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TITLE - Combating MDR P. aeruginosa through anti-virulence strategies: Targeting quorum sensing as an alternative to conventional antibiotics.

ABSTRACT

Treatment of MDR (Multidrug-Resistant) Pseudomonas aeruginosa infections has become clinically more challenging these days. This pathogen is listed on the World Health Organization's (WHO) ESKAPE group and critical priority list [1,2]. These bacteria use quorum sensing (QS), a density-dependent microbial communication system that form their biofilms, release virulence factors such as elastase and protease to evade host immune system [3]. The biggest clinical problem is that conventional antibiotics such as beta-lactams, carbapenems, and fluoroquinolones put lethal pressure on bacteria, forcing rapid mutation for survival that produces anti-microbial resistance (AMR) [4]. According to a 2019 report, antibiotic resistance was directly responsible for 1.27 million deaths globally [5].

This thesis explores a fundamentally different strategy that is anti-virulence therapy, in which the bacteria are not killed, but rather their survival is disrupted. We targeted the LasR-QslA complex (PDB ID: 4NG2), which is the master regulator of the las quorum sensing system of P. aeruginosa [6]. An in-silico virtual screening campaign was conducted in which 2,500 natural phytochemicals were screened using PyRx-integrated AutoDock Vina.and pharmacokinetic safety profiling was performed using the SwissADME web server, and binding interactions were analysed in the BIOVIA Discovery Studio Visualizer.

Screening results identified Glomeremophilane B (PubChem CID: 139589801) as the lead compound, with a binding affinity of −7.9 kcal/mol. It is 2.7 kcal/mol better than the native autoinducer 3-oxo-C12-HSL (−5.2 kcal/mol) and 2.4 kcal/mol superior to the validated reference beta-caryophyllene (−5.5 kcal/mol) [7]. Interaction analysis revealed that glomeremophilane B forms 6 hydrogen bonds with TRP75, TRP76, THR76, and GLN103 residues that is a dense polar engagement pattern that predicts high binding stability. SwissADME profiling confirmed: high GI absorption, zero CYP isoform inhibition, no BBB penetration, and full Lipinski's Rule of Five compliance. All these together establish Glomeremophilane B as a clinically relevant anti-virulence lead compound.

Keywords: Pseudomonas aeruginosa, Quorum Sensing Inhibition, LasR-QslA complex, Anti-virulence therapy, Glomeremophilane B, Molecular docking, ADME, Multidrug resistance, Natural compounds.

1. INTRODUCTION

1.1 Multidrug resistance in P. aeruginosa

Pseudomonas aeruginosa is a Gram-negative, aerobic, non-fermenting bacterium that is clinically important as a classic opportunistic pathogen, this means it infects people whose immune system is already compromised [1]. This Bacteria is identified as the major cause of ventilator-associated pneumonia (VAP), bloodstream infections, surgical site infections, and catheter-associated UTIs in the ICU (Intensive Care Units) of hospitals [2]. Globally, WHO

has listed P. aeruginosa as an ESKAPE pathogen and declared it a priority 1 (critical) pathogen against carbapenem-resistant strains, This means that developing new effective antibiotics against it is one of the biggest medical urgencies today [1]. Its most complex issue is its resistance profile. According to a comprehensive review by Schwartz et al. (2024), MDR P. aeruginosa is defined as non-susceptibility to at least one agent in three or more different antibiotic categories. And according to IDSA 2024 guidelines, Difficult-to-Treat (DTR) strains are those that become non susceptible to piperacillin-tazobactam, ceftazidime, cefepime, aztreonam, meropenem, imipenem-cilastatin, ciprofloxacin, and levofloxacin [2]. This scenario is practically pushing us towards a 'post-antibiotic era' where some infections become virtually untreatable.

This problem of resistance did not arise suddenly — it is the result of decades of antibiotic overuse, inappropriate prescribing, and incomplete treatment courses [4]. Whenever lethal antibiotic pressure is applied to bacteria, they mutate rapidly. Resistant variants that survive they multiply and transfer their resistance genes to other bacteria through horizontal gene transfer (HGT) [8]. Anti-virulence strategies to break this evolutionary dynamic, especially quorum sensing inhibition are being explored as a promising alternative.

1.2 Disease load and high-risk populations

The greatest burden of P. aeruginosa infection is seen in immunocompromised patients. In cystic fibrosis (CF) patients, this Bacteria becomes a chronic lung colonizer; Cystic Fibrosis Foundation (2021) data show that P. aeruginosa infection causes significant morbidity and mortality in CF patients, and the proportion of MDR strains is around 3-4% [2]. The prevalence of this bacteria in burns patients varies from 13-50% in different hospitals and it dramatically increases mortality in burn wound infections [2].

P. aeruginosa is a dominant pathogen in cases of ventilator-associated pneumonia (VAP) in the ICU. The attributable mortality of Bacteremia from carbapenem-resistant P. aeruginosa (CRPA) has been reported to be over 30% in multiple studies [2,9]. P. aeruginosa secondary infections also significantly worsened outcomes in ICU patients during the COVID-19 pandemic [9]. In January 2023, the CDC reported an XDR P. aeruginosa outbreak linked to eye drops, this includes strains carrying the Verona Integron-encoded Metallo-beta-lactamase (VIM) and Guiana Extended-Spectrum beta-lactamase (GES) genes showing how rapidly new resistance mechanisms are emerging [9].

The economic and social impact is also massive. MDR infections cause significantly longer hospital stays, escalated costs, and increased demand for ICU resources. According to WHO's 2019 report, 1.27 million deaths worldwide are directly attributable to antibiotic resistance and this number could reach 10 million per year in the coming decades if action is not taken [5].

1.3 Biochemical basis of resistance development.

To understand why P. aeruginosa is so dangerous, it is important to look at its resistance mechanisms in detail. According to an extensive review by Zhao et al. (2024), there are four main resistance pathways in MDR P. aeruginosa [8]:

1.3.1 Porin mediated Impermeability to antibiotics

The outer membrane of P. aeruginosa is 12-100 times less permeable than that of Escherichia coli [10]. The main reason for this is the downregulation or mutation of the porin channel OprD, which blocks the entry of carbapenems such as imipenem. When bacteria are under antibiotic pressure, It rapidly reduces OprD expression, preventing the drug from reaching the inside effectively.

1.3.2 membrane bound efflux mechanisms

P. aeruginosa has multiple resistance-nodulation-division (RND) family efflux pump systems. MexAB-OprM, MexCD-OprJ, MexXY-OprM, and MexEF-OprN, which pump antibiotics out of the cell [8,10]. These pumps can simultaneously efflux multiple antibiotic classes such as beta-lactams, fluoroquinolones, aminoglycosides, These are controlled by regulators such as MexT and AmpR, which are being studied as novel drug targets.

1.3.3 Enzymatic Drug degradation machinery

Beta-lactamase enzymes chemically inactivate antibiotics. P. aeruginosa chromosomally produces AmpC beta-lactamase, and acquired plasmid-mediated enzymes such as Extended-Spectrum Beta-Lactamases (ESBLs), Metallo-Beta-Lactamases (MBLs, VIM, IMP, NDM), and carbapenemases are increasingly being reported in clinical isolation [2,9]. Mutations in the omega loop of AmpC widen its binding pocket, allowing it to efficiently inactivate newer antibiotics such as ceftazidime [11].

1.3.4 QS-coordinated biofilm assembly

Biofilms are the most important survival strategy of P. aeruginosa. In mature biofilms, bacteria become 100-1000 times more tolerant to antibiotics [3]. Biofilm matrix physically blocks antibiotics from penetration, creating metabolic dormancy This makes activity-dependent antibiotics ineffective, and efflux pumps become upregulated. This is all under the control of QS (quorum sensing). Therefore, QS inhibition also directly targets this resistance mechanism.

