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Overview of Direct Thrombin Inhibitors for use in Staphylococcus Aereus Infections

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OVERVIEW OF DIRECT THROMBIN INHIBITORS FOR USE IN *STAPHYLOCOCCUS AUREUS* INFECTIONS

by

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ABSTRACT

The pathogenicity and intractable nature of the microorganism *Staphylococcus aureus* (SA) has been long documented and highlighted by many health care agencies, with emphasis on its ability to exploit the human coagulation system to deadly effect. Two drugs from a class of inhibitors known as Direct Thrombin Inhibitors (DTI) have been shown to have a substantial effect on the enzyme secreted by SA known as Staphylocoagulase (SC), but up until now the application of this potential treatment has been limited. This paper strives to supply an overview of these clinical studies and propose a novel protocol for testing DTI's on SA in an *in vitro* setting. Three DTIs have been identified, including two already tested in clinical trials, and computational molecular docking simulations have been applied to elucidate the mechanisms of action for the inhibition. An additional DTI has been developed using these mechanisms as principles and shows promise for future development. After conducting this preliminary protocol, it has been found that running a minimum inhibitory concentration test across several tubes with varying degrees of these DTIs demonstrated varying levels of coagulation consistent with the findings of clinical research papers. It is fair to conclude, then, that after development or discovery of new coagulase inhibitors, they can be quickly and accurately tested against existent DTIs to gauge their efficacy.

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LIST OF ABBREVIATIONS

DTI: Direct Thrombin Inhibitors

EDTA: Ethylenediaminetetraacetic acid

SA: *Staphylococcus aureus*

MRSA: Methicillin-Resistant *Staphylococcus aureus*

VRSA: Vancomycin-Resistant *Staphylococcus aureus*

SS: *Staphylococcus saprophyticus*

WHO: World Health Organization

SC: Staphylocoagulase

ST: Staphylothrombin

PT: Prothrombin

PyRx: Python Prescription Virtual Screening Tool

PyMol: Molecular Visualization Tool

CHAPTER 1: INTRODUCTION

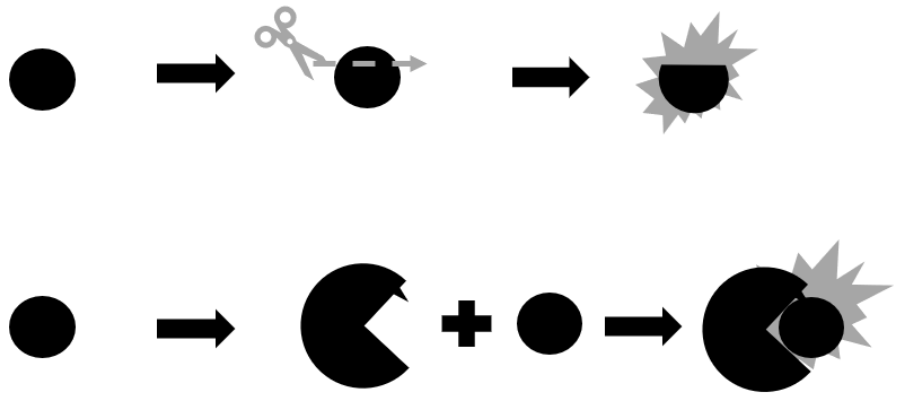
1.1 Statement of the Problem

Staphylococcus aureus (SA) is an opportunistic pathogen responsible for many nosocomial and post-operative infections. SA is a common human commensal that colonizes the upper nasopharynx in many normal, healthy humans. When transfected to the site of a wound or open sore, however, SA can cause painful and life-threatening infections, especially in the elderly and immunocompromised population. In a hospital setting, however, certain strains of SA have become resistant to the antibiotics used to treat them and can pose a threat to anyone in a hospital, immunocompromised or otherwise. These strains are commonly referred to as MRSA or VRSA (methicillin-resistant or vancomycin-resistant *Staphylococcus aureus*). It is for this reason that SA is on the High Priority list from the World Health Organization for the development of new antibiotics (Tacconelli, 2017).

SA possesses many virulence factors that either help it penetrate bodily tissue or evade the host's immune system. It has been demonstrated that one of the ways SA protects itself from the patient's immune system is through a protein called coagulase, which hijacks the natural coagulation process in the body, causing controlled clots to form around the bacterial colonies (McAdow, 2012). This allows SA to migrate around the body without risk of destruction from the body's immune system and attach to hard surfaces. When it attaches to a heart valve, this is called infective endocarditis, and SA is the leading causes of bacterial endocarditis worldwide (Fowler, 2005).

