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Pharmacokinetic Profiling and Molecular Docking of Carvacrol and Structural Analogs: Targeting Quorum Sensing Proteins to Disrupt Biofilm-Mediated Antimicrobial Resistance in Multidrug-Resistant Bacterial Pathogen

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Abstract

Antibiotic resistance, particularly in Pseudomonas aeruginosa, has become a major global health threat, exacerbated by the organism's ability to form biofilms and regulate virulence through quorum sensing (QS). The LuxR and LasR receptors are key regulators in this process, influencing both pathogenicity and antibiotic resistance. This study investigates potential QS inhibitors targeting these receptors as a strategy to mitigate antibiotic resistance. The primary objective was to identify novel ligands that can disrupt QS signaling in P. aeruginosa. Computational techniques, including molecular docking, pharmacological screening, and post-docking analysis, were employed to identify and evaluate potential QS inhibitors. A selection of ligands was docked into the LuxR and LasR receptor binding pockets to assess binding affinities. Compounds, such as homosalate, 2,2'-biphenol, and 2-methyl-4-oxo-1,4-dihydroquinolin-3-olate displayed strong binding affinities of –9.4 kcal/mol, –9.4 kcal/mol, and –8.8 kcal/mol, respectively. Post-docking analysis revealed significant interactions, including hydrogen bonds, hydrophobic interactions, and electrostatic forces, suggesting a robust binding mechanism. Pharmacological evaluations of these compounds indicated favorable properties, such as appropriate molecular weight, solubility, and lipophilicity, making them promising candidates for further development. The results indicate that these compounds could serve as effective QS inhibitors, potentially disrupting P. aeruginosa's ability to form biofilms and regulate virulence, thereby restoring antibiotic efficacy. But we still need experiments to prove these findings. The study's limitations include the need for in vitro and in vivo testing, as well as optimization of the compounds for improved pharmacokinetic profiles. This research highlights the potential of QS inhibition as an alternative strategy for combating antibiotic resistance, with promising future perspectives for drug development.

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INTRODUCTION

Antimicrobial resistance (AMR) is one of the most pressing global health concerns, exacerbated by the growing emergence of drug-resistant pathogens. Quorum sensing (QS), a critical bacterial communication mechanism, plays a pivotal role in regulating various virulence factors, biofilm formation, and antibiotic resistance. QS syreceptorsabsly those mediated by the LuxR and

LasR receptors, are involved in the regulation of virulence and pathogenicity in several bacteria, particularly *Pseudomonas aeruginosa*. These receptors, part of the LuxI/LuxR-type QS systems, control the expression of genes involved in antibiotic resistance, virulence, and biofilm formation, thus contributing significantly to the challenges in treating infections caused by these pathogens. The increasing ability of bacteria to resist treatment, through alterations in metabolic pathways and the regulation of genes responsible for drug resistance, complicates the management of infections. *Pseudomonas aeruginosa*, a gram-negative bacterium, is a model organism for studying QS mechanisms, and its involvement in chronic infections, particularly in immunocompromised patients, underscores the importance of understanding the role of LuxR and LasR receptors in disease pathogenesis and antibiotic resistance.

The ability of bacteria to modify their metabolic pathways and adapt to hostile environments leads to the development of multidrug resistance (MDR), which poses significant challenges to global healthcare. The dysregulation of QS mechanisms, through alterations in LuxR and LasR receptor signaling, is a key factor in enhancing drug resistance in bacteria. Finding new treatments that focus on these pathways could be a great way to fight bacterial resistance. However, the complexity of bacterial resistance mechanisms, coupled with the difficulty in developing drugs that can effectively inhibit QS without affecting host systems, remains a critical obstacle. Moreover, the emergence of bacterial biofilms, which are resistant to antibiotics, further complicates treatment and eradication strategies.

Given the limitations of traditional antimicrobial agents, there is growing interest in the use of natural compounds in drug development. Natural products, particularly plant-derived compounds, have shown promise as potential antibacterial agents due to their unique chemical structures and multifaceted mechanisms of action. Carvacrol, a phenolic compound predominantly found in oregano and thyme, has garnered attention for its antimicrobial, anti-inflammatory, and antioxidant properties. Studies show that carvacrol kills bacteria by breaking their cell membranes and blocking their communication. This makes it a promising option for developing new drugs to fight antibiotic-resistant infections. Additionally, carvacrol has been shown to modulate various metabolic pathways, offering a multifactorial approach to addressing drug resistance.

In the context of drug discovery, structural analogs of bioactive compounds, such as carvacrol, provide valuable insight into the design of more potent and selective therapeutic agents. Structural analogs are compounds that share similar core structures but differ in functional groups or other chemical features. This approach allows for the optimization of pharmacological properties, such as bioavailability, solubility, and binding affinity, while maintaining or enhancing the target activity. By modifying the structure of carvacrol, researchers can explore the potential of these analogs to improve the efficacy of treatment against antibiotic-resistant pathogens. Computational tools, including molecular docking and molecular dynamics simulations, are increasingly being employed in drug discovery to predict interactions between ligands and target proteins, assess the stability of these interactions, and optimize lead compounds. These computational approaches offer several advantages, such as cost-effectiveness, timesaving, and the ability to screen large compound libraries for potential drug candidates. In the present study, computational tools are utilized to explore the interactions of structural analogs of carvacrol with LuxR and LasR receptors, providing valuable insights into the potential of these compounds as inhibitors of quorum sensing and antibiotic resistance.

The aim of the present research is to explore the potential of carvacrol and its structural analogs as novel therapeutic agents targeting the LuxR and LasR receptors in *Pseudomonas aeruginosa*. Using computational tools, this study seeks to predict the binding affinities, interaction modes, and stability of these compounds, offering a rational basis for the design of new drugs to combat antibiotic resistance and bacterial infections. The objectives include the identification of promising carvacrol analogs, the analysis of their binding interactions with LuxR and LasR receptors, and the evaluation of their potential as inhibitors of quorum sensing and antibiotic resistance mechanisms.

MATERIALS AND METHODS

Protein Retrieval

The Protein Data Bank (PDB) is an important tool that gives scientists access to 3D structures of biological molecules, helping them understand how proteins work and interact. In this research, we focused on two protein-ligand complexes: the LUX R family protein –3IX3 and the LAS R family protein –6MVM. The 3IX3 structure, resolved by X-ray diffraction at a resolution of 1.40 Å, represents a transcription factor from *Pseudomonas aeruginosa*, which is essential for quorum sensing. The 6MVM structure, also determined by X-ray diffraction at a resolution of 1.90 Å, features a mutant form of LasR (L130F) complexed with 3OC14HSL. This methodology involved retrieving the structural data for both complexes from the PDB.

Ligand Retrieval

Carvacrol is a natural compound with anti-inflammatory, antioxidant, and immune-boosting effects, making it a great choice for virtual screening to find similar substances. The following steps were employed for the selection of carvacrol and its structural analogs.

Ligand-Based Virtual Screening with SwissSimilarity

SwissSimilarity (www.swisssimilarity.ch) is a web-based tool used for ligand-based virtual screening to identify compounds structurally like a given reference molecule. The tool compares molecular fingerprints and 3D structures using similarity metrics. The canonical SMILES of carvacrol was retrieved from PubChem, a chemical database (www.pubchem.ncbi.nlm.nih.gov), and input into SwissSimilarity for screening.

Compound Retrieval from ChEBI

After identifying potential candidates using SwissSimilarity, the results were cross-referenced with the ChEBI database (www.ebi.ac.uk), which provides detailed information on biologically relevant small molecules. 411 canonical SMILES of compounds like carvacrol were retrieved, and duplicates were removed, yielding 396 unique compounds for further analysis.

Data Quality Control

Duplicate entries were excluded to ensure the accuracy of the dataset, and compounds with low similarity to carvacrol were filtered out. Through SwissSimilarity and ChEBI, 396 compounds structurally like carvacrol were identified for further computational and experimental evaluation.