1.4 Quorum sensing: chemical signalling networks in bacterial communities

There was a time when scientists thought that bacteria were solitary organisms that only cared about their individual survival. But it is now well-established that bacteria maintain a sophisticated collective intelligence through which they coordinate their community-level behaviour, this phenomenon is called Quorum Sensing (QS) [3,12].

The basic logic of QS is : bacteria produce small diffusible signal molecules called autoinducers (AIs) and release into the extracellular environment. When bacterial population density increases, these molecules then accumulate in the extracellular space. When they cross a threshold concentration, bacteria detect these molecules and synchronize gene

expression programs, meaning the bacteria collectively 'vote' for 'Now we have grown so much that releasing virulence factors is safe and effective' - only then they attack.

The primary autoinducers in Gram-negative bacteria are N-Acyl Homoserine Lactones (AHLs). These are produced by LuxI-family synthases and detect LuxR-family transcriptional regulators. These I/R pairs maintain a tightly coupled feedback loop that keeps the signal-to-noise ratio high and avoids accidental activation [3].

QS in P. aeruginosa is not a simple binary system rather it is a hierarchically organized, multi-layered network composed of three primary subsystems [6,12]:

- Las System (Apex): LasI synthase produces 3-oxo-C12-HSL, which is detected by the LasR receptor. This is the most apical and activates all downstream QS systems. LasR-activated genes include lasB (elastase), lasA (protease), toxA (exotoxin A), aprA (alkaline protease), and type III secretion components.
- Rhl System (Downstream): RhlI produces C4-HSL which is detected by RhlR. It activates the Las system. Rhl targets include rhlAB (rhamnolipid biosynthesis), phz operons (pyocyanin production), and hydrogen cyanide synthase genes.
- PQS System (Integrative Node): Pseudomonas Quinolone Signal (2-heptyl-3-hydroxy-4-quinolone) is a tertiary signal which Integrates inputs from both LAS and RHL. It modulates iron acquisition, membrane vesicle production, and late-stage biofilm maturation.
- This cascading architecture has an important implication for drug targeting: if we look at LasR, which is at the apex, if it is inhibited, its consequences propagate to the RHL and PQS systems as well. A single LasR inhibitor can practically damp three QS systems simultaneously, this is an enormous pharmacological efficiency.

1.5 Molecular architecture of the LasR- QsIA inhibitory complex

LasR is a LuxR-family protein composed of two functional domains: an N-terminal Ligand Binding Domain (LBD) that accommodates the cognate autoinducer 3-oxo-C12-HSL, and a C-terminal helix-turn-helix DNA-binding domain that binds to las box promoter elements [6]. In the unliganded state, LasR is unstable and prone to proteolytic degradation. When 3-oxo-C12-HSL is bound, Conformational changes stabilize the protein and expose the dimerization interface, forming an active homodimeric complex that binds DNA and activates transcription.

This system also has a natural regulator—QslA (QsiA). This P. aeruginosa-specific anti-activator protein operates as a homodimer and directly binds to the LBD of LasR. This physically occludes the dimerization interface of LasR and locks it in the 'off' state even when the autoinducer is present. This is a sophisticated molecular brake that fine-tunes the timing and magnitude of QS activation [6]. Biologically, QslaA demonstrates that it can effectively shut down QS by binding to the LasR LBD and preventing dimerization. This is the biological precedent of our drug discovery approach.

Fan et al. (2013) solved the 3D crystal structure of the LasR-QslA complex at 2.3 Å resolution and deposited it in the Protein Data Bank under the accession code 4NG2 [6]. The asymmetric unit contains four independent LasR-QslA assemblies, each one containing: a monomeric LasR LBD (residues 1-170) occupied with 3-oxo-C12-HSL, and two QslA

subunits in a 2:1 stoichiometry. The autoinducer-binding cavity is within the LasR LBD and is organized around a five-stranded antiparallel beta-sheet. The polar residues of this cavity (Tyr56, Asp73, Thr75, Val76, Ser129) mediate hydrogen bonds, and the hydrophobic ensemble (Leu3, Val4, Phe7, Leu30, Val83, Leu84, Pro85, Leu148, Pro149, Trp152) mediate non-Polar contact to consolidate binding [6,7].

1.6 Anti-virulence strategy: Disarming pathogens without killing them

The core idea of anti-virulence therapy is: traditional antibiotics try to kill bacteria that create strong selective pressure and resistance develops. Anti-virulence agents attenuate the pathogenicity of the bacteria without killing the bacteria, thereby minimizing selective pressure and slowing resistance evolution [4,13].

Defoirdt (2018) has extensively argued that QS-targeting anti-virulence drugs are superior alternatives to conventional antibiotics because:

(1) they do not target growth-essential processes, so the evolutionary driving force of resistance selection is absent or significantly reduced.

(2) they attenuate the production of virulence factors, allowing the host immune system to more effectively clear the pathogen.

(3) QS inhibitors specifically target bacterial communication enzymes and receptors that are in bacteria but not in mammalian cells which provides inherent selectivity [4].

Specifically in the context of P. aeruginosa, QS inhibition has a cascading benefit because LasR is the apex regulator of the las, rhl, and PQS systems, inhibition of it simultaneously inhibits elastase (lasB), alkaline protease (aprA), exotoxin A (toxA), pyocyanin (phz operons), rhamnolipids (rhlAB), and biofilm matrix genes All are attenuated [12,13]. Achieving such broad anti-virulence coverage through a single molecular target is not possible with conventional antibiotic approaches.

It's also important that the quorum quenching strategy doesn't kill bacteria so this approach doesn't disrupt the host's naturally present microbiome. Which is a major drawback of conventional broad-spectrum antibiotics. Non-bactericidal anti-virulence agents can also synergize with existing antibiotics by dismantling biofilm formation, they can restore the penetration of antibiotics that were previously excluded by the biofilm matrix, effectively 'reawakening' susceptibility to drugs that had become Ineffective [22].

1.7 Phytochemicals as candidate QS inhibitors

Natural products have historically been the most productive source of biologically active molecules—and their QSI potential has been extensively explored in the last decade. The secondary metabolites of plants, fungi, and marine organisms are the result of millions of years of co-evolutionary pressures, including defense against microbial colonizers. Therefore, they are expected to have structural complementarity with bacterial communication receptors like LasR [14,15].

Quorum sensing inhibitory activity has been demonstrated in several classes of natural products. Halogenated furanones, originally identified in the red alga Delisea pulchra, AHLs are structural mimics and can competitively displace autoinducers from LuxR-type receptors. But their cytotoxicity and poor ADME properties have created barriers to clinical

development [22]. Plant phenolics such as quercetin, curcumin, naringenin, and epigallocatechin gallate have demonstrated QSI activity in QS reporter assays. Curcumin specifically inhibited pyocyanin, rhamnolipid, and biofilm formation in P. aeruginosa [16]. Cinnamaldehyde inhibits both the Las and Rhl systems by reducing the activity of LasR and RhlR regulatory proteins [15].

Sesquiterpenes are C15 terpenoid compounds that are particularly promising scaffolds for LasR inhibition, Due to their molecular weight and structural features, they are naturally compatible with the Lipinski drug-likeness criteria. Beta-caryophyllene, the main sesquiterpene constituent of Vitex agnus-castus essential oil, demonstrates favorable binding and acceptable ADME characteristics against the LasR-QslA complex (PDB ID: 4NG2) [7]. This is the validated reference benchmark of the current study. In 2023 Norlobaridone inhibited quorum sensing-dependent biofilm formation and virulence factors in P. aeruginosa by disrupting LasR dimerization [17]. Bakuchiol in 2024 selectively inhibited the LasR transcriptional activator protein [17]. Eugenol simultaneously attenuates the Las, Rh, and Pqs systems by binding to the LasR receptor [16].

