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IN SILICO DRUG REPURPOSING OF-FDA APPROVED COMPOUNDS AGAINST BURKHOLDERIA TARGETS ASSOCIATED WITH CYSTIC FIBROSIS

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Abstract: Cystic fibrosis patients commonly experience persistent lung infections caused by multidrug-resistant bacteria, with the Burkholderia cepacia complex being particularly severe and life-threatening. These infections accelerate the decline of lung function, respond poorly to conventional antibiotics, and negatively impact transplant success rates, highlighting the need for new treatment approaches. This study employs a structure-based drug repurposing strategy to identify FDA-approved drugs that can effectively target critical proteins of Burkholderia cepacia. Key essential and virulence-related proteins were identified and assessed for their suitability as drug targets using both structural and functional analyses. Selected high-priority targets were then screened virtually against a library of approved drugs to identify compounds with strong binding affinity. Further evaluation through molecular docking and interaction analysis helped to understand how these drugs interact with the target proteins, while molecular dynamics simulations confirmed the stability of these interactions under physiological conditions. This comprehensive computational approach enables the identification of promising therapeutic candidates that can interfere with vital bacterial processes, reducing the time and cost associated with early drug development. Overall, the study provides a strong scientific foundation for repurposing existing drugs to treat Burkholderia cepacia infections and presents an efficient strategy to address antimicrobial resistance in cystic fibrosis.

KEYWORDS – Cystic Fibrosis, Burkholderia cepacia complex, Drug Repurposing, Multidrug Resistance, Molecular Docking, Virtual Screening , FDA-approved Drugs, Antimicrobial Resistance.

I. INTRODUCTION

Cystic fibrosis (CF) is a life-limiting autosomal recessive disorder caused by mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene, which encodes a chloride and bicarbonate channel expressed on epithelial surfaces of multiple Cystic organs. Defective CFTR disrupts ion transport, leading to dehydration of airway surface liquid, impaired mucociliary clearance, and accumulation of thick mucus that obstructs airways and ducts (Elborn, Ratjen et al. , 2015). More than 2,000 CFTR variants have been identified, with mutations classified into six functional categories based on their molecular effects (Cutting, 2015, Sosnay et al. , 2013). Although CFTR modulator therapies have significantly improved outcomes, chronic pulmonary infection and progressive lung damage remain major causes of morbidity and mortality.

Globally, approximately 105,000 individuals have been diagnosed with CF across 94 countries, while epidemiological modeling suggests that the true number may exceed 160,000 worldwide (Burgel et al. , 2015). In the United States, around 1,000 new cases are diagnosed annually, and median survival now exceeds 50 years in high-income countries due to advances in treatment. However, increased longevity

has led to prolonged exposure to chronic infections and repeated antimicrobial therapy, intensifying challenges related to resistance and treatment burden (Nichols and Chmiel, 2015).

Pulmonary disease remains the principal determinant of survival in CF. Persistent bacterial colonization triggers exaggerated neutrophilic inflammation, characterized by elevated IL-8 levels, protease release, and progressive airway destruction (Nichols and Chmiel, 2015). Among CF-associated pathogens, the *Burkholderia cepacia* complex (Bcc) is particularly concerning. Although less prevalent than *Pseudomonas aeruginosa*, Bcc infections are associated with rapid pulmonary decline, intrinsic multidrug resistance, biofilm formation, and, in severe cases, "cepacia syndrome," a fulminant necrotizing pneumonia(Mahenthiralingam et al. , 1995). Bcc species exhibit intrinsic and acquired resistance mechanisms, including efflux pumps and reduced membrane permeability, and can survive intracellularly within macrophages, contributing to persistent infection (Saini et al., 1999).

Despite modern antimicrobial strategies and CFTR modulators such as Elexacaftor/Tezacaftor/Ivacaftor (Middleton et al. , 2019), therapeutic options for multidrug-resistant Bcc infections remain limited. Conventional antibiotic development is costly and slow, highlighting the need for alternative strategies. Drug repurposing of FDA-approved compounds offers a promising approach due to established safety profiles, reduced development timelines, and lower regulatory barriers.

Therefore, the present study aims to perform in silico drug repurposing of FDA-approved compounds against selected *Burkholderia* targets associated with cystic fibrosis. By integrating structure-based virtual screening and molecular docking approaches, this work seeks to identify potential inhibitors targeting essential or virulence-associated proteins of *Burkholderia cepacia* complex. This strategy may accelerate identification of candidate therapeutics to combat multidrug-resistant infections in CF patients and improve clinical outcomes.

II. COMPUTATIONAL DETAILS

genome of *Burkholderia cepacia* strain BC16 was retrieved from the National Center for Biotechnology Information genome database https://www.ncbi.nlm.nih.gov/home/genomes/ (Assembly accession: GCF_009586235.1; BioProject: PRJNA561256) https://www.ncbi.nlm.nih.gov/home/genomes/. The dataset corresponds to a complete genome assembly generated using Oxford Nanopore sequencing technology. Antimicrobial resistance (AMR)-associated proteins were identified using the Comprehensive Antibiotic Resistance Database (https://card.mcmaster.ca), enabling annotation of resistance determinants based on curated ontologies. Protein–protein interaction (PPI) networks were constructed and analyzed using Cytoscape (Shannon et al. , 2003). Hub proteins were identified using the cytoHubba plugin based on topological parameters.

The three-dimensional structure of the selected target protein PEN-B2 was obtained through UniProt (UniProt ID: C1J897) and AlphaFold (AF-C1J897-F1). FDA-approved compounds were retrieved from the ChEMBL (Zdrazil et al. , 2024) and screened using AutoDock Vina (Trott and Olson, 2010). Docking results were ranked based on binding affinity, and interaction analysis was performed using PyMOL.

III. RESULTS

3.1 Identification of AMR genes

A total of ten antimicrobial resistance (AMR)-associated proteins were identified in *Burkholderia cepacia*, representing multiple resistance mechanisms (Table 1). The resistance profile was dominated by efflux-mediated mechanisms, primarily involving resistance-nodulation-division (RND) transport systems. Key efflux-related genes included ceoA, adeF, amrA, and gacG, which encode components of multidrug efflux pumps responsible for the extrusion of diverse antibiotic classes such as fluoroquinolones, tetracyclines, aminoglycosides, macrolides, and disinfectants. Among these, adeF was consistently identified across multiple entries, indicating its central role in multidrug resistance. In addition to efflux systems, target modification was observed through the vanH gene (vanO cluster), associated with glycopeptide resistance. Furthermore, enzymatic antibiotic inactivation was represented by PEN-B2, a class A beta-lactamase conferring resistance to penicillin-class antibiotics. Overall, the results indicate that *B. cepacia* exhibits a multifactorial resistance strategy, with efflux pumps as the dominant mechanism supplemented by enzymatic inactivation and target alteration.

The sunburst plot (Figure 1) illustrates the hierarchical organization of identified AMR genes in *Burkholderia cepacia*. The central node represents the overall AMR classification, from which individual resistance-associated genes radiate outward, including gacG, ceoA, adeF, vanH, amrA, and PEN-B2.

A prominent observation from the figure is the dominance of efflux-related genes, particularly adeF, ceoA, amrA, and gacG, which are distributed across major segments of the plot. This indicates that efflux-mediated resistance is the primary defense mechanism in *B. cepacia*, enabling the bacterium to expel a wide range of antimicrobial agents, including fluoroquinolones, tetracyclines, aminoglycosides, and disinfectants.

In contrast, vanH and PEN-B2 occupy smaller segments, representing secondary resistance mechanisms. The vanH gene contributes to resistance through target modification, particularly against glycopeptide antibiotics, while PEN-B2, a class A beta-lactamase, mediates antibiotic inactivation by hydrolyzing beta-lactam antibiotics.

The hierarchical structure of the plot highlights a multi-layered resistance architecture, where efflux systems form the core mechanism, supported by enzymatic degradation and target alteration pathways. This distribution underscores the multidrug-resistant nature of *B. cepacia* and suggests that targeting efflux pumps could be a more effective therapeutic strategy compared to single-target approaches.

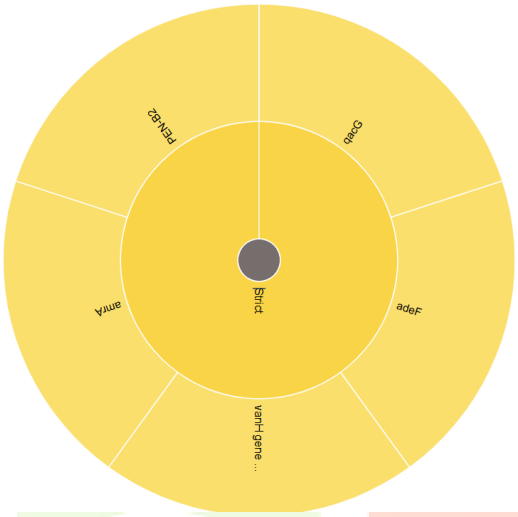

Fig1: Hierarchical distribution of key antimicrobial resistance determinants in *Burkholderia cepacia* highlighting dominant efflux-associated genes.

Table 1. Identification and characterization of antimicrobial resistance-associated proteins in *Burkholderia cepacia* based on CARD analysis

Protein	Gene	Drug Class	Resistance Mechanism
DMT family transporter [Burkholderia]	qacG	disinfecting agents and antiseptics	antibiotic efflux
Multidrug efflux RND transporter periplasmic adaptor subunit CeoA [Burkholderia]	ceoA	fluoroquinolone antibiotic; aminoglycoside antibiotic	antibiotic efflux
Efflux RND transporter permease BpeB [Burkholderia]	adeF	fluoroquinolone antibiotic; tetracycline antibiotic	antibiotic efflux
Multidrug efflux RND transporter permease subunit CeoB [Burkholderia cepacia]	adeF	fluoroquinolone antibiotic; tetracycline antibiotic	antibiotic efflux

Hydroxyacid dehydrogenase [Burkholderia cepacia]	vanH gene in vanO cluster	glycopeptide antibiotic	antibiotic target alteration
MexX/AxyX family multidrug efflux RND transporter periplasmic adaptor subunit [Burkholderia cepacia]	amrA	macrolide antibiotic; aminoglycoside antibiotic	antibiotic efflux
PEN family class A beta-lactamase, Bcc-type [Burkholderia cepacia]	PEN-B2	penicillin beta-lactam	antibiotic inactivation
Efflux RND transporter permease subunit [Burkholderia cepacia]	adeF	fluoroquinolone antibiotic; tetracycline antibiotic	antibiotic efflux
Efflux RND transporter permease subunit [Burkholderia cepacia]	adeF	fluoroquinolone antibiotic; tetracycline antibiotic	antibiotic efflux
Efflux RND transporter permease subunit [Burkholderia cepacia]	adeF	fluoroquinolone antibiotic; tetracycline antibiotic	antibiotic efflux

3.2. PPI network and identification of hub of genes

The protein–protein interaction (PPI) network analysis identified the top 50 hub genes in *Burkholderia cepacia* based on centrality measures. The network exhibited a highly interconnected architecture, with hub genes spanning multiple essential biological processes.

Consistent with previous observations, several ribosomal proteins (rpl, rps, rpm families) such as rplK, rplP, rplC, rplL, rpsA, rpsB, and rpmF were identified as central nodes, confirming the importance of the translational machinery. However, the network also revealed significant contributions from non-ribosomal functional modules.

Key transcription-related genes, including rpoB, rpoC, rpoA, rpoH, and rpoZ, were among the top hubs, indicating strong coupling between transcription and translation processes. Additionally, DNA replication and cell division proteins such as dnaA, dnaB, dnaE, dnaN, ftsQ, and ftsW were identified, highlighting their critical roles in bacterial proliferation.

