit for an hour. After this time, the product will be evaporated using a rotary evaporator (evaporates within a short amount of time) and then inorganic impurities will be rinsed off using dichloromethane and water. We will then combine 1-thiol-3-proponal (0.0004 mols,0.036 grams) with ethylene glycol in ethyl acetate to protect the aldehyde group. The protected aldehyde will then be combined with the purified product (0.0004 mols or 0.1 g) of the previous step in the solvent dichloromethane (Fig. 8). The aldehyde will then be unprotected using the method outlined by Gregg et al (2007). Then the organic layer will be dried of water using magnesium sulfate, and the solvent will be evaporated off, using the rotary evaporator, to create the TL4.

TL2 Synthesis: 4-iodobenzonitrile (0.00198 mols, 0.4535 g) will first be combined with the binding group (0.0158 mols, 2.28 g) in anhydrous (water free) benzene (25 mL) and then heated while being refluxed (which distills the product) for 48 hours. After two days, the reaction mixture will then be cooled to room temperature and purified using liquid

chromatography (which separates the product and its impurities by its weight) (Fig. 9). An NMR (Nuclear Magnetic Resonance), which identifies what compound was created using the hydrogens in the product, will be taken to check that TL2 was indeed synthesized.

Aldehyde transformation to acetal- First, chlorobenzaldehyde (0.1406 g, 0.001 mols) and ethylene glycol (0.0621 g, 0.001 mol) will be combined in the solvent ethyl acetate (25 mL). Then the solution will be refluxed for about an hour. TLC (Thin layer chromatography), which separates the different materials by weight, will be taken every 15 minutes to check the progress of the reaction.

Synthesis of TL3- First, the protected chlorobenzyaldehyde (0.001 mols, 0.184 g) will be combined with ethynylmagnesium bromide (0.0015 mols, 0.194 g) and the solvent system of n-methyl-2-pyrrolidone and tetrahydrofuran (7 mL), which are combined in a 4:3 ratio and then stirred for 16 hours. The compound will then be purified by liquid chromatography (which separates the different products by weight) giving the purified TL3 (Fig. 10).

Acetal reversion to aldehyde- To transform the acetal in chlorobenzyaldehyde back into an aldehyde, a catalyst, indium (III) trifluoromethanesulfonate (0.008 mols, 4.49 g), will be added to TL3 along with 15 mL of acetone at room temperature. The mixture will then be stirred 8 hours to produce the chlorobenzaldehyde. An NMR will again be taken to ensure that the aldehyde containing TL3 was created.

Synthesis of TL1- TL2 (0.215 g, 0.000577 mols), TL3 (0.147 g, 0.00126 mmols) and trimethylamine (0.0025 mols, 350 microliters) will be mixed together. A small amount of the catalysts Bis(triphenylphosphine)palladium(II) dichloride (0.02 g, 0.000028 mols), and copper iodide (0.0106 g, 0.000055 mols) will then be added (Fig. 11). Subsequently, the solution will be stirred at room temperature for 22 hours and excess reagents will be evaporated by rotovap

(rotary evaporation). The raw TL1 will then be purified by silica gel chromatography to give a purified TL1. TL1 synthesis will then be checked using NMR spectroscopy.

Literature cited

Anderson, N. H., Bowman, J., Erwin, A., Harwood, E., Kline, T., Mdluli, K., Ng, S., Pfister, K. B., Shawar, R., Wagman, A., & Yabannavar, A. (n.d.). Washington, DC: U.S. Patent and Trademark Office.

Centers for Disease Control and Prevention. (2019, April). *Antibiotic/Antimicrobial Resistance (AR / AMR)*. https://www.cdc.gov/drugresistance/about.html.

Cheung, C. W., Ren, P., & Hu, X. (2014). ChemInform Abstract: Mild and Phosphine-Free Iron-Catalyzed Cross-Coupling of Nonactivated Secondary Alkyl Halides with Alkynyl Grignard Reagents. *ChemInform*, 45(46), 2566-2569. doi:10.1002/chin.201446058

Graham, L. P. (2013) *An Introduction to Medicinal Chemistry.* Oxford.