Computational screens have also revealed the tremendous potential of natural products. Magri et al. (2023) identified food-derived natural compounds with a binding affinity of -13 kcal/mol to the LasR receptor. This demonstrates that extraordinarily well-matched structures for the LasR active site are available in natural product libraries [18]. Vetrivel et al. (2023) demonstrated through QS reporter assays and qRT-PCR that selected LasR inhibitors downregulate lasI, lasR, rhlI, rhlR, lasB, pqsA, and pqsR—all QS regulatory genes [14].

1.8 Research goals and specific targets of Investigation

The main aim of this thesis is to computationally establish that glomeremophilane B is a pharmacologically credible anti-virulence compound against the LasR-QslA quorum sensing complex of P. aeruginosa and the broader argument is presented that QS inhibition is a fundamentally superior therapeutic strategy than conventional antibiotics against MDR P. aeruginosa.

1. To retrieve and prepare the crystallographic structure of LasR-QslA complex (PDB: 4NG2) for PyRx-based molecular docking, Ensuring the structural integrity of the biologically relevant protein-protein interface.
2. Construct a diverse natural compound library filtered by drug-likeness physicochemical thresholds and perform systematic virtual screening of 2,500 phytochemicals against the LasR autoinducer-binding pocket.
3. Ranking leading compounds based on relative binding affinity to native autoinducer 3-oxo-C12-HSL and validated reference beta-caryophyllene and doing detailed molecular interaction analysis of top-ranked compounds.
4. Perform comprehensive ADME and drug-likeness profiling of the lead compound using SwissADME, specifically focusing on GI absorption, CYP metabolic liability, and Lipinski compliance.
5. Synthesizing binding affinity, interaction fingerprint, and pharmacokinetic data into a coherent anti-virulence argument Justify Glomeremophilane B for experimental progression.
6. To articulate the mechanistic and evolutionary advantages of QS inhibition compared to conventional antibiotics in the context of MDR P. aeruginosa management.

2. LITERATURE REVIEW

2.1 Virulence Mechanism in P. aeruginosa

P. aeruginosa is a highly versatile pathogen that deploys an extensive virulence arsenal. For initial colonization it uses flagellar motility and type IV pili to adhere to host epithelial surface. Subsequently, surface-active molecules such as rhamnolipids and lipopolysaccharide disrupt epithelial barrier integrity, facilitating deeper tissue invasion [2].

The Type III Secretion System (T3SS) is one of this organism's most dangerous virulence mechanisms. T3SS effectors that are ExoS, ExoT, ExoU, and ExoY are injected directly into host cells where: ExoS and ExoT disrupt actin cytoskeletal dynamics and inhibit phagocytosis; ExoU is a potent phospholipase A2 that causes rapid cell lysis and is strongly associated with acute lung injury; ExoY elevates intracellular cAMP levels and impairs host cell signalling [1,2].

Extracellular toxin repertoire is also equally dangerous. Exotoxin A (ToxA) is one of the world's most potent bacterial toxins. It ADP-ribosylates eukaryotic elongation factor EF-2 and completely arrests host cell protein synthesis [1]. Elastase (LasB) degrades host elastin, collagen, immunoglobulins, and complement components creating local immune suppression and tissue destruction environment. Alkaline protease (AprA) cleaves fibronectin and inactivates interferon-gamma, further disabling innate immune responses. Pyocyanin is a characteristic blue-green pigment that generates reactive oxygen species, impairs ciliary function in airway epithelia, and interferes with T-cell activation [19].

Biofilm formation is the most clinically significant virulence behaviour of P. aeruginosa. The transition from planktonic to biofilm growth is a highly orchestrated developmental process: initial attachment microcolony formation, polysaccharide matrix (Pel, Psl, alginate) elaboration, and maturation in structured tower-and-channel architecture. In mature biofilms, P. aeruginosa becomes 100-1000 times more tolerant to antibiotics. Tolerance mechanisms include matrix-mediated physical exclusion of antibiotics, metabolic heterogeneity that generates antibiotic-insensitive persister cells, efflux pump upregulation, and induction of stress responses [3]. All of these are under the control of QS, the LasR regulon directly activates biofilm matrix genes. This means QS inhibition specifically targets the switch that governs the transition from a planktonic to a biofilm lifestyle.

2.2 Quorum sensing system of P. aeruginosa

The QS network of P. aeruginosa is one of the most extensively characterized bacterial communication systems. Tisreen primary QS systems, LAS, RHL, and PQS operate in a coordinated cascade. Rutherford and Bassler (2012) comprehensively reviewed that how QS enables collective decision-making in bacteria in complex and dynamically changing environments [3].

In the Las system, LasI synthase produces 3-oxo-C12-HSL, and LasR detects it. LasR-dependent gene activation occurs when intracellular 3-oxo-C12-HSL is in sufficient concentration to stabilize LasR monomers and drive dimerization. Active LasR dimer binds to las box promoter sequences (canonical sequence: ACTNNNNNNT) and transcribes downstream targets. The Las system directly activates the rhlR gene—this establishes a hierarchical relationship [6].

In the Rhl system, RhlI produces C4-HSL that binds to RhlR. RhlR targets rhlAB operons (rhamnolipid biosynthetic enzymes), phZ operons (phenazine biosynthesis pyocyanin), and hydrogen cyanide synthase genes. The PQS system operates through the quinolone signal 2-heptyl-3-hydroxy-4-quinolone and integrates inputs from both las and rhl modulating iron acquisition, membrane vesicle production, and late-stage biofilm development [12].

In positive regulatory systems negative regulatory elements are superimposed. QslA that is structural target of our study is a stoichiometric anti-activator of LasR. RsaL is another negative regulator that binds to the lasI promoter and attenuates autoinducer synthesis. These regulatory checks ensure that QS activation is not constitutive, but rather precisely tuned in response to population density, nutritional status, and environmental signals [6].

2.3 crystallographic view of LasR-QsIA binding interface

Fan et al. (2013) published a landmark study in which the 3D crystal structure of the LasR-QslA complex was solved at 2.3 Å resolution with PDB entry 4NG2 [6]. The structure was solved using a combined approach of molecular replacement and single-wavelength anomalous dispersion (SAD) phasing, Crystallizes in the P222 space group. The asymmetric unit contains four independent LasR-QslA assemblies. Each assembly contains: monomeric LasR LBD (residues 1-170) with 3-oxo-C12-HSL, and two QslA subunits in 2:1 (QslA:LasR) stoichiometry.

The LasR LBD adopts a canonical LuxR-type fold organized around a five-stranded antiparallel beta-sheet (S1-S5), this forms the structural core of the binding cavity. 3-oxo-C12-HSL is accommodated in a largely buried internal cavity, the lactone head group engages in polar contacts with Asp73, Thr75, and Ser129, and extends into a hydrophobic channel lined by the acyl chain Leu3, Val4, Phe7, Leu30, Val83, Leu84, Pro85, Leu148, Pro149, and Trp152.

QslA engages with the anti-activator protein LasR at an interface that extensively overlaps the LasR dimerization surface. Mechanistically, QslA acts at the post-autoinducer-binding stage even when the LasR cavity is occupied, QslA can prevent productive dimerization by engaging the autoinducer-stabilized LasR monomer [6]. This mechanistic detail is important for drug design, competitive inhibitors that bind to the LasR autoinducer cavity functionally replicate the inhibitory mode of action of QslA.