Pharmacology Studies

ADME stands for Absorption, Distribution, Metabolism, and Excretion. These are important factors that explain how a drug enters the body, spreads, breaks down, and gets removed (www.swissadme.ch). ADME studies are essential for understanding the behaviour of drug compounds in living organisms, providing insights into their effectiveness, safety, and bioavailability. In this study, the 396 canonical SMILES of carvacrol were retrieved from the CHEBI database. The SwissADME tool was used to analyze different properties, including chemical traits, how the drug moves through the body, its suitability as a medicine, and its overall effectiveness in drug development. The compound screening was based on established criteria, including Lipinski's rule of five, gastrointestinal (GI) absorption, bioavailability, PAINS (Pan Assay Interference Compounds), Brenk's rules, Silicos IT, Ghose, Veber, and P-glycoprotein (Pgp) substrate considerations. After filtering based on these criteria, 63 potential ligands were selected for further analysis.

Protein Purification and Validation

Protein purification is a crucial step in ensuring the stability, solubility, and functional activity of proteins for drug discovery. The BIOVIA Discovery Studio is employed to refine the protein structure by removing non-structural water molecules and unwanted heteroatoms, which may interfere with subsequent analysis. Additionally, polar groups are added to the protein structure to enhance solubility, stability, and interaction properties. These modifications optimize the protein's ability to bind with potential ligands and facilitate its purification, making it suitable for use in drug development processes.

Protein Validation Using SAVES Tool

Following protein purification, the structure is validated using the SAVES Tool (saves.mbi.ucla.edu), a comprehensive web-based platform that integrates five distinct programs for protein quality assessment. One of the key validation methods is ERRAT, which evaluates non-bonded interactions between atom types in the protein. ERRAT generates a quality factor by comparing the protein's structural data to those of highly refined experimental structures. This helps identify any discrepancies or structural errors that could affect the protein's functional integrity.

Another important validation method is PROCHECK, which checks the structural quality of the protein. PROCHECK utilizes the Ramachandran plot to evaluate the distribution of the protein's amino acid residues based on their phi (ϕ) and psi (ψ) dihedral angles. The plot categorizes residues into favourable, allowed, or unfavourable regions. A high percentage of residues in the favourable region indicates a structurally sound protein, while those in the unfavourable region may suggest errors in protein folding. This assessment aids in confirming the accuracy and reliability of the protein structure before further analysis.

Molecular Docking

Molecular docking is a computer-based method that helps predict how ligands interact with target proteins, playing a key role in drug discovery. In this study, molecular docking was performed using PyRx, a virtual screening tool designed for computational drug discovery. The aim was to predict the binding modes and affinities of ligands to the receptor proteins 3IX3 (LuxR family protein) and 6MVM (LasR family protein).

Protein and Ligand Preparation

To begin, both ligand and receptor structures were first energy-minimized to optimize their geometries and ensure stable configurations. The energy minimization process involves adjusting the structure to minimize steric clashes and unfavourable interactions. Afterward, both the ligands and receptors were converted into the PDBQT format, which includes necessary information, such as atomic charges and torsion angles. This format is essential for docking simulations, as it enables proper representation of ligand flexibility and interaction properties.

Docking Setup

A grid was generated around the active sites of the receptor proteins, which ensures that the docking calculations focus on the relevant regions of the receptor where ligand binding is most likely to occur. The grid centers and dimensions were carefully defined for each protein: for 3IX3, the grid center was positioned at coordinates (X: 12.0874, Y: 1.2318, Z: 16.5948), and for 6MVM, the coordinates were (X: 6.3776, Y: 5.2406, Z: 65.8354). These grid parameters are critical for accurately simulating the ligand-receptor interactions.

Docking Simulation

AutoDock Vina, a popular and fast molecular docking software, was used for docking due to its high accuracy. AutoDock Vina creates different shapes of the ligand inside the receptor's active site, mimicking possible ways they can bind together. For each ligand, nine conformations were generated, offering various binding poses to explore.

Analysis of Docking Results

The docking results were analysed based on Root Mean Square Deviation (RMSD) and binding affinity. RMSD is a measure of the deviation between the predicted binding poses and the reference structure, with lower values indicating a better alignment of the ligand within the receptor's binding site. Binding affinity, measured in kcal/mol, shows how strongly the ligand attaches to the receptor. More negative binding affinity values correlate with stronger binding interactions.

Post Molecular Docking

After docking, BIOVIA Discovery Studio was used to study how ligands interact with their target proteins. Ligands with the best binding affinities from the docking studies were subjected to further molecular interaction analysis within the protein's binding pocket. This process involved identifying the specific amino acid residues involved in ligand binding and examining the nature, type, and length of these interactions. By visualizing the binding complex in both 2D and 3D, key interactions, such as hydrogen bonds, hydrophobic contacts, and electrostatic forces were assessed. The detailed analysis helped in understanding the stability of the ligand-protein complex, providing critical insights into the mechanisms of binding, and contributing to the selection of promising drug candidates for further development.

RESULTS

Protein Retrieval

Proteins are sourced from the RCSB Protein Data Bank (PDB) in PDB format, which provides access to three-dimensional structures of biological macromolecules. These structures are determined through various experimental techniques, such as X-ray crystallography, NMR spectroscopy, X-ray diffraction, and cryo-electron microscopy. The PDB serves as a comprehensive repository, where researchers worldwide deposit their structural data along with relevant metadata, making this valuable information publicly accessible to the global scientific community. The structures of the LuxR family protein (3IX3) and the LasR family protein (6MVM) are specifically derived from X-ray diffraction data.

Protein Purification and Validation

Protein purification is an essential process in molecular docking studies to ensure the protein is both pure and functionally active. This process involves several critical steps to prepare the protein for precise docking simulations (Figure 1).

Elimination of Non-Structural Components

a. *Water Molecules:* Unnecessary water molecules that do not contribute to the protein's active site are removed.
b. *Heteroatoms and Ligands:* Non-protein elements, such as metal ions and bound ligands, which are not part of the protein's natural conformation, are excluded. These components may be treated separately in docking simulations if needed.

Retention of the Biological Chain

a. *Removal of Extra Chains:* Proteins may contain additional chains due to crystallization artifacts, which are discarded. Only the biologically relevant A chain is retained for the docking process.

Addition of Polar Hydrogen Atoms

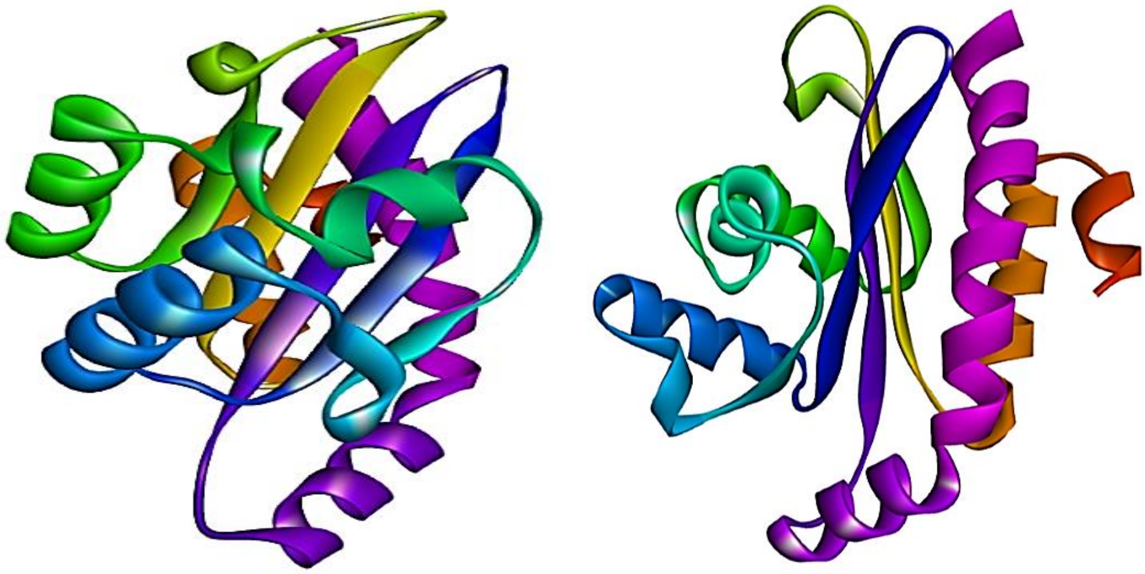
a. *Hydrogen Addition:* Polar hydrogen atoms are computationally added to fill in missing hydrogens in polar groups (e.g., hydroxyl, amine, and carbonyl groups). This step is crucial to ensure accurate hydrogen bonding during docking, as hydrogen atoms are integral to ligand binding and protein stability.