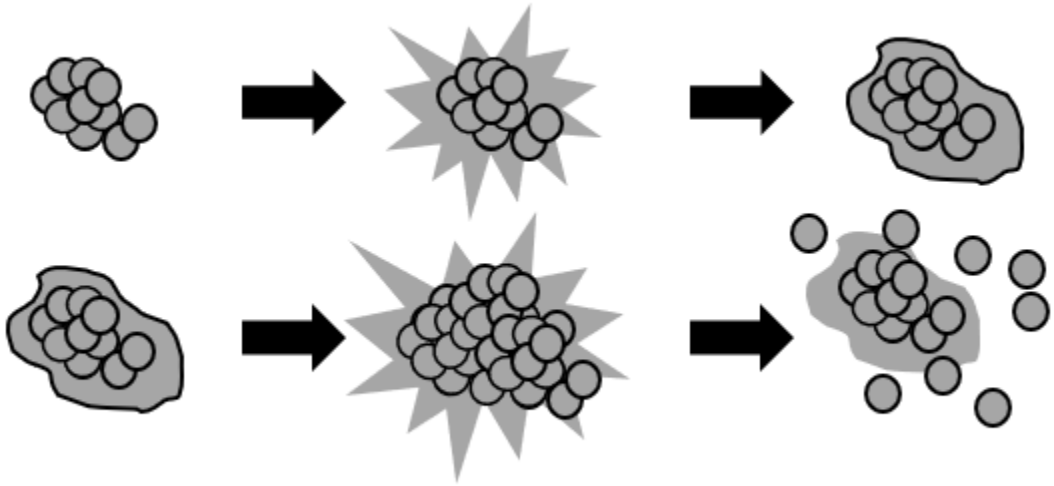
As a result of this high virulence, SA's coagulase property has been the target of much investigation to elucidate the underlying mechanism. As it turns out, the clotting is caused by an enzyme called Staphylocoagulase (SC) binding to the human pro-enzyme prothrombin, causing it to behave like its activated form, thrombin (Liesenborghs, 2018). This is done by coagulase through inserting a portion of itself into prothrombin, illustrated in Figure 1 by a "tooth" piercing the enzyme, and activating it, without cleavage.

Figure 1: Diagram showing a cartoon representation of activation of prothrombin by prothrombinase (top), activation of prothrombin by coagulase (bottom)



This new enzyme complex is known as Staphylothrombin (ST). In the normal coagulation cascade, thrombin is activated to convert the monomer fibrinogen to the active polymer fibrin, which is the primary constituent of a healthy clot. In this inappropriate coagulase cascade, however, SC diffuses from the SA colonies, attaches itself to the host prothrombin enzyme, and begins depositing large quantities of fibrin locally around the colony. SA also contains "sticky" proteins that allow fibrin to bind more readily to its surface, coating the bacterial colonies in a tough, thick biofilm made from host proteins, indistinguishable to the host's immune system.

Figure 2: *Staphylococcus aureus* can form biofilms that break off to form metastatic colonies



If any part of the colony breaks off from the rest, it can very easily find its way into another part of the host's circulatory system, and lodge into the vascular endothelium. If this occurs, it presents as bacterial metastasis. Bacterial metastasis refers to the inability of the immune system to keep up with the bacterial population, resulting in circulating bacteria that can establish colonies in dangerous places in the body. This can be indicated and even exasperates septic shock, an often-fatal complication of bacterial metastasis. This turns a local, uncomplicated infection of a wound site into a systemic, complex infection with multiple sites. If the SA subtype happens to be one of the antibiotic resistant organisms mentioned previously, the infection is all but untreatable and has a 20-50% mortality rate (Kaye, 2008).

1.2 Proposed Solution

While the outlook for treatment of these antibiotic-resistant strains may be bleak, an interesting consequence of the structural relationship between ST and activated thrombin is that it has been theorized that an inhibitor of thrombin can also inhibit coagulase. Such a class of drugs would have no interaction with the normal forms of antibiotic resistance that MRSA and VRSA possess, instead targeting a specific virulence factor. There exists a class of drugs known as Direct Thrombin Inhibitors (DTI) that were developed to prevent thrombosis in patients who for various reasons could not have heparin or warfarin administered to them (Nisio, 2005). As their name suggests, DTIs are direct, competitive inhibitors of the active enzyme thrombin, and act by binding to the active site of the enzyme, preventing its function. While the other drugs mentioned target parts of the pathways upstream of thrombin, which can have detrimental effects to other parts of the coagulation and inflammatory response, DTIs exclusively target the enzyme responsible for the conversion of soluble fibrinogen to insoluble, polymeric fibrin. Drugs in this class can be subdivided into the univalent DTIs, which contain Dabigatran, Argatroban, and Melagatran, and the bivalent DTIs, which include Hirudin, Bivalirudin, and others. For the purposes of simplicity, this paper will refer to univalent DTIs as simply DTIs, while referring to bivalent DTIs specifically.

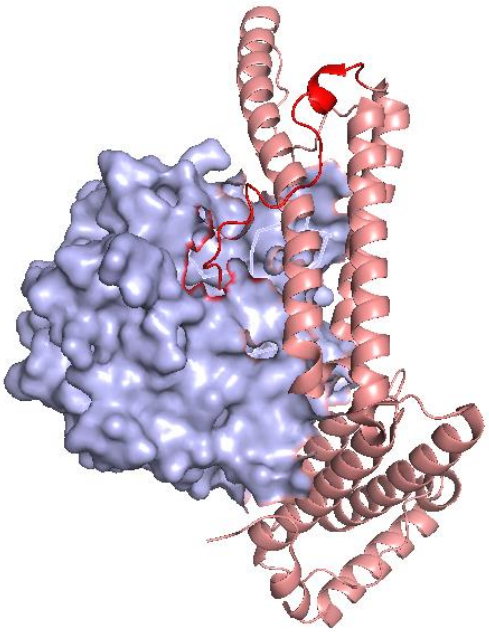
As previously stated, ST has a striking structural and functional similarity to thrombin, lending itself to inhibition by this class of drugs. If inhibited, SA would no longer be able to protect itself with coagulation and would be exposed to the host's immune response. A potential downside of this therapy however, is that DTIs inhibit both ST and normal, activated thrombin in response to the coagulation cascade, as a result, a patient given a DTI for a SA infection may be at an increased bleeding risk, which could compound mortality in an already dire situation.