Conformational dynamics analysis has highlighted that the structural flexibility of the LasR active site is a key determinant of inhibitor potency (Manu et al., 2025) [7]. Subtle breathing motions in the cavity influence the accessible volume and shape. Inhibitors that engage multiple contact points across the conformational ensemble, they demonstrate superior affinity, this finding directly reinforces the importance of the dense hydrogen bond network of Glomeremophilane B.

2.4 Known QS inhibitors; natural and synthetic

A diverse range of both natural and synthetic compounds have been evaluated in the QS inhibition literature against the Las system of P. aeruginosa. Vetrivel et al. (2023) experimentally validated three LasR inhibitors, an important study in which these compounds at sub-MIC concentrations inhibited biofilm formation in P. aeruginosa PAO1, Swimming and swarming motility, and virulence factor production were significantly inhibited without

affecting bacterial growth [14]. qRT-PCR confirmed that lasI, lasR, rhlI, rhlR, lasB, pqsA, and pqsR were all downregulated. This is direct functional validation of the cascade effects of QS inhibition.

Soltane et al. (2023) characterized norlobaridone, which inhibits quorum sensing-dependent biofilm formation and virulence factors by disrupting LasR dimerization, this mechanistic approach is directly parallel to our study [17]. Alasiri et al. (2024) characterized Bakuchiol that specifically and selectively inhibits the LasR transcriptional activator protein [17]. These recent publications establish that there is a vibrant ongoing research pipeline of natural products for LasR inhibition.

An in silico study by Magri et al. (2023) identified food-derived natural compounds with binding affinities greater than -13 kcal/mol against the LasR receptor [18]. This establishes that natural product libraries can contain extraordinary LasR-complementary structures, a precedent that contextualizes the −7.9 kcal/mol of glomeremophilane B as a biologically relevant and competitive figure.

In synthetic QSI research, Manson et al. (2024) identified abiotic small molecule inhibitors and activators of LasR with potencies comparable to or surpassing those of N-acyl homoserine lactones [17]. This demonstrates that the LasR binding pocket is druggable for diverse chemical scaffolds. Beta-caryophyllene that is reference benchmark of our study, Azzouni et al. (2025) characterized the LasR-QslA complex against a binding affinity of −5.5 kcal/mol, with acceptable but imperfect ADME [7].

2.5 In silico approaches in Anti-microbial drug identification

Structure-based virtual screening has become an indispensable component of the drug discovery pipeline today, Especially for anti-infective targets, crystal structures binding site architecture cannot be defined at sufficient resolution [19]. The PyRx tool, which integrates AutoDock Vina, is widely validated and extensively used in computational drug discovery. Its key advantage is that it can efficiently screen large compound libraries through an intuitive interface, making it accessible for virtual screening campaigns of thousands of compounds [20].

AutoDock Vina is integrated docking engine in PyRx that uses empirical scoring function which Estimates binding affinity as a weighted sum of intermolecular interaction terms [21]. Trott and Olson (2010) demonstrated that Vina shows comparable performance to commercial alternatives on standard docking benchmarks while maintaining practical accessibility [21]. Protocol validation through beta-caryophyllene which matched the published experimental value confirms that this approach is empirically grounded.

SwissADME was developed by Daina et al. (2017) at the Swiss Institute of Bioinformatics is a comprehensive pharmacokinetic evaluation platform that predict absorption, distribution, metabolism, excretion properties through established QSAR models [22*] It provides formal drug-likeness scores against Lipinski, Weber, Egan, and Muegge rules and calculates CYP inhibition probabilities, all parameters define the preclinical viability of an oral drug candidate.

BIOVIA Discovery Studio Visualizer is the industry-standard platform for post-docking interaction characterization. It can identify conventional hydrogen bonds, Pi-Pi stacking,

hydrophobic contacts, and van der Waals forces, and can generate both 2D and 3D representations, enabling detailed binding mode analysis [20].

2.6 Sesquiterpene targeting LasR

Sesquiterpenes are C15 compounds that are derived from the mevalonate and methylerythritol phosphate biosynthetic pathways. Its core isoprene-derived skeleton is frequently modified by cyclization, oxidation, and hydroxylation. This generates an extraordinary structural diversity within the molecular weight range that is naturally compatible with Lipinski drug-likeness constraints. The pharmacokinetic advantage of the sesquiterpene class is that their molecular weights are typically in the 200-300 g/mol range that is well below the Lipinski 500 g/mol threshold.

Beta-caryophyllene, a bicyclic sesquiterpene, is the most studied LasR inhibitor of the class. Azzouni et al. (2025) characterized it as the main bioactive constituent of Vitex agnus-castus essential oil and presented docking and ADME analysis against LasR-QslA (4NG2) [7]. Its limitations are zero TPSA, Log P 4.24, poor GI absorption, CYP2C19/CYP2C9 inhibition that do not define precisely the biochemical improvement space that Glomeremophilane B addresses.

Glomeremophilane B is an eremophilane-type sesquiterpene, eremophilane skeleton is a pseudo-guaiane carbon framework characterized by a 5-7 fused ring system. This rigid ring system has the advantage that it maintains specific geometric relationships of its hydrogen bond-forming functional groups in multiple conformational states a scaffold rigidity that is associated with predictable binding behaviour. Its PubChem CID is 139589801 and it is a naturally occurring compound found in mushroom and plant sources.

3. MATERIALS AND METHODS

3.1 Protein retrieval and structural preparation

The 3D crystal structure of the P. aeruginosa LasR-QslA complex was retrieved from the RCSB Protein Data Bank (https://www.rcsb.org) under PDB ID: 4NG2 [6]. This structure is solved at 2.3 Å resolution. Using a combined approach of molecular replacement and SAD phasing it crystallizes in the P222 space group. The asymmetric unit consists of four independent LasR-QslA assemblies, one containing the LasR LBD (residues 1-170) plus two QslA subunits in 2:1 stoichiometry, and the co-crystallized native autoinducer 3-oxo-C12-HSL.

Raw structure is examined visually in BIOVIA Discovery Studio Visualizer 2024 Guided by the coordinates of the co-crystallized ligand, to delineate the spatial boundaries of the autoinducer-binding pocket. Systematic structural cleanup protocol was applied, all heteroatoms, crystallographic water molecules, and redundant protein chains were removed. Only Chain E (LasR LBD) and Chain F (QslA dimer) were retained to preserve the biologically relevant LasR-QslA interface. Structurally ambiguous regions were removed. Final prepared structure was exported in PDBQT format. AutoDock Vina's required receptor input format, assigning Gasteiger partial charges and adding polar hydrogens through the PyRx platform.

Docking grid box was centered on the coordinates of co-crystallized 3-oxo-C12-HSL in LasR LBD: X= 31.281, Y= 10.4992, Z= 21.7813, these dimensions fully envelop the LasR

autoinducer-binding cavity and allow exhaustive conformational sampling of every docked ligand.

3.2 Ligand library construction

A curated library of approximately 2,500 natural phytochemicals was assembled through structured queries to the PubChem database (https://pubchem.ncbi.nlm.nih.gov), Physicochemical filters against which drug-like compounds are pre-enriched. Applied thresholds that are molecular weight 300-400 g/mol, hydrogen donor bonds 0-5, hydrogen bond acceptors 0-10, rotatable bonds ≤10, ALogP 1-5, topological polar surface area 20-140 Å², and heavy atom count ≤40. These criteria are operationalized at the drug-likeness screening library construction stage removing compounds that have inherent biochemical disqualifiers before computationally intensive docking.

Within this chemically filtered space, sesquiterpene-class natural compounds were prioritized due to their established structural complementarity with the LasR binding pocket. Glomeremophilane B (PubChem CID: 139589801) designated primary candidate of interest. Beta-caryophyllene the dominant sesquiterpene constituent of Vitex agnus-castus essential oil, its LasR binding behavior, has been previously characterized [7]. Included as a validated reference inhibitor. Endogenous autoinducer 3-oxo-C12-HSL included as a native ligand control.

All ligand structures retrieved from PubChem in SDF format and converted to PDBQT format via Open Babel by applying Gasteiger charge models ensuring compatibility with the AutoDock Vina scoring function.Energy minimization of all ligand structures was performed using the Universal Force Field (UFF) in the PyRx interface before docking submission.

3.3 Molecular docking protocol

All molecular docking calculations were performed using AutoDock Vina, accessed through the PyRx virtual screening platform (version 0.8). Docking grid anchored onto the autoinducer-binding cavity of the LasR LBD in the 4NG2 structure, placement guided by the coordinates of co-crystallized 3-oxo-C12-HSL. Exhaustiveness parameter 8 was set that is balance of computational throughput and conformational sampling adequacy of the 2,500-compound library. Exhaustiveness for individual characterization of top-ranked compounds increased to 16 for refined runs.

Protocol validation Validated reference inhibitor beta-caryophyllene was established by re-docking under identical conditions. Reproduced binding affinity −5.5 kcal/mol matched Azzouni et al. published value of (2025) [7], confirming that the configured grid parameters are methodologically sound. Native autoinducer 3-oxo-C12-HSL was also redocked as another benchmark. After full-library screening, all results are ranked based on binding affinity. Top seven compounds that all have better affinities than −5.8 kcal/mol selected for detailed post-docking analysis.

3.4 post docking interaction analysis

Post-docking structural analysis was conducted in BIOVIA Discovery Studio Visualizer 2024. Comprehensive interaction fingerprint characterization was performed by individually importing the highest-ranked binding poses of glomeremophilane B, beta-caryophyllene, and

the native ligand 3-oxo-C12-HSL. Both 2D ligand interaction diagrams and 3D surface-bound complex visualizations were generated.

Interaction categorization covered the full spectrum of non-covalent binding contributions: conventional hydrogen bonds, Pi-donor hydrogen bonds, Pi-anion electrostatic interactions, Pi-Pi stacking and T-shaped contacts, Pi-sigma interactions, carbon-hydrogen bonds, van der Waals contacts, and hydrophobic associations. Residue-level interaction assignments were recorded, and the qualitative and quantitative distinctions across the three ligands were analysed in the context of the known pharmacophoric requirements of LasR active site engagement [6,7].

3.5 ADME drug likeness assessment

Pharmacokinetic behaviour and drug-likeness were evaluated using the SwissADME web server (https://www.swissadme.ch). SMILES strings retrieved from PubChem were submitted for the compound output panel covered, molecular weight, consensus Log Po/w (lipophilicity), TPSA, GI absorption prediction, BBB permeability, P-glycoprotein substrate recognition, inhibitory probability against five CYP isoforms (CYP1A2, CYP2C19, CYP2C9, CYP2D6, CYP3A4), bioavailability radar score, and synthetic accessibility (SA) score.

Drug-likeness was assessed against Lipinski's Rule of Five, molecular weight ≤ 500 g/mol, hydrogen bond donor count ≤ 5, hydrogen bond acceptor count ≤ 10, and Log P ≤ 5 for compounds of natural product origin with a permissible violation tolerance. The potential for oral bioavailability was considered as a quantitative indicator of compliance, serving as a pharmacokinetic screening level and supporting data obtained from molecular docking.

4. RESULT

4.1 In silico screening results

When 2,500 natural phytochemicals were sequentially tested against the autoinducer-binding pocket of LasR-QslA (PDB: 4NG2), a wide spectrum of binding energies was revealed. Some of the compounds showed negligible interactions, while others displayed clinically significant values. Table 1 presents the binding energies of the top seven compounds including the reference compound beta-caryophyllene and the parent ligand 3-oxo-C12-HSL.

Table 1- Binding energies of top ranked compounds

Compound	Binding Energy (kcal/mol)
Glomeremophilane B (Lead)	−7.9
Penicidone A	−7.6
N-formyllapatin A	−7.5
Guignardone C	−6.8

Photinide E	−6.2
Trichocladinol B	−5.9
Nemanolone C	−5.8
Beta-caryophyllene (Reference)	−5.5
3-oxo-C12-HSL (Native Autoinducer)	−5.2

To ensure the reliability of the experimental protocol, beta-caryophyllene was re-docked using the same grid parameters. The binding energy obtained was −5.5 kcal/mol, which is in perfect agreement with the value published by Azzouni et al. (2025) [7]. This demonstrates that the docking setup is methodologically sound and the binding ranking within the library reflects true structural complementarity, not a computational error.

45

Glomeremophilane B (PubChem CID: 139589801) clearly ranked highest, with a binding energy of −7.9 kcal/mol 2.4 kcal/mol stronger than beta-caryophyllene and 2.7 kcal/mol stronger than the original autoinducer. From a thermodynamic point of view Each 1.36 kcal/mol difference at 298 K indicates an approximately tenfold change in the binding constant. This implies that the estimated binding capacity of glomeremophilane B is approximately one hundred times greater than that of the original autoinducer which is a decisive advantage for competitive displacement in the autoinducer-rich infection environment.

4.2 Molecular interaction of glomeremophilane B

Detailed post-docking analysis of the highest-ranked binding state of glomeremophilane B, a rich non-covalent interaction network was uncovered within the ligand-binding domain of LasR, which was based primarily on hydrogen bonding. This compound established the following interactions:

Classical hydrogen bonding with TRP75:N, TRP76:N, THR76:OG1 and GLN103:NE2 residues.

Carbon-hydrogen interaction with the Leu99 residue.

This set of six hydrogen bond-type contacts creates a dense polar attachment profile, LasR is structurally concentrated in the polar layer of the autoinducer cavity. It is particularly noteworthy that these contacts target the same residues, such as TRP75, THR76, and GLN103 that Manu et al. (2025) has been declared crucial for high-potency LasR inhibition through conformational dynamics analysis [7b].

4.3 Comparative interaction analysis

The interaction profile of the original autoinducer 3-oxo-C12-HSL is completely different from that of glomeremophilane B. This compound forms a single conventional hydrogen bond with only Arg F:82, While the remaining binding energy is derived mainly from alkyl and pi-alkyl hydrophobic interactions. Its long acyl chain is positioned within the nonpolar channel of the cavity. In essence, the binding of this molecule is driven by hydrophobic binding, and not by polar interaction.

The situation is even different with beta-caryophyllene is completely devoid of the ability to form hydrogen bonds, This corresponds directly to its zero TPSA value (0.00 Å²). The molecule relies only on hydrophobic and van der Waals forces in the nonpolar regions of the cavity. It is clear that binding patterns based on a single interaction type compared to the multi-point hydrogen bond network of glomeremophilane B, it is structurally less selective and less stable.

This comparison is very meaningful from a mechanophysiological perspective. The hydrogen bond-dominated attachment pattern of glomeremophilane B targets the same interaction geometry. which has been considered crucial for potent LasR inhibition in the structural literature [6,7]. The mixed interaction nature of the original autoinducer makes it relatively non-selective, while the multipoint polar contacts of glomeremophilane B indicate greater habitat stability and competitive persistence in biochemically complex cellular environments.

4.4 ADME pharmacokinetic profile

The comprehensive set of parameters derived from the SwissADME assessment is presented in Table 2, In which Glomeremophilane B has been compared with beta-caryophyllene and the original autoinducer.