The Ramachandran Plot Analysis

The Ramachandran plot is a graphical tool used to assess the torsion angles (φ and ψ) of amino acid residues in a protein structure, providing insights into the stability and quality of the protein. It illustrates which conformations are sterically favourable or unfavourable based on the energetically favourable combinations of these angles required for proper protein folding (Table 1; Figure 2).

- *Most Favoured Regions [A, B, L]:* These areas represent the most observed and energetically stable torsion angle combinations. A high percentage of residues in these regions suggests a more reliable and stable protein structure.
- *Additional Allowed Regions [a, b, l, p]:* These conformations are less common but still energetically permissible. While not as ideal as the most favoured regions, they still contribute to a stable structure.

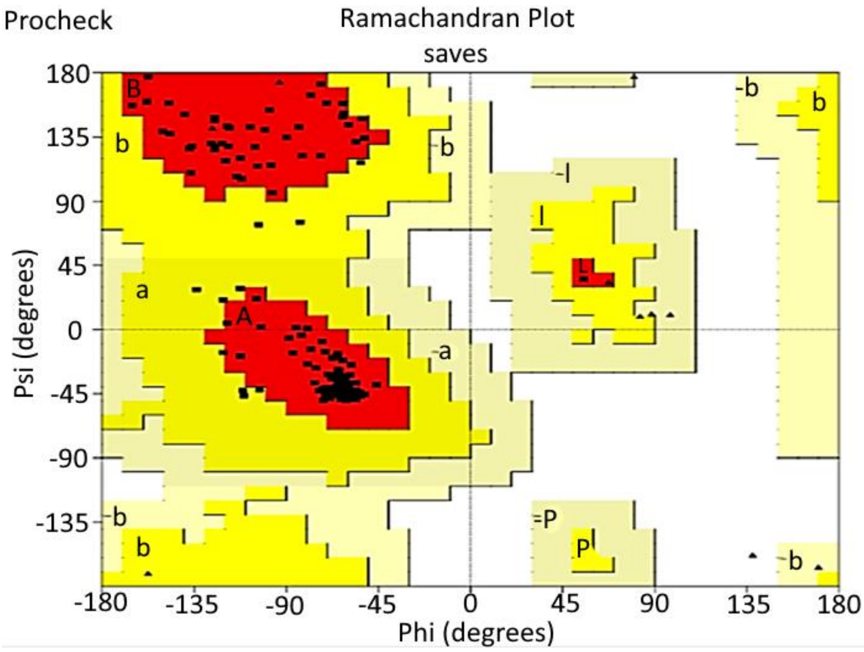
- *Generously Allowed Regions [~a, ~b, ~l, ~p]:* These regions indicate slightly less favourable conformations, which are still energetically feasible but may point to areas of potential strain or less common folding patterns.
- *Disallowed Regions:* These areas correspond to energetically unfavourable conformations, typically signalling issues with protein folding.



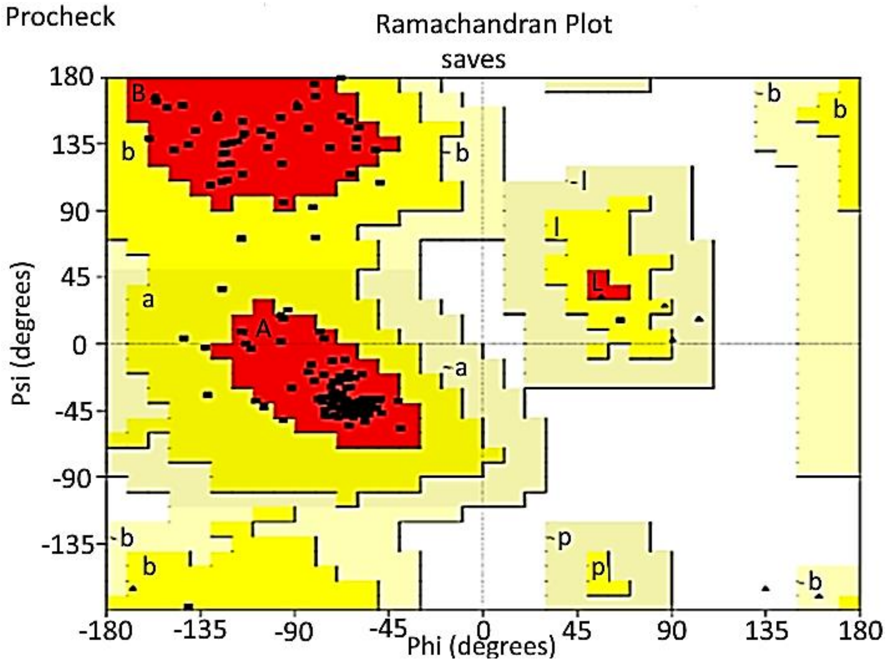
(a) LUX R PROTEIN-(3IX3) (b) LAS R PROTIEN-(6MVM)

Figure 1. Purified structures of the target proteins (a) LuxR receptor and (b) LasR receptor.

For the LuxR family protein (3IX3) (Figure 2(a)), 93.0% of its residues fall within the most favoured regions, suggesting a highly stable structure. The complete absence of residues in disallowed regions further confirms the high quality of the model. Similarly, the LasR family protein (6 MVM) shows 90.8% of its residues in the most favoured regions, with no residues in disallowed regions, indicating a reliable and well-folded structure (Figure 2(b)).



(a) Lux R Protein-(3IX3).



(b) Las R Protien-(6MVM).

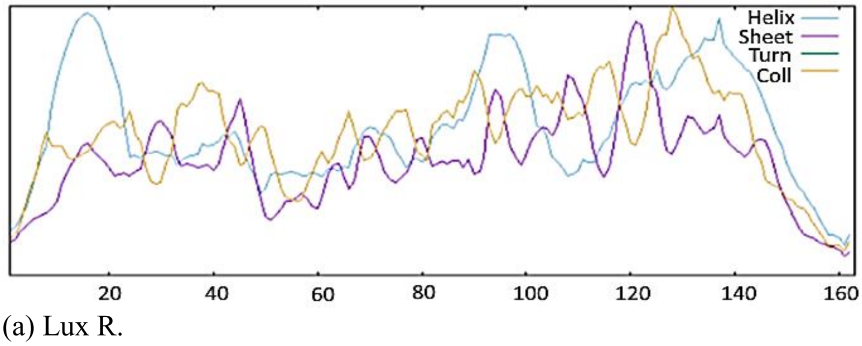
Figure 2. Ramachandran plots for (a) LuxR and, (b) LasR receptors.

SECONDARY STRUCTURE PREDICTION

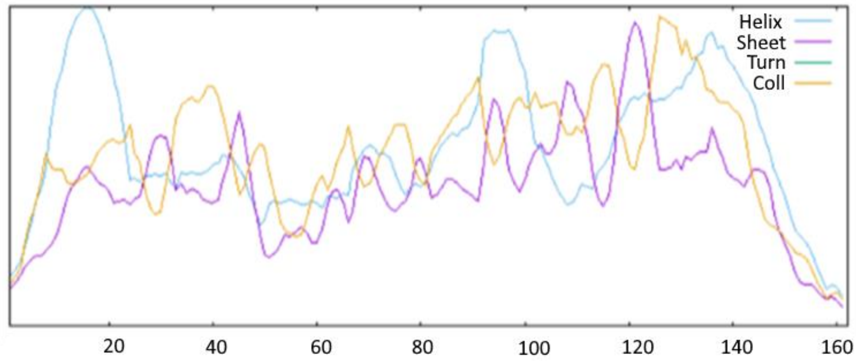
Secondary structure prediction focuses on identifying the local folding patterns of a protein, such as alpha-helices, beta-strands, and random coils. These structural elements are crucial for understanding the protein's function and its potential interactions with ligands. The graphical representations of carbonic anhydrase and pendrin are shown in Table 1 and Figure 3.

- *Alpha Helix (Hh):* A right-handed spiral shape held together by hydrogen bonds linking every fourth amino acid. Alpha helices play essential roles in protein stability and function.
- *Extended Strand (Ee):* Beta-strands that form part of beta-sheets, contributing to the structural stability and often participating in protein-protein interactions.
- *Random Coil (Cc):* Flexible regions without a defined secondary structure, typically involved in protein flexibility or functional interactions.

For the LuxR family protein (3IX3), the secondary structure is characterized by 41.10% random coils, suggesting regions of flexibility and potential functional interactions. It also contains 44.17% alpha helices and 14.72% extended strands, contributing to the overall structural integrity of the protein (Figure 3(a)). In contrast, the LasR family protein (6MVM) is predominantly composed of 43.21% alpha helices, indicating a more stable and structured protein. The secondary structure also includes 12.96% extended strands and 43.83% random coils, reflecting a balance of stability and flexibility within the protein (Figure 3(b)).



(a) Lux R.



(b) Las R.

Figure 3. Secondary structures for (a) LuxR and, (b) LasR receptors.

ERRAT Structure Validation

ERRAT is a software tool used to assess the quality of protein models by analysing the high-resolution environment of each amino acid within the structure. The ERRAT score helps evaluate the realism and accuracy of a predicted structure by comparing it to known high-resolution experimental data [1]. The ERRAT score reflects the overall quality of the model, with higher scores indicating fewer structural flaws and greater reliability. Generally, a score above 80 is considered acceptable for a high-quality protein model (Table 1).

- The LuxR family protein (3IX3) received a perfect ERRAT score of 100, indicating an excellent model with well-placed residues and minimal structural discrepancies.
- Similarly, the LasR family protein (6MVM) also achieved a score of 100, signifying a high-quality model with correctly positioned residues and negligible structural issues.

Table 1. Statistical validation analysis of structural protein.

Protein	RC Plot	Errat Score	2d Structure
3IX3	Residues in most favoured regions [A, B, L] 132 93.0% Residues in additional allowed regions [a,b,l,p] 10 7.0% Residues in generously allowed regions [~a,~b,~l,~p] 0 0.0% Residues in disallowed regions 0 0.0%	100	Alpha helix (Hh): 72 is 44.17%. Extended strand (Ee): 24 is 14.72%. Random coil (Cc): 67 is 41.10%.
6MVM	Residues in most favoured regions [A, B, L] 128 90.8% Residues in additional allowed regions [a,b,l,p] 13 9.2% Residues in generously allowed regions [~a,~b,~l,~p] 0 0.0% Residues in disallowed regions 0 0.0%	100	Alpha helix (Hh): 70 is 43.21%. Extended strand (Ee): 21 is 12.96%. Random coil (Cc): 71 is 43.83%.

Pharmacological Studies

Macromolecules, such as proteins, to identify promising drug candidates. An ADME (Absorption, Distribution, Metabolism, and Excretion) analysis is performed using SwissADME to assess the pharmacokinetic properties of the ligands. Key criteria for selecting viable drug candidates include gastrointestinal absorption, adherence to the Lipinski Rule of Five, the presence of PAINS (Pan Assay Interference Compounds), Brenk rules, Silicos IT, Ghose, Veber, P-glycoprotein (pgp) substrate interactions, and overall bioavailability. After this screening, 63 compounds were shortlisted for further investigation.

Physicochemical Properties

The physicochemical properties of the compounds play a pivotal role in determining their potential as drug candidates. These properties influence the ADME processes and are critical for evaluating a compound's suitability as a therapeutic agent. Key factors include molecular weight (M.W.), the fraction of sp3 hybridized carbons (Csp3), topological polar surface area (TPSA), solubility, lipophilicity (log P), and hydrogen bond donor/acceptor counts.

Molecular weight influences both absorption and distribution in the body, while the fraction of sp3 carbons reflects a compound's flexibility, which can impact its interaction with biological targets. TPSA measures how well a molecule can pass through cell membranes and bind to receptors. Solubility affects absorption, whereas lipophilicity (log P) determines the ease with which a compound crosses lipid membrane. The number of hydrogen bond donors and acceptors is vital for interactions with proteins and other macromolecules in the body.

Table 2 presents the physicochemical properties of five best ligands for LUXR, which are critical for evaluating their drug-like characteristics. The molecular weight values range from 174.18 to 262.34, indicating the size of the molecules. The fraction of sp3 hybridized carbons (Csp3) varies between 0 and 0.56, reflecting the flexibility and structural complexity of the compounds. The topological polar surface area (TPSA) values range from 40.46 to 55.92, influencing the compounds' ability to cross biological membranes. Lipophilicity (Mlog P) values range from 0.53 to 3.16, suggesting the compounds' varying affinity for lipid and aqueous environments. All ligands are soluble, indicating favorable characteristics for absorption and bioavailability. These properties collectively guide the potential of these ligands as viable drug candidates.

Table 3 outlines the physicochemical properties of five ligands with respect to the 6 MVM (LASR) protein. The molecular weights range from 174.18 to 262.34, indicating variability in size. The fraction of sp3 hybridized carbons (Csp3) varies from 0 to 0.56, influencing the flexibility of the compounds. TPSA values range from 40.46 to 55.92, which are important for predicting the compounds' permeability across biological membranes. The lipophilicity (Mlog P) values, spanning from 0.53 to 3.16, suggest how well these compounds can interact with lipid environments. All the ligands are soluble, indicating favorable absorption properties [2, 3].

Table 2. Physiochemical properties of the LUXR compound.

Ligand Name	**Molecular Weight**	**Fractioncsp3**	**TPSA**	**Lipophilicity (Mlog P)**	**Solubility (Silicos IT)**
2,2'-Biphenol	186.21	0	40.46	2.41	Soluble
Homosalate	262.34	0.56	46.53	3.16	Soluble
2-Methyl-4-oxo-1,4-dihydroquinolin-3-olate	174.18	0.1	55.92	0.53	Soluble
3-Methyl-quinolin-2,8-diol	175.18	0.1	53.09	1.34	Soluble
1,8-Dihydroxy-3-methylnaphthalene	174.2	0.09	40.46	2.17	Soluble

Table 3. Physiochemical properties of the 6 mvm compound.

Ligand Name	**Molecular Weight**	**Fraction csp3**	**TPSA**	**Lipophilicity (Mlog P)**	**Solubility (Silicos IT)**
Homosalate	262.34	0.56	46.53	3.16	Soluble
3-Methyl-quinolin-2,8-diol	175.18	0.1	53.09	1.34	Soluble
9-(3-Methylbutanoyl)-8,10-Dehydrothymol	248.32	0.4	46.53	2.95	Soluble
2-Methyl-4-oxo-1,4-dihydroquinolin-3-olate	174.18	0.1	55.92	0.53	Soluble
3,5-Dihydroxybiphenyl	186.21	0	40.46	2.41	Soluble

The Lipinski Rule

Lipinski's Rule of Five is a guideline used to determine whether a compound has the right properties to be a potential drug. The rule states that for a compound to be considered a viable oral drug, it should adhere to the following criteria:

1. Molecular weight ≤500 Da.
2. No more than 5 hydrogen bond donors.
3. No more than 10 hydrogen bond acceptors.
4. LogP (lipophilicity) ≤ 5.

These guidelines help assess the compound's absorption, distribution, and bioavailability. If a compound meets most or all these parameters, it is likely to be orally active. SwissADME follows this rule as part of its ADME/Tox screening, providing insights into the drug-likeness and pharmacokinetic properties of compounds, thus aiding in the identification of promising drug candidates.

Table 4 provides the physicochemical properties of five ligands in relation to the 3IX3 protein. The molecular weight ranges from 174.18 to 262.34, with most compounds falling under the commonly accepted threshold of 500 Da for drug-likeness [4, 5]. Hydrogen bond acceptors range from 2 to 3, and hydrogen bond donors vary between 1 and 2, which is consistent with Lipinski's Rule of Five, as it recommends fewer than 5 hydrogen bond donors and fewer than 10 acceptors for good oral bioavailability. The lipophilicity values (Mlog P) range from 0.53 to 3.16, with values between 1 and 5 being ideal for oral bioavailability. These compounds largely meet Lipinski's criteria, suggesting their potential as viable drug candidates.

Table 5 outlines the physicochemical properties of five ligands for the 6MVM protein. Molecular weights range from 174.18 to 262.34, all falling below the 500 Da threshold for ideal drug candidates. The hydrogen bond acceptor count is 2–3, and the donor count ranges from 1 to 3, aligning with Lipinski's recommendations. Lipophilicity values (Mlog P) vary from 0.53 to 3.16, all within the acceptable range for drug absorption and bioavailability. These properties indicate that these compounds adhere to the Lipinski Rule of Five.

Table 4. The Lipinski screening of the top phytocompounds for 3XI3.

Ligand Name	Molecular Weight	H-Bond Acceptor	H-Bond Donor	Lipophilicity (mlog P)
2,2'-Biphenol	186.21	2	2	2.41
Homosalate	262.34	3	1	3.16
2-Methyl-4-oxo-1,4-dihydroquinolin-3-olate	174.18	2	1	0.53
3-Methyl-quinolin-2,8-diol	175.18	2	2	1.34
1,8-Dihydroxy-3-methylnaphthalene	174.2	2	2	2.17

Table 5. The Lipinski screening of the top phytocompounds for 6 MVM.

Ligand Name	Molecular Weight	H-Bond Acceptor	H-Bond Donor	Lipophilicity (mlog P)
Homosalate	262.34	3	1	3.16
3-Methyl-quinolin-2,8-diol	175.18	2	2	1.34
9-(3-Methylbutanoyl)-8,10-Dehydrothymol	248.32	3	1	2.95
2-Methyl-4-oxo-1,4-dihydroquinolin-3-olate	174.18	2	1	0.53
3,5-Dihydroxybiphenyl	186.21	2	2	2.41

Pharmacokinetic Properties

Pharmacokinetic properties describe the behaviour of a drug within the body, encompassing its absorption, distribution, metabolism, and excretion (ADME). Understanding these properties is essential for evaluating a compound's effectiveness, safety, and bioavailability. Key parameters critical to assessing the pharmacokinetic profile of a compound include:

- *Gastrointestinal (GI) Absorption:* This describes how well a compound is absorbed into the bloodstream after being taken by mouth. High GI absorption is crucial for oral drugs, as it suggests the compound will be efficiently absorbed from the digestive tract into systemic circulation.
- *Blood-Brain Barrier (BBB) Penetration:* This factor determines if a compound can pass through the blood-brain barrier and reach the central nervous system (CNS). BBB penetration is important for drugs targeting neurological conditions, but compounds capable of crossing the BBB may also cause unintended neurological side effects [6, 7].
- *Bioavailability:* Bioavailability measures how much of an orally taken drug enters the bloodstream in its active form. A higher bioavailability generally correlates with a more effective drug, as it suggests better absorption and therapeutic potential.
- *PAINS (Pan Assay Interference Compounds):* PAINS are compounds that often yield false positives in bioassays due to their tendency to interfere with assay systems. A PAINS score of 0 indicates that a compound is less likely to interfere with assay results, making it a more reliable candidate for further study.
- *Brenk's Rules:* This parameter evaluates whether a compound violates known chemical principles regarding toxicity or problematic structural features. A score of 0 signifies that the compound adheres to these rules, suggesting a safer chemical profile and fewer potential safety concerns.

Table 6 presents pharmacokinetic properties of five ligands for the 3IX3 protein. All ligands exhibit high gastrointestinal (GI) absorption, suggesting that they are effectively absorbed when administered orally [8, 9]. All compounds can pass through the blood-brain barrier (BBB), suggesting they may affect the central nervous system (CNS). Bioavailability values range from 0.55 to 0.85, implying that these ligands are moderately bioavailable, with 2-Methyl-4-oxo-1,4-dihydroquinolin-3-olate exhibiting the highest bioavailability (0.85). None of the compounds display PAINS (Pan Assay Interference Compounds) or violate Brenk's rules, suggesting that they are free from common assay interferences and structural issues that could hinder drug development.

Table 6. Pharmacokinetic properties of the top phytocompounds for 3XI3.

Ligand Name	GI Absorption	BBB	Bioavailability	PAINS	Brenk
2,2'-Biphenol	High	Yes	0.55	0	0
Homosalate	High	Yes	0.55	0	0
2-Methyl-4-oxo-1,4-dihydroquinolin-3-olate	High	Yes	0.85	0	0
3-Methyl-quinolin-2,8-diol	High	Yes	0.55	0	0
1,8-Dihydroxy-3-methylnaphthalene	High	Yes	0.55	0	0

Table 7 provides pharmacokinetic data for five ligands for the 6MVM protein. Like Table 6, all ligands show high gastrointestinal (GI) absorption and the ability to cross the blood-brain barrier (BBB), suggesting that they have good potential for oral administration and CNS activity. Bioavailability values are consistent across most ligands (0.55), except for 2-Methyl-4-oxo-1,4-dihydroquinolin-3-olate, which has a higher bioavailability of 0.85 [10, 11]. This indicates that the latter compound may be more efficiently absorbed and remain active in the system for a longer duration. None of the compounds exhibit PAINS or violate Brenk's rules, indicating that they are likely to have reliable assay outcomes and structurally safe profiles.

Table 7. Pharmacokinetic properties of the top phytocompounds for 6MVM.

Ligand Name	GI Absorption	BBB	Bioavailability	PAINS	Brenk
Homosalate	High	Yes	0.55	0	0
3-Methyl-quinolin-2,8-diol	High	Yes	0.55	0	0
9-(3-Methylbutanoyl)-8,10-Dehydrothymol	High	Yes	0.55	0	0
2-Methyl-4-oxo-1,4-dihydroquinolin-3-olate	High	Yes	0.85	0	0
3,5-Dihydroxybiphenyl	High	Yes	0.55	0	0

Molecular Docking

Molecular docking was performed using PyRx, followed by energy minimization to ensure stable interactions between the ligands and the target proteins. The ligands were then converted into PDBQT format, incorporating Kollman charges and torsion angles. This step helps neutralize the system and allows for ligand flexibility during subsequent docking analyses. A docking grid was generated to ensure correct atom positioning (Table 8) [12, 13].

Table 8. Grid Dimensions in Armstrong for Docking (3IX3) and (6 mvm).

Proteins	Grid Dimensions In (Å)		
	X-Axis	*Y-Axis*	*Z-Axis*
3IX3	33.2199	47.2029	54.7226
6MVM	54.3188	46.7998	33.0039

The docking process produced nine distinct conformations for each ligand, which were screened and filtered based on a Root Mean Square Deviation (RMSD) value of 0. RMSD values represent the degree of deviation in the protein's backbone dihedral angles (phi and psi) from their ideal conformations, with lower values indicating better structural alignment and stability. The conformations with the highest binding affinity are presented in Table 9 [14, 15].

Binding affinity measures how strongly a ligand attaches to its target protein and is usually shown as a negative value (in kcal/mol). A more negative binding affinity indicates stronger binding. For instance, a ligand with a binding affinity of –9 kcal/mol will bind more tightly to the protein than one with a binding affinity of –6 kcal/mol. The negative value reflects that the binding energy of the ligand-protein complex is lower than the combined energies of the separate components. A more negative binding affinity suggests a more stable interaction, making it less likely for the ligand to dissociate. In drug discovery, a high binding affinity is often associated with the potential effectiveness of the ligand, influencing its therapeutic potential. Ligands with lower (more negative) binding affinities are typically prioritized as promising drug candidates [16, 17].

- 2,2'-Biphenol and Homosalate exhibited a binding affinity of –9.4 kcal/mol with the Lux R protein (3IX3), indicating a strong binding interaction.
- Homosalate demonstrated a binding affinity of –9.6 kcal/mol with the Las R protein (6MVM), also indicating a robust binding interaction.

Table 9. Binding affinity of the protein retrieved from Py Rx.

Ligand Name	PubChem Id	Binding Affinity
Protein	*Lux R Protein (3ix3)*	
2,2'-Biphenol	5731	–9.4
Homosalate	8362	–9.4
2-Methyl-4-oxo-1,4-dihydroquinolin-3-olate	25202294	–8.8
Protein	*Las R Protein (6 mvm)*	
Homosalate	8362	–9.6
3-Methyl-quinolin-2,8-diol	440986	–8.8
9-(3-Methylbutanoyl)-8,10-Dehydrothymol	53262899	–8.8

Post Docking Analysis

Molecular visualization is essential for understanding the nature and strength of interactions between ligands and their target proteins (Figures 4 and 5). After conducting molecular docking, visualization tools, such as BIOVIA Discovery Studio are employed to explore the specific binding modes and atomic-level interactions between the ligand and the protein (Tables 10 and 11) and (Figures 6 and 7).

