Grenze International Journal of Engineering and Technology, June Issue

Insights into the Inhibition Mechanism of Lipoteichoic Acid Synthase (LtaS) Enzyme by Endophytic Fungal Metabolites: A Molecular Docking and Dynamics Simulation Study for Combatting Drug Resistance

Arif Khan[1], Suman Yadav[2] and Jai Gopal Sharma[3]
[1-3]Department of Biotechnology, Delhi Technological University, Delhi-110042, India
Email: akrathor786123@gmail.com, chinu60000@gmail.com, sharmajaigopal@dce.ac.in

***Abstract*— Antimicrobial Resistance (AMR) has spread throughout the world and caused well-known antibiotics to repeatedly fail to treat common infections. *Staphylococcus aureus* is a highly concerning pathogen among drug-resistant bacteria. It is the primary cause of life-threatening infections acquired in healthcare settings and the community, particularly those linked to implanted medical devices. The synthesis of lipoteichoic acid has been identified as a potential target for antibacterial treatment due to its essential functions in the proliferation and survival of Gram-positive bacteria. In this research work, we have employed secondary metabolites, particularly coumarins and iso-coumarins which are polyketides derived from endophytic fungi in the inhibition of Lipoteichoic acid synthase (LtaS) enzyme through molecular docking. The objective was to identify a potential drug candidate that could be used as an alternative to antibiotics in treating drug-resistant infections. Pestalustaine B displayed the highest docking score of -8.5 Kcal/mol out of the eleven polyketides that were examined. The ADMET study of Pestalustaine B and Pestalotiopisorin B revealed that both of these metabolites followed Lipinski's rule of five which is an important parameter while considering a compound as a drug candidate. Thus, polyketides indicated their potential as a drug candidate for addressing drug resistance.**

***Index Terms*— Antimicrobial resistance, Lipoteichoic acid synthase, polyketides, molecular docking, Pestalustaine B, and Pestalotiopisorin B**

I. INTRODUCTION

Antimicrobial resistance (AMR) is widely recognized as a major concern in the healthcare industry. As antibiotics are being used at increasing rates worldwide, there is a greater chance that bacteria are becoming resistant to these drugs. New genetically altered strains have thereby made it less likely that patients would receive adequate therapy, which can have serious consequences including illness, death, or medical issues.[1], [2]. If new antibiotics are not produced by 2050, antibiotic resistance (AMR) would take the lives of 10 million people yearly and the global healthcare expenditure will approach $1 trillion [3]. According to the Centres for Disease Control and Prevention (CDC), around 2 million illnesses and 23,000 fatalities occur each year due to antibiotic resistance bacteria [4]. Antimicrobial resistance has significantly increased over the past 80 years as a result of the widespread use, misuse, and overuse of antibiotics. A frightening example of a deadly member of the drug-resistant bacterial class is

Grenze ID: 01.GIJET.10.2.738

Staphylococcus aureus. It is the main reason why potentially lethal infections are emerging in hospitals and among the general public. With time, the available treatments became less effective as their scope limited. When the prevalence of drug resistance increases, patients ultimately have worse outcomes. Antibiotic-resistant bacteria are becoming more and more common in water bodies due to the discharge of untreated waste into water and the growing use of antimicrobial agents in agriculture [5]. Endophytic fungi form associations with plants. The fungi are abundant reservoirs of distinctive secondary metabolites. They commonly produce polyketides, a prevalent form of secondary metabolites. Polyketides include coumarins, iso-coumarins, chromens, α-pyrones, anthrones, and quinones. The primary objective of the current research is to explore the application of coumarins and iso-coumarins which are derived from different endophytic fungi in combating drug-resistant bacteria. Pestalustaine B is a compound that belongs to the coumarin class and is obtained from *Pestalotiopsis adusta*, a type of fungus that lives inside the *Sinopodophyllum hexandrum* plant. Pestalotiopisorin B, an isocoumarin is isolated from *Pestalotiopsis sp*., an endophyte of Rhizophora stylosa plant. Nine novel isocoumarins, designated as Peniisocoumarins A, B, C, D, E, F, G, I, and J, were derived from endophytic fungi, *Penicillium commune* QQF-3 of the plant *Kandelia candel*. These polyketides were evaluated for their antibacterial property through molecular docking studies.

Teichoic acid is found in gram-positive bacteria's cell wall specifically in the form of lipoteichoic acids and wall teichoic acids[6]. *Staphylococcus aureus* synthesizes type I lipoteichoic acid, which is the most commonly found and extensively studied polymer[7]. Lipoteichoic acid synthase (LtaS) is an crucial enzyme in the biosynthesis of Lipoteichoic acid. LtaS enzyme primarily aids in the degradation of the glycerolphosphate head group of the membrane lipid phosphatidylglycerol (PG), which forms the polyglycerolphosphate backbone of lipoteichoic acid.This is followed by the sequential addition of glycerol-phosphate units to diglucosyl-diacylglycerol. The lack of human homologues of LtaS, the positioning of the LtaS catalytic domain on the surface of bacteria, and the crucial need for LTA synthesis in bacterial growth and cell division, meet the essential criteria for developing new antibiotics to combat multi-drug resistant Gram-positive bacteria. Lipoteichoic acid is essential for cell viability, growth, and division, LtaS-deficient mutants are vulnerable to osmotic lysis and can only proliferate in environments that are osmotically stable. So teichoic acid biosynthesis inhibition is a potential therapeutic intervention approach. There are some studies in which the enzyme LtaS was targeted to halt the growth of S. aureus. In a study LtaS was irreversibly inactivated with the help of SpsB[8]. In another study, Congo red was used to inhibit the activity of LtaS and it was found that it effectively inactivated the enzyme in both cellular and in vitro experiments[9]. In the present work molecular docking has been performed targeting LtaS enzyme using coumarins and iso-coumarins isolated from endophytic fungi. ADMET study was also performed in order to determine the properties such as BBB permeant, GI absorption, drug-likeness, and pharmacokinetics to validate whether these secondary metabolites obtained from endophytic fungi possess the necessary characteristics to be considered as drugs.

II. Methodology

A. Protein and Ligand Preperation

By utilizing PDB (https://www.rcsb.org/) three- dimensional configration of protein was downloaded in PDB format. Initially the protein contains water molecules, ligands, and heteroatoms. Because of these elements, the protein cannot be used for molecular docking. these compounds don't have a critical role in the functioning of proteins, they can prevent certain ligands from attaching themselves to their intended targets. So, for their elimination Biovia Discovery Studio was employed. Hydrogen bonds were added, and protein was saved in PDB format.

B. Construction of ligand Library

A list of Polyketides, coumarins and iso-coumarins that are secondary metabolites produced by the endophytic fungi having antimicrobial properties was gathered from the literature. Every Polyketides structure was sourced from the database PubChem (https://pubchem.ncbi.nlm.nih.gov/) and was acquired in SDF format

C. Molecular Docking Studies of Secondary Metabolites of Endophytic Fungi

Molecular docking was carried out using PyRx- Python Prescription 0.8 for the prediction of binding modes of polyketides with lipoteichoic acid synthase (LtaS) target enzyme. Proteins are accepted in the PDB layout and ligands in SDF format. The SDF format of polyketides and PDB layout of target protein in PyRx was converted to PDBQT format. The dimensions (in Angstrom) of the grid box were X: 61.4214, Y: 65.8659, Z: 65.9049. The possible binding affinity between the ligand and target protein was determined. Following the completion of

docking procedure, the obtained interaction between ligand and target protein was saved in PDB format and was then analyzed using Biovia Discovery Studio and PyMOL where a 2D graphic depicting the interaction between the protein and ligand was created and saved as image files.