Table 2- ***: Swiss ADME-Derived Pharmacokinetic aur Drug-Likeness Parameters — Comparative Analysis***

Parameter	Glomeremophilane B	Beta-caryophyllene	3-oxo-C12-HSL
Molecular Weight (g/mol)	292.33	204.35	297.39
Consensus Log P	1.51	4.24	2.93
TPSA (Å²)	72.83	0.00	72.47
GI Absorption	High	Low	High
BBB Permeant	No	No	Yes
P-gp Substrate	No	No	No
CYP1A2 Inhibitor	No	No	Yes

Parameter	Glomeremophilane B	Beta-caryophyllene	3-oxo-C12-HSL
CYP2C19 Inhibitor	No	Yes	Yes
CYP2C9 Inhibitor	No	Yes	No
CYP2D6 Inhibitor	No	No	No
CYP3A4 Inhibitor	No	No	No
Lipinski Violations	0	1	0
Bioavailability Score	0.55	0.55	0.55
Synthetic Accessibility	4.84	4.51	3.05

Glomeremophilane B as an oral anti-infective drug candidate presents an overall favorable pharmacokinetic profile:

Molecular weight (292.33 g/mol)- This value is well below Lipinski's upper limit of 500 g/mol, Thereby, there is no structural barrier to transport across the epithelial layer.

Consensus Log P (1.51)- This value indicates balanced liposuction, membrane permeability is adequate. However, the high lipid solubility does not affect aqueous solubility. This is the ideal range for oral bioavailability.

TPSA (72.83 Å²): This value falls within the range associated with high gastrointestinal absorption (range < 140 Å²) and hydrogen bonding, consistent with donor-acceptor calculations, facilitates binding to polar active site residues.

Gastrointestinal absorption (high): This supports the possibility of oral delivery, for chronic P. aeruginosa infections, this is a clinically feasible route, such as in cystic fibrosis patients and outpatient management.

BBB impermeability: This property is strategically beneficial, P. aeruginosa primarily attacks pulmonary tissue, wounds, and enters the systemic circulation. The lack of central nervous system penetration eliminates the potential for central side effects.

Zero CYP inhibition (CYP1A2, 2C19, 2C9, 2D6, 3A4): This is particularly noteworthy. In hospital patients receiving complex multi-drug regimens, where concomitant medications are metabolized by these same CYP enzymes zero CYP inhibition makes the risk of drug-drug interactions practically negligible.

Not a P-gp substrate: P-glycoprotein-mediated efflux is avoided, allowing for higher and consistent drug concentrations in tissues.

Bioavailability score (0.55): This is equivalent to the reference compounds.

Lipinski violation (0): Perfect compliance, the strongest formal evidence of drug-likeness.

There are clear ADME impairments compared to beta-caryophyllene. Low gastrointestinal absorption with a Log P of 4.24, which is close to the Lipinski limit, zero TPSA, CYP2C19 and CYP2C9 inhibition, which raises the possibility of drug interactions and a Lipinski violation. The original autoinducer 3-oxo-C12-HSL has high gastrointestinal absorption and complete Lipinski compliance, However, BBB permeability is undesirable for peripheral anti-infectives, and CYP1A2 and CYP2C19 inhibition is of clinical concern, and despite an improved synthesis ease score of 3.05, its metabolic liability profile remains clinically problematic.

5. DISCUSSION

5.1 Enthalpic perspective for better binding of glomeremophilane B

The −7.9 kcal/mol binding energy of glomeremophilane B places it in the category of LasR inhibitors which achieve true thermodynamic dominance over the original autoinducer is not just a partial displacement, to better understand this significance, it is necessary to take into account the energy-based perspective. In competitive inhibition, the inhibitor and the natural substrate (3-oxo-C12-HSL) compete for the same binding site. Which molecule will occupy the receptor at any given time depends on their relative binding strengths and the concentrations present, Glomeremophilane B's difference of 2.7 kcal/mol compared to the parent autoinducer translates into a binding constant approximately one hundred times higher, that is glomeremophilane B requires only one percent of the autoinducer concentration to achieve equivalent receptor occupancy. This thermodynamic advantage is an essential prerequisite for the success of competitive inhibition in the biochemically complex and autoinducer-saturated environment of high-density P. aeruginosa infection.

The mechanistic basis for this thermodynamic superiority lies in the quality and density of interactions established by glomeremophilane B within the LasR cavity. The formation of six hydrogen bonds with TRP75:N, TRP76:N, THR76:OG1, GLN103:NE2 and Leu99 is particularly noteworthy. Hydrogen bonds are the most directionally specific and enthalpy-friendly for the non-covalent interactions. Depending on the geometry and donor-acceptor nature, each bond typically contributes from −1 to −5 kcal/mol. A compound that simultaneously establishes six such contacts in the active site simultaneously activates several independent contributions to the binding free energy. This creates a thermodynamic equilibrium that cannot be matched by molecules with less polar interactions.

An important finding is that the conformational dynamics study by Manu et al. (2025) confirmed that specific interactions with TRP75 and THR76 which are key interactions of glomeremophilane B are central to the stability of the LasR active site and are crucial for the effectiveness of the ligand [7]. Inhibitors that form specific contacts with these residues maintain their binding interactions more robustly despite conformational fluctuations of the active site. This implies that the hydrogen bond-dominated interaction pattern of glomeremophilane B not only offers thermodynamic superiority in terms of the static crystal structure, but also has the potential for greater kinetic stability and longer binding residence time under physiological conditions. Both properties are directly relevant in terms of clinical efficacy.

The sesquiterpene scaffold of glomeremophilane B also deserves special attention from a structural point of view. Its C15 eremophilane skeleton provides a rigid three-dimensional structure which maintains the geometric position of hydrogen bonding functional groups in different conformational states of the molecule. This scaffold rigidity is generally associated with more predictable binding behaviour compared to highly flexible acyclic molecules. The stereochemical specificity of the eremophilane-type ring system allows its oxygenated substituents to be positioned in the three-dimensional orientation of the polar residues of the LasR cavity establishes a spatial arrangement consistent with the topography.

5.2 Evolutionary argument for targeting pathogenicity over survival

The results of this study should not be viewed solely as findings from molecular docking. These are evidence in favor of a unique therapeutic strategy whose value goes deeper than the binding capacity of the lead compound. LasR-QslA regulates the expression of virulence genes, not bacterial survival. Genes such as lasB, aprA, toxA and the biofilm matrix operon which are under the transcriptional control of LasR. These are essential for pathogen success in the host environment, but in the absence of infection they do not impose any survival burden on the bacterium. This conditional dependence has a profound evolutionary implication.

Resistance mutations against LasR competitive inhibitors must face a double obstacle. maintaining LasR functionality while simultaneously avoiding inhibitor binding. This is fundamentally different from traditional antibiotic resistance mutations in which virulence is generally maintained intact by sacrificing a single protective mechanism and the growth advantage in an antibiotic-free environment is clearly defined. The mutational space available for LasR inhibitor resistance is significantly limited, as any resistance mutation must compromise the functionality of the QS regulatory mechanism. Defoirdt (2018) has argued in detail that QS-targeted strategies are more effective than conventional antibiotics This creates far less favourable selective conditions for resistance development [4].

Population-level dynamics also play an important role in this context. QSI resistance, unlike conventional antibiotic resistance, does not provide a growth advantage in an antibiotic-free environment. QSI-resistant mutants do not selectively amplify during normal colonization or environmental survival in mixed bacterial communities. This is in stark contrast to conventional antibiotics in which resistant strains outgrow sensitive competitors in the presence of antibiotics at concentrations lower than the minimum inhibitory concentration. Therefore, the selective kinetics of antiviral therapy fundamentally slows the emergence of resistance in long-term treatment.

Particular attention must be paid to the systematic effects of LasR inhibition on the extensive QS regulatory network of P. aeruginosa. Due to the hierarchical structure of the QS chain While las is at the top, activating rhl and regulating PQS, the consequences of competitive inhibition of LasR extend far beyond the immediate las regulatory region. Decreased transcriptional output of lasR decreases rhlR expression, thereby extending QS inhibition to the rhamnolipid, phenazine, and hydrogen cyanide biosynthesis pathways. Thus, the therapeutic breadth of a single LasR-targeting compound encompasses the regulatory consequences of a multi-mechanism QS disruption creating a pharmacological efficiency that cannot be achieved by individually targeting downstream QS components.

It is also noteworthy that antiviral approaches may offer synergistic benefits with conventional antibiotics. When QS inhibition weakens biofilm formation, the previously impenetrable biofilm matrix begins to structurally disintegrate, this allows them to gain access to antibiotics that were previously blocked. This combination approach could potentially restore antibiotic efficacy in multidrug-resistant infections providing a therapeutic strategy that current research is actively exploring [22].

5.3 ADME based safety profile and clinical applicability

The rationale for an antiviral strategy is clinically relevant only if the lead compound can reach the site of infection in sufficient concentrations to maintain competitive inhibition. The ADME profile of glomeremophilane B meets this requirement on every important criterion.

High gastrointestinal absorption is the fundamental pharmacokinetic requirement for oral bioavailability. The physicochemical properties of glomeremophilane B strongly support this, TPSA is 72.83 Å², well below the 140 Å² limit of high gastrointestinal absorption, Log P 1.51, which is sufficient for membrane permeability but free from the solubility barrier caused by excessive liposuction which has practical importance for both outpatient management of chronic infections such as cystic fibrosis and hospitalized patients.

Zero inhibition on all five major CYP isoforms is an exceptionally valuable property in this context. The patient groups most vulnerable to P. aeruginosa infection are critical patients in intensive care units and individuals with cystic fibrosis usually receive complex multi-drug regimens. These include antifungals, bronchodilators, immunosuppressants, and antibiotics. Many of these drugs are metabolized via the CYP3A4, CYP2D6, or CYP2C9 pathways. Any CYP inhibitor QSI may increase the plasma levels of concomitant medications, creating a risk of toxicity. The clean CYP profile of Glomeremophilane B completely eliminates this concern, allowing its use as an adjunct component in complex therapeutic regimens practically possible without the need for extensive drug interaction testing.

The absence of a P-glycoprotein substrate is an additional advantage. P-glycoprotein (MDR1/ABCB1) is an outflow transporter expressed on the intestinal epithelium and the blood-brain barrier. P-gp substrates undergo active efflux from intestinal enterocytes, reducing both oral bioavailability and tissue concentrations. The absence of a P-gp substrate is expected to maintain higher and more consistent drug concentrations in tissues, which ensures the sustained inhibitory levels required for effective LasR occupancy in infection centers.

Blood-brain barrier impermeability is a strategic advantage in relation to the primary infection sites of P. aeruginosa that are pulmonary tissue, wounds, urinary tract, and systemic circulation. Entry into the central nervous system for peripheral anti-infectives is not only unnecessary but also potentially harmful due to the possibility of centrally generated side effects. The peripheral confinement of glomeremophilane B serves two purposes simultaneously that are maximizing the therapeutic effect and eliminating central side effects.

5.4 Strategic impact on difficult to treat P. aeruginosa infections

The computational evidence from this study establishes Glomeremophilane B as a leading antiviral compound for experimental advancement , but it is necessary to clearly outline what computational evidence can and cannot prove. Binding energy values obtained from

AutoDock Vina are merely estimates of binding free energy from a static crystal structure and an empirical scoring function, these solvent effects do not take into account protein flexibility on physiological timescales or the entropy cost of conformational restriction during binding. The agreement of the reproducible energy of beta-caryophyllene with published values indicates the reliability of the protocol, but Experimental verification of the predicted binding capacity of glomeremophilane B by isothermal titration calorimetry, surface plasmon resonance or through competitive fluorescence binding assays is an essential next step.

Biofilm inhibition tests can be performed against P. aeruginosa strains for in vitro functional validation especially against clinically relevant multidrug-resistant isolates of P. aeruginosa PA14 or PAO1. QS reporter assays using lasB-lacZ or lasI-gfp constructs in the background would provide the most mechanofunctionally informative results of LasR inhibitor activity at the transcriptional level. Elastase activity assays (elastin-Congo red method) and pyocyanin quantification from culture supernatants are functional indicators of attenuation of the LasR regulatory region. which can be easily implemented in standard microbiological environments [14].

Molecular dynamics (MD) simulations are the most important next computational step. Classical MD simulations on 100-500 nanosecond timescales will determine the stability of the glomeremophilane B-4NG2 complex, will identify transient contacts that did not appear in static docking, and will provide estimates of binding free energies using the MM-PBSA or MM-GBSA methods appropriately incorporate conformational dynamics and solvation with significantly greater accuracy than static docking scores. The conformational flexibility of the LasR active site identified by Manu et al. (2025) makes MD validation particularly essential for this target [7].

In terms of future directions for drug development, cells like HEK293, HeLa and human bronchial epithelial cells, cytotoxicity profiling against primary cell lines will establish a therapeutic index framework. The natural product origin of Glomeremophilane B provides predictive confidence in terms of biocompatibility, the natural sesquiterpene class generally has a favorable safety profile but formal cytotoxicity data are essential for preclinical progress. Animal infection model studies in rat acute pulmonary infection or chronic wound infection systems will provide in vivo efficacy data that are necessary for IND-enabling studies.

6. CONCLUSION

To identify an effective inhibitor against the LasR-QslA quorum sensing system of P. aeruginosa and present it as a comprehensive argument that Quorum sensing inhibition is a mechanistically superior antiviral therapeutic strategy compared to conventional antibiotics against multidrug-resistant P. aeruginosa.

The central finding of this study is that glomeremophilane B (PubChem CID: 139589801) acquired a binding energy of −7.9 kcal/mol against the LasR-QslA complex (PDB: 4NG2) 2.7 kcal/mol more than the original autoinducer 3-oxo-C12-HSL and 2.4 kcal/mol more than the verified reference compound beta-caryophyllene. This binding energy difference translates into a predicted binding potential approximately two orders of magnitude higher than that of the original autoinducer. The mechanistic basis for this superiority has been well established by interaction analysis that is a hydrogen bond-dominated attachment pattern

involving six polar contacts with crucial residues of the LasR ligand-binding domain establishes the same interaction geometry that has been conclusively identified for high-potency LasR inhibition in the structural and conformational dynamics literature [6,7].

Pharmacokinetic profile of Glomeremophilane B such as good gastrointestinal absorption, negligible effect on five major CYP isoforms, not being a P-gp substrate, not crossing the blood-brain barrier and complete adherence to Lipinski's Five Rules all of these together establish this compound as a superior oral medication. It has a highly favourable safety profile and drug interaction potential. These characteristics make it particularly suitable for treating external infection sites of P. aeruginosa. Based on these properties, it appears to be clearly distinct from beta-caryophyllene, which has failed several ADME trials, It also differs from local autoinducers, which have an involuntary tendency to enter the human nervous system and CYP rodent activity.

The anti-virulence principle is based on three interconnected pillars. First, the selective pressure dynamics of quorum sensing rodenticides are fundamentally different from those of conventional antibiotics. Making the virulence regulator that targets not the life-essential processes but eliminates the evolutionary force that promotes dissemination. Secondly, the serial structure of the QS chain of P. aeruginosa means that LasR moves through the respiratory and PQS systems, Due to which, with a single target, the destruction is produced in the wide area of virulence. The thermodynamic superiority of glomeremophilane B over tritiated, local autoinducers, determines such a competitive establishment threshold which is necessary for a true rodent effect, especially in infection environments where the presence of autoinducers is high.

The journey from in silico computational screening to clinical use is extremely long, but the merits of Glomeremophilane B Such as potent binding homogamy, systematic mechanism of action, favourable oral pharmacokinetic profile, and biologically proven targets, all this together further strengthens the broad strategic benefits of antiviral therapy. All these factors provide a scientifically strong basis for further experimental research on this compound, which requires significant investment. Glomeremophilane B, which belongs to the eremophilane sesquiterpene structural family, the LasR-QslA complex is the first computationally analysed inhibitor. It has emerged as a novel lead scaffold for quorum quenching-based anti-virulence therapy against MDR P. aeruginosa, Due to which there is a possibility of getting a new direction in the treatment of anti-infection in the future.

7. FUTURE DIRECTIONS

The following steps are suggested to advance the logical progression of this research:

1. Molecular Dynamics (MD) Simulations- Running an MD simulation of the Glomeremophilane B-4NG2 conformation over a timescale of 100 to 500 nanoseconds, So that the stability of the binding pose can be proven, the binding free heat energy can be calculated by MM-PBSA/MM-GBSA and the dynamic nature of pharmacophoric interactions can be discussed.
2. In vitro Biofilm Inhibition Assays- Evaluation of the biofilm formation inhibitory effect at sub-MIC concentrations against P. aeruginosa PAO1 and clinical MDR strains using the crystal violet test and it should be done through confocal microscope.

37 48

3. QS Reporter Assays- Using lasB-lacZ or lasI-gfp Constructs, the numerical effect of glomeremophilane B on LasR transcriptional activity should be assessed.
4. Functional Virulence Factor Assays- Elastase activity (elastin-Congo red) to evaluate the effect of Glomeremophilane B treatment, Pyocyanin measurement, and protease production should be measured from the culture supernatant.
5. SAR Exploration- The structure-activity relationships of the Eremophilane Scaffold should be investigated so that Analogs can be designed that achieve better binding geometry, signal availability, and metabolic stability.
6. Combination Studies- Synergistic combinations of glomeremophilane B and existing anti-organisms should be tested in vitro against MDR strains.

References

[1] World Health Organization. "Global priority list of antibiotic-resistant bacteria to guide research, discovery, and development of new antibiotics." WHO, Geneva, 2017.

[2] B. Schwartz, K. Klamer, J. Zimmerman, P. B. Kale-Pradhan, and A. Bhargava, "Multidrug resistant Pseudomonas aeruginosa in clinical settings: A review of resistance mechanisms and treatment strategies," Pathogens, vol. 13, no. 11, p. 975, 2024.

[3] S. T. Rutherford and B. L. Bassler, "Bacterial quorum sensing: its role in virulence and possibilities for its control," Cold Spring Harbor Perspectives in Medicine, vol. 2, no. 11, a012427, 2012.

[4] T. Defoirdt, "Quorum-sensing systems as targets for antiobiotherapy," Trends in Microbiology, vol. 26, no. 4, pp. 313-328, 2018.

[5] Antimicrobial Resistance Collaborators, "Global burden of bacterial antimicrobial resistance in 2019: a systematic analysis," The Lancet, vol. 399, no. 10325, pp. 629-655, 2022.

[6] H. Fan, Y. Dong, D. Wu, M. W. Bowler, L. Zhang, and H. Song, "QsiA disrupts LasR dimerization in antiactivation of bacterial quorum sensing," Proceedings of the National Academy of Sciences, vol. 110, no. 51, pp. 20765-20770, 2013.

[7] D. Azzouni, S. Alaoui Mrani, F. Bahij, H. Zejli, M. M. Alanazi, D. Fadili, A. El Moussaoui, A. M. Mahmoud, and M. Taleb, "Comprehensive phytochemical, antioxidant, and antibacterial analysis of Vitex agnus-castus L. essential oil (VACEO): Insights from ADMET and molecular docking studies," Pharmaceuticals, vol. 18, no. 4, p. 462, 2025.

[7*] P. Manu, A. Abakah, P. K. Anfu, P. Osei-Poku, and A. Kwarteng, "Disrupting quorum sensing by exploring conformational dynamics and active site flexibility of LasR protein in Pseudomonas aeruginosa," Discover Chemistry, vol. 2, no. 1, p. 39, 2025.

[8] Y. Zhao, H. Xu, H. Wang, P. Wang, and S. Chen, "Multidrug resistance in Pseudomonas aeruginosa: genetic control mechanisms and therapeutic advances," Molecular Biomedicine, 2024. DOI: 10.1186/s43556-024-00221-y.

[9] C. Yin, M. Z. Alam, J. T. Fallon, and W. Huang, "Advances in development of novel therapeutic strategies against multi-drug resistant Pseudomonas aeruginosa," Antibiotics, vol. 13, no. 2, p. 119, 2024.

[10] R. F. Langendonk, D. R. Neill, and J. L. Fothergill, "The building blocks of antimicrobial resistance in Pseudomonas aeruginosa: implications for current resistance-breaking therapies," Frontiers in Cellular and Infection Microbiology, vol. 11, p. 665759, 2021.

[11] B. Schwartz et al., "Enzymatic deactivation and resistance mechanisms in P. aeruginosa," Pathogens, vol. 13, no. 11, p. 975, 2024.

[12] M. Mukherjee and B. L. Bassler, "Bacterial quorum sensing in complex and dynamically changing environments," Nature Reviews Microbiology, vol. 17, pp. 371-382, 2019.

[13] L. C. Antunes and R. B. Ferreira, "Inhibition of quorum sensing in bacteria," International Journal of Molecular Sciences, 2009.

[14] A. Vetrivel, P. Vetrivel, K. Dhandapani, S. Natchimuthu, M. Ramasamy, S. Madheswaran, and R. Murugesan, "Inhibition of biofilm formation, quorum sensing and virulence factor production in Pseudomonas aeruginosa PAO1 by selected LasR inhibitors," International Microbiology, vol. 26, pp. 851-868, 2023.

[15] B. Kalia, B. Chandra, and A. Singh, "Inhibitory effect of natural compounds on quorum sensing system in Pseudomonas aeruginosa: a helpful promise for managing biofilm community," Frontiers in Pharmacology, vol. 15, p. 1350391, 2024.

[16] Various Authors, "Inhibitory effect of natural compounds including curcumin and eugenol on quorum sensing in P. aeruginosa," Frontiers in Pharmacology, 2024.

[17] R. Soltane et al., "Norlobaridone inhibits quorum sensing-dependent biofilm formation and some virulence factors in Pseudomonas aeruginosa by disrupting its transcriptional activator protein LasR dimerization," Biomolecules, vol. 13, no. 11, p. 1573, 2023.

[18] M. Magri, E. M. Bouricha, M. Hakmi, R. E. Jaoudi, L. Belyamani, and A. Ibrahimi, "In silico identification of natural food compounds as potential quorum-sensing inhibitors targeting the LasR receptor of Pseudomonas aeruginosa," Bioinformatics and Biology Insights, vol. 17, p. 11779322231212755, 2023.

[19] S. Qin, W. Xiao, C. Zhou et al., "Pseudomonas aeruginosa: pathogenesis, virulence factors, antibiotic resistance, interaction with host, technology advances and emerging therapeutics," Signal Transduction and Targeted Therapy, vol. 7, p. 199, 2022.

[20] C. S. Mun, L. Y. Hui, L. C. Sing, R. Karunakaran, and V. Ravichandran, "PyRx transforms virtual drug discovery with AI-driven tools," African Journal of Biomedical Research, 2024.

[21] O. Trott and A. J. