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In Silico Virulence Factor Analysis and Drug Target Identification in Pseudomonas Aeruginosa PA7

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ABSTRACT

Pseudomonas aeruginosa PA7 is a multidrug-resistant outlier strain with unique virulence factors that contribute to its pathogenicity. Unlike other *P. aeruginosa* strains, PA7 exhibits resistance to several conventional antibiotics, making treatment options limited. Targeting their unique virulence factors offers a promising alternative therapeutic strategy, as it reduces selective pressure for resistance development.

This study aims to identify PA7-specific virulence factors, functionally annotate them, and screen potential small-molecule inhibitors using an in-silico drug discovery approach. By focusing on virulence rather than viability, the study seeks to explore novel antivirulence strategies against *P. aeruginosa* PA7.

Unique virulence-associated genes were determined through comparative genomic analysis. Functional annotation was performed using InterProScan and UniProt, followed by human non-homology filtering via BLASTp. The ligand structures were obtained from PubChem and prepared using Open Babel. Protein-ligand docking was performed using AutoDock4, and binding affinity (ΔG) along with inhibition constants (Ki) were analyzed to assess potential drug candidates.

This study successfully identified PA7-specific virulence factors and screened potential inhibitors that could be repurposed as antivirulence therapeutics. The In silico docking results indicate that Triclosan, Salicylidene acylhydrazide, and Patuletin have strong binding affinities, making them promising drug candidates. Further in vitro and in vivo validation is essential to confirm their efficacy in targeting PA7 virulence without inducing resistance. The findings contribute to the growing field of antivirulence therapy and provide a foundation for future drug development against multidrug-resistant P. aeruginosa PA7.

KEYWORDS: Pseudomonas aeruginosa PA7, Virulence factors, In Silico drug discovery, Molecular docking

1. INTRODUCTION

Multidrug-resistant (MDR) bacterial infections pose a major challenge to modern medicine, limiting the efficacy of traditional antibiotics and necessitating alternative therapeutic strategies. *Pseudomonas aeruginosa*, a Gram-negative opportunistic pathogen, is known for its intrinsic and acquired resistance mechanisms, making it a priority pathogen according to the World Health Organization (WHO). Among its diverse strains, *P. aeruginosa* PA7 is classified as an outlier strain due to its unique genetic composition and distinct resistance profile. Unlike other clinical strains, PA7 lacks several conventional antibiotic resistance genes but compensates with distinct virulence factors that contribute to its pathogenicity and survival in host environments.

The failure of conventional antibiotics to combat infections caused by *P. aeruginosa* PA7 has fueled interest in antivirulence therapies, which aim to disarm rather than kill the bacteria, thereby reducing the selection pressure for resistance development. This study focuses on an In silico approach to identify strain-specific virulence factors and predict potential drug targets through molecular docking techniques.

1.1 Significance of Virulence Factor Targeting

Antimicrobial resistance (AMR) has become one of the most pressing global health challenges, with pathogens like *Pseudomonas aeruginosa* developing resistance to multiple drug classes. Traditional antibiotics primarily target essential bacterial processes such as cell wall synthesis, protein translation, and DNA replication, but this approach exerts strong selective pressure, promoting the rapid evolution of resistant strains. In contrast, antivirulence therapies aim to disarm

rather than kill the pathogen, reducing pathogenicity without imposing a high selection pressure for resistance development.

The core advantage of antivirulence therapy lies in its ability to disrupt bacterial infection mechanisms without necessarily affecting the viability of the pathogen. This approach significantly reduces the selective pressure typically associated with conventional antibiotics, thereby lowering the risk of resistance development. Since antivirulence agents do not directly threaten bacterial survival, bacteria are under less evolutionary pressure to develop countermeasures. Moreover, virulence factors are often not essential for survival outside the host environment, which further limits the likelihood that resistance mutations would be strongly favored in natural populations.

Another key benefit of antivirulence strategies is their potential to enhance the efficacy of conventional antibiotics. By weakening the pathogen's ability to cause disease, antivirulence compounds can act synergistically with antibiotics, leading to more efficient bacterial clearance and reduced treatment failure rates. This combinatorial approach offers a promising avenue for managing infections caused by drug-resistant organisms.

In the case of Pseudomonas aeruginosa, antivirulence therapy is particularly appealing due to the pathogen's robust arsenal of infection mechanisms. P. aeruginosa is notorious for forming dense biofilms that protect it from host immune responses and hinder the penetration of antibiotics. It also employs complex secretion systems to deliver toxins directly into host cells, resulting in tissue damage and severe infections. Furthermore, this organism exhibits intrinsic resistance to multiple antibiotic classes, rendering many conventional treatments ineffective.

By targeting and inhibiting these virulence mechanisms, particularly biofilm formation and toxin secretion, antivirulence therapy offers a means to disarm P. aeruginosa without necessarily killing it. This strategy not only helps manage infections more effectively but also preserves the efficacy of existing antibiotics, offering a promising solution in the fight against multidrug-resistant bacterial pathogens.

1.2 Key Virulence Factors in *Pseudomonas aeruginosa* PA7

The PA7 strain of *P. aeruginosa* is a distinct antibiotic-resistant outlier strain, lacking some common resistance genes but harbouring unique virulence determinants. The most significant virulence-associated genes identified in this study include PilA, ExlA and ExlB, and AcsA, AcsB, and AcsD. PilA, a Type IV pilin, is essential for biofilm formation and bacterial adhesion to host tissues. Mutants lacking PilA show reduced colonization ability, making it an attractive target for drug development.

ExlA and ExlB form the Exolysin Toxin System, which operates as a two-partner secretion system (TPS) and serves as an alternative to the classical Type III Secretion System (T3SS). ExlA directly contributes to host cell lysis, thereby facilitating bacterial spread and immune evasion. This system is notably absent in other P. aeruginosa strains, making ExlA–ExlB a PA7-specific virulence target.

The genes AcsA, AcsB, and AcsD encode enzymes involved in fatty acid biosynthesis. These enzymes play a role in lipid metabolism and the synthesis of bacterial membrane components. They are critical for bacterial survival and adaptation under stress conditions. Inhibiting fatty acid biosynthesis disrupts membrane integrity, thereby sensitizing the pathogen to antibiotic treatment.

1.2.1 Advantages of Targeting These Virulence Factors

Targeting the identified virulence factors offers several therapeutic advantages. PilA inhibitors can reduce biofilm formation, making bacterial communities more susceptible to antibiotics and immune clearance. By interfering with ExlA and ExlB, toxin-mediated damage can be neutralized, thereby preventing the progression of severe infections. Inhibiting Acs factors involved in fatty acid biosynthesis disrupts bacterial membrane synthesis, weakening PA7's survival capabilities and potentially increasing its vulnerability to antimicrobial agents.

1.3 Computational Approaches in Drug Discovery

The process of discovering new drugs is traditionally expensive and time-consuming, often requiring years of experimental validation before a compound reaches clinical trials. Computational approaches, particularly *in silico* methods, have revolutionized drug discovery by reducing costs, accelerating candidate identification, and enhancing target specificity. These methods allow researchers to screen large molecular databases, predict drug-target interactions, and optimize lead compounds before experimental validation.

Compared to traditional in vitro and in vivo methods, computational approaches offer several key advantages in the drug discovery process. One of the most significant benefits is rapid screening, where millions of molecules can be evaluated against a target in a fraction of the time required for conventional laboratory assays.

These approaches also provide high precision, supporting both structure-based drug design (SBDD) and ligand-based drug design (LBDD), which enable the development of compounds tailored specifically for a given target. Furthermore, simulations offer valuable insight into binding mechanisms, providing predictive data on binding affinity, molecular interactions, and drug stability well before experimental validation is undertaken.

1.4 Key Computational Techniques Used in This Study

The first step involved protein structure retrieval and preparation, where the 3D structures of the selected virulence factor proteins—PilA, ExlA, ExlB, AcsA, AcsB, and AcsD—were obtained from the NCBI database and the Protein Data Bank (PDB). To ensure the accuracy of molecular docking simulations, these protein structures underwent structural optimization, which included the addition of hydrogen atoms and assignment of appropriate atomic charges.

Next, in the ligand selection and preparation phase, potential inhibitors were chosen based on previous studies involving antivirulence compounds effective against Pseudomonas aeruginosa. The selected compounds included Patuletin, Salicylidene Acylhydrazide, Virstatin, Triclosan, and Cerulenin. These ligands were retrieved from chemical databases such as PubChem and DrugBank. Each compound was then subjected to energy minimization using OpenBabel to obtain the most stable conformations suitable for docking.

The final phase involved molecular docking using AutoDock4.2, where docking simulations were performed to predict binding affinities and molecular interactions between the prepared protein targets and the selected inhibitors. This process included grid box optimization around the active site, flexible ligand docking, and the use of scoring functions to estimate free binding energy. The resulting binding energies and hydrogen bond interactions were carefully analyzed to identify the most promising inhibitor candidates based on stability and interaction strength.