1.3 Literature Review

In order to further understand the breadth of research conducted in this area, a literature review has been conducted. First, in a previously cited paper, "*Staphylococcus aureus, master manipulator of the human hemostatic system*" (Liesenborghs, 2018), points out the mechanism of action that is unique to coagulase. In normal thrombotic activation, an enzyme known as prothrombinase enzymatically cleaves prothrombin from a 72 kiloDalton (kDA) proenzyme, to a 36 kDa enzyme. This results in a freshly cleaved, "loose" N-terminal region, which then reinserts into thrombin, thereby becoming activated. Coagulase, on the other hand, does not cleave prothrombin into the active form, instead inserting its own N-Terminal region that closely resembles the thrombin N-Terminus, thereby activating it. This creates a large (136 kDa) coagulase-prothrombin complex, with a similar active site.

This complex is not completely identical to thrombin, however, and this is demonstrated in the effect that no inhibitory ligand other than univalent direct thrombin inhibitors that bind to thrombin can bind to the coagulase-prothrombin complex. In addition, many bivalent DTIs have limited FDA approval, and in preliminary testing demonstrated inefficacy with ST, due to the binding of SC blocking a regulatory site called Exosite 1 on the surface of prothrombin. Note in Figure 3 the process from Coagulase into prothrombin, connecting the two, the "tooth" previously noted. This is where in normal states prothrombin would be cleaved and restructured, but in this case no cleavage is necessary for Staphylothrombin to become enzymatically activated.

Figure 3: Molecular visualization of the insertion of N-Terminus region (red) into Prothrombin (blue) by Coagulase (salmon). PDB ID: 1NU9 *(Friedrich, 2003)*

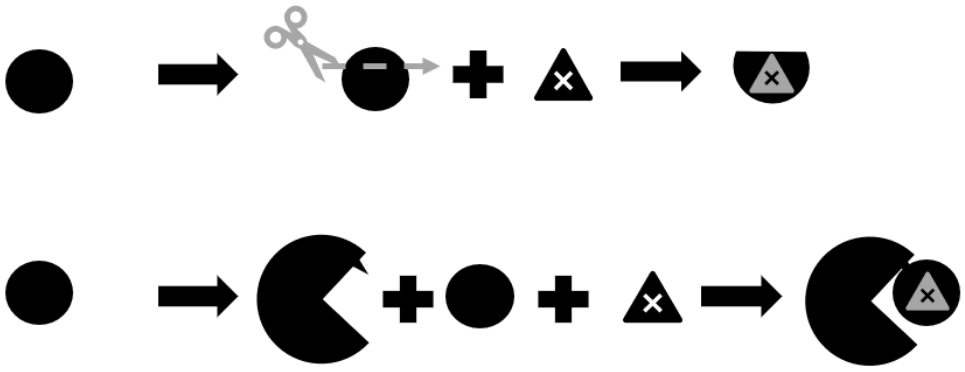


Now that the underlying mechanism in the literature has been reviewed, the clinical literature shows even greater promise. Two groundbreaking studies of this potential treatment have been performed, both with promising results.

One, titled "*Targeting Coagulase Activity in Staphylococcus aureus Bacteraemia: A Randomized Controlled Single-Centre Trial of Staphylothrombin Inhibition"* (Peetermans, 2018) identified 94 patients who experienced SA bacteremia. They were split into 2 equal groups of 47, one group received the standard treatment of antibiotics, and in addition received a Low Molecular Weight Heparin, which has no interaction with ST. This group acted as a control group. The testing group of another 47 received a DTI, either Dabigatran orally or Argatroban intravenously, until the level in their blood stream was about equal and clinically relevant.

The results showed that patients who were treated with DTIs showed lower levels of D-dimer (an indicator for inflammation and coagulation) as well as lower positive blood cultures, when compared to a control. In addition, there were no differences in any other inflammatory factors, clinically relevant bleeding, and thrombotic complications. The paper concluded that: "Targeting Staphylothrombin with DTIs is feasible in a subset of *S. aureus* bacteraemic patients, with comparable safety to standard thromboprophylaxis. In future studies of Staphylothrombin inhibition, feasibility can be further improved by rapid diagnostics."

Figure 4: Diagram showing a cartoon representation of inhibition of thrombin by a univalent DTI (top), and inhibition of Staphylothrombin by a DTI (bottom)



Another study explored the potential protective nature that DTIs exhibit with respect to medical implants, such as venous catheters. The paper titled "The Role of Staphylothrombin-Mediated Fibrin Deposition in Catheter-Related Staphylococcus aureus Infections" notes that fibrin deposition facilitates the adhesion of *S. aureus* to catheters, resulting in a disease complication called catheter-related bloodstream infections (CRBSIs), which can have a fatality rate of up to 35%. When catheters were submerged in a solution containing plasma, SA, and either Dabigatran or Argatroban, there was no measurable increase in fibrin deposition when

compared to samples lacking the DTIs, or samples that contained non-DTI anticoagulants, such as low molecular weight heparin (LMWH).

The researchers went on to test catheters that had a thin layer of fibrin already deposited, which still showed decreased fibrin deposition when compared to the controls. This further demonstrates that catheter infections could be prevented and significantly slowed with the administration of DTIs.

The study also used *in vivo* mouse models to elucidate the effect of these catheter related infections on other organ systems, in order to determine the ability of DTIs to prevent the infectious metastasis of SA. They found that the absence or inhibition of ST resulted in significantly reduced distant infectious complications, which they tested by measuring bacterial load on the kidneys in mouse models. (Vanassche, 2013)

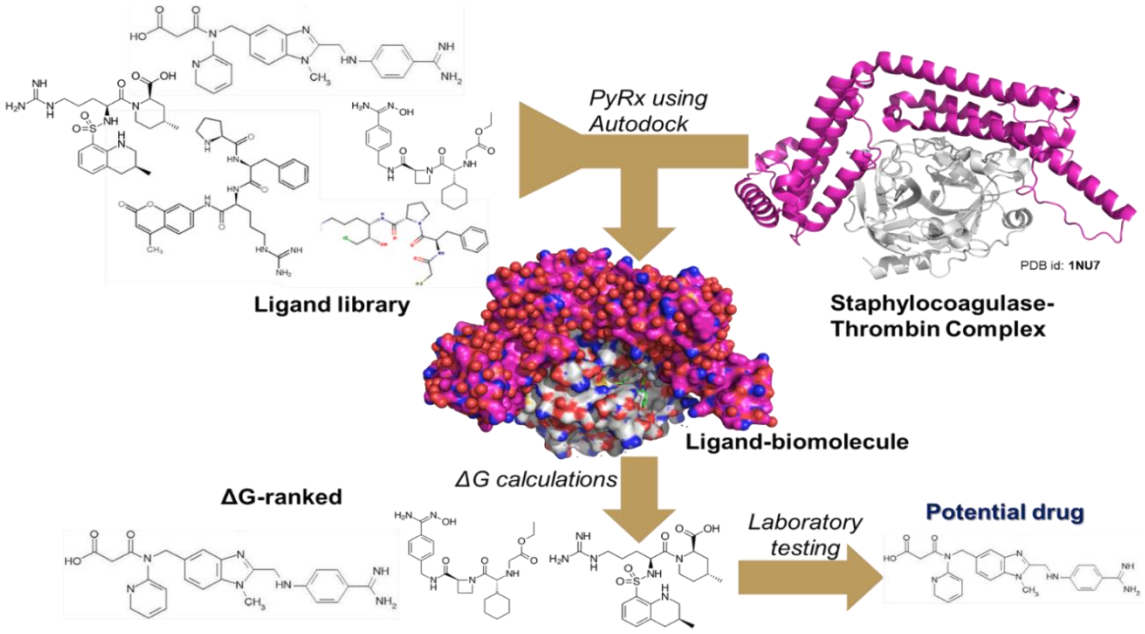
The literature on SA coagulase is extensive, but to understand the nature of binding and inhibition, it is necessary to look in depth at the structure of ST through molecular studies, and to do that it is useful to form a broad library of substances that can bind and inhibit ST. This will provide an overview of the substances that bind to ST, how they bind, and what can be done to help develop more molecules like them in the future.

CHAPTER 2: METHODS AND MATERIALS

2.1 Molecular Docking Studies

In order to further explore the mechanism of the binding of DTIs to ST, thus causing inhibition, a molecular docking review was conducted using several DTIs as potential ligands. Figure 5 details the encompassing theory behind this process. The resources available from the National Center for Biotechnology Information, specifically the PubChem database and the built-in derivative function, were used to develop a large ligand library (Kim, 2019). Several of these promising potential ligands were identified in a screening process. The program Autodock, in the parent program PyRx was used to perform molecular docking protocols (Dallakyan, 2015), which produced comparable ΔG values, also known as binding Affinity, in kcal/mole. It was found that the three DTIs tested exhibited nearly identical binding patterns when bound to Staphylothrombin, with varying binding affinity. Table 1 shows the various direct thrombin inhibitors, with their molecular weight, structure, and binding affinities, as well as the active organic groups that engaged in hydrogen bonding with Staphylothrombin.

Figure 5: Identification of Potential Drugs Using Molecular Docking, Affinity, and Laboratory Testing

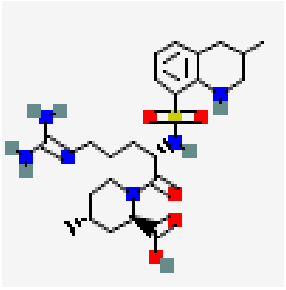
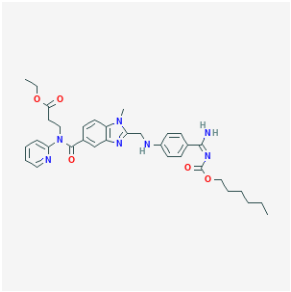
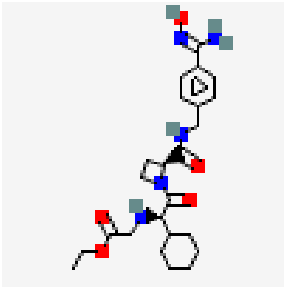
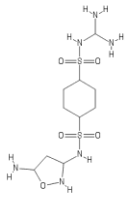


Dabigatran and Argatroban were selected as the model ligand, because they have proven clinical relevancy as previously noted. They also have widespread FDA approval for existent thrombotic diseases, and they are easily obtained for research purposes. Several additional derivatives of Dabigatran and Argatroban were tested, and a ligand that showed the greatest promise was Ximelagatran, which is converted into the DTI melagatran in the presence of physiological conditions. Ximelagatran was developed to specifically circumvent the detrimental dietary and drug interactions that patients on warfarin (a rat poison) experienced (Thijssen, 1995). Warfarin is a long-term therapy in response to thrombosis, either from strokes, heart attacks, or embolism. As a result, Ximelagatran was tested in long-term patients, and was approved by the FDA for use in warfarin substitution therapy. However, after such long-term use, Ximelagatran was pulled from marketing by the manufacturing company AstraZeneca due to concerns over liver toxicity (Harenberg, 2009). In an acute setting, however, it would be reasonable to administer Ximelagatran until a *S. aureus* infection is resolved. As shown on the

table, it has a respectable binding affinity (8.1 kcal/mol), falling squarely between Dabigatran (7.8) and Argatroban (8.4).

