Volume 3, Issue 5, 2025

Frontier in Medical & Health Research

ISSN: (e) 3007-1607 (p) 3007-1593

MOLECULAR DOCKING INVESTIGATION OF VANILLIN-SCHIFF BASES AS A ARABINOSYLTRANSFERASE-C INHIBITORS

Husna Iqbal[1], Asad Ullah Khan[2], Sidratul Muntaha[3], Muhammad Omar[4], Rahman Ali Khan[5], Salma[6], Sunbal Khan[7], Abdul Latif[8], Farhat Iqbal[9], Naila[10], Adnan Shahzad[*11], Izharullah Mian[12]

*[1,2,4,6,7,8,9,10, *11,12]Institute of Chemical Sciences, University of Swat, Swat, Pakistan*
[3]Center for Plant Science and Biodiversity, University of Swat, Swat,
[5]Center for Microbiology and Biotechnology, University of Swat, Swat,

*[*11]adnanshahzad@uswat.edu.pk*

DOI: https://doi.org/10.5281/zenodo.16742352

Keywords
Molecular Docking, Arabinosyltransferase-C, Vanillin, Schiff Bases

Article History
Received: 28 April, 2025
Accepted: 16 July, 2025
Published: 31 July, 2025

Corresponding Author: *
Adnan Shahzad

Abstract

Based on their biological pharmacophore activity, schiff bases are potent. As a result, we chose different Schiff bases SB1–SB4 based on vanillin for their ability to bind to the MTB Arabinosyltransferase- C target enzyme. Molecular docking software AutoDock vina in the protein's Ligand Binding site pocket, Avogadro, Gaussian09 software, B3LYP theory, and 6-31g basis were used to investigate the inhibitory potential of a subset of vanillin-based Schiff bases, SB1-SB4. According to the findings of the molecular docking research, the compounds SB1–SB4 show encouraging binding interactions with Arabinosyltransferase-C, with favourable binding energies ranging from -8.46 kcal/mol to -8.85 kcal/mol. Because of their significant binding energies, which indicate that they can effectively alter the activity of the Mycobacterium enzyme, these inhibitor chemicals are great candidates for the creation of novel therapeutics. With more investigation and testing of these medications, potent Mycobacterium inhibitors with enhanced effectiveness and fewer adverse effects might be developed. According to the findings, compounds based on vanillin-based Schiff bases exhibit superior inhibitory efficacy against specific enzymes Arabinosyltransferase-C

INTRODUCTION

About one-fourth of the world's population is infected with Mycobacterium tuberculosis (MTB), the pathogen that causes one of the most deadly and contagious diseases, tuberculosis (TB). [1] Around 10.4 million people had TB in 2016, according to the WHO, and it is a leading cause of death for those who have HIV. [2] One of the most common public health problems that causes havoc globally is multidrug-resistant (MDR) bacteria. 4,90,900 of the 6,00,000 TB patients are multidrug resistant (MDR-TB). Antimicrobial medications EMB (ethambutol) and INH (isoniazid) are widely used to treat tuberculosis; however, in recent years, isoniazid (INH) has been proven to be resistant. [3] According to the most recent assessment, EMB resistance affects 2.3% and 3.8% of patients with new and recurrent tuberculosis, respectively, in the United States. [4-6] According to a list of 63 surveys on antituberculosis medication resistance carried out between 1985 and 1994, a small number of nations had a rate of acquired EMB resistance higher than 13.7%. A transferase enzyme called arabinosyltransferase C operates on arabinose, which is found in furanose form, and aids in the polymerisation of arabinogalactan, a crucial component of the cell wall of mycobacteria. [7]

Frontier in Medical & Health Research

ISSN: (e) 3007-1607 (p) 3007-1593

EMB inhibits the biosynthesis of cell walls by blocking the synthesis of arabinogalactan and lipoarabinaomannan (LAM) through three arabinosyl transferases. [8] It is used in combination with isoniazid, pyrazinamide, and rifampin (rifampicin). i.eEmbA(Rv3794), EmbB(Rv3795) and EmbC(Rv3793). EmbA, EmbB and EmbC belongs to glycosyltransferase superfamily C(GT-C). While EmbC is linked to the production of LAM, EmbA and EmbB are involved in the biosynthesis of arabinogalactan. [3] LAM plays a significant role in several perspectives on the interactions between host cells and Mycobacterium species. [9] Since there is a considerable correlation between EmbC activity and the amount of LAM species generated from overexpression of the embC mutant of M. smegmatis with embC Mtb, EmbC is regarded as a high-quality target for ethambutol. [10] The transmembrane helices that make up the characteristic architecture of these M. tuberculosis Emb enzymes are synchronised with a hydrophilic C-terminal domain that comprises residues ranging from 719 to 1094 in the full-length enzyme. [11] While ligand-based computational virtual screening methods, when coupled with other techniques, are found to be an important part that is used for de novo characterisation, identification of potential inhibitors, and drug repurposing, structure-based drug discovery and computational approaches provide a valuable alternative to the costly and time-consuming process of random screening. [12-14] Using molecular docking, the current study aims to assess the binding affinities of several functional derivatives of ethambutol and the already available medication isoniazid against Arabinosyltransferase C of Mycobacterium TB.[15-17]

1. Methodology

The binding interaction mechanism of the synthesized vanillin-based Schiff bases were evaluated for competitive and noncompetitive inhibition of the mark enzymes of *Mycobacterium tuberculosis* (DNA gyrase subunit A and DprE1) using molecular docking studies. The crystal structures of enzymes of interest were obtained from the Protein Data Bank. Protein was theoretically be prepared by using AutoDock MGL tools (1.5.6 version) and optimized by removing all water molecules, heteroatoms, and cofactors for simplicity of calculations [18]. The best docking conformer with the lowest binding energy were selected for further investigations. The protein-ligand interaction results analysis were concluded from highly stable confirmer images, and the docking poses of selective vanillin-based Schiff bases against target enzymes were visualized by PyMol (3.1), AutoDock Vina (1.5.6 version), BIOvia Discovery Studio visualization (V 4), and LigPlot (2.2)**.**[19, 20]

2. Result and discussion

2.1. Molecular docking of vanillin-based Schiff bases:

The selection procedures of receptor proteins were purely based on the literature survey. To assess the multi-domain antimycobacterial activity of ligands, different types of receptor proteins (mycobacterial protein) were taken into consideration that had different 3D structures and different functions as well; specifically, they all must have had some critical functions required for the survival of the target bacterial cell and its infection [21]. The selected receptor protein Arabinosyltransferase-C (PDB ID: 3pty) have important roles in carrying out essential cellular processes. The Arabinosyltransferase-C enzyme is essential for the mycobacteria's cell wall production [22] To inhibit the function of selected enzymes in our present study the synthesized vanillin-based Schiff bases **(SB1-SB4)** were selected for inhibition potential against Arabinosyltransferase-C and one standard **Macozinone** [23] (as shown in Scheme 1). Also test against these enzymes for comparative studies.

Volume 3, Issue 5, 2025

Frontier in Medical & Health Research

ISSN: (e) 3007-1607 (p) 3007-1593

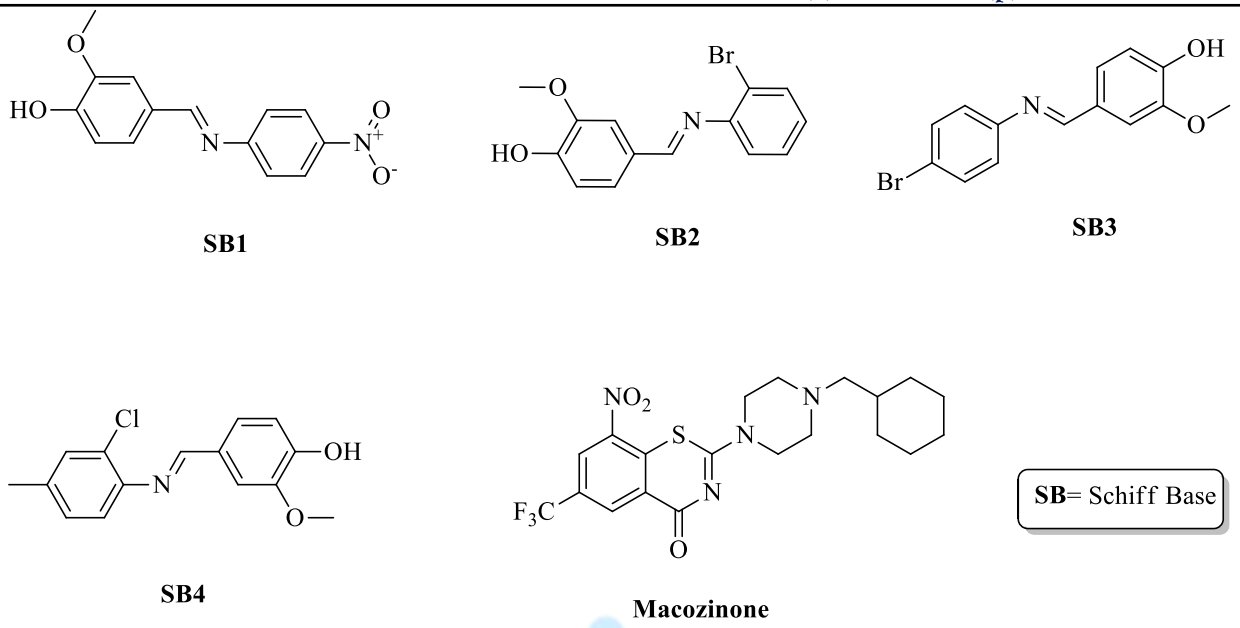

*Figure 1. Structural formulae of vanillin-based Schiff bases **(SB1-SB4)** and Standard **S1-S2***

The binding analysis of **SB1** with Arabinosyltransferase-C shows that compound **(SB1)** possesses a great binding potential with docking score of -8.85 kcal/mol (shown in **Table 1**) and inhibition constant is 326.9nM. Compound **(SB1)** show interaction with Arabinosyltransferase-C enzyme in active site TYR-841, ARG-930, SER-1047, TRP-1057, ASP-1056 and GLY-1058. The title compound S1 was fitted in the active pocket of Arabinosyltransferase-C enzyme which allows, N-O and N-H groups to make hydrogen bonding interaction with ARG-930 (N-O...H-N), TRP-1057 (N-O...H-N), and GLY-1058 (N-O...H-N) residues of Arabinosyltransferase-C enzyme. With a bond distance is 2.2Å each (shown in **table 2**) respectively. Beside these Hydrogen-bonds in active site, **SB1** also exhibit hydrophobic, weak Vander Wall interaction and π-π interaction with, TYR-841 (N...O-H) amino acid of Arabinosyltransferase-C represented in **Figure 2**. The Binding poses of compound **(SB1)** with above mention Arabinosyltransferase-C enzyme 3D structures are illustrated in **Figure 2**. The selected compound **(SB2)** show inhibition potential against to Arabinosyltransferase-C possess a docking score of -8.49 kcal/mol (shown in **Table 1**) and inhibition constant is 601.56nM. Compound (S2) show interaction with Arabinosyl transferase-C enzyme in active site, ARG-930, ASN-928, SER-1047, ASP-1056.The title Compound (S2) was fitted in active pocket of Arabinosyltransferase-C enzyme which allow C-O and O-H group to form hydrogen bond interaction with ARG-930(C-O...HN), SER-1047(OH...O), ASN-928(HO...HN) and SER-1047(HO...HN) residue of Arabinosyl transferase-C enzyme with a bond distance is 1.8Å, 1.9Å, 2.2Å, 2.2Å (shown in **table 2**) respectively. Beside these hydrogen bonds in active site, **SB2** also exhibit hydrophobic, weak Vander Wall interaction and π-π interaction with ASP-1056(N...O) in **Figure 3.** In 2021, Manu Kumar and Sandeep Kumar Singh tested the effects of aloin, a key component of aloe vera, on the enzyme arabinosyltransferase-C. While our experiment was similar to theirs, our compound's results were much different, coming in at -8.49 kcal/mol as opposed to their -6.3 kcal/mol [21]. The Binding poses of compound **(SB2)** with Arabinosyltransferase-C enzyme 3D structures are illustrated in **Figure 2**. Compound **(SB3)** was selected for their inhibition potential against the Arabinosyltransferase-C enzyme docking score is -8.46kcal/mol (shown in **Table 1**) and inhibition constant is 629.76nM. Compound **(SB3)** interact with residues TRP-926, TYR-841, SER-1047 of Arabinosyltransferase-C enzyme. The compound **(SB3)** was able to be docked deeply within the binding pocket region of the Arabinosyl-transferase-

Frontier in Medical & Health Research

ISSN: (e) 3007-1607 (p) 3007-1593

C enzyme. Among these hydrogen bonds Participating in the interaction via the SER-1047 (O-H...O), SER-1047 (H-O...H-N), SER-1047 (C=O...H-N) (given in **Figure 4)** with a bond distance is 1.8Å, 2.1Å, 2.1Å (shown in **table 2)** Beside these hydrogen bonds in active site **SB3** also exhibit hydrophobic, weak Vander wall interaction and π-π interaction with TYR-841 (N...O-H) and TRP-926(N...O-C) amino acid of Arabinosyltransferase-C enzyme represent in **(Figure 4)**. The Binding poses of compound **(SB3)** with Arabinosyltransferase-C enzyme 3D structures are illustrated in **Figure 3**. The selected compound **(SB4)** show excellent potential of inhibition against the Arabinosyl transferase-C enzyme with a Docking score -8.70kcal/mol (shown in **Table 1)** and inhibition constant is 417.41μM. Compound (SB4) show interaction with Arabinosyltransferase-C enzyme in active site GLY-1058, ARG-930, SER-1047 and ASN-928. The title compound S4 was fitted in the active pocket of Arabinosyltransferase-C enzyme which allow C-O and H-O group to make hydrogen bonding interaction with ARG-930 (-C-O...H-N), SER-1047 (O-H...O) and ASN-928 (H-O...H-N) residues of Arabinosyltransferase-C enzyme with a bond distance is .1.9Å,1.9Å and 2.0Å (shown in **table 2)** respectively. Beside these hydrogen bonds in active site **SB4** also exhibit hydrophobic, weak Vander wall interaction and π-π interaction with GLY-1058 (N...O) amino acid of Arabinosyltransferase-C enzyme represent in **(Figure 5)**. The Binding poses of compound **(SB4)** with Arabinosyltransferase-C enzyme 3D structures are illustrated in **Figure 5**

The standard compound **macozinone** show inhibitor opposed to Arabinosyltransferase-C possess a docking score -9.12 kcal/mol (shown in **Table 1)** and inhibition constant is 204.93 nM. Compound **macozinone** show interaction with the active site of Arabinosyltransferase-C enzyme is VAL-920 and TYR-841. Compound **macozinone** form a hydrogen bond with the active amino site of Arabinosyltransferase-C enzyme is TYR-841 (O...H-N) with a bond distance is 2.3Å. Beside these hydrogen bond in active site, **macozinone** also exhibit hydrophobic, weak Vander Wall interaction and π-π interaction with VAL-920 (N...O) amino acid of Arabinosyltransferase-C enzyme.

Table 1. Binding energy in (kcal/mol) of compound (SB1-SB4) with Arabinosyltransferase-C

Compounds	*Binding energy in (kcal/mol)*	*Inhibition constant*
SB1	-8.85	326.9nM
SB2	-8.49	601.56nM
SB3	-8.46	629.76nM
SB4	-8.70	417.41μM
Macozinone	-9.12	204.93 nM

Table 2. Residues involved in interactions and their distances of selected compounds with Arabinosyltransferase-C

Compounds	Interacting amino acids	Interactions	Types of interactions	Distances(Å)
SB1	ARG-930, TRP-1057, GLY-1058	NH...O=N, NH...O=N, NH...O=N	H-Bonding	2.2, 2.2, 2.2
SB2	ARG-930, ASN-928, SER-1047, SER-1047	C-O...H-N, H-O...H-N, H-O...H-N, O-H...O	H-Bonding	1.8, 2.2, 2.2, 1.9
SB3	SER-1047, SER-1047, SER-1047	O...HN, O...HN, O...HN	H-Bonding	1.8, 2.1, 2.1
SB4	ARG-930, SER-1047, ASN-928	C-O...H-N, O-H...O, H-O...H-N	H-Bonding	1.9, 1.9, 2.0
Macozinone	SER-1047, ASN-928, ARG-930	O...HN, NH..O, O...HN	H-Bonding	1.8, 2.1, 2.3

Volume 3, Issue 5, 2025

Frontier in Medical & Health Research

ISSN: (e) 3007-1607 (p) 3007-1593

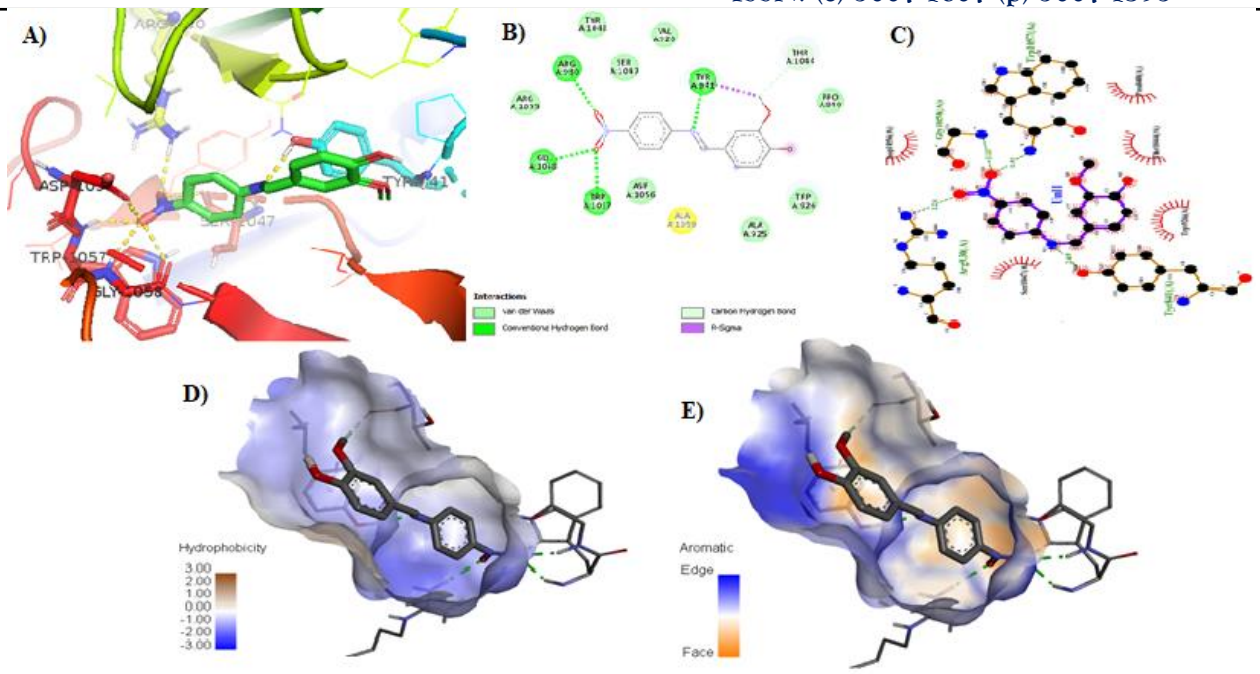

Figure 2. Binding poses of Compound (**SB1**) with Arabinosyltransferase-C enzyme (A), 2D interactions (B & C) (Hydrophobic interactions (D) and Aromatic interactions (E).

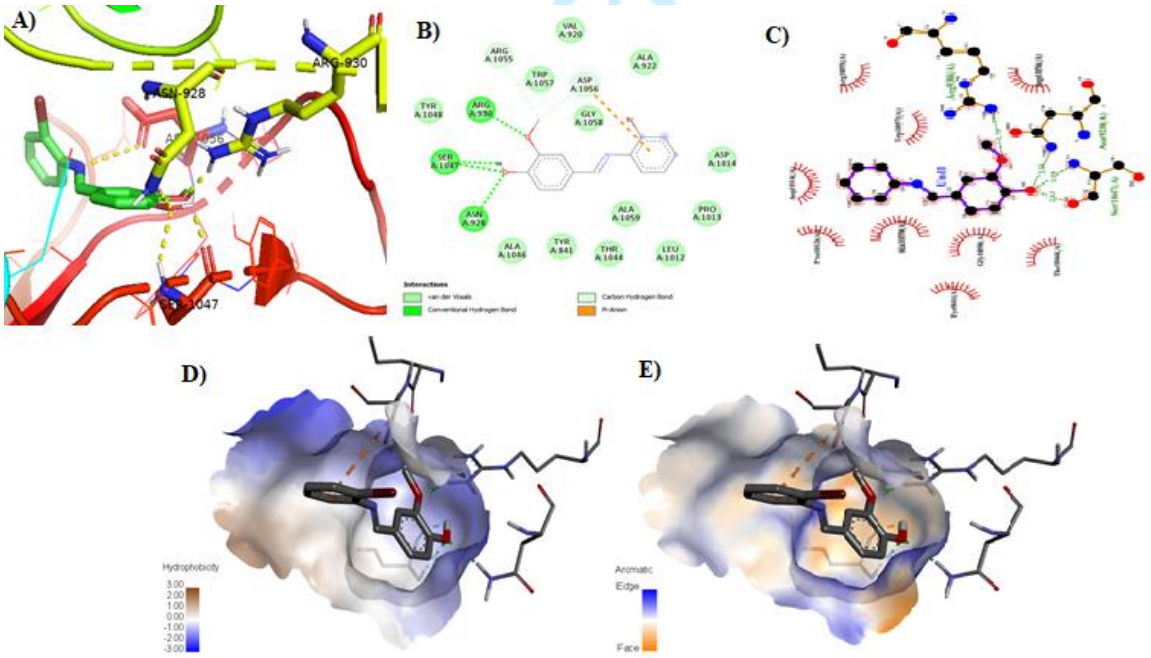

Figure 3. Binding poses of Compound (**SB2**) with Arabinosyltransferase-C enzyme (A), 2D interactions (B & C) (Hydrophobic interactions (D) and Aromatic interactions (E).

Volume 3, Issue 5, 2025

Frontier in Medical & Health Research

ISSN: (e) 3007-1607 (p) 3007-1593

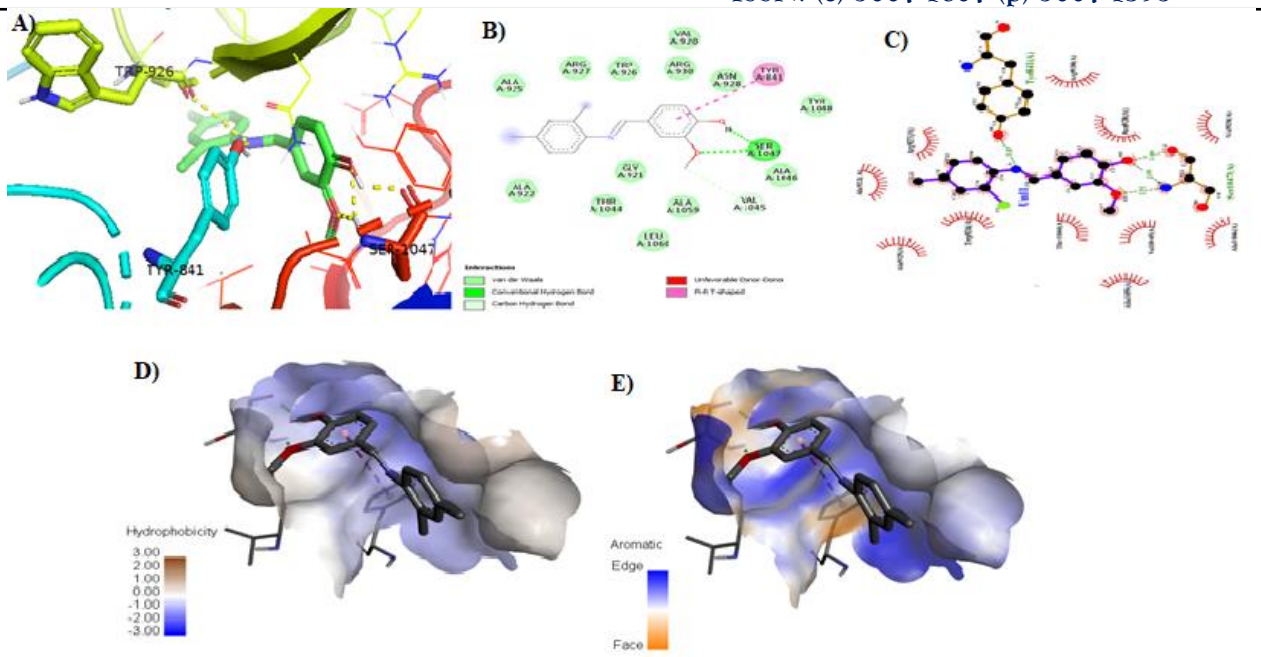

Figure 4. Binding poses of Compound **(SB3)** with Arabinosyltransferase-C enzyme (A), 2D interactions (B & C) (Hydrophobic interactions (D) and Aromatic interactions (E).

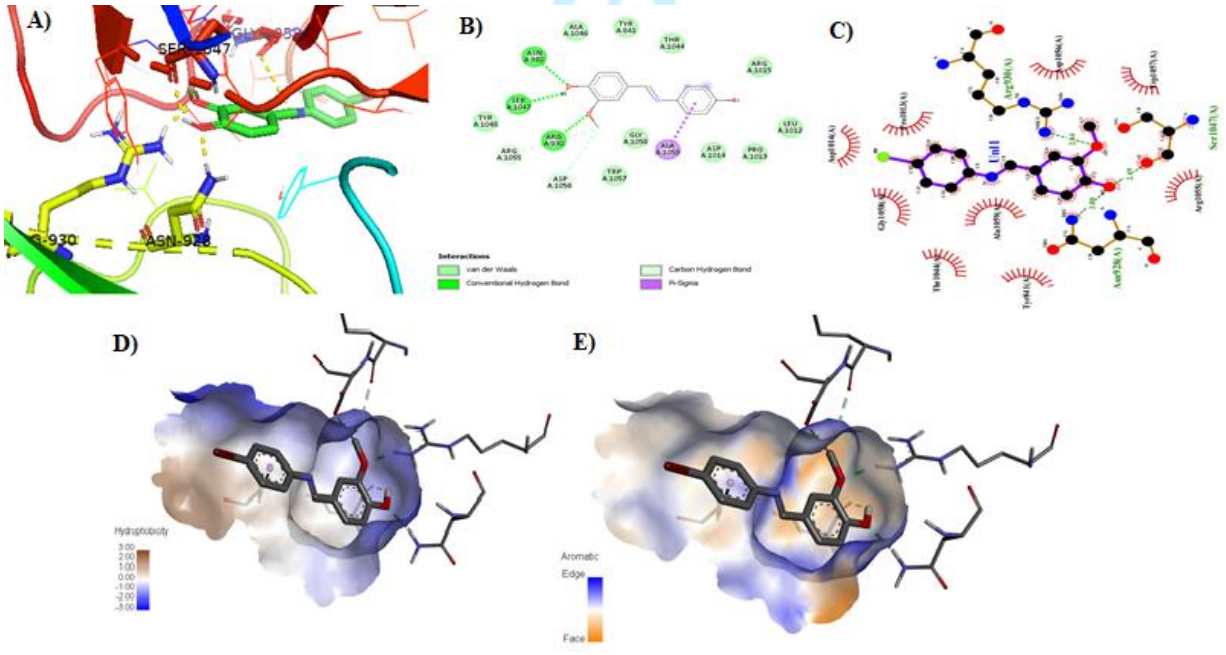

Figure 5. Binding poses of Compound **(SB4)** with Arabinosyltransferase-C enzyme (A), 2D interactions (B & C) (Hydrophobic interactions (D) and Aromatic interactions (E).

Frontier in Medical & Health Research

ISSN: (e) 3007-1607 (p) 3007-1593

Conclusion

In this study, we investigated the inhibitory potential of Vanillin Schiff bases SB1-SB4 and standard compound Macozinone against Arabinosyltransferase-C by employing molecular docking techniques. These compounds were subjected to treatment with enzyme Arabinosyltransferase-C. Our results indicate that compounds SB1-SB4 exhibit significant inhibitory activity against Arabinosyltransferase-C, surpassing the reported compounds (Isoniazid, bedaquiline, pyrazinamide). Furthermore, we compared the binding energy of these compounds with that of the Macozinone and it was found that the binding energy of compounds SB1-SB4 was significantly comparable. Consequently, compounds SB1-SB4 hold great potential as leading candidates for further pharmacological investigations aimed at developing treatments for Mycobacterium tuberculosis

Acknowledgments.

Institute of Chemical Sciences, University of Swat, Swat, Pakistan

Conflict of Interest

The authors declare no conflict of interest

Funding:

No Financial Funding is received for the title study from any funding agency.

REFERENCES

Kiziltaş, Ş. and A. Babalik, *Tuberculosis: An Overview*. Airway diseases, 2023: p. 635-659.

Glaziou, P., K. Floyd, and M.C. Raviglione. *Global epidemiology of tuberculosis*. in *Seminars in respiratory and critical care medicine*. 2018. Thieme Medical Publishers.

Das, N., P.K. Jena, and S.K. Pradhan, *Arabinosyltransferase C enzyme of Mycobacterium tuberculosis, a potential drug target: An insight from molecular docking study*. Heliyon, 2020. **6**(2).

Sun, Q., et al., *Mutations within embCAB are associated with variable level of ethambutol resistance in Mycobacterium tuberculosis isolates from China*. Antimicrobial agents and chemotherapy, 2018. **62**(1): p. 10.1128/aac. 01279-17.

Xu, Y., et al., *Mutations found in embCAB, embR, and ubiA genes of ethambutol-sensitive and-resistant Mycobacterium tuberculosis clinical isolates from China*. BioMed research international, 2015. **2015**(1): p. 951706.

Ekeleme, U.G., *Antibacterial-Resistant Genes (ABRG) Associated With Bloodstream Infections In Patients Receiving Treatment: A Case Study Of Nigeria Airforce Medical Center Onikan, Lagos State*. 2025.

Assam-Assam, J.-P., et al., *Mycobacterium tuberculosis complex drug resistance pattern and identification of species causing tuberculosis in the West and Centre regions of Cameroon*. BMC infectious diseases, 2011. **11**(1): p. 94.

Jankute, M., et al., *Genetics of mycobacterial arabinogalactan and lipoarabinomannan assembly*. Molecular Genetics of Mycobacteria, 2014: p. 535-557.

Decter, K.L.U., *An anthropological approach to immunogenetic variation in Manitoba First Nation populations: implications for tuberculosis*. 2013: University of Manitoba (Canada).

Tan, Y.Z., et al., *Cryo-EM structure of arabinosyltransferase EmbB from Mycobacterium smegmatis*. Nature communications, 2020. **11**(1): p. 3396.

Birch, H.L., *Molecular and biochemical characterisation of novel glycosyltransferases in Mycobacterium tuberculosis*. 2011, University of Birmingham.

Vázquez, J., et al., *Merging ligand-based and structure-based methods in drug discovery: an overview of combined virtual screening approaches*. Molecules, 2020. **25**(20): p. 4723.

Stahura, F.L. and J. Bajorath, *New methodologies for ligand-based virtual screening*. Current pharmaceutical design, 2005. **11**(9): p. 1189-1202.

Frontier in Medical & Health Research

ISSN: (e) 3007-1607 (p) 3007-1593

Bhunia, S.S., M. Saxena, and A.K. Saxena, *Ligand-and structure-based virtual screening in drug discovery*, in *Biophysical and computational tools in drug discovery*. 2021, Springer. p. 281-339.

Salgado-Moran, G., et al., *Docking Studies of Binding of Ethambutol to the C-Terminal Domain of the Arabinosyltransferase from Mycobacterium tuberculosis.* Journal of Chemistry, 2013. **2013**(1): p. 601270.

Satish, S., et al., *Drug Discovery and Computational Strategies in Multi-Drug-Resistant Tuberculosis*, in *Applications of Computational Tools in Drug Design and Development*. 2025, Springer. p. 965-997.

Pedelacq, J.D., et al., *A comprehensive review on mycobacterium tuberculosis targets and drug development from a structural perspective.* Structural Biology in Drug Discovery: Methods, Techniques, and Practices, 2020: p. 545-566.

Rahman, F.U., et al., *Thiourea Derivatives, Simple in Structure but Efficient Enzyme Inhibitors and Mercury Sensors.* Molecules, 2021. **26**(15): p. 4506.

Venugopal, S., *Molecular docking and molecular dynamic simulation studies to identify potential terpenes against Internalin A protein of Listeria monocytogenes.* Frontiers in Bioinformatics, 2024. **4**: p. 1463750.

Kurniatin, P.A., F.E. Putri, and U.M.S. Purwanto, *Virtual Screening of Flavonoid Compounds as Potential Antibiofilm Agents Targeting Glucosyltransferase.* Jurnal Jamu Indonesia, 2025. **10**(2): p. 102-109.

Kumar, M., et al., *Potential anti-mycobacterium tuberculosis activity of plant secondary metabolites: insight with molecular docking interactions.* Antioxidants, 2021. **10**(12): p. 1990.

Sammartino, J.C., et al., *Functional investigation of the antitubercular drug target Decaprenylphosphoryl-β-D-ribofuranose-2-epimerase DprE1/DprE2 complex.* Biochemical and Biophysical Research Communications, 2022. **607**: p. 49-53.

Boni, F.G., et al., *The Gene and Regulatory Network Involved in Ethambutol Resistance in Mycobacterium tuberculosis.* Microbial Drug Resistance, 2022