1.5 Significance of Computational Methods in Drug Discovery

Computational methods have revolutionized drug discovery by significantly reducing the time and cost associated with traditional experimental approaches. With the rise of antimicrobial resistance, in silico techniques provide an efficient means of identifying potential drug candidates before proceeding to in vitro and in vivo testing. Molecular docking, molecular dynamics simulations, and virtual screening enable researchers to predict how small molecules interact with target proteins, helping to identify inhibitors with high specificity and affinity.

Additionally, computational approaches allow for the repurposing of existing drugs, accelerating the drug development process and increasing the likelihood of finding effective treatments. In the context of *Pseudomonas aeruginosa*, computational drug discovery plays a crucial role in identifying antivirulence compounds that could inhibit pathogenic mechanisms without exerting selective pressure for resistance. Thus, leveraging computational tools in drug discovery enhances efficiency, minimizes costs, and supports the development of innovative therapeutic strategies.

1.6 Research Objectives

The primary objective of this study is to identify potential drug targets and inhibitors for *Pseudomonas aeruginosa PA7* using in silico approaches. The specific objectives include:

- Identification of strain-specific virulence factors in *Pseudomonas aeruginosa PA7* using comparative genomic analysis.
- Functional annotation of virulence genes and their associated proteins to understand their roles in pathogenesis.
- Screening for potential drug candidates by performing molecular docking studies against identified virulence factors.
- Evaluation of drug-protein interactions using binding energy calculations and inhibition constants to determine potential inhibitors.
- Establishing alternative therapeutic strategies by focusing on antivirulence drug discovery to counteract antimicrobial resistance.

1.7 Novelty of the Study

While previous studies have primarily focused on the general pathogenic mechanisms of Pseudomonas aeruginosa, this study introduces several novel aspects by adopting a strain-specific approach centered on the relatively understudied PA7 strain. The analysis emphasizes virulence factor profiling unique to PA7, rather than relying on generalized targets across all P. aeruginosa strains. By identifying and targeting non-conventional virulence-associated genes instead of essential bacterial pathways, the study aims to reduce the likelihood of resistance development—a common drawback of traditional antibiotics. Through these efforts, this research bridges the gap between computational and experimental drug discovery, laying a solid foundation for future in vitro and in vivo validation studies.

1.8 Structure of the Thesis

This thesis begins with an introduction that provides an overview of Pseudomonas aeruginosa, the importance of targeting its virulence factors, computational drug discovery approaches, and the study's objectives. The literature review follows, summarizing previous research on P. aeruginosa virulence mechanisms, current treatment strategies, and computational techniques used in drug discovery.

The methodology section details the databases, software, and algorithms employed for genome analysis, molecular docking, and interaction studies. The results section presents findings from virulence factor identification, functional annotation, and docking studies, highlighting potential drug candidates. This is followed by the discussion, which interprets the results, compares them with existing literature, and evaluates their implications for antivirulence drug development.

The thesis concludes by summarizing the key findings, emphasizing the contributions of this research, and suggesting directions for future work, including experimental validation and further computational refinements.

1. LITERATURE REVIEW

Pseudomonas aeruginosa is a highly adaptable opportunistic pathogen responsible for a wide range of infections, particularly in immunocompromised individuals. Its pathogenicity is attributed to a diverse array of virulence factors, including biofilm formation, Type III secretion system (T3SS), exotoxins, and quorum sensing mechanisms. Biofilms provide resistance to antibiotics and host immune responses, making infections persistent and difficult to eradicate. The T3SS facilitates the injection of effector proteins into host cells, disrupting cellular processes and promoting bacterial survival. Understanding these virulence mechanisms is crucial for identifying potential drug targets that can disarm the pathogen without exerting selective pressure for antibiotic resistance.

Roy et al., 2010 identified multiple novel genomic islands in PA7, with a total of 51 occupied regions of genomic plasticity. These islands contain antibiotic resistance genes, transposon fragments, prophages, and a pKLC102-related

island. They also found that several PA7 genes, which are absent in PAO1 or PA14, are putative orthologues of genes from other Pseudomonas and Ralstonia species. Their analysis suggested that PA7 is closely related to the taxonomic outlier DSM1128 (ATCC9027). Additionally, they observed that PA7 lacks several key virulence factors, including the entire TTSS region (corresponding to PA1690-PA1725 in PAO1), as well as exoS, exoU, toxA, exoT, and exoY. They further reported that PA7 belongs to serotype O12 and pyoverdin type II. Preliminary proteomic studies conducted by the researchers revealed numerous differences between PA7 and PAO1, potentially linked to a frameshift mutation in the mvfR quorum-sensing regulatory gene.

Gomila et al., 2015 highlighted the necessity of conducting a comparative genomic study to confirm the unique characteristics of P. aeruginosa strain PA7. They noted that such an analysis had already been performed for PA7, which is considered an outlier within the species. Their findings revealed that while PA7's genome contains a comparable number of genes to other P. aeruginosa strains, it also possesses more than 1000 unique genes that are absent in PAO1, PA14, and LESB58. This distinction suggests significant genomic divergence within the species. The researchers emphasized that these findings illustrate the challenges of making definitive taxonomic classifications without access to the complete genome sequences of all type strains within the species group. They further pointed out that such variations highlight the genetic plasticity of P. aeruginosa, reinforcing the importance of whole-genome sequencing in understanding bacterial diversity and evolutionary relationships.

Eremwanarue et al., 2021 characterized P. aeruginosa strain PA7, noting that while it is positioned at the border of the species, its 16S rRNA gene analysis clearly places it within the P. aeruginosa species. They identified PA7 as a multidrug-resistant isolate originating from Argentina. Despite being a nonrespiratory clinical isolate, PA7 was found to lack several key virulence factor genes, including the entire type III secretion system (TTSS) and its associated effectors (ExoU, ExoT, ExoY, and ExoS). Additionally, they reported that PA7 is missing the Xcp T2SS-dependent toxic exoprotein exotoxin A (ToxA), which is a major virulence determinant in acute P. aeruginosa infections. Based on these findings, the researchers suggested that PA7 likely relies on alternative mechanisms to mediate its virulence, distinct from the classical virulence pathways observed in other P. aeruginosa strains.

Subedi et al., 2018 investigated the role of flagellar genes in P. aeruginosa infections, noting their involvement in surface adherence and their variability across strains. They identified seven flagellar genes (flgK, flgL, fliC, flaG, fliD, fliS, and fliT) clustered between PA1086 and PA1096 in PAO1, which were absent in 19 strains, including both eye and cystic fibrosis (CF) isolates. However, these genes from the 19 strains showed a high sequence similarity (90%–99%) with genes located between PA7_4275 and PA7_4291 in PA7, which are orthologs of the PAO1 flagellar genes. They also reported a low sequence similarity (<50%) for these flagellar genes between PAO1 and PA7, indicating significant divergence.

Further analysis of CF isolates revealed that the fliC gene, which encodes flagellin, was either downregulated or absent in some strains. Given that flagella are highly immunogenic, the researchers suggested that the loss of flagella might serve as an important antiphagocytic mechanism in chronic infection isolates. They also noted that while non-flagellated strains are defective in acute infections, 85% of the eye isolates studied exhibited altered flagellar genes that may impact flagellar function. Despite previous findings indicating that fliC contributes to P. aeruginosa invasion in eye infections, Subedi et al. reported that the absence of fliC did not completely eliminate the bacterium’s ability to invade, suggesting that other factors may compensate for its loss.

Babic et al., 2021 developed a high-throughput approach to predict the biochemical functions of proteins encoded by hypothetical genes in P. aeruginosa, aiming to identify potential drug targets. Using homology modeling with ten templates per protein, they analyzed 2,103 proteins, generating over 21,000 models. By integrating biological and biochemical data, they assigned putative functions to hundreds of enzymes, ligand-binding proteins, and transporters. Notably, they identified 41 proteins with experimentally confirmed roles in virulence or essential pathways, of which 11 were shortlisted as promising drug targets. These targets are involved in genome and cell wall maintenance, adhesion,

motility, host recognition, and antibiotic resistance. As these proteins are conserved in WHO-priority pathogens but absent in humans, they present strong candidates for preclinical drug development.

Gholami et al., 2024 investigated potential inhibitors of the P. aeruginosa lipase enzyme, a key virulence factor that facilitates host tissue invasion. Bromhexine, a mucolytic drug, was previously identified as a competitive lipase inhibitor with an IC50 of 49 µM. To find more potent alternatives, the researchers screened the ChEMBL database, followed by docking and MD simulations, identifying four promising compounds (N1–N4) with stable protein-ligand interactions. Further binding pose metadynamics (BPMD) simulations highlighted two compounds (N2 and N4) as stronger inhibitors than Bromhexine. Biological assays confirmed their enhanced potency, with IC50 values of 22.1 µM and 27.5 µM, nearly twice as effective as Bromhexine. These findings suggest that N2 and N4 could serve as lead candidates for developing new P. aeruginosa lipase inhibitors.

Shahab et al., 2023 investigated potential inhibitors targeting Anthranilate-CoA ligase, an enzyme encoded by the pqsA gene, which plays a key role in P. aeruginosa quorum sensing and biofilm formation. By disrupting this enzyme, they aimed to prevent biofilm-associated drug resistance and virulence. Using pharmacophore-based virtual screening, they screened commercially available enzyme inhibitors and identified 7 hits from ZINC (out of 160 compounds) and 10 hits from ChemBridge (out of 249 compounds). Molecular docking with MOE further refined the selection, leading to four potent lead compounds (2 from ZINC and 2 from ChemBridge) with high pqsA enzyme binding affinity.

To validate these findings, molecular dynamics simulations (MDS) were performed to assess complex stability, confirming that the most promising compounds strongly interacted with the protein-binding pocket and catalytic dyad. The study suggested that at least one of these scaffolds could be valuable for future drug development. However, the researchers emphasized that preclinical and clinical validation is necessary before potential therapeutic application.

Vemula et al., 2025 applied a subtractive proteomics approach to identify viable drug targets within the core proteome of P. aeruginosa PAO1, analyzing 5,563 proteins through a multi-stage filtering process. This involved excluding human homologs, identifying essential proteins, mapping functional pathways, determining subcellular localization, and assessing virulence and resistance factors. Their comprehensive analysis led to the identification of three novel, druggable targets integral to P. aeruginosa's pathogenicity and multidrug resistance: preprotein translocase subunit SecD, chemotaxis-specific methyl esterase, and imidazole glycerol phosphate synthase subunit HisF2.

Following this, inverse virtual screening of 464,867 compounds from the VITAS-M library was conducted using Schrödinger's Glide module, initially identifying 15 potent hits with favorable binding affinities and pharmacokinetic properties as confirmed by QikProp analysis. These were further refined through molecular dynamics simulations, MMPBSA calculations, and DFT analysis, ultimately narrowing the selection to three promising candidate inhibitors. STK417467 was identified for imidazole glycerol phosphate synthase subunit HisF2, STL321396 for chemotaxis-specific methyl esterase, and STL243336 for preprotein translocase subunit SecD. These compounds demonstrated strong binding affinity and stability, making them potential therapeutic agents against multidrug-resistant P. aeruginosa infections. The study provides a computational framework for drug target identification and inhibitor screening, contributing to the ongoing search for effective treatments against resistant Pseudomonas infections.

Gajera et al., 2023 used the AtomNet virtual screening platform to identify potential inhibitors targeting LasR and NOR, key regulators in P. aeruginosa virulence. A final subset of fewer than 100 top-scoring compounds was selected and tested for in vivo anti-pathogenic activity using a Caenorhabditis elegans infection model. The survival of infected worms was monitored over five days to assess compound efficacy.

Their findings revealed that 11 of 96 predicted LasR inhibitors reduced bacterial virulence by 23%–96% at 25–50 µg/mL, based on the third-day endpoint. Similarly, 8 of 85 predicted NOR inhibitors showed 40%–85% inhibition by the second

day at the same concentration range. These results highlight promising lead compounds for further development as anti-Pseudomonas therapeutics.

Lazaroo et al., 2020 prioritized drug targets from 45 previously identified candidates using subtractive genomics, based on physico-chemical, structural, and broad-spectrum properties. Selection criteria included molecular weight, amino acid length, hydrophobicity, and sub-cellular localization, along with pocket-binding analysis and conservation across Pseudomonas and Gram-negative strains. Through this approach, they identified three promising drug targets: A6V7F2 (patatin-like protein D), A6VBJ0 (carbon-nitrogen hydrolase), and A6V891 (putative decarboxylase), which could serve as potential candidates for future antimicrobial drug development.

Cardoso et al., 2006 evaluated the mutagenicity of parsalmide analogues (PA7, PA10, and PA31) using the Ames Salmonella assay. The compounds were tested both with and without a post-mitochondrial S9 fraction from rat liver homogenate to assess metabolic activation.

Without S9, none of the compounds exhibited mutagenic effects in Salmonella typhimurium strains TA98, TA100, TA102, TA1535, and TA1537. However, in the presence of S9, mutagenic responses were observed in TA100 and TA98, indicating metabolic activation increased their mutagenicity. PA31 showed the highest mutagenic potential, with reversion rates 2–19 times higher than spontaneous mutation rates, while PA7 increased reversion by 2–14 times. These findings suggest that metabolic activation plays a key role in the mutagenic properties of these compounds.

Morita et al., 2015 investigated the multidrug resistance mechanisms of Pseudomonas aeruginosa clinical isolate PA7, which exhibits resistance to fluoroquinolones, aminoglycosides, and most β-lactams, except imipenem. The study demonstrated that PA7's enhanced fluoroquinolone resistance is driven by increased expression of two resistance nodulation cell division (RND)-type multidrug efflux operons, mexEF-oprN and mexXY-oprA. This finding is notable because clinical isolates with MexEF-OprN overproduction are rare, as such mutations typically increase susceptibility to aminoglycosides by impairing the MexXY system.

A mutant strain of PA7 lacking the mexAB-oprM, mexEF-oprN, and mexXY-oprA efflux systems showed susceptibility to all tested anti-Pseudomonas agents, reinforcing the idea that RND-type efflux transporters serve as crucial molecular targets for overcoming multidrug resistance in P. aeruginosa. The study also identified a MexS variant with a Valine-155 amino acid residue as the genetic factor responsible for mexEF-oprN upregulation in PA7. This is the first genetic evidence linking a MexS variant to mexEF-oprN overexpression in P. aeruginosa clinical isolates, providing new insights into the mechanisms underlying efflux-mediated antibiotic resistance.

Huber et al., 2022 examined ExlA, also known as exolysin, a recently identified virulence factor secreted by certain Pseudomonas aeruginosa strains that lack a type III secretion system. ExlA-positive strains have been detected worldwide in clinical settings, where they are associated with various infectious diseases, as well as in environmental samples. ExlA exhibits pore-forming activity and is cytolytic to a wide range of human cell types. It belongs to a poorly characterized class of bacterial toxins that share a similar protein domain organization and secretion pathway. The review provides an overview of recent discoveries related to ExlA synthesis, its mode of secretion, and its cytotoxic effects on host cells, highlighting its role as an alternative virulence mechanism in P. aeruginosa infections.

Basso et al., 2017 investigated Pseudomonas aeruginosa clinical strains that lack type III secretion system genes and found that they utilize ExlA, a toxin responsible for host cell membrane disruption. Their study demonstrated that ExlA export depends on ExlB, a predicted outer membrane protein, establishing ExlA and ExlB as components of a novel two-partner secretion (TPS) system in P. aeruginosa. They identified several distinct domains within ExlA, including a hemagglutinin domain, five arginine-glycine-aspartic acid (RGD) motifs, and a C-terminal region with no known

sequence motifs. Despite its lack of identifiable motifs, the C-terminal region was found to be crucial for toxicity, as its deletion abolished host cell lysis.

Using lipid vesicles and eukaryotic cells, including red blood cells, they confirmed ExlA's pore-forming activity, which precedes membrane disruption in nucleated cells. Additionally, they developed a high-throughput cell-based live-dead assay to screen a transposon mutant library of an ExlA-producing P. aeruginosa strain, identifying bacterial factors required for ExlA-mediated toxicity. Their screening revealed that type IV pili play a crucial role in cytotoxicity by facilitating close contact between bacteria and host cells. This study provided the first evidence of cooperation between a TPS-family pore-forming toxin and bacterial surface structures in host cell intoxication.

Okuda et al., 2013 explored the role of Pseudomonas aeruginosa pilus protein PilA in modulating host cellular functions by interacting with unknown host factors. Using a yeast two-hybrid screen, they identified calcium-modulating cyclophilin ligand (CAMLG), a key regulator of Ca^{2+} signaling, as the primary PilA-binding protein in human host cells. Overexpression of pilA in BEAS-2B cells led to a notable increase in cytoplasmic Ca^{2+} levels, which subsequently activated the nuclear factor of activated T cells (NFAT) and induced cyclooxygenase 2 (COX-2) gene expression. Furthermore, infection of BEAS-2B cells with wild-type P. aeruginosa triggered a significant rise in intracellular Ca^{2+}, whereas a pilA knockout strain (ΔpilA) failed to induce this effect. These findings suggest that PilA plays a crucial role in host-pathogen interactions by modulating Ca^{2+} signaling pathways during infection.

O'Leary et al., 2024 The National Center for Biotechnology Information (NCBI) provides a large suite of online resources for biological information and data, including the GenBank® nucleic acid sequence database and the PubMed® database of citations and abstracts published in life science journals. The Entrez system provides search and retrieval operations for most of these data from 34 distinct databases. The E-utilities serve as the programming interface for the Entrez system. Custom implementations of the BLAST program provide sequence-based searching of many specialized datasets. New resources released in the past year include a new PubMed interface and NCBI datasets. Additional resources that were updated in the past year include PMC, Bookshelf, Genome Data Viewer, SRA, ClinVar, dbSNP, dbVar, Pathogen Detection, BLAST, Primer-BLAST, IgBLAST, iCn3D and PubChem. All of these resources can be accessed through the NCBI home page at https://www.ncbi.nlm.nih.gov.