Gregg, B. T., Golden, K. C., & Quinn, J. F. (2007). Indium(III) Trifluoromethanesulfonate as an Efficient Catalyst for the Deprotection of Acetals and Ketals. ChemInform, *38(49),* 5890-5893. doi:10.1002/chin.200749044

Kurth, M. J., Rodriguez, M. J., & Olmstead, M. M. (1990). ChemInform Abstract: Tandem 1,3-Dipolar Cycloaddition and Electrophilic Cyclization Reactions: Cyclic Ether Subunits of Polyether Antibiotics from Unsaturated Isoxazolines. *ChemInform*, *21*(28), 283-288. doi:10.1002/chin.199028060

Lee, P. S., Lapointe G., Madera, A. M., Simmons, R. L., Wenjian X., Yifru, A., Tjandra, M., Karur, S., Rico, A., Thompson, K., Bojkovic, J., Xie, L., Uehara, K., Liu, A., Shu, W., Bellamacina, C., McKenny, D., Morris, L., Tonn, G. R., Osborne, C., Benton, B. M., McDowell, L., Fu, J., & Sweeney, Z.K. (2018). Application of Virtual Screening to the Identification of New LpxC Inhibitor Chemotypes, Oxazolidinone and Isoxazoline. *J. Med. Chem, 61*, 9360-9370. doi:10.1021/acs.jmedchem.8b01287

McCall, K. A., Huang, C., & Fierke, C. A. (2000). Function and Mechanism of Zinc Metalloenzymes. *The Journal of Nutrition*, *130*(5), 1437S-1446S. doi:10.1093/jn/130.5.1437s

McClerren, A. L., Endsley, S., Bowman, J. L., Andersen, N. H., Guan, Z., Rudolph, J., & Raetz, C. R. (2005). A Slow, Tight-Binding Inhibitor of the Zinc-Dependent Deacetylase LpxC of Lipid A Biosynthesis with Antibiotic Activity Comparable to Ciprofloxacin. *Biochemistry*, *44*(50), 16574-16583. doi:10.1021/bi0518186

Montecalvo, M. A., Horowitz, H., Gedris, C., Carbonaro, C., Tenover, F. C., Issah, A., . . . Wormser, G. P. (1994). Outbreak of vancomycin-, ampicillin-, and aminoglycoside-resistant Enterococcus faecium bacteremia in an adult oncology unit. *Antimicrobial Agents and Chemotherapy*, 38(6), 1363-1367. doi:10.1128/aac.38.6.1363

Neu, H. C. (1992). The Crisis in Antibiotic Resistance. *Science*, 257(5073), 1064-1073. doi:10.1126/science.257.5073.1064

Pirrung, M. C., Tumey, L. N., Raetz, C. R., Jackman, J. E., Snehalatha, K., McClerren, A. L., . . . Rusche, K. M. (2002). Inhibition of the Antibacterial Target UDP-(3-O-acyl)-N-acetylglucosamine Deacetylase (LpxC): Isoxazoline Zinc Amidase Inhibitors Bearing Diverse Metal Binding Groups. *Journal of Medicinal Chemistry,* 45(19), 4359-4370. doi:10.1021/jm020183v

Tomaras, A. P., McPherson, C. J., Kuhn, M., Carifa, A., Mullins, L., George, D., Desbonnet, C., Eidem, T.M., Montgomery, J.I., Brown, M.F., Reilly, U., Miller, A.A., & O'Donnell, J.P.(2014). LpxC Inhibitors as New Antibacterial Agents and Tools for Studying Regulation of Lipid A Biosynthesis in Gram-Negative Pathogens. *American Society for Microbiology*, *5*(5), pp. 1-13.

Whittington, D. A., Rusche, K. M., Shin, H., Fierke, C. A., & Christianson, D. W. (2003). Crystal structure of LpxC, a zinc-dependent deacetylase essential for endotoxin biosynthesis. *Proceedings of the National Academy of Sciences*, *100*(14), 8146-8150. doi:10.1073/pnas.1432990100

World Health Organization. (2018, April). *Antibiotic Resistance*. https://www.who.int/news-room/fact-sheets/detail/antibiotic-resistance

Wright, G. D. (2000). Resisting resistance: new chemical strategies for battling superbugs. *Chemistry & Biology, 7*(6), 127-132. doi:10.1016/s1074-5521(00)00126-5