Types of Interactions Observed

- *Electrostatic Interactions:* These occur between oppositely charged atoms, such as the interaction between Homosalate (ASP73:OD2) and the ligand (UNK1). Electrostatic interactions are long-range forces that help guide the ligand into the correct orientation within the active site, facilitating its initial binding to the protein. These interactions contribute to the specificity and stability of the ligand-protein complex.
- *Hydrogen Bonds:* Hydrogen bonding happens when a hydrogen atom, attached to a highly electronegative atom, like oxygen or nitrogen, is drawn to another electronegative atom. For example, the hydrogen bond between ARG61: HE and UNK1:O plays a critical role in stabilizing the ligand within the protein's binding pocket. These bonds are key to maintaining the ligand's proper orientation and ensuring the specificity of its interaction with the protein [18, 19].
- *Hydrophobic Interactions:* These interactions occur between nonpolar residues of the ligand and the protein, such as between TYR56 or LEU36. Hydrophobic regions tend to interact with each other rather than with surrounding water molecules, helping to stabilize the ligand-protein complex. By minimizing the exposure of nonpolar surfaces to the aqueous environment, these interactions reinforce the overall binding, contributing to the stability of the complex [20].

DISCUSSION

The rise of drug-resistant bacteria, especially MDR and XDR strains, is a major public health challenge today. The widespread overuse and misuse of antibiotics have accelerated this problem, leading to a situation where conventional antimicrobial treatments are no longer effective against many infections. Drug resistance is a phenomenon that occurs when microorganisms evolve mechanisms to evade the effects of drugs designed to kill or inhibit them. These mechanisms can include mutation of target sites, modification of metabolic pathways, increased drug efflux, and the formation of biofilms, which serve as protective environments for bacterial colonies, making them resistant to both immune defense and drug therapy. The rise of antibiotic-resistant infections has been further compounded by the ability of bacteria to transfer resistance genes through horizontal gene transfer, which can occur across different species and strains [21].

Table 10. Non-bond interactions for the target protein and top ligands (3IX3).

Ligand	Interaction (Amino Acid – Atom)	Distance (Å)	Category
Homosalate (3IX3 – 8362)	A:TYR64 – N:UNK1	4.8	Hydrophobic
	A:ALA50 – N:UNK1	5.25	Hydrophobic
	A:VAL76 – N:UNK1	4.73	Hydrophobic
	A:ALA127 – N:UNK1	4.78	Hydrophobic
	A:ASP73:OD2 – N:UNK1	3.63	Electrostatic
	N:UNK1 – A:LEU36	4.68	Hydrophobic
2,2'-Biphenol (3IX3 – 15731)	A:ARG61:HH21 – N:UNK1:O	2.13	Hydrogen Bond
	A:ARG61:HE – N:UNK1:O	2.28	Hydrogen Bond
	N:UNK1:H – A:ARG61:OG1	2.31	Hydrogen Bond
	A:LEU36:CD1 – N:UNK1	3.97	Hydrophobic
	A:LEU36:CD2 – N:UNK1	3.91	Hydrophobic

	A:TYR64 – N:UNK1	4.37	Hydrophobic
	N:UNK1 – A:ALA50	5.13	Hydrophobic
	N:UNK1 – A:ALA70	5.07	Hydrophobic
	N:UNK1 – A:VAL76	4.82	Hydrophobic
	N:UNK1 – A:ALA127	5.36	Hydrophobic
2-Methyl-4-oxo-1,4-dihydroquinolin-3-olate (3IX3 – 25202294)	A:SER129:HG – N:UNK1:O	2.85	Hydrogen Bond
	N:UNK1:O – A:SER129:OG	2.8	Hydrogen Bond
	A:TRP88:HE1 – N:UNK1:O	2.94	Hydrogen Bond
	A:ASP73:OD2 – N:UNK1	3.28	Electrostatic
	A:ASP73:OD2 – N:UNK1	4.11	Electrostatic
	A:TYR56 – N:UNK1	5.19	Hydrophobic
	N:UNK1:C – A:LEU36	4.03	Hydrophobic
	A:TYR56 – N:UNK1:C	5.36	Hydrophobic
	A:TYR64 – N:UNK1:C	4.31	Hydrophobic
	N:UNK1 – A:ALA105	4.67	Hydrophobic
	N:UNK1 – A:LEU110	4.67	Hydrophobic

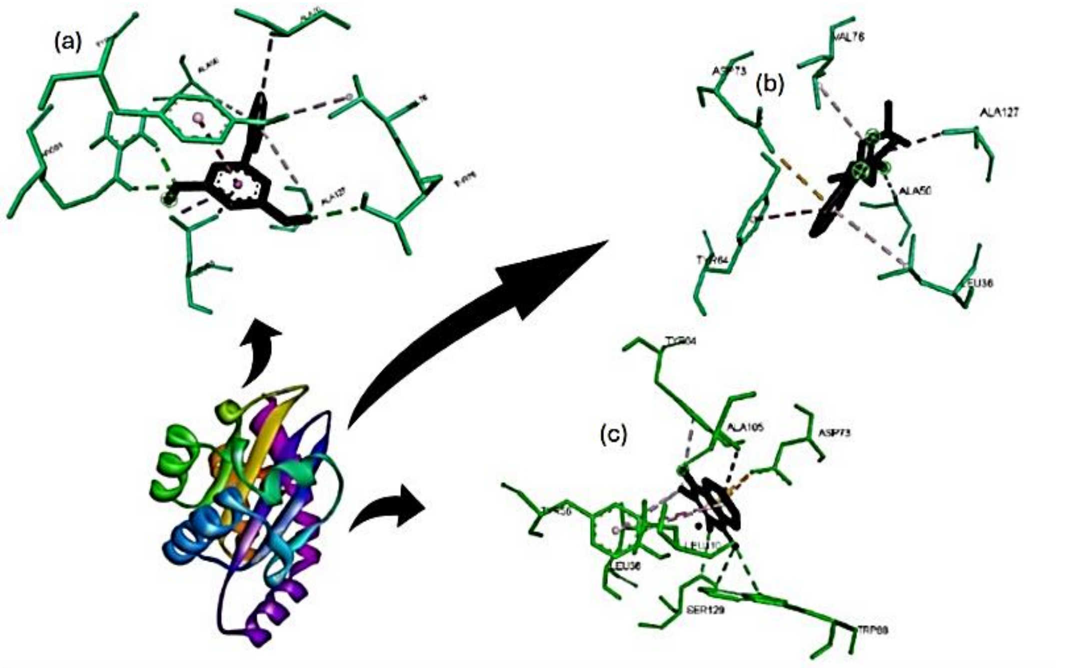


Figure 4. Top three ligands interaction with Lux R protein (3IX3) protein (3D Structure). (a) 15731 ligand (black) Interaction with protein (green), (b) 8362 ligand (black) interaction with protein (green), (c) 2520229 ligand (black) Interaction with protein (green).

One critical player in the development of drug resistance is bacterial quorum sensing (QS). QS is a complex way bacteria communicate with each other to sense and react to their population size. This system regulates the expression of genes involved in various bacterial behaviors, including biofilm formation, virulence factor production, and resistance mechanisms. Through QS, bacteria can coordinate their actions, ensuring that these behaviors are only expressed when beneficial to the bacterial community, such as in the case of biofilm formation, which provides physical protection against antibiotics. In both gram-positive and gram-negative bacteria, QS is a key determinant of pathogenicity and resistance to therapeutic interventions [22].

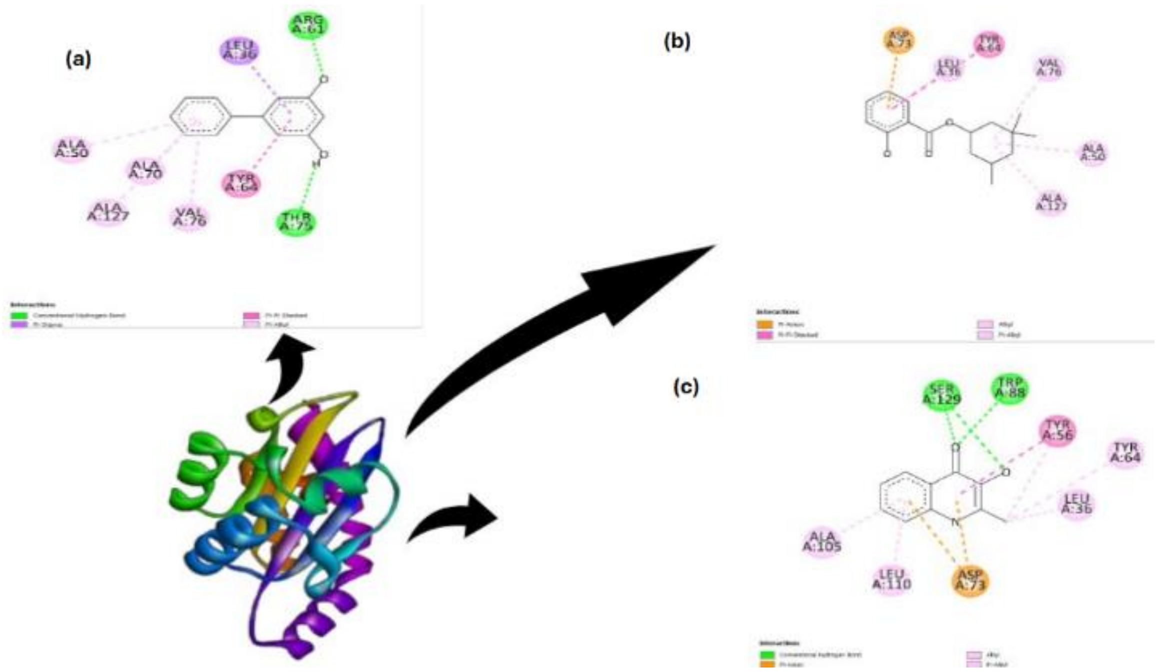


Figure 5. Top three ligands interaction with Lux R protein (3IX3) protein (a) 15731, (b) 8362, and (c) 2520229.

Table 11. Non-bond interactions for the target protein and top ligands (6 MVM).

Ligand	**Interaction (Amino Acid – Atom)**	**Distance (Å)**	**Category**
Homosalate (6MVM – 8362)	N:UNK1:H – A:THR75:OG1	2.49	Hydrogen Bond
	A:TYR64:HH – N:UNK1:O	2.65	Hydrogen Bond
	A:ASP73:OD2 – N:UNK1	3.36	Electrostatic
	A:TYR56 – N:UNK1	5.24	Hydrophobic
	A:LEU36 – N:UNK1	4.85	Hydrophobic
	A:VAL76 – N:UNK1	5.36	Hydrophobic
	A:ALA127 – N:UNK1	5.17	Hydrophobic
	N:UNK1:C – A:ILE52	4.68	Hydrophobic
	A:TYR64 – N:UNK1:C	5.25	Hydrophobic
3-Methyl-quinolin-2,8-diol (6MVM – 440986)	N:UNK1:H – A:TYR93:OH	2.37	Hydrogen Bond
	N:UNK1:H – A:THR75:OG1	2.28	Hydrogen Bond
	A:ASP73:OD1 – N:UNK1	4.53	Electrostatic
	A:ASP73:OD2 – N:UNK1	3.16	Electrostatic
	A:TYR64 – N:UNK1	5.56	Hydrophobic
	A:TRP88 – N:UNK1	5.77	Hydrophobic
	A:TRP88 – N:UNK1	5.3	Hydrophobic
	A:TRP88 – N:UNK1	5.23	Hydrophobic
	A:TRP88 – N:UNK1	4.54	Hydrophobic
	N:UNK1 – A:ALA105	4.57	Hydrophobic
	N:UNK1 – A:LEU110	4.97	Hydrophobic
	A:THR115:HG1 – N:UNK1:O	2.71	Hydrogen Bond
	N:UNK1:H – A:THR75:OG1	2.93	Hydrogen Bond
	N:UNK1:H – N:UNK1:O	2.87	Hydrogen Bond
	A:TYR64:HH – N:UNK1	2.98	Hydrogen Bond
	A:LEU36:CD2 – N:UNK1	3.98	Hydrophobic

	A:VAL76:CG2 – N:UNK1	3.91	Hydrophobic
	N:UNK1:C – A:TRP88	3.65	Hydrophobic
	A:TYR64 – N:UNK1	5.48	Hydrophobic
	A:ALA105 – N:UNK1:C	4.24	Hydrophobic
	N:UNK1:C – A:VAL76	4.47	Hydrophobic
	N:UNK1:C – A:LEU110	4.56	Hydrophobic
	A:TRP88 – N:UNK1:C	4.51	Hydrophobic
	N:UNK1 – A:ALA127	4.91	Hydrophobic

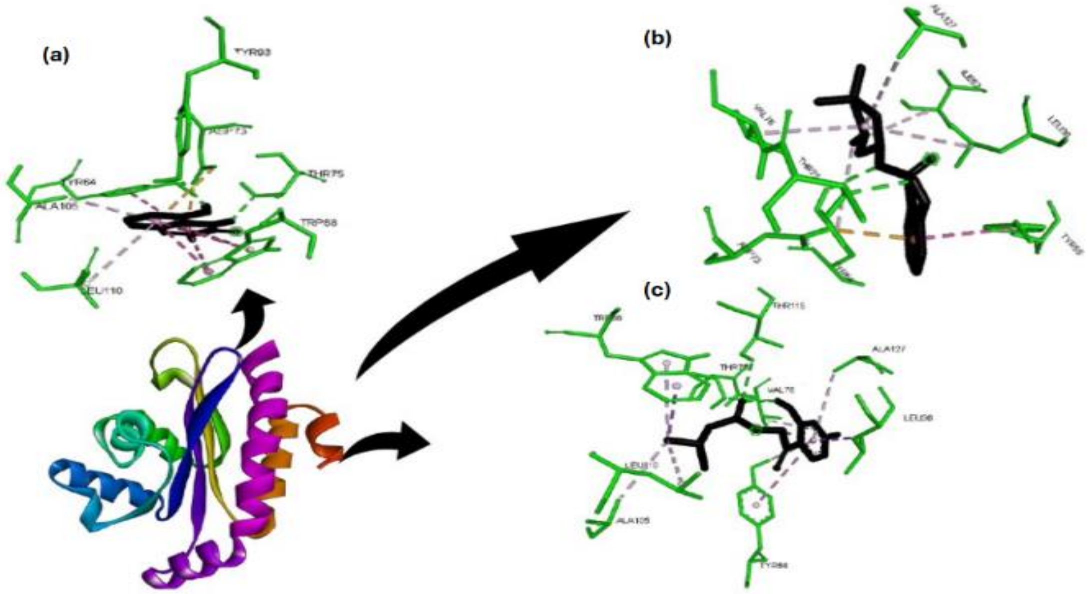


Figure 6. Top three ligands interaction with LAS R protein (6MVM) protein. (a) 440986 ligand (black) Interaction with protein (green), (b) 8362 ligand (black) interaction with protein (green), (c) 53262899 ligand (black) Interaction with protein (green).

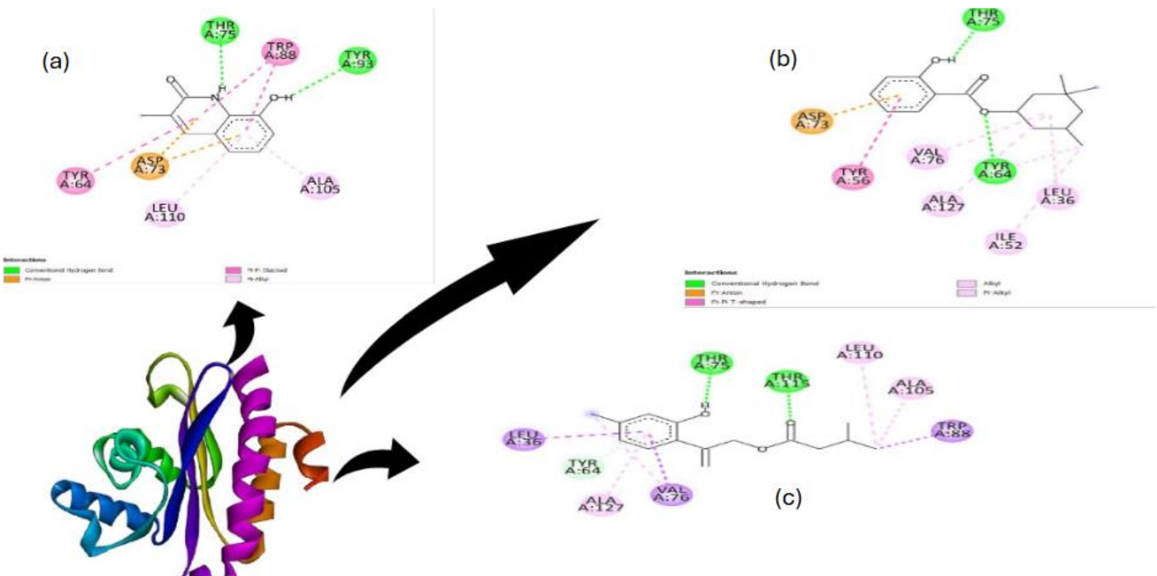


Figure 7. Top three ligands interaction with LAS R protein (6 MVM) protein (a) 440986, (b) 8362, (c) 53262899.

In gram-negative bacteria, such as *Pseudomonas aeruginosa*, QS is regulated by acyl-homoserine lactones (AHLs) that bind to specific transcription factors, such as LuxR and LasR receptors. These receptors help regulate genes that control antibiotic resistance and biofilm growth. The LasR receptor regulates the expression of genes responsible for producing exopolysaccharides and proteases that aid in biofilm formation and increase the bacteria's resistance to both host immune responses and antibiotics. Similarly, LuxR, another key receptor in *P. aeruginosa*, is responsible for regulating genes involved in virulence and the formation of biofilms. Inhibition of QS signaling, particularly through targeting the LuxR and LasR receptors, represents a promising strategy to combat the virulence and resistance mechanisms in *P. aeruginosa* and other bacterial pathogens.

In our study, we explored the potential of disrupting bacterial QS to mitigate drug resistance in *P. aeruginosa* by targeting the LuxR and LasR receptors. Through molecular docking simulations and pharmacological screening, we identified several natural and synthetic compounds with high binding affinities for these receptors, including homosalate, 2,2'-biphenol, and 2-methyl-4-oxo-1,4-dihydroquinolin-3-olate. The results of our docking studies revealed strong binding interactions between these compounds and the LuxR and LasR receptors, with binding affinities ranging from –8.8 to –9.6 kcal/mol, indicating their potential as QS inhibitors.

The high binding affinities of these compounds suggest that they could effectively bind to the active sites of the LuxR and LasR receptors, potentially blocking their ability to regulate QS-controlled genes involved in virulence and antibiotic resistance. This disruption of QS signaling could, in turn, reduce biofilm formation, enhance bacterial susceptibility to antibiotics, and decrease the production of virulence factors. The binding interactions between the compounds and the receptors were primarily mediated by hydrogen bonds, hydrophobic interactions, and electrostatic forces, which are known to stabilize ligand-receptor complexes and contribute to the specificity of these interactions. For instance, homosalate showed interactions with key residues of the LuxR receptor, such as hydrogen bonds with the receptor's hydrophilic residues, which likely contribute to its inhibitory effect. A closer examination of the chemical structures of the identified compounds provides further insights into their potential mechanisms of action. Homosalate, for example, contains a hydroxyl group, which may form hydrogen bonds with specific amino acid residues in the binding pocket of the LuxR and LasR receptors [23]. The presence of hydrophobic functional groups, such as the phenyl ring in 2,2'-biphenol, suggests that these compounds can interact with the hydrophobic regions of the receptors, contributing to the stabilization of the ligand-receptor complex. Similarly, 2-methyl-4-oxo-1,4-dihydroquinolin-3-olate possesses a quinoline ring structure, which is known to participate in aromatic stacking interactions with aromatic residues in the receptor. These structural features are significant in optimizing the binding affinity and specificity of these compounds for the LuxR and LasR receptors.

Our study also highlighted the importance of molecular docking simulations and pharmacological screening in identifying potential drug candidates. Computational techniques, such as these have become increasingly essential in modern drug discovery, allowing researchers to predict the binding affinity of compounds for target proteins and screen vast compound libraries for promising candidates. By simulating ligand-receptor interactions, molecular docking helps to identify the most likely binding modes of compounds, which can be further optimized for higher potency and selectivity. This method is a cheaper and faster alternative to traditional experiments, which usually require a lot of resources.

In our study, we applied molecular docking techniques to screen a range of natural and synthetic compounds, identifying several that exhibited strong binding to LuxR and LasR receptors. These compounds were tested to assess their potential as drugs.Using Lipinski's Rule of Five and other ADMET (Absorption, Distribution, Metabolism, Excretion, and Toxicity) predictions, we assessed the pharmacokinetic profiles of these compounds, ensuring that they met the necessary criteria for further development as potential therapeutic agents. These computational methods are indispensable in modern drug design, allowing researchers to efficiently identify lead compounds with desirable pharmacological properties [24].

The compounds identified in our study, including homosalate, 2,2'-biphenol, and 2-methyl-4-oxo-1,4-dihydroquinolin-3-olate, showed promising results in terms of their ability to bind to the LuxR and LasR receptors and disrupt QS signaling. However, there are several limitations to our study. First, while computational methods, such as molecular docking are powerful tools, they are based on predictions and assumptions that may not always align with real-world conditions. Experimental validation through *in vitro* and *in vivo* assays is necessary to confirm the efficacy of these compounds in disrupting QS signaling, preventing biofilm formation, and enhancing bacterial susceptibility to antibiotics. Before these compounds can be used in medicine, their possible side effects and toxicity must be carefully studied.

Despite these limitations, our study provides important insights into the potential of targeting QS receptors as a strategy to combat antibiotic resistance in *P. aeruginosa*. The identification of novel QS inhibitors opens new avenues for drug development, especially in the context of antibiotic-resistant infections. Future research should focus on experimental validation, including biofilm inhibition assays, bacterial growth inhibition studies, and toxicity assessments. In vivo studies using animal models will also be crucial to determine the therapeutic potential of these compounds in treating infections caused by resistant pathogens.

CONCLUSIONS

The present study underscores the growing importance of quorum sensing (QS) inhibition as a promising therapeutic strategy to combat antibiotic resistance in *Pseudomonas aeruginosa* and potentially other multidrug-resistant pathogens. By targeting the Lux R and Las R receptors, which regulate critical processes, such as biofilm formation, virulence factor production, and antibiotic resistance mechanisms, we have identified several compounds, including homosalate, 2,2'-biphenol, and 2-methyl-4-oxo-1,4-dihydroquinolin-3-olate, with promising binding affinities. These compounds identified through computational techniques, such as molecular docking and pharmacological screening, show the potential to disrupt QS signaling, reduce biofilm formation, and enhance bacterial susceptibility to antibiotics, offering an innovative approach to tackling the pressing issue of antimicrobial resistance. Although the computer results look promising, lab and animal studies are needed to confirm the compounds' effectiveness and safety. Future research should focus on optimizing these compounds for better pharmacokinetic properties, evaluating their in vivo performance, and testing their synergistic effects with existing antibiotics. The findings of this study lay the groundwork for the development of novel QS inhibitors and contribute to the advancement of strategies to mitigate the global challenge of antibiotic resistance.

List of Abbreviations

ADME	Absorption, Distribution, Metabolism, Excretion
AHL	Acyl-Homoserine Lactones
AMR	Antimicrobial Resistance
BioVIA	BIOVIA Discovery Studio
ChEBI	Chemical Entities of Biological Interest
ERRAT	Evaluation of Resection of Atom Types
GI	Gastrointestinal
MDR	Multidrug Resistance
PAINS	Pan Assay Interference Compounds
PDB	Protein Data Bank
Pgp	P-glycoprotein
QS	Quorum Sensing
RMSD	Root Mean Square Deviation
SAVES	Structure, Activity, Validation, and Evaluation Software
SMILES	Simplified Molecular Input Line Entry System
XDR	Extensively Drug-Resistant

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