D. ADMET Analysis and Toxicity Profile

ADMET analysis of selected polyketides was evaluated to determine their suitability as a potential drug candidate. The analysis was evaluated using SwissADME, a web tool available at http://www.swissadme.ch/. The selected compounds were converted to SMILE format and subsequently analyzed using PreADMET and SwissADME tools. The drug-likeness of the secondary metabolite was assessed using two rules, Lipinski's rule and Veber's rule. The compound's toxicity profile was predicted using the ProTox-II Web tool. Organ toxicity and toxicity endpoint predictions were also made using ProTox-II Web tool.

III. Results And Discussion

A. Molecular Docking Studies of Secondary Metabolites of Endophytic Fungi

Molecular docking was carried out to evaluate the binding interactions between polyketides and the target protein, lipoteichoic acid synthase (PDB ID: 2W5S). Pestalustaine B recorded the highest binding affinity of -8.5 Kcal/mol followed by Pestalotiopisorin B with -7.8 Kcal/mol binding affinity. The binding affinities of polyketides are mentioned in Table 1. Congo red was taken as the control molecule. It has been reported that Congo red is the reported inhibitor of LtaS enzyme. The binding affinity of control was recorded to be -7.2 Kcal/mol. Fig. 1 illustrates the 2D interaction of the control and target protein and also highlights the interactions that took place while binding to the target protein. Seven polyketides recorded their docking score more than that of the control, while two of them recorded the same docking score as -7.2 Kcal/mol. 2D interactions of polyketides and 2W5S with interactions that took place between them are shown in Fig. 2.

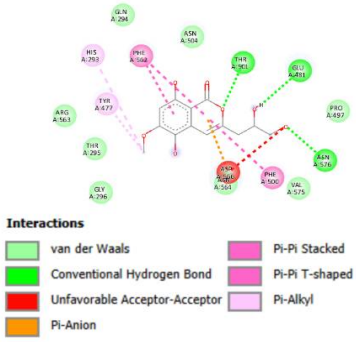
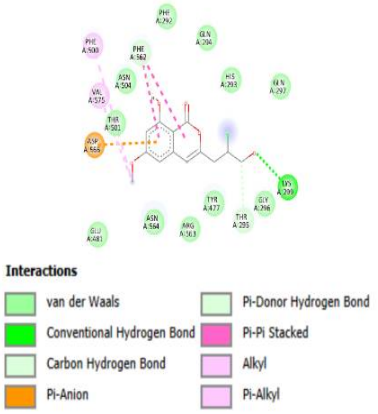
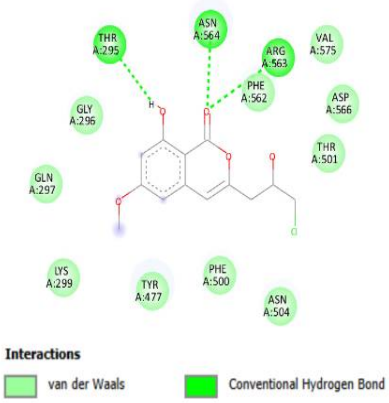


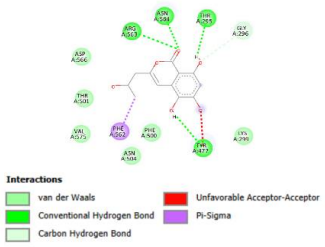
Figure 1. 2D interaction of Congo red (Control) with 2W5S target protein.



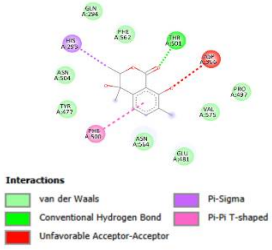
Peniisocoumarin E



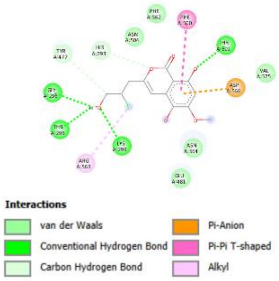
Peniisocoumarin D



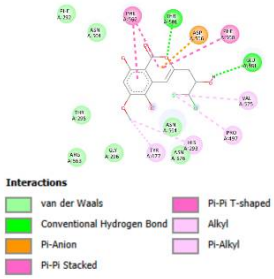
Peniisocoumarin G



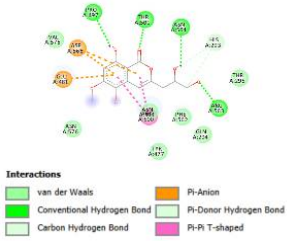
Pestalotiopisorin B



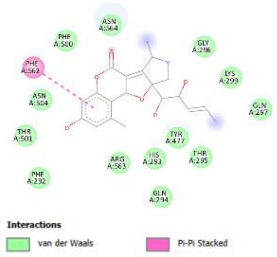
Peniisocoumarin F



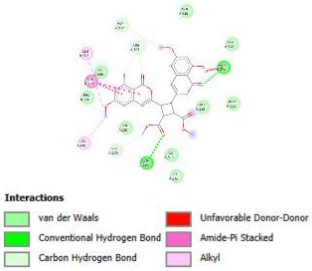
Peniisocoumarin J



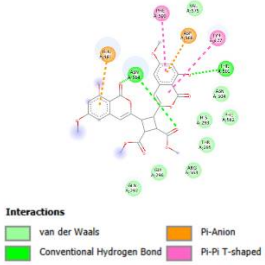
Peniisocoumarin I



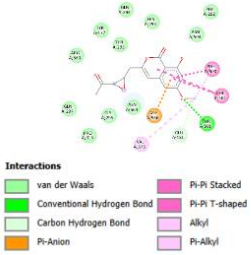
Pestalotiopisorin B



Peniisocoumarin A



Peniisocoumarin B



Peniisocoumarin C

Figure 2. 2D interactions between polyketides and 2W5S.

TABLE I. BINDING AFFINITY OF POLYKITIDES

Polyketide	Binding Affinity (Kcal/mol)
Pestalustaine B	-8.5
Pestalotiopisorin B	-7.8
Peniisocoumarin B	-7.8
Peniisocoumarin J	-7.4
Peniisocoumarin C	-7.4
Peniisocoumarin A	-7.3
Peniisocoumarin G	-7.3
Peniisocoumarin E	-7.2
Peniisocoumarin F	-7.2
Peniisocoumarin D	-7.1
Peniisocoumarin I	-7.0

B. ADMET Analysis and Toxicity Profile

The ADMET analysis of the top 2 polyketides, Pestalustaine B and Pestalotiopisorin B with the highest binding affinity was carried out. The molecular weight of Pestalustaine B and Pestalotiopisorin B was found to be 370.40 g/mol and 222.24 g/mol respectively. Gastrointestinal absorption of both the secondary metabolites was predicted to be high and Pestalotiopisorin B was also found to be BBB permeant. The number of H-Bond acceptors and H-Bond donors in the case of Pestalustaine B was found to be 3 and 6 respectively while in the case of Pestalotiopisorin B, it was found to be 2 and 4 respectively. Log Po/w (MLOGP) values in both the cases did not exceed 5 thus the SwissADME prediction reveals that both the polyketides follow Lipinski's rule of five. Both the metabolites also followed Veber's rule as the number of rotatable bonds was less than 10 in both the cases and the Topological Polar Surface Area was also predicted to be less than 140 A2. Various properties of compounds predicted by ADMET analysis are mentioned in Table 2. Further compound's toxicity profile was predicted using the ProTox-II Web tool. LD50 of Pestalustaine B was predicted to be 600 mg/kg and 4 was the predicted toxicity class while LD50 of Pestalotiopisorin B was found to be 1000kg/mg and its predicted toxicity class was also 4. Further organ toxicity and toxicity endpoints were also examined and are mentioned in Tables 3 and 4.

Pestalustaine B Pestalotiopisorin B

Figure 3. Bioavaibility RADAR of Pestalustaine B and Pestalotiopisorin B

TABLE II. ADMET ANALYSIS OF PESTALUSTAINE B AND PESTALOTIOPISORIN B

Parameter	Properties	Pestalustaine B	Pestalotiopisorin B
Pharmacokinetics	GI absorption	High	High
	BBB Permeant	No	Yes
	P-gp substrate	Yes	No
	CYP1A2 inhibitor	No	No
	CYP3A4 inhibitor	No	No
	CYP2C9 inhibitor	No	No
	CYP2D6 inhibitor	Yes	No
	CYP2C19 inhibitor	No	No
Physicochemical	Formula	$C_{21}H_{22}O_6$	
	Molecular Wt	370.40(g/mol)	$C_{12}H_{14}O_4$
		3	

	.Num. of rotatable bonds Num. of H donors Num. of H acceptors TPSA	3 6 100.13 A^2	222.24(g/mol) 0 2 4 66.76 A^2
Solubility	Log S (Ali) Class	-3.66 Soluble	-2.90 Soluble
Lipophilicity	Log $P_{o/w}$ (iLOGP) Log $P_{o/w}$ (MLOGP) Log $P_{o/w}$ (WLOGP)	3.02 1.74 2.53	2.06 1.27 1.36
Drug-likeness	Bioavailability score Lipinski	0.55 Yes; 0 violation	0.55 Yes; 0 violation

TABLE III. TOXICITY PROFILE OF PESTALOTIOPISORIN B

Classification	Target	Probability	Prediction
Toxicity endpoints	Immunotoxicity Carcinogenicity Mutagenicity Ecotoxicity Cytotoxicity Clinical-toxicity Nutritional toxicity	0.88 0.55 0.60 0.54 0.83 0.53 0.5	Inactive Inactive Inactive Inactive Inactive Inactive Inactive
Organ toxicity	Respiratory toxicity Neurotoxicity Nephrotoxicity Hepatotoxicity	0.68 0.85 0.61 0.72	Active Inactive Inactive Inactive

TABLE IV. TOXICITY PROFILE OF PESTALUSTAINE B

Classification	Target	Probability	Prediction
Toxicity endpoints	Immunotoxicity Carcinogenicity Mutagenicity Ecotoxicity Cytotoxicity Clinical toxicity Nutritional toxicity	0.61 0.61 0.57 0.53 0.83 0.52 0.55	Active Active Active Inactive Inactive Inactive Active
Organ toxicity	Respiratory toxicity Neurotoxicity Nephrotoxicity Hepatotoxicity	0.73 0.88 0.74 0.82	Active Inactive Inactive Inactive

IV. CONCLUSION

There is a wide range of antibiotics currently being used in the clinical field to target various aspects of bacteria in order to kill them or prevent their growth. However, bacteria will persistently acquire resistance to these antibiotics through various mechanisms. The high occurrence of antimicrobial resistance requires the implementation of proactive measures to identify and address existing environmental risks, as well as the careful evaluation of alternative approaches. In the present work, we have employed polyketides, particularly coumarins and iso-coumarins in inhibiting lipoteichoic acid synthase (LtaS) through molecular docking studies. Pestalustaine B recorded the highest docking score of -8.5 Kcal/mol. The binding affinity of Congo red which is taken as control was recorded to be -7.2 Kcal/mol. It was found that seven polyketides recorded their docking score higher than control. Further ADMET analysis and toxicity profile of Pestalustaine B and Pestalotiopisorin B revealed that both of them followed Lipinski's rule and Veber's rule according to the prediction that was made through SwissADME tool. Thus, these compounds can be considered potential drug candidates and can inhibit the spread of drug resistance infections by inhibiting the enzyme LtaS. Further research is needed to evaluate the interaction between LtaS produced by Staphylococcus aureus and secondary metabolites produced by endophytic fungi in a controlled laboratory environment. This is necessary to confirm their potential as antimicrobial agents against MRSA, with the aim of addressing drug resistance.

ACKNOWLEDGMENT

We are grateful for the kind support of Prof. Jai Gopal Sharma, Dr. Megha Bansal, and our lab mates, who have inspired and supported our academic endeavors. The completion of this paper has been equally contributed by both authors. We express our gratitude the Department of Biotechnology, DTU, for providing the essential resources.

REFERENCES

[1] B. Khameneh, R. Diab, K. Ghazvini, and B. S. Fazly Bazzaz, "Breakthroughs in bacterial resistance mechanisms and the potential ways to combat them," Microbial Pathogenesis, vol. 95. Academic Press, pp. 32–42, Jun. 01, 2016. doi: 10.1016/j.micpath.2016.02.009.

[2] G. Zhou, Q. S. Shi, X. M. Huang, and X. B. Xie, "The three bacterial lines of defense against antimicrobial agents," International Journal of Molecular Sciences, vol. 16, no. 9. MDPI AG, pp. 21711–21733, Sep. 09, 2015. doi: 10.3390/ijms160921711.

[3] O'Neill, J. (2014) Antimicrobial resistance: tackling a crisis for the health and wealth of nations. Wellcome Trust and HM Government, London, United Kingdom, Antimicrobial resistance: tackling a crisis for the health and wealth of nations. Wellcome Trust and HM Government, London, United Kingdom.

[4] M. U. Okwu, M. Olley, A. O. Akpoka, and O. E. Izevbuwa, "Methicillin-resistant staphylococcus aureus (MRSA) and anti-MRSA activities of extracts of some medicinal plants: A brief review," AIMS Microbiology, vol. 5, no. 2. AIMS Press, pp. 117–137, 2019. doi: 10.3934/microbiol.2019.2.117.I. S. Jacobs and C. P. Bean, "Fine particles, thin films and exchange anisotropy," in Magnetism, vol. III, G. T. Rado and H. Suhl, Eds. New York: Academic, 1963, pp. 271–350.

[5] Young, H.-K. Antimicrobial Resistance Spread in Aquatic Environments. Journal of Antimicrobial Chemotherapy 1993, 31 (5), 627–635. https://doi.org/10.1093/jac/31.5.627.R. Nicole, "Title of paper with only first word capitalized", J. Name Stand. Abbrev., in press.

[6] L. W. Pasquina, J. P. Santa Maria, and S. Walker, "Teichoic acid biosynthesis as an antibiotic target," Current Opinion in Microbiology, vol. 16, no. 5. pp. 531–537, Oct. 2013. doi: 10.1016/j.mib.2013.06.014.

[7] O. Schneewind and D. Missiakas, "Lipoteichoic acids, phosphate-containing polymers in the envelope of gram-positive bacteria," Journal of Bacteriology, vol. 196, no. 6. pp. 1133–1142, Mar. 2014. doi: 10.1128/JB.01155-13.

[8] M. E. Wörmann, N. T. Reichmann, C. L. Malone, A. R. Horswill, and A. Gründling, "Proteolytic cleavage inactivates the Staphylococcus aureus lipoteichoic acid synthase," J Bacteriol, vol. 193, no. 19, pp. 5279–5291, Oct. 2011, doi: 10.1128/JB.00369-11.

[9] C. R. Vickery, B. M. K. Wood, H. G. Morris, R. Losick, and S. Walker, "Reconstitution of Staphylococcus aureus Lipoteichoic Acid Synthase Activity Identifies Congo Red as a Selective Inhibitor," J Am Chem Soc, vol. 140, no. 3, pp. 876–879, Jan. 2018, doi: 10.1021/jacs.7b11704.