Metabolic enzymes, including gapA, pyk, eno, and mdh, were also present among the hub genes, suggesting that central carbon metabolism contributes significantly to network stability and connectivity. Furthermore, protein quality control and stress-response proteins, such as dnaK, dnaJ, and clpP, were identified, reflecting their importance in maintaining cellular homeostasis under stress conditions.

Notably, lptD, a key outer membrane protein involved in lipopolysaccharide transport, was identified as a hub gene, linking membrane integrity and potential antimicrobial resistance mechanisms to the core network.

Overall, the results indicate that the PPI network is not solely dominated by ribosomal proteins but represents a multi-functional hub architecture integrating translation, transcription, replication, metabolism, and membrane processes, providing a more comprehensive view of essential bacterial survival pathways.

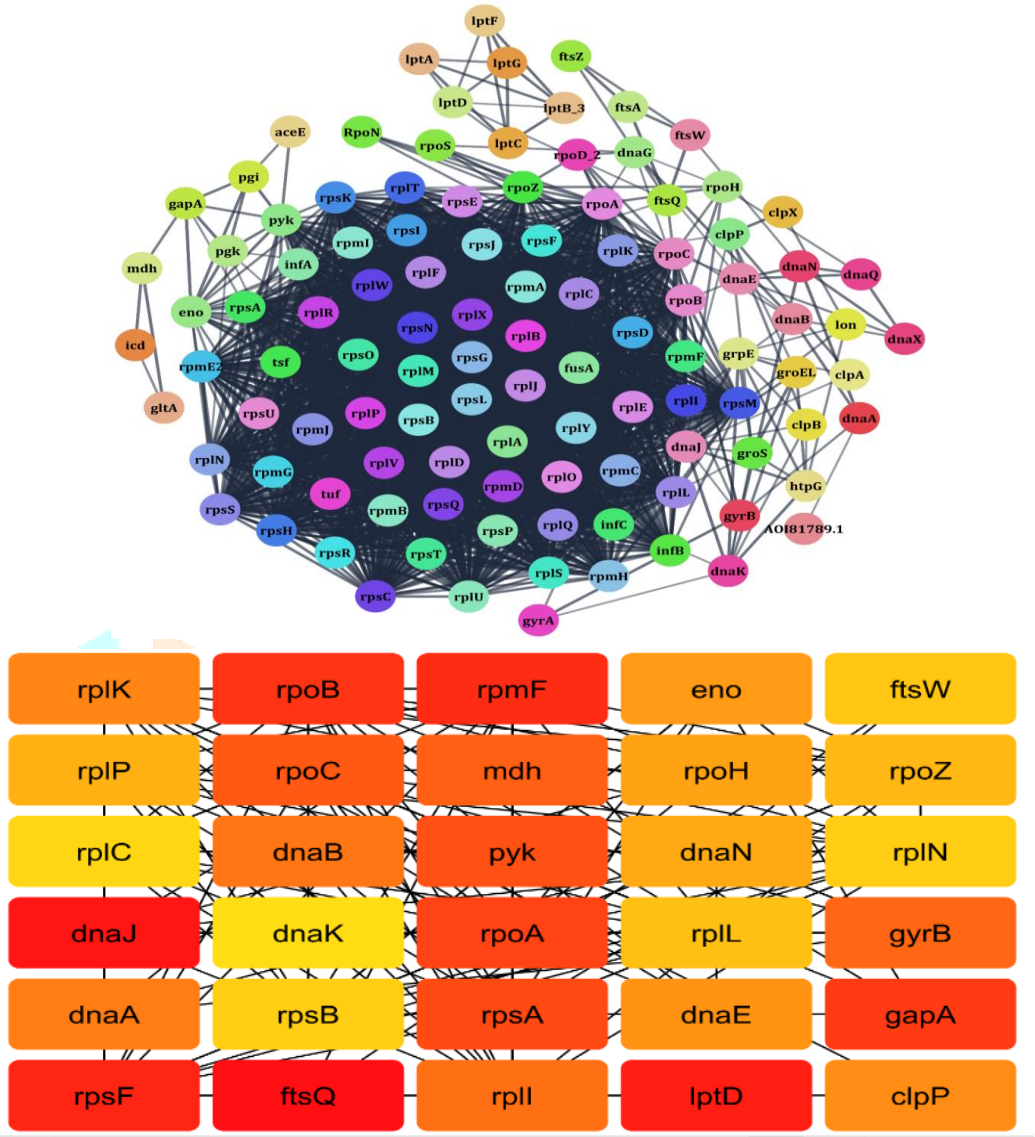

Fig2: PPI network and top30 genes present in the *B.*Cepacia

3.3 Virtual Screening and Binding Energy Distribution

Virtual screening of 1895 FDA-approved compounds against the target protein PEN-B2 was performed using AutoDock Vina. The binding affinities of the screened compounds ranged from −10.4 to −3.0 kcal/mol, indicating variable interaction strengths.

A total of 74 compounds exhibited strong binding affinity (≤ −9.0 kcal/mol), representing the top-ranking candidates. Additionally, 365 compounds showed binding energies between −8.9 and −8.0 kcal/mol, while 652 compounds fell within the range of −7.9 to −7.0 kcal/mol, indicating moderate binding potential. The majority of compounds (745 molecules) demonstrated binding energies between −6.9 and −5.0 kcal/mol, suggesting weak to moderate interactions. A small subset of 59 compounds exhibited low binding affinity (−4.9 to −3.0 kcal/mol). Overall, the distribution of binding energies indicates that a subset of FDA-approved drugs exhibits strong interaction potential with PEN-B2, highlighting promising candidates for drug repurposing against *Burkholderia cepacia*.

3.4 Intermolecular interactions of top 4 compounds

The intermolecular interactions of the top-ranked compounds with the PEN-B2 protein were analyzed to elucidate their binding mechanisms (Table 9). The CHMBL ids of the compounds CHEMBL3137309, CHEMBL1683590, CHEMBL500576 and CHEMBL4204794 refers to the drug molecules venetoclax, eribulin, temoporfin and avapritinib, respectively secured lowest energy among 1895 drug molecules. The selected compounds— venetoclax (−10.4 kcal/mol), eribulin (−10.3 kcal/mol), temoporfin (−10.0 kcal/mol), and avapritinib (−10.0 kcal/mol)— demonstrated strong binding affinities supported by extensive hydrogen bonding and hydrophobic interactions.

CHEMBL3137309 exhibited the highest binding affinity and formed multiple hydrogen bonds with key residues such as Ser153, Asn155, Tyr190, Val239, Arg243, Lys257, Thr258, Thr260, Gly261, Asp262, Ala291, and Arg292. Hydrophobic interactions with Tyr128, Asn193, Val239, Thr260, and Arg294 further stabilized the complex.

CHEMBL1683590 also showed strong binding, interacting through hydrogen bonds with Asn127, Asn155, Asn193, Val239, Lys242, Arg243, Asp262, Ala295, and Asp297, along with hydrophobic contacts involving Tyr128, Thr260, and Arg294.

CHEMBL500576 formed hydrogen bonds with Ser93, Asn155, Val239, Thr260, Tyr263, Gly264, Ala293, Arg294, and Ala295, supported by hydrophobic interactions with Tyr128, Thr260, and Arg294.

CHEMBL4204794, despite having comparable binding energy, exhibited relatively fewer hydrophobic interactions but maintained stability through hydrogen bonding with Tyr128, Ser153, Asn155, Tyr190, Asn193, Thr258, Thr260, and Asp262, and a hydrophobic interaction with Asn193.

Notably, residues such as Tyr128, Asn155, Thr260, Asp262, and Arg294 were consistently involved across multiple complexes, indicating their critical role in ligand binding within the PEN-B2 active site. The intermolecular interactions, along with cartoon and surface representations of the protein–ligand complexes, are illustrated in Figures 3–6, confirming stable ligand accommodation within the binding pocket.

Table : intermolecular interactions of drugs with lowest binding energy with PEN-B2

Complex	**Binding energy (kcal/mol)**	**Hydrogen bonding interactions (Å)**	**Hydrophobic interactions (Å)**
Venetoclax with PEN-B2	-10.4	Ser153 (2.6), Asn155 (2.0), Tyr190 (2.5), Val239 (3.0), Arg243 (2.8), Lys257 (3.0), Thr258 (2.6), Thr260 (1.8), Gly261 (2.5), Asp262 (2.7), Ala291 (3.4), Arg292 (2.6)	Tyr128 (3.7), Asn193 (3.9), Val239 (3.8), Thr260 (3.7), Arg294 (3.8)
Eribulin with PEN-B2	-10.3	Asn127 (2.1), Asn155 (2.1), Asn193 (2.0), Val239 (2.9), Lys242 (2.2), Arg243 (2.6), Asp262 (3.3), Ala295 (2.6), Asp297 (2.6)	Tyr128 (3.9), Thr260 (3.7), Arg294 (3.9)
Temoporfin with PEN-B2	-10.0	Ser93 (2.3), Asn155 (2.7), Val239 (2.7), Thr260 (2.5), Tyr263 (1.9), Gly264 (2.0), Ala293 (3.2), Arg294 (3.2), Ala295 (3.1)	Tyr128 (3.9), Thr260 (3.6), Arg294 (3.8)
Avapritinib with PEN-B2	10.0	Tyr128 (3.2), Ser153 (2.4), Asn155 (2.8), Tyr190 (3.4), Asn193 (2.3), Thr258 (2.3), Thr260 (2.1), Asp262 (2.3)	Asn193 (3.9)

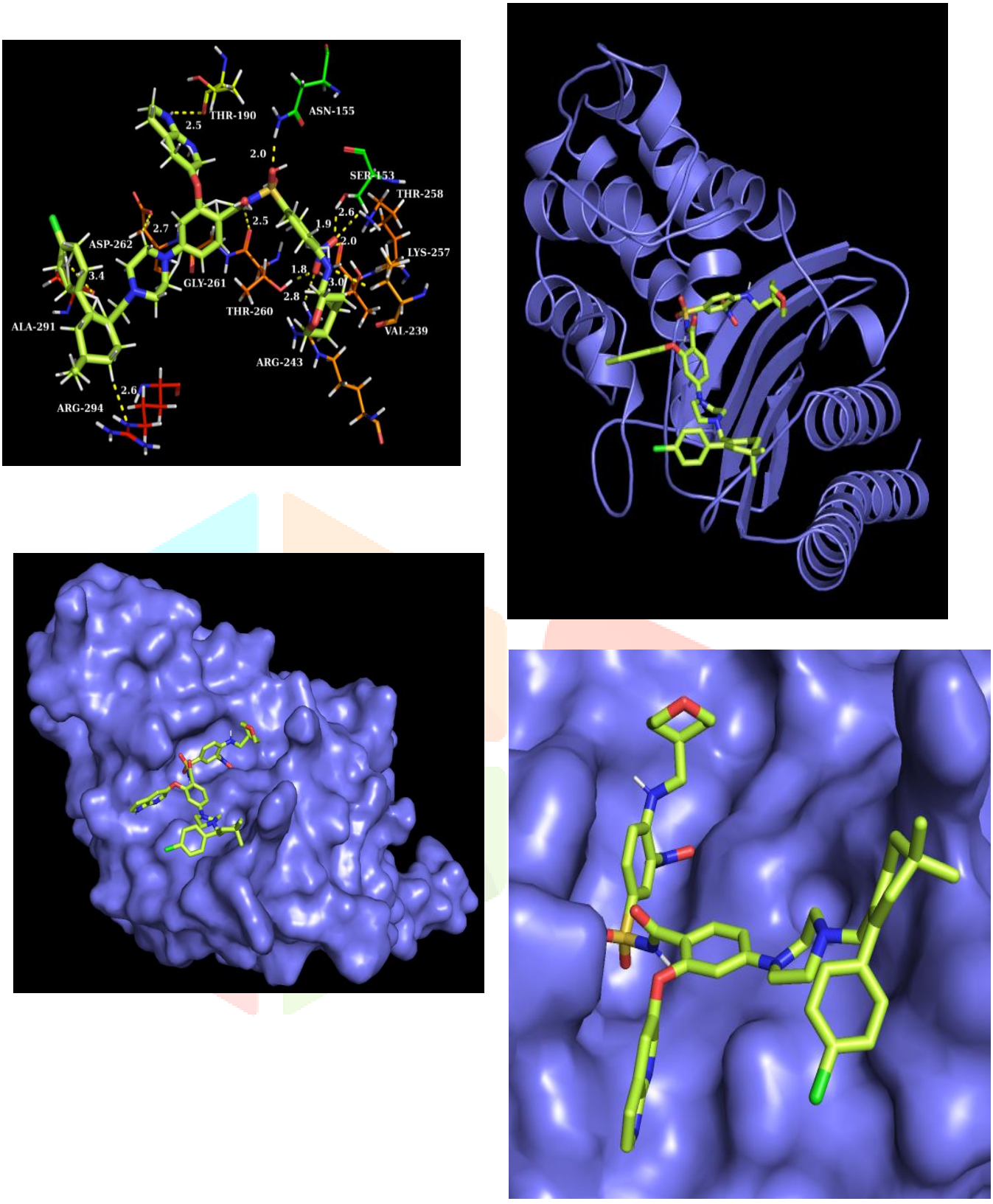

Fig 3: Intermolecular interactions of venetoclax with PEN-B2

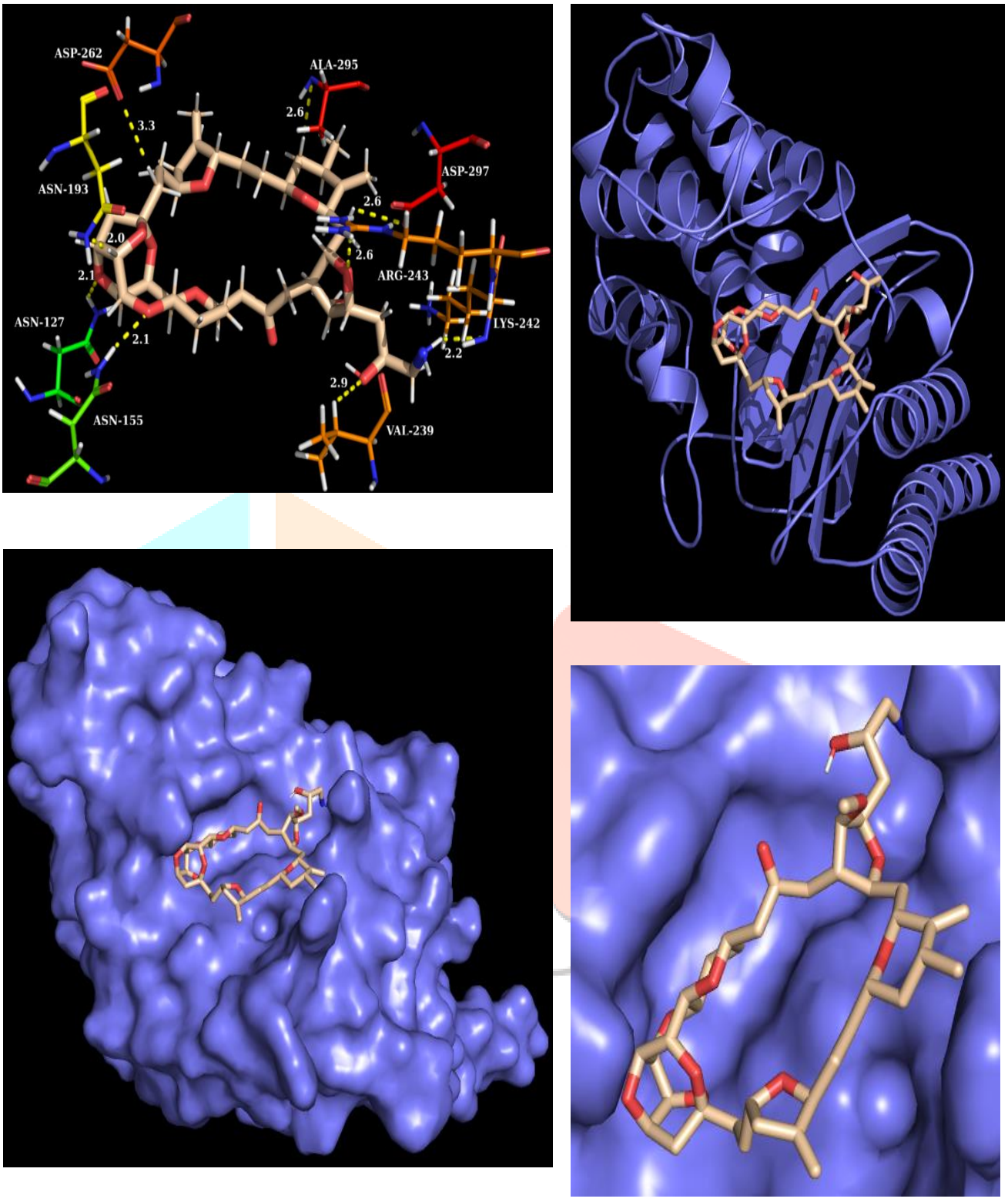

Fig 4: Intermolecular interactions of eribulin with PEN-B2

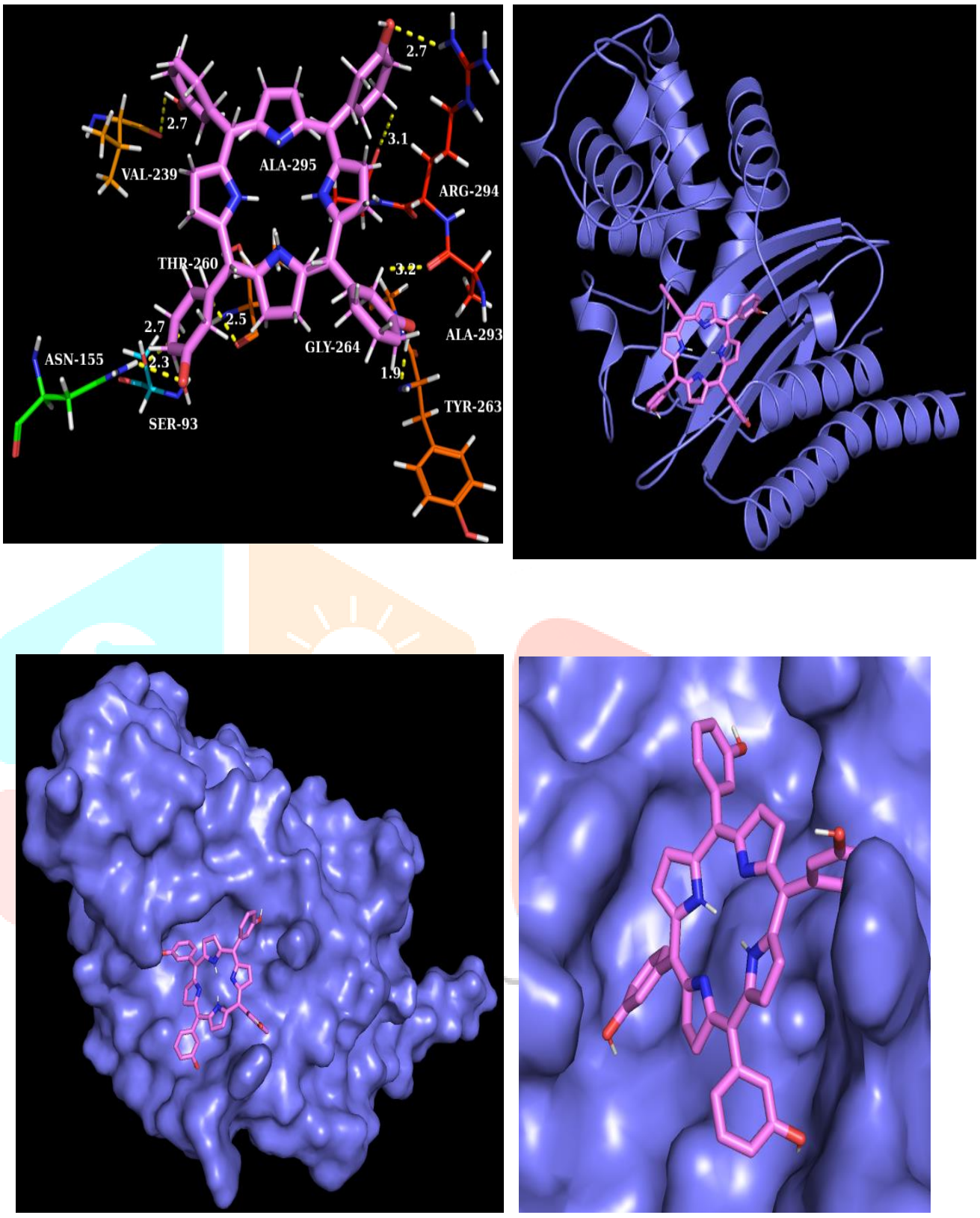

Fig 5: Intermolecular interactions of temoporfin with PEN-B2

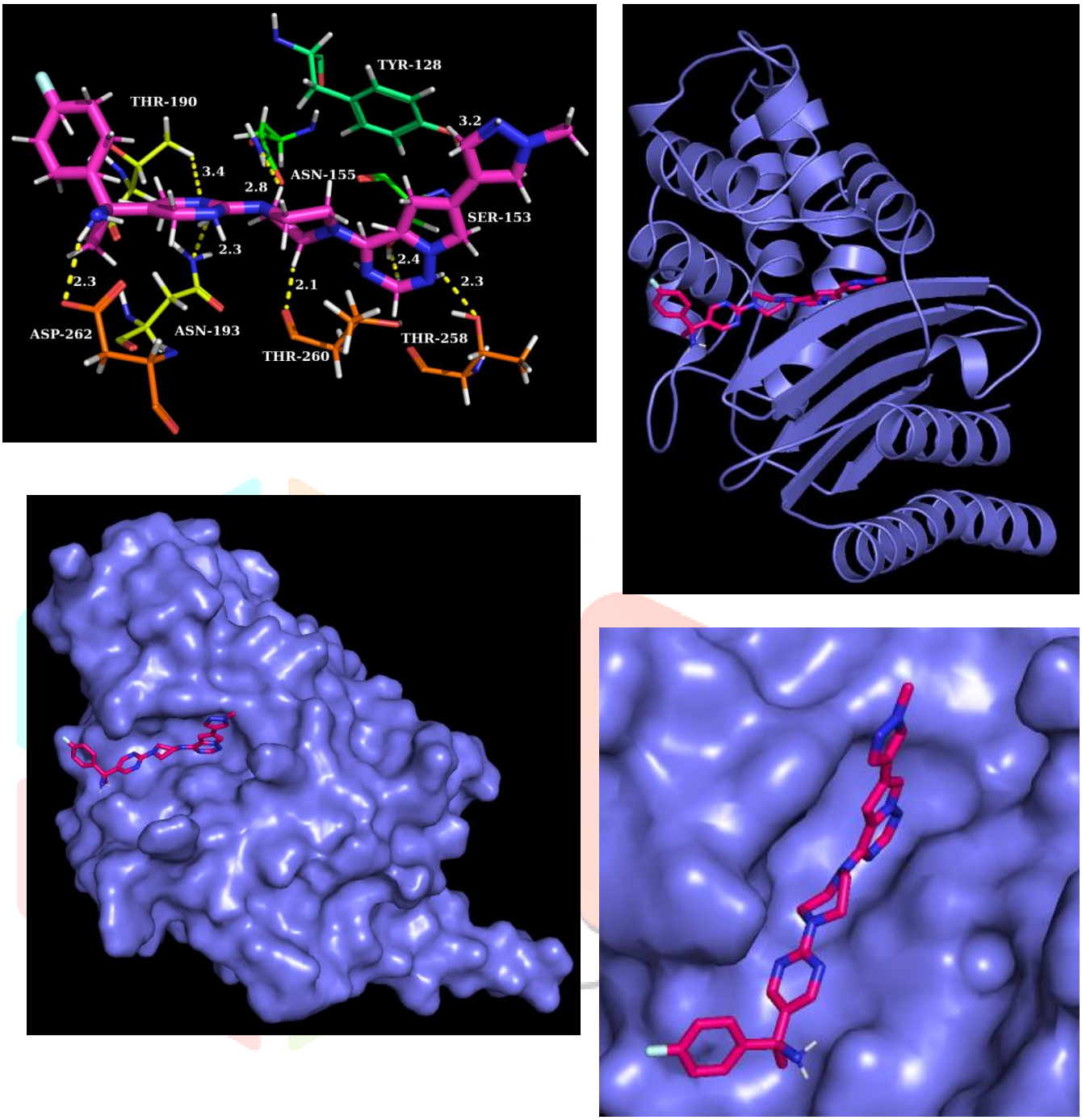

Fig 6: Intermolecular interactions of avapritinib with PEN-B2

IV. DISCUSSION

The present study integrates antimicrobial resistance (AMR) profiling, protein–protein interaction (PPI) analysis, and structure-based virtual screening to identify potential therapeutic targets against *Burkholderia cepacia*, a clinically challenging multidrug-resistant pathogen. The findings collectively highlight the central role of β-lactamase-mediated resistance, efflux systems, and essential cellular networks in sustaining bacterial survival and drug resistance. The antimicrobial resistance (AMR) profiling of *Burkholderia cepacia* in the present study revealed a multi-mechanistic resistance architecture, primarily dominated by efflux-mediated resistance, along with target modification and enzymatic antibiotic inactivation. This observation is consistent with previously reported studies, which

describe *B. cepacia* complex as an intrinsically multidrug-resistant organism due to the combined action of multiple resistance systems (Alnimr et al. , 2026, Rhodes and Schweizer, 2016).

A major finding of this study is the prevalence of efflux pump-associated genes, including ceoA, adeF, amrA, and qacG, which belong to the Resistance-Nodulation-Division (RND) and Small Multidrug Resistance (SMR) families. These systems are responsible for the active extrusion of structurally diverse antibiotics such as fluoroquinolones, tetracyclines, aminoglycosides, and disinfectants. This result strongly aligns with previous reports indicating that RND efflux pumps are the most clinically significant resistance determinants in *Burkholderia* species, contributing to multidrug resistance phenotypes (Scoffone et al. , 2021).

Furthermore, experimental studies have demonstrated that overexpression of RND efflux systems is directly correlated with increased resistance levels in clinical isolates, confirming their dominant role in antibiotic resistance (Podnecky et al. , 2015). The presence of qacG, associated with disinfectant resistance, further supports the ability of *B. cepacia* to survive in hospital environments, contributing to its persistence and nosocomial transmission.

In addition to efflux systems, the identification of the vanH gene within the vanO cluster indicates the presence of target modification-based resistance, particularly against glycopeptide antibiotics. This mechanism alters the antibiotic binding site, thereby reducing drug efficacy. Such target modification strategies have been previously reported as secondary resistance mechanisms in *Burkholderia*, often acting in combination with efflux systems and permeability barriers (Schweizer, 2012).

A key finding of this study is the identification of PEN-B2, a class A β-lactamase, which mediates resistance through enzymatic hydrolysis of β-lactam antibiotics. This result is consistent with prior studies showing that β-lactam resistance in *Burkholderia cepacia* complex is largely driven by β-lactamase production, including PenA/PenB-type enzymes (Somprasong et al. , 2020). However, previous reports also indicate that β-lactamase activity alone is often insufficient to confer high-level resistance, and is typically complemented by efflux pumps and membrane permeability barriers (Everaert and Coenye, 2016).

This supports the findings of the present study, where PEN-B2 is part of a broader resistance network rather than a standalone mechanism. The combined presence of:

- Efflux pumps (ceoA, adeF, amrA, qacG)
- Target modification (vanH)
- Enzymatic inactivation (PEN-B2)

demonstrates that *B. cepacia* employs a multi-layered resistance strategy, where different mechanisms act synergistically to ensure survival under antimicrobial pressure.

This is consistent with current understanding that multidrug resistance in Gram-negative pathogens is not driven by a single mechanism but by the interplay of multiple systems, including efflux, enzymatic degradation, and reduced permeability (Arzanlou et al. , 2017).

The AMR profile identified in this study closely mirrors previously reported resistance mechanisms in *Burkholderia cepacia*, particularly the dominance of RND efflux systems and the contribution of β-lactamases such as PEN-B2. However, the simultaneous identification of multiple resistance pathways highlights a more integrated and synergistic resistance network, reinforcing the complexity of targeting this pathogen.

The PPI network analysis revealed a highly interconnected hub gene architecture, dominated not only by ribosomal proteins but also by transcriptional regulators, replication proteins, metabolic enzymes, and membrane-associated components.

While ribosomal proteins are commonly identified as hubs due to their essential nature, the inclusion of genes such as rpoB, dnaA, gapA, clpP, and lptD indicates a broader functional integration of survival pathways, including:

- Protein synthesis (ribosomal proteins)
- Transcription (RNA polymerase subunits)
- DNA replication and cell division
- Central metabolism
- Membrane integrity and transport

Such multi-system integration is consistent with previous genomic and systems biology studies, which emphasize that antimicrobial resistance and virulence in *Burkholderia* species are governed by complex, interconnected pathways rather than single targets (Magizhvannan and Veerappapillai, 2025).

Importantly, the presence of lptD and efflux-related proteins in the hub network strengthens the link between cell envelope integrity and resistance mechanisms, which are critical for Gram-negative pathogens.

Virtual Screening and Drug Repurposing Potential

The virtual screening of 1895 FDA-approved drugs resulted in the identification of 74 high-affinity compounds (≤ −9 kcal/mol), indicating strong interaction potential with the PEN-B2 target. This hit rate (~3.9%) is consistent with previous large-scale docking studies, where only a small fraction of compounds typically demonstrates strong binding.

Similar in silico studies targeting *Burkholderia* proteins have also reported limited but significant high-affinity hits, emphasizing the feasibility of drug repurposing approaches for resistant pathogens (Dare et al. , 2025). The distribution of binding energies observed in this study further supports the reliability of the screening pipeline.

Compared to reported β-lactamase inhibitor studies, where compounds such as avibactam and relebactam restore antibiotic susceptibility by inhibiting PenA activity, our results identify structurally diverse FDA-approved molecules with comparable or stronger predicted binding affinities. This suggests that non-classical inhibitors may also effectively target β-lactamase active sites, expanding the scope of therapeutic options.

Previous docking and structural studies on β-lactamases, including PenA variants, have focused primarily on known inhibitor scaffolds and β-lactam analogs. These studies emphasize the importance of active site residues and conformational flexibility in determining inhibitor binding and efficacy (Nukaga et al. , 2021).

In contrast, the present study adopts a drug repurposing approach using FDA-approved compounds, enabling the identification of novel chemical scaffolds with potential inhibitory activity. The observed binding energies (up to −10.4 kcal/mol) are comparable or better than those reported for several known inhibitors in computational studies, suggesting strong binding stability.

Moreover, the interaction analysis revealed consistent involvement of key residues (Tyr128, Asn155, Thr260, Asp262, Arg294) across multiple ligand complexes, supporting the presence of a conserved active binding pocket, similar to previously characterized β-lactamases.

The combined analysis of AMR profiling, PPI network, and virtual screening provides a comprehensive understanding of *B. cepacia* resistance mechanisms:

- β-lactamase (PEN-B2) → enzymatic antibiotic degradation
- Efflux systems → drug extrusion
- Core cellular machinery (PPI hubs) → survival and adaptation
- Metabolic and stress pathways → resilience under antimicrobial pressure

This multi-layered system explains the intrinsic and acquired resistance observed in *B. cepacia*, making it a difficult pathogen to treat.

The present study demonstrates that β-lactamase-mediated resistance, particularly through PEN-B2, plays a central role in *Burkholderia cepacia* antimicrobial resistance. The integration of network biology and structure-based virtual screening identified functionally critical targets and venetoclax, eribulin, temoporfin and avapritinib, as promising repurposable drug candidates. These findings highlight the importance of multi-target therapeutic strategies and provide a strong computational foundation for the development of novel interventions against multidrug-resistant *B. cepacia* infections.

V.CONCLUSION

The present study employed an integrative computational approach to investigate antimicrobial resistance mechanisms and identify potential therapeutic candidates against *Burkholderia cepacia*. AMR profiling revealed a multi-layered resistance system, predominantly driven by efflux pump mechanisms (ceoA, adeF, amrA, qacG), along with target modification (vanH) and β-lactamase-mediated antibiotic inactivation (PEN-B2). These findings are consistent with previous reports indicating that *B. cepacia* exhibits intrinsic multidrug resistance through the combined action of efflux systems, β-lactamases, and permeability barriers (Tavares et al. , 2020).

Protein–protein interaction (PPI) network analysis identified functionally diverse hub genes, including ribosomal proteins, transcriptional regulators (rpoA, rpoB, rpoC), replication proteins (dnaA, dnaE), metabolic enzymes (gapA, pyk), and membrane-associated proteins (lptD). This indicates that bacterial

survival is supported by a highly interconnected network integrating translation, transcription, metabolism, and membrane integrity, rather than isolated pathways.

Virtual screening of 1895 FDA-approved compounds against the PEN-B2 protein identified 74 high-affinity candidates (≤ −9 kcal/mol), demonstrating strong interaction potential. The top compounds exhibited binding energies up to −10.4 kcal/mol, with stable interactions involving key residues such as Tyr128, Asn155, Thr260, Asp262, and Arg294, confirming the presence of a conserved and druggable active site.

Intermolecular interaction analysis further validated these findings by demonstrating extensive hydrogen bonding and hydrophobic interactions, supporting the stability of protein–ligand complexes. The structural representations (Figures 3–6) confirmed proper ligand accommodation within the active site of PEN-B2.

This study provides a comprehensive computational framework for understanding antimicrobial resistance and identifying potential therapeutic strategies against *Burkholderia cepacia*. The results demonstrate that resistance in this organism is governed by a synergistic interplay of efflux systems, β-lactamase activity, and target modification, rather than a single dominant mechanism. This observation is strongly supported by previous studies emphasizing the central role of efflux pumps and β-lactamases in *B. cepacia* multidrug resistance (Nair et al. , 2004, Tseng et al. , 2014).

The identification of PEN-B2 as a druggable β-lactamase target, combined with the discovery of multiple high-affinity FDA-approved compounds, highlights the potential of drug repurposing approaches for overcoming antibiotic resistance. Importantly, the integration of PPI network analysis revealed that resistance mechanisms are closely linked with essential cellular processes, suggesting that effective therapeutic strategies must target multiple pathways simultaneously.

Overall, this study advances current understanding by:

- Identifying key AMR determinants in *B. cepacia*
- Revealing a multi-system interaction network underlying bacterial survival
- Prioritizing high-affinity repurposable drug candidates targeting PEN-B2

The findings of this study emphasize that combating *Burkholderia cepacia* requires a multi-target therapeutic approach, combining inhibition of β-lactamase activity with disruption of efflux systems and essential cellular networks.

Further validation through molecular dynamics simulations, binding free energy calculations, and experimental assays (MIC and enzyme inhibition studies) is necessary to confirm the therapeutic potential of the identified compounds.

VI. Acknowledgment

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