Finally, in addition to the three DTIs, a novel ligand was designed, and showed early promise in the binding affinity, as well as the number of hydrogen bonds holding it in place. This prototype molecule was preliminarily called Sulfamido, due to the inclusion of two sulfamide active groups in its structure. Its structure can be found on the table. The most promising aspect of this ligand is two-fold. First, it has a very high binding affinity to molecular weight ratio, because of its very small profile. Second, it is largely constituted by amino acids, allowing for readily available testing and further exploration.

Table 1: Compounds and Their Binding Affinities

Compound name	Molecular Weight	Image	Binding Functional Group	Number of Hydrogen Bonds	Binding Affinity (kcal/mol)
Argatroban	508.6 g/mol		Sulfonamide, (sulfoxyl) Amino, Acid.	6	-8.4
Dabigatran	626.3 g/mol		Acid, Amide (carboxyl), Amide (amino)	4	-7.8
Ximelagatran	473.6 g/mol		Amine, Ketone, Aldehyde	3	-8.1
Sulfamido	363.4 g/mol		Amine (Carbamine), Ketone, sulfonyl,	8	-8.3

The molecular program PyMol was used to visualize the intricacies of the bound molecules after the docking protocol was run. In addition, to obtain the binding groups and number of hydrogen bonds connecting the ligand to the active site, a hydrogen bond search was conducted using PyMol's built in function. The result is these fantastic visualizations of bound inhibitors in action. Shown in yellow, are the hydrogen bonds, going to acidic or basic residues respectfully. While more hydrogen bonds tend to lend towards higher affinities, in the case of Ximelagatran, there are only a few hydrogen bonds, but still a relatively stable binding affinity, due to factors such as London Dispersion forces and overall fit of the enzyme.

Figure 6: Argatroban bound to the inhibitory pocket of Staphylothrombin

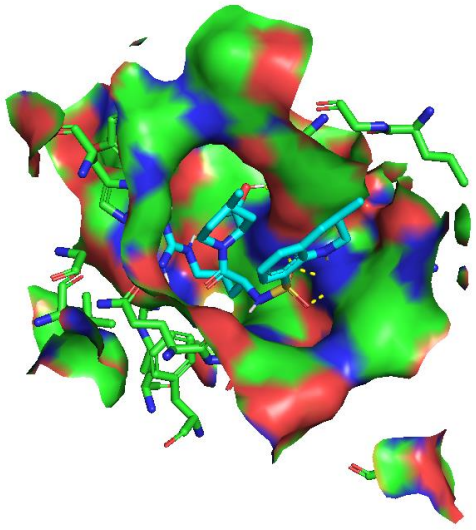


Figure 7: Dabigatran bound to the inhibitory pocket of Staphylothrombin

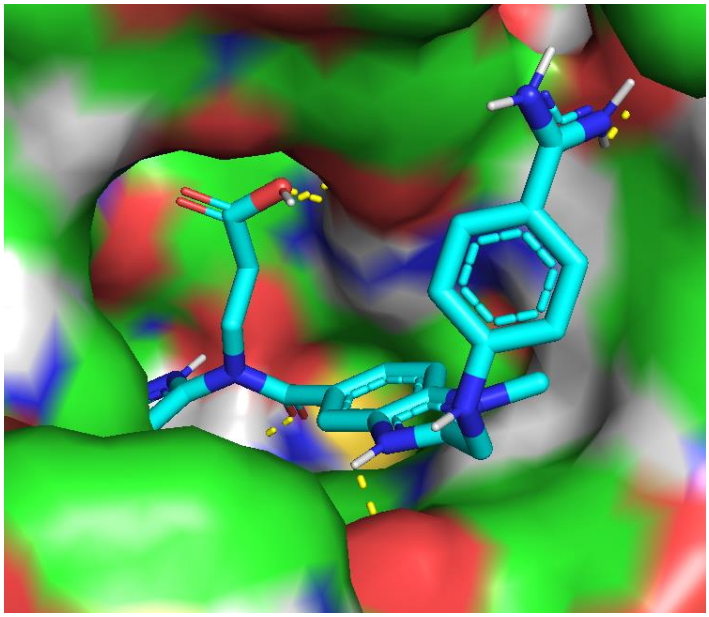


Figure 8: Ximelagatran Bound to the inhibitory pocket of Staphylothrombin

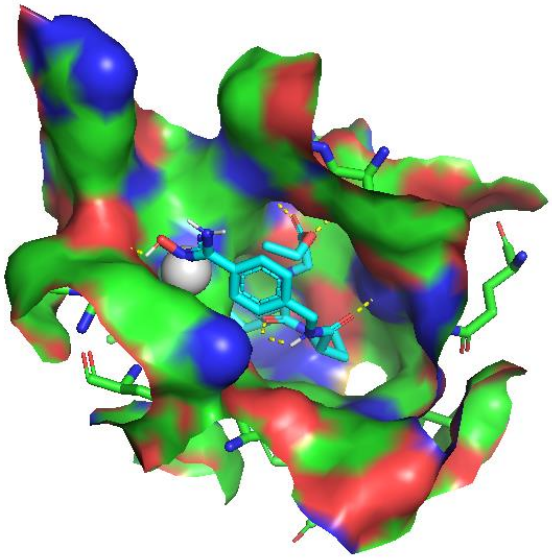
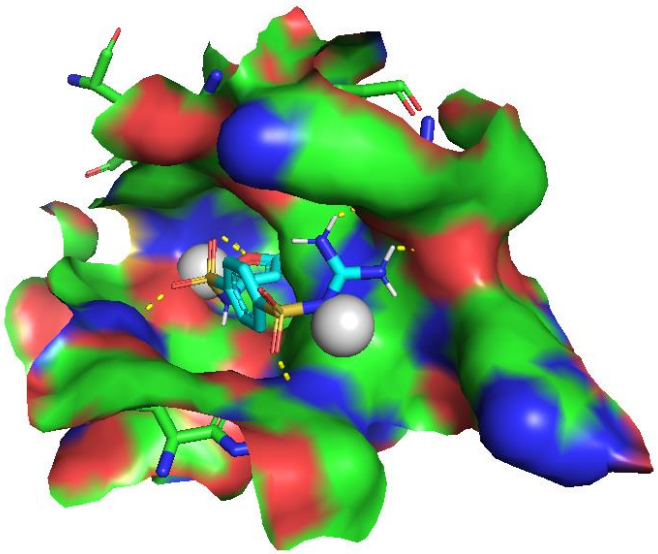


Figure 9: Sulfamido bound to the inhibitory pocket of Staphylothrombin



The binding energies and the overall fit of the enzyme will allow for an estimate of the minimum inhibitory concentration required in the following section of the test. The general prediction based off the binding affinities is that as the binding affinity to molecular weight ratio increases, the lower the minimum inhibitory concentration required to prevent coagulation. Therefore, this ligand, if synthesized, may have the lowest minimum inhibitory concentration of all the drugs previously mentioned.

2.2 Minimum Inhibitory Concentration

Much of this research revolves around the practical application of a commonly used differential test known as the coagulase test (Siegrist, 2010). This test is used differentiate *Staphylococcus aureus* from other, less pathogenic commensals in a clinical or diagnostic setting. The coagulase test is made up of a few key ingredients. The first is lyophilized rabbit plasma, which provides the prothrombin and fibrinogen necessary for coagulation. The second major constituent is an EDTA solution. EDTA, or Ethylenediaminetetraacetic acid, is a polyvalent chelating agent that sequesters calcium in the solution that would start an inappropriate coagulation cascade. Coagulase, as previously noted, activates prothrombin (a zymogen) and causes it to behave like the activated form, thrombin. Coagulase tests are usually conducted with a positive and negative control, in this case a known quantity of *S. aureus*, a known coagulase positive organism, and *Staphylococcus saprophyticus*, a known coagulase negative organism.

Notably absent from this test was the ligand developed in the previous section. The reason for this was an unfortunate time constraint, which meant the only ligands able to be tested were those that were commercially available for purchase from Sigma-Aldrich. 5 mg of each were purchased, excluding Dabigatran, where 10mg were purchased, in order to hone the technique and protocol.

Before beginning the test, several determinations of the protocol were made experimentally. First, it was experimentally tested if Dabigatran inhibited the growth or metabolism of SA by adding 1 mL of a 1.8 mg/L solution to 1 mL of broth SA. This solution was then plated and left overnight with a control containing just water and SA broth, and the next morning the two produced an indistinguishable number of colonies. The same process was

performed for DMSO, to ensure the minuscule volume of DMSO used to dissolve the drugs would not confound the inhibition of the enzyme. After these preliminary tests were performed, 0.5 milliliters (mLs) of 85% rabbit plasma was used as the basis for the test. 0.1 mLs of a 0.5 McFarland Standard (McFarland, 1907), or approximately 1.5x10^8 S. aureus colonies, as this was experimentally determined to be the least volume required for coagulation within a reasonable time period. Aliquots of 3 different direct thrombin inhibitors were added, dissolved in DMSO, with varying concentrations, to 30 tubes, 10 for each drug, with $1/5^{th}$ dilutions serially. A total of 0.4mL of the diluted ligand solution was added, bringing the whole solution in the tube to 1mL. After 14 hours, in an incubator at 37 degrees Celsius, the results were collected. "None" refers to no coagulation, VC stands for variable, or slight, coagulation, and C stands for normal coagulation. Note the concentrations in Table 2 are in mg/ml for ease of calculations, however in Table 3, they have been converted to Molarity.

Table 2: Concentration of ligands and inhibition of coagulation in mg/ml

Concentration	0.4 mg/ml	0.08 mg/ml	.016 mg/ml	3.2 µg/ml	.64 µg/ml	.128 µg/ml	25.6 pg/ml	5.12 pg/ml	1.024 pg/ml	.205 pg/ml
Argatroban	None	None	None	None	None	None	VC	C	C	C
Dabigatran	None	None	None	None	None	VC	C	C	C	C
Ximelagatran	None	None	VC	VC	VC	C	C	C	C	C

Table 3: Concentration of ligands and inhibition of coagulation in Molarity

Argatroban Results:	None	None	None	None	None	None	VC	C	C	C
Concentration in Moles/L	7.4E-4	1E-4	3E-05	6.1E-06	1.2E-06	2.43E-08	4.865E-11	9.7E-12	1.9E-12	3.91E-13
Dabigatran Result:	None	None	None	None	None	VC	C	C	C	C
Concentration in Moles/L	6.4E-4	1.3E-4	2.6E-5	5.1E-6	1.0E-6	2.04E-8	4.1E-11	8.2E-12	1.6E-12	3.3E-13
Ximelagatran Results	None	None	VC	VC	VC	C	C	C	C	C
Concentration in Moles/L	8.4E-4	1.7E-4	3.38E-05	6.8E-06	1.4E-06	2.7E-08	5.4E-11	1.1E-11	2.2E-12	4.4E-13

Dabigatran is shown in Table 4 to have about a 400x higher minimum inhibitory concentration when compared to Argatroban, which is consistent with the binding affinity theory, however on a much more significant scale. Ximelagatran in Table 5 has a much larger minimum inhibitory concentration compared even to Dabigatran and would be at a concentration impractical for clinical relevancy.

Table 4: Minimum Inhibitory concentration for Dabigatran as Compared to Argatroban

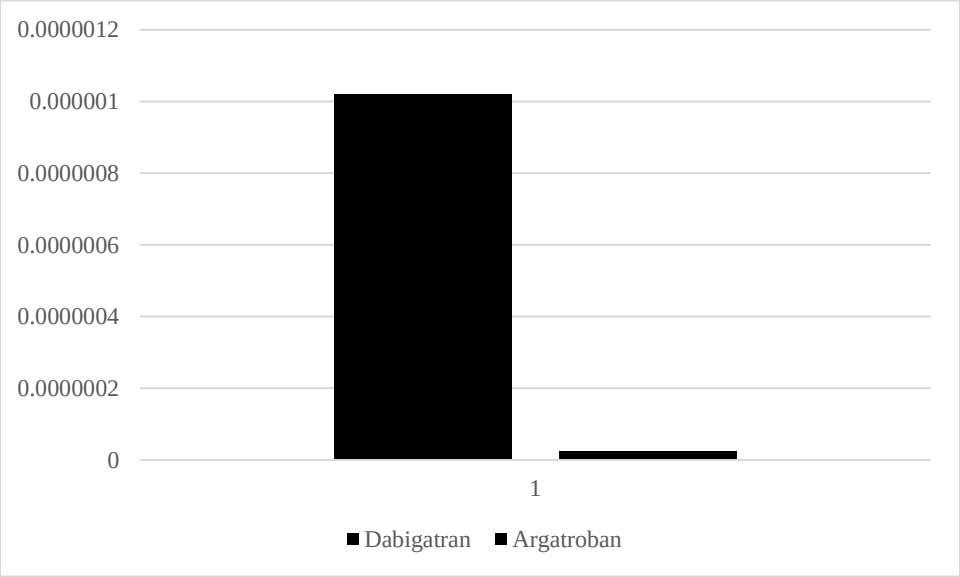
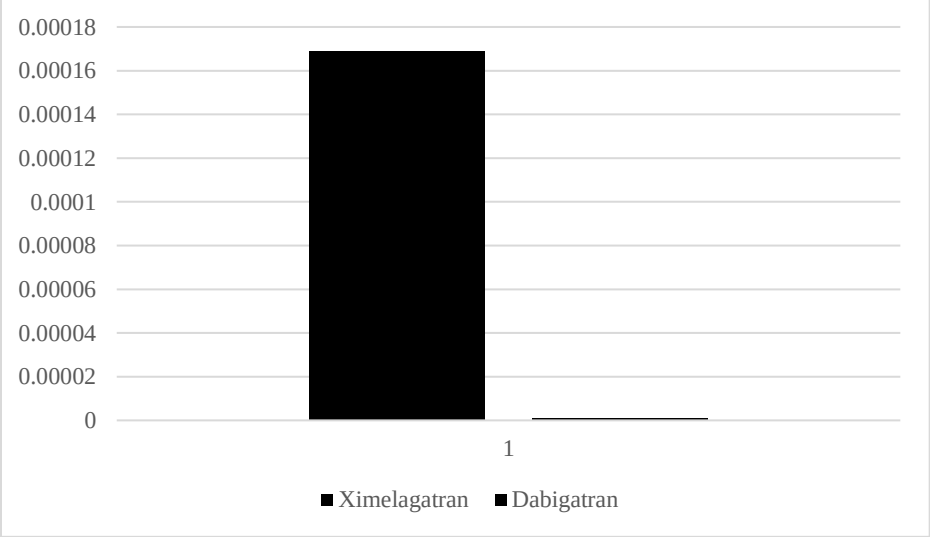


Table 5: Minimum inhibitory Concentration for Ximelagatran as Compared to Argatroban



CHAPTER 3: DISCUSSION AND CONCLUSION

3.1 Discussion

These results were largely within an expected range. Argatroban and Dabigatran behaved within reason, with the reservation that minimum inhibitory concentration assays tend to be a crude instrument for determining the relative effectiveness for an inhibitor. The major disappointment was Ximelagatran, which showed promise in a molecular docking setting, however *in vitro* demonstrated bizarre inhibition characteristics, even when standardized by molarity in the solution. Despite the lack of resolution in this test, however, this can still hold clinical relevance for one reason: Cost. Coagulase tests are regularly run in a hospital setting, using patient plasma, and patient SA samples. The only additional variable is the addition of a DTI solution, which is readily available in a hospital setting, and relatively available for a laboratory. The benefit is that it adds in the ability to determine the ideal drug to use to best fit the patient's strain of SA. If the protocol was to be further standardized using a specific concentration of thrombin, it could be used to track subtypes of MRSA or VRSA while also providing a substantial chance of recovery for the patient.

Regarding the expected trend of binding affinity to molecular weight ratio being related to requiring less of the drug to inhibit the coagulase enzyme, it seems to apply readily to Dabigatran and Argatroban (Dabigatran has a lower affinity to molecular weight ratio than Argatroban, therefore it requires more to cause inhibition) however, this did not apply to Ximelagatran, which despite in a molecular docking program having a higher affinity to weight ratio, as was seen previously required far more in order to obtain inhibition.

Some possible confounders are first that both Ximelagatran and Dabigatran have an intermediary step in the body before being deposited into the bloodstream. For Ximelagatran it is

its conversion from Ximelagatran to a smaller molecule melagatran, and for Dabigatran, it is its conversion from Dabigatran Etexilate, which is lipid soluble and readily digested and absorbed into the intestines, to the much more water-soluble Dabigatran. While this may have played a small role, it would have also played a role in the two molecule's binding affinity, so the net effect should be marginal.

An additional confounder for this protocol is the use of live *S. Aureus* species. SA is known to produce several exoenzymes such as Staphylokinase, hemolysins, and leucocidins (Tam, 2019). These all have the potential to play a possible role in partially deactivating or rendering the drugs less effective *in vitro*. While this factor may seem to render this test useless due to its unreliability, this is part of the goal of this test: to test drugs that work on live SA virulence factors, not just the enzyme held in isolation. If a certain drug is enzymatically broken down in the presence of SA species, this would be key information to know before it is given to a patient in a clinical setting.

Finally, due to the time constraints the experiment had to exclude the ligand outlined in section 2.1. This ligand showed promise in the molecular docking tests, and it would have been ideal to have tested it using the methods outlined in the minimum inhibitory concentration section. While unsuccessful, this screening strategy could theoretically be used to test a larger quantity of ligands.

3.2 Conclusion

The intent of this research was to provide an overview of the existing literature on testing DTIs on *Staphylococcus Aureus* infections, as well as provide a background and context for the mechanism of both Staphylothrombin activation and inhibition. While there are several cases in the literature where DTIs were tested in a clinical setting, the final intent of this thesis was to examine the possibility of using an existent test in order to titrate a dose and type of DTI to use on certain subtypes of SA. Using a molecular screening process against a ligand library, several potential drugs were identified based on their relative affinity. Finally, they were tested in a laboratory setting, to mixed results.

This paper has contributed to the literature by proposing a new protocol for use of the coagulase test in the context of a clinical setting and provided a useful set of chemical guideline and potential pitfalls to avoid. Future work should involve refining the process to eliminate any potential confounding factors, as well as add additional layers of quantification, such as time, refractive index, or absorption in order to increase resolution of this test. Future work should also include a preliminary ligand like the one outlined in the paper, in order to test the efficacy of this protocol in a laboratory setting. Finally, it would be ideal after obtaining a promising MIC to use X-ray crystallography to see the exact binding of DTIs to Staphylothrombin.

Staphylococcus aureus is a dangerous and robust infectious microorganism, and alternative treatments to traditional antibiotics must be considered in order to treat and cull its spread in hospitals and the population at large. MRSA and VRSA are a ubiquitous threat in a nosocomial setting, and this paper has hopefully contributed to the body of data that can be called upon for further study.

REFERENCES

Costerton, J. W. (2005). “Biofilm in Implant Infections: Its Production and Regulation.”. *The International Journal of Artificial Organs*, 1062-1068.

Dallakyan, S. (2015). Small-Molecule Library Screening by Docking With PyRx. *Methods in Molecular Biology*, 243-50.

Fowler, V. J. (2005). Staphylococcus aureus endocarditis: a consequence of medical progress. *Journal of the American Medical Association*, 3014-21.

Friedrich, R. (2003). Staphylocoagulase is a prototype of cofactor-induced zymogen activation. *Nature*, 535-539.

Harenberg, J. (2009). Drugs Effecting Blood Coagulation, Fibrinolysis, and Hemostasis. *Side Effects of Drugs Annual 31*, pp. 553-571.

Kaye, K. (2008). The deadly toll of invasive methicillin-resistant Staphylococcus aureus infection in community hospitals. *Clinical Infectious Diseases*, 1568-77.

Kim, S. (2019). Pubchem 2019 update: improved access to chemical data. *Nucleic Acids Research*, 1102-9.

Liesenborghs, L. (2018). Staphylococcus aureus, master manipulator of the human hemostatic system. *Journal of Thrombosis and Haemostasis*, 441-54.

McAdow, M. (2012). Staphylococcus aureus Secretes Coagulase and von Willebrand Factor Binding Protein to Modify the Coagulation Cascade and Establish Host Infections. *Journal of Innate Immunity*, 141-8.

McFarland, J. (1907). Nephelometer: an instrument for media used for estimating the number of bacteria in suspensions for calculating the opsonic index and for vaccines. *JAMA*, 1176-8.

Nisio, M. D. (2005). Direct Thrombin Inhibitor [review]. *The New England Journal of Medicine*, 1028-40.

Peetermans, M. (2018). Targeting Coagulase in Staphylococcus aureus Bacteraemia: A Randomized Controlled Single-Centre Trial of Staphylothrombin Inhibition. *Thombosis and Haemostasis*, 818-29.

Siegrist, J. (2010). *sigmaaldrich.com*. Retrieved from Sigma-Aldrich: https://www.sigmaaldrich.com/technical-documents/articles/analytix/staphylococcus-aureus.html

Tacconelli, E. (2017, February). *Global Priority List of Antibiotic-Resistant Bacteria to Guide Research, Discovery, and Development of New Antibiotics*. Retrieved from World Health Organization: http://www.who.int

Tam, K. (2019). Staphylococcus Aureus Secreted Toxins and Extracellular Enzymes. *Microbiology Spectrum*, 7.

Thijssen, H. H. (1995, January). Warfarin-based roedenticides: Modes of action and mechanism of resistance. *Pest Management Science*, pp. 73-78.

Vanassche. (2013). The Role of Staphylothrombin-Mediated Fibrin Deposition in Catheter Related Staphylococcus aureus Infections. *The Journal of Infectious Diseases*, 208.