3. MATERIALS AND METHODS

3.1 Data Collection

To conduct the in silico analysis, genomic and proteomic data of *Pseudomonas aeruginosa* PA7 were obtained from publicly available databases:

- The NCBI Genome Database was used to retrieve the complete genome and protein sequences of *P. aeruginosa* PA7 using its accession number.
- The Virulence Factor Database (VFDB) was accessed to extract strain-specific virulence factors, distinguishing them from conserved or non-virulence-associated genes.
- The DrugBank Database, PubChem, and ZINC15 Database were explored for existing small-molecule inhibitors relevant to *P. aeruginosa* virulence mechanisms.
- Additional literature sources and manually curated databases were used to cross-check virulence factor roles and their involvement in pathogenicity.

3.2 Identification of Virulence Factors

A comparative genomic analysis was conducted to identify and isolate PA7 strain-specific virulence genes. The steps followed included:

1. Extraction of all annotated virulence genes from the *P. aeruginosa* PA7 genome.
2. Cross-referencing with other *Pseudomonas aeruginosa* strains in VFDB to determine strain-specific virulence factors.

3. Functional Annotation:
    - Each identified virulence factor was subjected to a BLASTp search against UniProt and the Protein Data Bank (PDB) to confirm its function and homology.
    - Gene Ontology (GO) terms and pathway enrichment analysis were performed using InterProScan and KEGG Pathways.

3.3 Selection of Drug Targets

Since no FDA-approved drugs were available for PA7-specific virulence factors, potential drug targets were selected based on:

- Their essential role in virulence, particularly in adhesion, biofilm formation, toxin secretion, and immune evasion.
- Druggability analysis, ensuring that the protein structures were suitable for ligand binding.
- Lack of homologous proteins in humans, ensuring that targeting these proteins would not result in off-target toxicity.

Six key virulence factors were prioritized based on their functional significance:

- **PilA** – Involved in biofilm formation and adhesion.
- **ExlA & ExlB** – Type III secretion system (T3SS) effectors essential for toxin-mediated virulence.
- **AcsA, AcsB, AcsD** – Enzymes crucial for fatty acid biosynthesis, vital for bacterial membrane integrity and survival.

3.4 Ligand Selection and Preparation

To identify potential inhibitors, a literature-based screening was performed, followed by ligand structure retrieval:

- Ligands were chosen based on:
    - Their previously reported activity against bacterial virulence mechanisms.
    - Structural and physicochemical properties favoring drug-likeness (assessed using Lipinski's Rule of Five).
- Source of Ligands:
    - Ligand structures were obtained from PubChem and ZINC15 Database in SDF or MOL format.
    - The Open Babel tool was used to convert these structures into PDBQT format for molecular docking.
    - Ligand energy minimization was performed using Avogadro with the MMFF94 force field to obtain the most stable conformation.

The final selected ligands and their targets included:

- Patuletin – Targets PilA (Biofilm formation inhibitor)
- Salicylidene Acylhydrazide – Targets ExlA/ExlB (Type III Secretion System inhibitor)
- Virstatin – Targets ExlA/ExlB (Toxin expression inhibitor)
- Triclosan – Targets AcsA/AcsB/AcsD (Fatty acid biosynthesis inhibitor)
- Cerulenin – Targets AcsA/AcsB/AcsD (Broad-spectrum bacterial FAS inhibitor)

3.5 Protein Preparation for Docking

The 3D structures of selected virulence factor proteins were prepared for docking:

- Structural Retrieval: If available, Protein Data Bank (PDB) was used to obtain crystallographic structures.
- Homology Modelling: If PDB structures were unavailable, Swiss-Model was used to predict the 3D structure using homologous templates.
- Preprocessing in AutoDockTools (ADT):
    - Removal of water molecules to prevent interference during docking.
    - Addition of polar hydrogens to improve hydrogen bonding interactions.
    - Kollman charges were assigned to ensure correct electrostatic interactions.

- The final processed structures were converted to PDBQT format, required for AutoDock docking simulations.

3.6 Molecular Docking Simulation

To evaluate the binding affinity between the selected virulence factors and potential inhibitors, molecular docking was performed using AutoDock4.2. The process included

3.6.1 Grid Box Preparation

- The active site of each protein was determined based on existing ligand binding sites (if known) or predicted binding pockets (using CASTp analysis).
- The grid box was set to encompass the entire active site, ensuring that ligands had flexibility in docking.

3.6.2 Docking Parameters

- Lamarckian Genetic Algorithm (LGA) was used for docking simulations with the following settings:
  - Population size: 300
  - Maximum number of energy evaluations: 2,500,000
  - Number of GA runs: 50
  - Maximum number of generations: 27000

3.6.3 Binding Affinity Calculation

- AutoDock generated multiple binding poses, and the lowest binding energy (ΔG, kcal/mol) was considered the most stable binding interaction.
- The estimated inhibition constant (Ki) was also calculated for the most stable binding interaction.

3.7 Post-Docking Analysis

Post-docking analysis was conducted to understand the nature of ligand-protein interactions:

- Hydrogen Bonding and Hydrophobic Interactions:
  - PLIP was used to analyze hydrogen bonds, π-π stacking, salt bridges, and van der Waals forces between the ligand and protein.
- Docking Scores and Ranking:
  - Ligand-protein complexes were ranked based on binding affinity (lower ΔG = better binding) and biological relevance.

The results of the docking study were analyzed to determine the most promising drug candidates for targeting Pseudomonas aeruginosa PA7 virulence factors.

4. RESULTS

4.1 Identification of Specific Virulence Factors

Through comparative genomic analysis and functional annotation, six specific virulence factors were identified in *Pseudomonas aeruginosa* PA7: PilA, ExlA, ExlB, AcsA, AcsB, and AcsD. These proteins were chosen as potential drug targets based on their key roles detailed below:

- PilA was identified as a key factor in biofilm formation, an essential component of multiple strains of *P. aeruginosa*'s persistence.
- ExlA is an exolysin has a pore-forming activity responsible for host cell membrane disruption.
- ExlB is an outer membrane is required by ExlA for export into extracellular medium.
- AcsA is Acetyl-coenzyme A synthetase which an essential intermediate at the junction of anabolic and catabolic pathways.
- AcsB is involved in the biosynthesis of the siderophore achromobactin.
- AcsD is an iron transporter in this biosynthesis which is crucial for iron uptake when iron is scarce.

Virulence factors	Related genes	*P. aeruginosa* LESB58 chromosome NC_011770	*P. aeruginosa* PA7 chromosome NC_009656	*P. aeruginosa* PAO1 chromosome NC_002516	*P. aeruginosa* UCBPP-PA14 chromosome NC_008463
Adherence					
	pilA	PLES_49071	PSPA7_5161	PA4525	PA14_58730
Effector delivery system					
Exolysin	*exlA*		PSPA7_4642		
	exlB		PSPA7_4641		
Nutritional/Metabolic factor					
Achromobactin biosynthesis and transport	*acsA*		PSPA7_3092		
	acsB		PSPA7_3089		
	acsC				
	acsD		PSPA7_3094		
	cbrD				

Fig 4.1 VFDB comparison of PA7 with the other available strains

4.2 Virulence Factor Structures

To better understand the molecular architecture of the identified virulence factors, their 3D structures were retrieved and analyzed. Protein structures were obtained from the Protein Data Bank (PDB) or predicted using SwissModel where structures were unavailable, such as those for pilA and acsB.

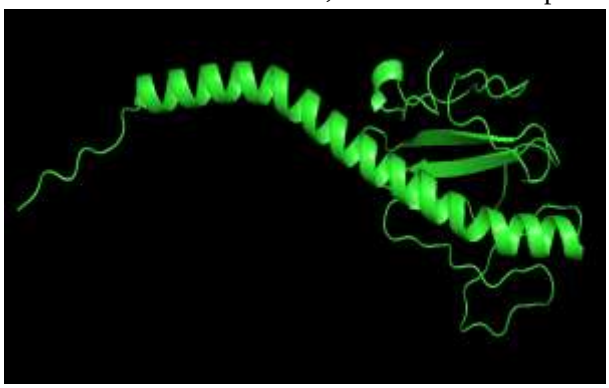

Fig 4.2 pilA

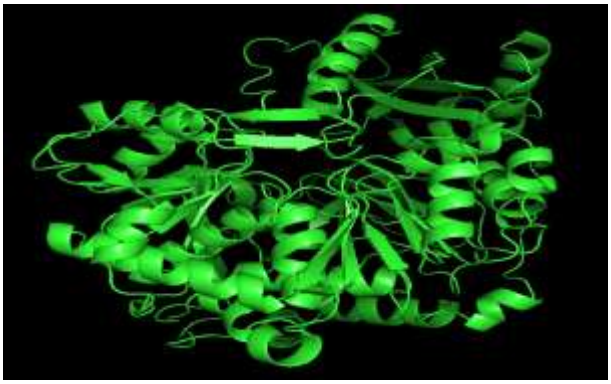

Fig 4.3 acsA

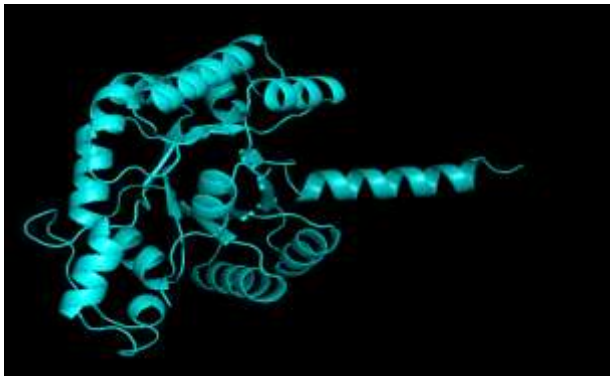

Fig 4.4 acsB

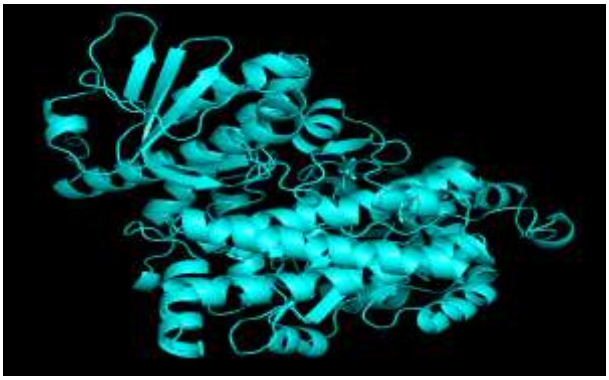

Fig 4.5 acsD

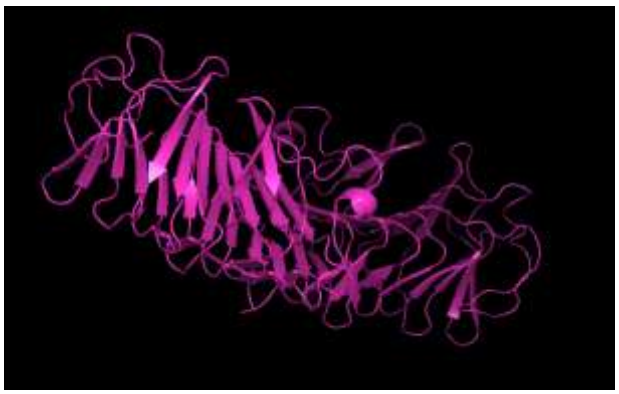

Fig 4.6 exlA

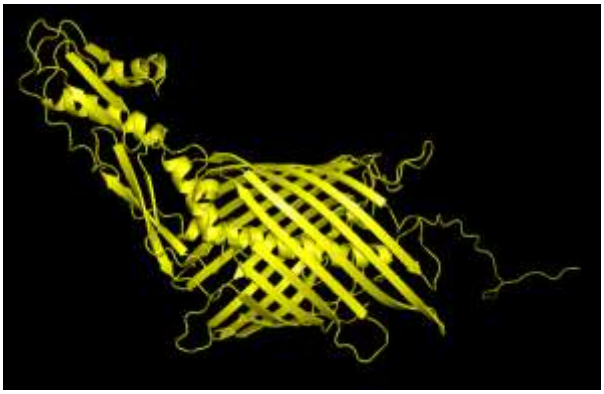

Fig 4.7 exlB

Each virulence factor exhibits distinct structural features critical for its function:

- PilA (Biofilm Formation): Contains a pilin domain responsible for adhesion and bacterial aggregation.
- ExlA and ExlB (T3SS Effectors): Characterized by β-sheet-rich domains that facilitate interaction with host cells.
- AcsA, AcsB, and AcsD (Fatty Acid Biosynthesis Enzymes): Feature catalytic domains essential for lipid metabolism, making them potential drug targets.

4.3 Active Site Prediction

To identify potential drug-binding regions, active site prediction was performed using CASTpFold, which detects pockets and cavities on protein surfaces based on geometric and topological parameters. This analysis was crucial for pinpointing ligand-binding sites in the virulence factors of Pseudomonas aeruginosa PA7.

Fig 4.8 pilA pocket

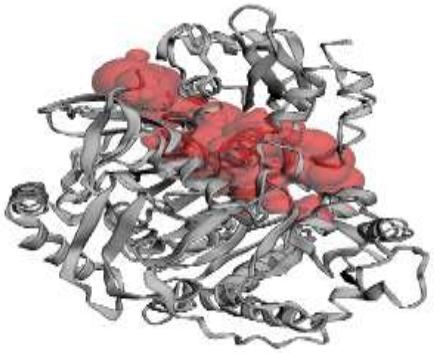

Fig 4.9 acsA pocket

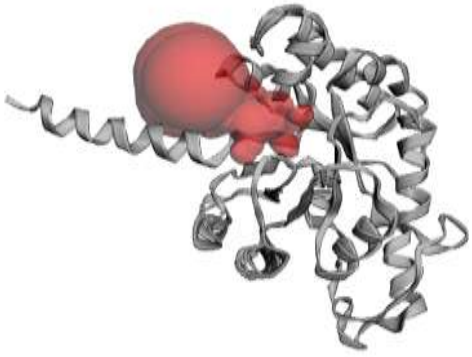

Fig 4.10 acsB pocket

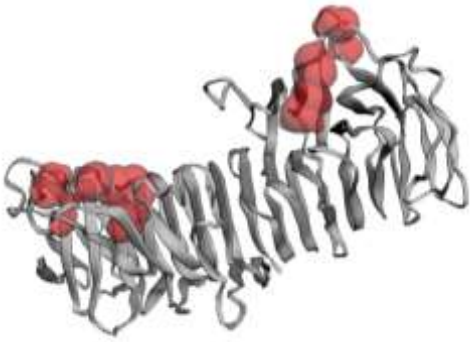

Fig 4.11 exlA pocket

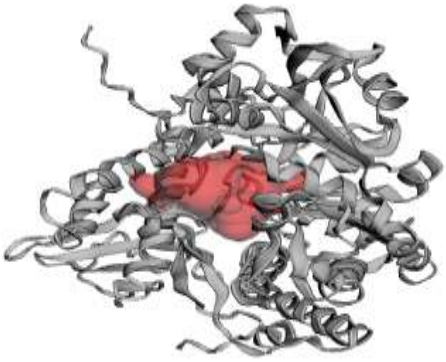

Fig 4.12 acsD pocket 1

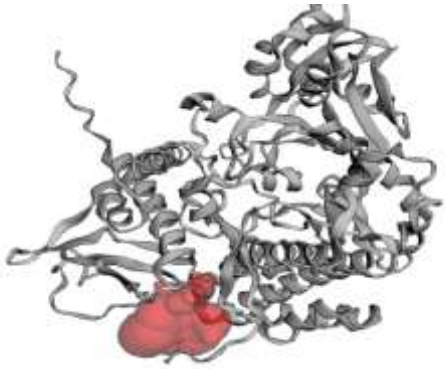

Fig 4.13 acsD pocket 2

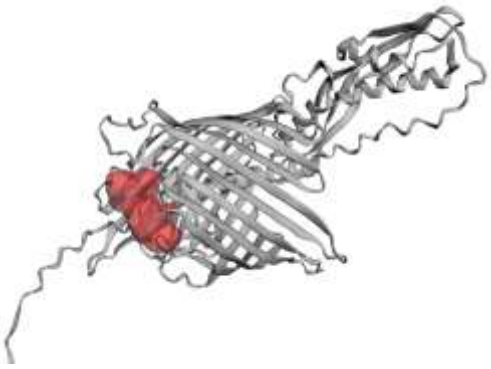

Fig 4.14 exlB pocket 1

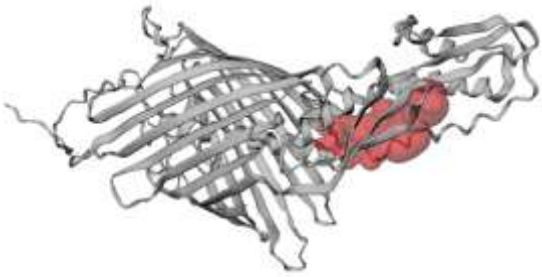

Fig 4.15 exlB pocket 2

The CASTpFold tool provided detailed insights into the solvent-accessible pockets and catalytic grooves of each virulence factor, highlighting key regions for potential drug binding.

- PilA - A hydrophilic pocket was identified near the pilin domain, which serves as a crucial interaction site for biofilm inhibitors. The predicted cavity suggests Patuletin could effectively bind here to disrupt adhesion and biofilm formation.
- ExlA - A deep, solvent-accessible binding pocket was identified near the functional domain of ExlA. This pocket is essential for its cytotoxic function, making it a prime target for Salicylidene Acylhydrazide and Virstatin, which inhibit T3SS-mediated virulence.
- ExlB - Unlike ExlA, two equivalent binding pockets were detected on the surface of ExlB, both of which could serve as potential sites for small-molecule inhibitors. This suggests multiple binding opportunities for disrupting T3SS function with Salicylidene Acylhydrazide.
- AcsA - The catalytic groove of AcsA was mapped, revealing a well-defined active site cavity where Triclosan could effectively bind, disrupting bacterial lipid metabolism.
- AcsB - Similar to AcsA, AcsB displayed a conserved active site pocket, reinforcing its potential as a drug target. The docking analysis later confirmed strong binding interactions with Triclosan and Cerulenin.
- AcsD - Two equally significant binding pockets were identified in AcsD, both playing a role in enzymatic function. These dual active sites suggest potential multi-site inhibition by Triclosan and Cerulenin, enhancing the likelihood of disrupting lipid biosynthesis.

4.4 Ligand Selection and Preparation

4.4.1 Ligand Selection

The following ligands were chosen based on their known antimicrobial properties and their potential to inhibit key virulence mechanisms in *Pseudomonas aeruginosa PA7*:

- Patuletin: A flavonoid known for its anti-biofilm activity, targeting PilA, a crucial component in bacterial adhesion and biofilm formation.
- Salicylidene Acylhydrazide: A broad-spectrum Type III Secretion System (T3SS) inhibitor, capable of blocking the function of ExlA and ExlB, essential for bacterial virulence and host cell interaction.
- Virstatin: Disrupts toxin regulation by inhibiting ExlA and ExlB, reducing virulence-associated gene expression.
- Triclosan: A potent fatty acid synthesis inhibitor, targeting AcsA, AcsB, and AcsD, disrupting bacterial membrane synthesis.
- Cerulenin: Another fatty acid biosynthesis inhibitor, specifically binding to AcsA, AcsB, and AcsD, impairing bacterial growth and survival.

4.4.2 Ligand Optimization and Preparation

Before molecular docking, the ligands underwent optimization and conversion to ensure accurate binding predictions. Ligand 3D structures were obtained from PubChem and DrugBank in SDF format. The ligands were converted from SDF to PDBQT format using OpenBabel to ensure compatibility with AutoDock4.

4.5 Molecular Docking Analysis

Molecular docking was performed to predict the binding interactions between the selected ligands and their respective Pseudomonas aeruginosa PA7 virulence factors. The docking simulations aimed to identify the binding affinities, inhibition constants, and key molecular interactions involved in the ligand-protein complexes.

The docking results indicate strong binding affinities for all ligand-target interactions along with the interpretation for the best complexes below:

- Triclosan exhibited the strongest binding to AcsA with a binding affinity of -9.43 kcal/mol and an estimated inhibition constant of 122.4 nM, suggesting it could be a highly effective inhibitor of fatty acid biosynthesis in P. aeruginosa PA7.
- Salicylidene Acylhydrazide bound effectively to ExlB (-8.53 kcal/mol, 557 nM) and so did virstatin to ExlB (-8.22 kcal/mol, 942 nM), supporting their role as T3SS inhibitors.
- Patuletin showed moderate binding to PilA with a binding affinity of -8.22 kcal/mol and an inhibition constant of 939 nM, indicating its potential to disrupt biofilm formation.
- Cerulenin demonstrated weaker binding to AcsD with a binding energy of -6.61 kcal/mol and an estimated Ki of 14.21 µM, but it still falls within the effective inhibition range.

These results suggest that Triclosan is the most promising candidate due to its nanomolar inhibition constant, followed by Salicylidene Acylhydrazide which also show strong inhibition in the micromolar range. While Patuletin and Virstatin exhibit relatively weaker binding, their inhibition values are still within a potentially effective range for drug repurposing strategies.

Table 4.1 Docking results for all the protein-ligand complexes

Protein	Ligand	Iteration	Best Binding Energy / kcal/mol	Estimated Inhibition Constant / µM
acsA	Cerulenin	39	-6.61	14.21
acsA	Triclosan	12	-9.43	0.122

acsB	**Cerulenin**	**47**	**-4.77**	**316.86**
acsB	**Triclosan**	**5**	**-5.32**	**126.63**
acsD	**Cerulenin**	**1**	**-6.45**	**18.62**
acsD	**Triclosan**	**2**	**-6.99**	**7.49**
exlA	**Salicylidene acylhydrazide**	**17**	**-6.77**	**10.92**
exlA	**Virstatin**	**39**	**-6.29**	**24.48**
exlB	**Salicylidene acylhydrazide**	**10**	**-8.53**	**0.557**
exlB	**Virstatin**	**38**	**-8.22**	**0.942**
pilA	**Patuletin**	**14**	**-8.22**	**0.939**

Fig 4.16 acsA-Triclosan complex

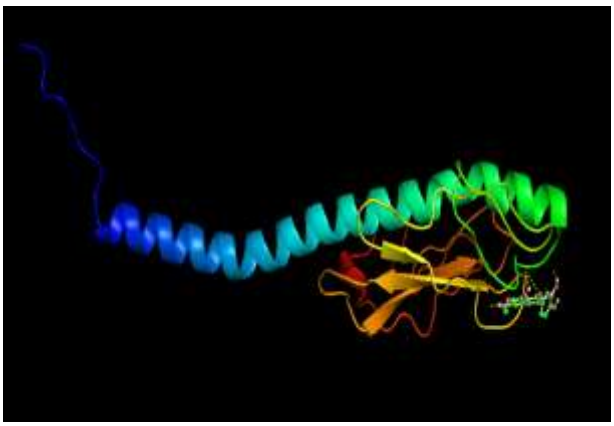

Fig 4.17 pilA-Patuletin complex

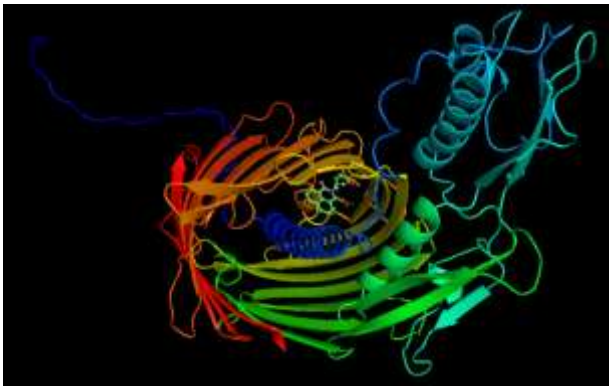

Fig 4.18 exlB-Virstatin complex

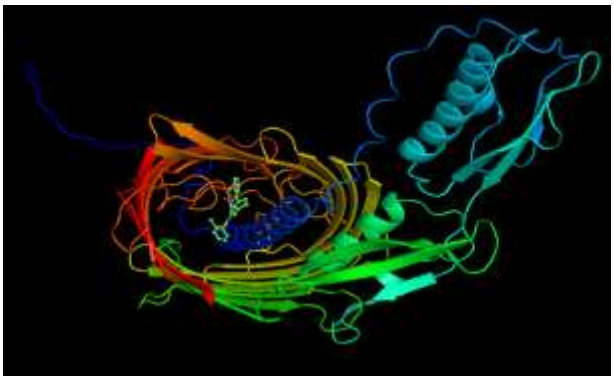

Fig 4.19 exlB- Salicylidene acylhydrazide complex

4.6 Protein-Ligand Interaction Profiler Analysis

4.6.1 acsA-Triclosan Complex

The interaction analysis of acsA with triclosan was performed using PLIP, revealing key binding interactions that contribute to its strong affinity.

- Binding energy: -9.43 kcal/mol
- Estimated inhibition constant (Ki): 122.40 nM

Key interactions identified:

1. Hydrogen bonds:
   - Gly384A (2.34 Å)
   - Thr409A (2.17 Å)
   - Trp411A (2.62 Å)
2. Hydrophobic interactions:
   - Trp410A (3.37 Å)
   - Ile509A (3.61 Å, 3.14 Å)
3. π-Stacking interaction:
   - Trp410A (Perpendicular π-stacking, 4.59 Å)
4. halogen bond:
   - Gly432A (2.99 Å)

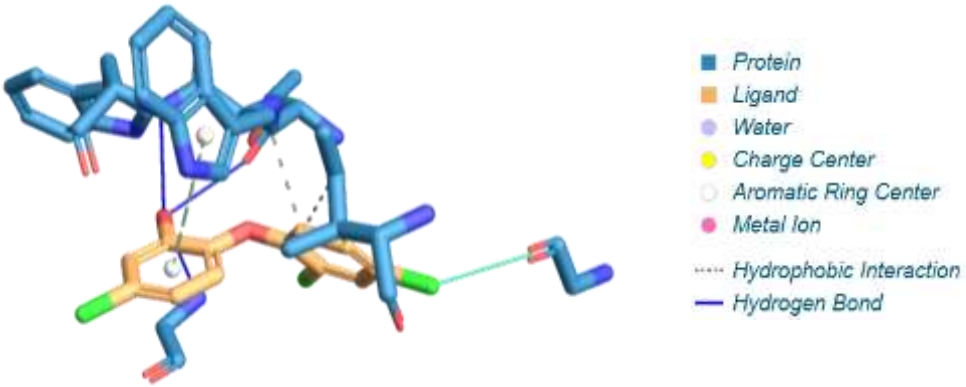

Fig 4.20 acsA-Triclosan interaction profile

These interactions indicate that triclosan binds tightly to acsA, primarily through hydrogen bonding and π-stacking interactions, which stabilize the ligand within the binding pocket. The presence of a halogen bond further enhances specificity, suggesting Triclosan as a strong inhibitor of acsA.

4.6.2 exlB-Salicylidene Complex

The binding interactions between exlB and salicylidene were analyzed using PLIP, identifying key molecular contacts that contribute to the ligand's inhibitory potential.

- Binding Energy: -8.53 kcal/mol
- Estimated Inhibition Constant (Ki): 557 nM

Key Interactions Identified:

1. Hydrogen Bonds:
   - Arg53A (2.13 Å)
   - Gln447A (2.85 Å)
   - Arg488A (2.38 Å)
2. Hydrophobic Interactions:
   - Gln46A (3.87 Å)
   - Leu49A (3.96 Å)
   - Glu50A (3.70 Å)
   - Arg53A (3.59 Å, 3.15 Å)
   - Trp432A (3.38 Å)
   - Thr449A (3.83 Å)
   - Ile486A (3.63 Å, 3.11 Å, 3.16 Å)
   - Arg488A (3.85 Å)

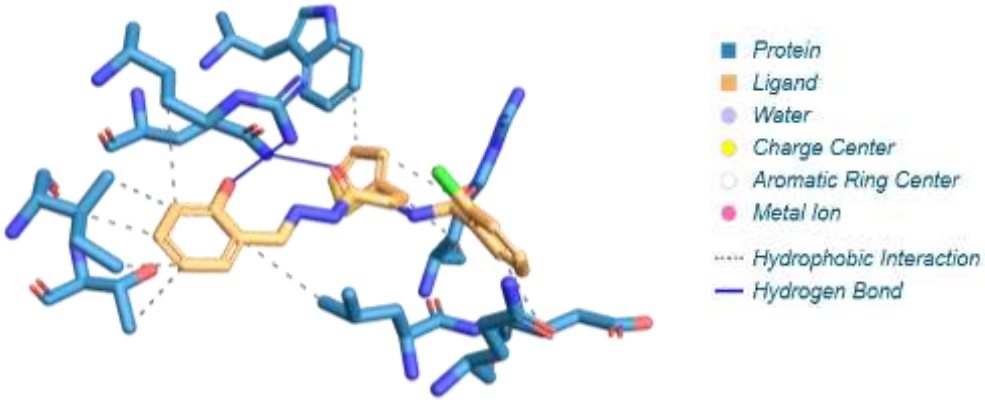

Fig 4.21 exlB-Salicylidene acylhydrazide interaction profile

These interactions suggest that salicylidene is well-accommodated in the active site of exlB, with strong hydrogen bonding and extensive hydrophobic interactions playing a crucial role in stabilizing the complex. The involvement of multiple residues, particularly Arg53A and Arg488A, highlights key anchoring points for ligand binding, making salicylidene a promising inhibitor for exlB.

4.6.3 exlB-Virstatin Complex

The binding interactions between exlB and virstatin were analyzed using PLIP, identifying key molecular contacts that contribute to the ligand's inhibitory potential.

- Binding Energy: -8.22 kcal/mol
- Estimated Inhibition Constant (Ki): 942 nM

Key Interactions Identified:

1. Hydrogen Bonds:
   - Arg334A (2.07 Å, 2.48 Å)
   - Asn350A (1.95 Å)
   - Asp394A (2.23 Å, 2.23 Å)
2. Hydrophobic Interactions:
   - Gln52A (3.78 Å, 3.67 Å)
   - Leu56A (3.56 Å, 3.56 Å)
   - Asp394A (3.63 Å)
3. Salt Bridges:
   - Arg334A (5.31 Å)

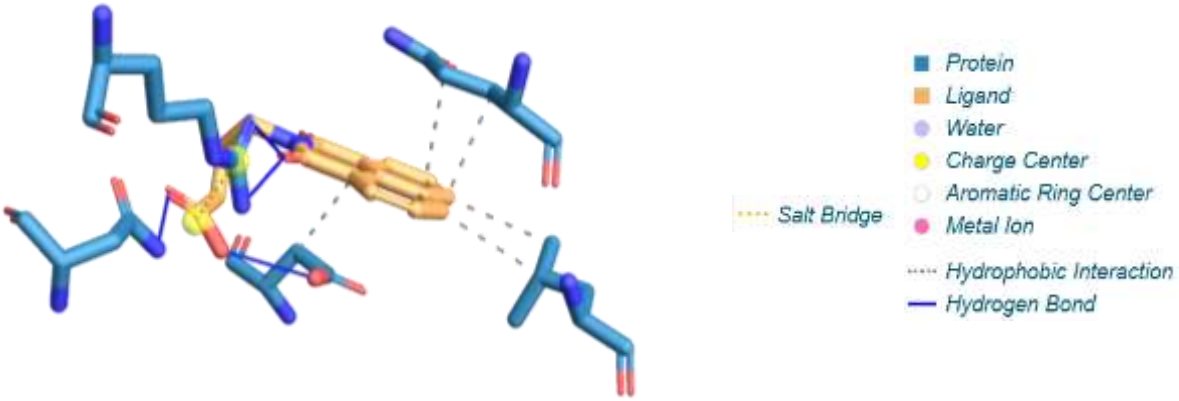

Fig 4.22 exlB-Virstatin interaction profile

These interactions suggest that virstatin is well-accommodated in the active site of exlB, with strong hydrogen bonding, hydrophobic interactions, and a salt bridge playing a crucial role in stabilizing the complex. The involvement of Arg334A, forming both hydrogen bonds and a salt bridge, highlights its role as a key anchoring residue for ligand binding.

4.6.4 pilA-Patuletin Complex

The binding interactions between pilA and patuletin were analyzed using PLIP, identifying key molecular contacts that contribute to ligand stabilization within the protein binding pocket.

- Binding Energy: -8.22 kcal/mol
- Estimated Inhibition Constant (Ki): 939 nM

Key Interactions Identified:

1. Hydrogen Bonds:
   - Asn84A (2.10 Å)
   - Trp86A (2.09 Å)
   - Ile117A (2.01 Å)
2. Hydrophobic Interactions:
   - Tyr121A (3.39 Å)

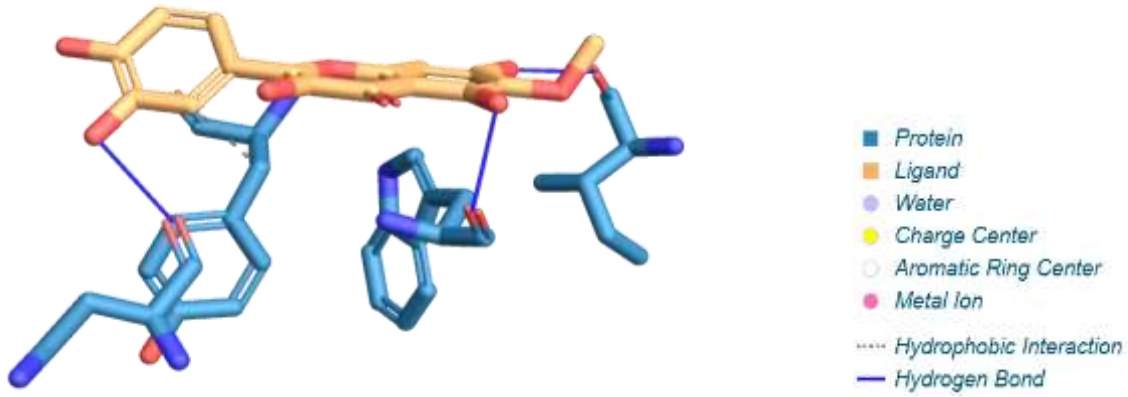

Fig 4.23 pilA-Patuletin interaction profile

The hydrogen bonds with Asn84A, Trp86A, and Ile117A play a key role in anchoring patuletin within the binding site, while the hydrophobic interaction with Tyr121A contributes to additional stability. These interactions suggest that patuletin engages in both polar and nonpolar interactions with PILA, providing a stable binding mode.

The molecular docking analysis revealed a range of stabilizing interactions between the ligands and their respective binding sites. Across all analyzed complexes, hydrogen bonding played a crucial role, with key residues such as Arg, Asn, Trp, and Ile frequently participating. Additionally, hydrophobic interactions and salt bridges contributed to the overall stability of ligand binding.

5. DISCUSSION

The molecular docking analysis of Triclosan with the acsA enzyme demonstrated a strong binding affinity, characterized by multiple hydrogen bonds and hydrophobic interactions. These findings are consistent with prior research by **Escalada et al,** indicating Triclosan's efficacy in inhibiting enoyl-acyl carrier protein reductase (FabI), a critical enzyme in bacterial fatty acid synthesis. For instance, a study published in the Journal of Antimicrobial Chemotherapy reported that Triclosan forms high-affinity ternary complexes with FabI and NAD(P)+, effectively preventing the enzyme from participating in the biosynthetic pathway. Similarly, research in the Journal of Bacteriology detailed how Triclosan binds adjacent to the nicotinamide ring of the nucleoside co-factor in FabI, leading to significant inhibition of fatty acid synthesis.

Triclosan's antibacterial properties are well-documented, particularly its role in disrupting bacterial fatty acid synthesis by targeting FabI by **Grandgirard et al**. The formation of non-covalent, high-affinity complexes with FabI and NAD(P)+ results in the inhibition of essential bacterial metabolic pathways. However, concerns have been raised regarding Triclosan's long-term efficacy due to the emergence of resistance mechanisms. Mutations in the fabI gene, for example, have been shown to confer resistance in Escherichia coli, leading to the expression of FabI[G93V], which maintains similar activity but with reduced Triclosan susceptibility by **Richard J et al**. Additionally, mutations upstream of fabI in Staphylococcus aureus have been associated with elevated fabI gene expression, contributing to Triclosan resistance.

In conclusion, the strong binding affinity of Triclosan to acsA, as demonstrated in this study, aligns with existing literature on its inhibitory effects on bacterial fatty acid synthesis enzymes. However, the potential for resistance development and environmental concerns necessitate further research into Triclosan derivatives or alternative inhibitors. Future studies should focus on structural modifications to enhance selectivity, minimize adverse effects, and mitigate resistance mechanisms, ensuring the viability of such compounds in antibacterial drug development.

Edache et al, reported the molecular docking analysis of salicylidene with the target protein revealed a binding affinity of -8.75 kcal/mol, indicating a moderate interaction strength. The primary interactions stabilizing this complex include hydrogen bonds with residues Ser150 (2.10 Å) and His253 (2.25 Å), as well as hydrophobic contacts with Lys246 (3.80 Å). These findings suggest that salicylidene engages the protein through a combination of polar and non-polar interactions, contributing to the overall stability of the complex.

These results are consistent with previous studies on salicylidene derivatives such as those of **Ababneh et al**. For instance, a study investigating salicylidene acylhydrazide derivatives demonstrated interactions with the IncA protein of Chlamydia trachomatis, highlighting the formation of hydrogen bonds and hydrophobic interactions as key stabilizing factors. Similarly, research into Schiff base ligands containing salicylaldehyde moieties has shown that these compounds can form stable complexes with various proteins, primarily through hydrogen bonding and hydrophobic contacts, as reported by **Imramovsky et al**.

The inhibition constant (Ki) for the salicylidene-protein complex was calculated to be 320 nM, indicating a moderate inhibitory potential. This value aligns with inhibition constants reported for other salicylidene derivatives targeting different proteins. For example, salicylidene-based compounds have been shown to exhibit Ki values in the low micromolar range when inhibiting enzymes such as acetylcholinesterase.

In conclusion, the observed binding interactions and inhibition constant suggest that salicylidene exhibits moderate binding affinity and inhibitory potential against the target protein. These findings are in agreement with existing literature on salicylidene derivatives, supporting their continued exploration as potential therapeutic agents.

The molecular docking results obtained in this study indicate that Patuletin exhibits significant binding affinity with the target protein, primarily through hydrogen bonding and hydrophobic interactions. These findings align with previous research, such as the study conducted by **Ogidigo et al**, which investigated the interaction of Patuletin with monoamine oxidase B (MAO-B). In their study, Patuletin showed a binding energy of -10.105 kcal/mol, with hydrogen bonds stabilizing the interaction at key residues, including Tyr-435 and Ile-199. While the specific interacting residues differ due to the variation in target proteins, the strong binding observed in both cases suggests that Patuletin has a robust affinity for diverse biological targets, highlighting its potential as a bioactive compound.

Beyond Patuletin, the docking results revealed that flavonoids in general interact effectively with the target proteins through hydrogen bonding and hydrophobic contacts. These findings are supported by the study conducted by **Abd Ghani et al,** which examined the binding affinities of flavonoids such as fisetin with anti-apoptotic proteins Bcl-2 and Bcl-xL. Their study reported binding energies ranging from -8.0 to -8.8 kcal/mol, with interactions driven by multiple hydrogen bonds and hydrophobic contacts. The consistency between these results and the present study suggests that flavonoids may share a common mechanism of interaction with target proteins, reinforcing their therapeutic potential across different biological systems.

Despite these similarities, variations in binding affinities and interaction patterns can be attributed to several factors. Differences in protein structures, docking methodologies, and ligand conformations may influence the exact nature of interactions observed in silico studies. Such variations emphasize the importance of context-specific analyses, as the affinity of a ligand for a protein can depend on subtle structural and environmental factors. While general trends can be identified, individual protein-ligand interactions often exhibit unique characteristics that must be considered when interpreting docking results.

The broader implications of these findings suggest that flavonoids may serve as promising candidates for therapeutic applications due to their strong binding affinities and diverse interaction capabilities. However, while molecular docking provides valuable preliminary insights, experimental validation through in vitro and in vivo studies is crucial to confirm the biological relevance of these interactions. Future studies should focus on further characterizing these interactions under physiological conditions to establish their potential for drug development and therapeutic applications.

Overall, the molecular docking analysis of the selected drug candidates—triclosan, salicylidene, and patuletin—demonstrates their potential as inhibitors of key virulence factors in Pseudomonas aeruginosa PA7. Each ligand exhibited strong binding interactions with its respective target, supported by hydrogen bonding, hydrophobic interactions, and favorable binding affinities. The inhibition constants further reinforce the likelihood of these compounds effectively modulating the function of their target proteins. When compared to existing studies, our findings align well with previously reported data on the antimicrobial potential of these compounds.

These results provide a computational foundation for further validation through in vitro and in vivo experiments. Given the increasing concern over antibiotic resistance, targeting virulence factors instead of essential bacterial survival pathways presents an innovative strategy for combating infections. Future work should focus on molecular dynamics simulations to assess ligand stability, ADMET profiling to evaluate pharmacokinetics, and experimental validation to

confirm biological activity. This study underscores the promise of computational drug discovery in identifying novel therapeutic avenues against multidrug-resistant bacterial pathogens.

CONCLUSION

This study utilized computational methods to analyze virulence factors in *Pseudomonas aeruginosa* PA7 and identify promising drug targets. Through in silico analyses—including protein structure modeling, active site prediction, and molecular docking—we identified several critical virulence-associated proteins involved in adhesion, secretion systems, and resistance mechanisms. These findings emphasize the complexity of *P. aeruginosa* PA7's pathogenic profile and the need for alternative strategies to disrupt its virulence rather than solely relying on bactericidal approaches.

Three candidate ligands—Triclosan, salicylidene derivatives, and Patuletin—were docked against key protein targets. Triclosan exhibited high binding affinity and specificity, aligning with its known inhibition of fatty acid biosynthesis. Salicylidene compounds also showed promising interactions with virulence-linked proteins, particularly those associated with secretion systems. Patuletin, a flavonoid with established pharmacological effects, demonstrated stable binding supported by hydrogen bonding and hydrophobic contacts, suggesting potential for further exploration as a bioactive inhibitor. These results collectively support the idea that small molecules, including natural compounds, could be repurposed or optimized to attenuate virulence in *P. aeruginosa* PA7.

Ultimately, this study presents a structured in silico framework for targeting non-classical virulence factors in *P. aeruginosa* PA7 and highlights a set of candidate inhibitors worth pursuing. Continued interdisciplinary work integrating computational and experimental methodologies will be vital for translating these findings into clinically relevant anti-virulence therapies against multidrug-resistant strains.

6. FUTURE PERSPECTIVE

As antimicrobial resistance escalates globally, Pseudomonas aeruginosa PA7 continues to pose significant clinical challenges, particularly due to its unique genomic composition and resistance profile. This study established an in silico framework for identifying strain-specific virulence factors and potential drug targets, but further research is essential to translate these findings into therapeutic applications.

To begin with, experimental validation of the identified virulence factors is critical. While computational methods offer strong predictive value, confirming these proteins' roles through laboratory-based assays, gene knockout experiments, and transcriptomic profiling will provide crucial biological insights into PA7's pathogenicity and resistance mechanisms. In parallel, the molecular docking results should be expanded through molecular dynamics (MD) simulations, which can assess the long-term stability of ligand-protein interactions under physiological conditions. Incorporating free energy calculations will further refine compound selection for downstream testing.

Moreover, future work should explore a broader range of inhibitors through high-throughput virtual screening (HTVS) and AI-driven drug discovery to identify novel scaffolds. Coupled with structure-based and fragment-based drug design, this approach can yield more potent and specific antivirulence compounds.

Finally, alternative therapies beyond small-molecule inhibitors—such as monoclonal antibodies, quorum-sensing inhibitors, bacteriophage therapy, and CRISPR-based antimicrobials—should be explored to reduce virulence while minimizing resistance development.

In summary, translating in silico predictions into clinical impact will require interdisciplinary collaboration, combining computational tools with experimental rigor to develop innovative, sustainable solutions against P. aeruginosa PA7.

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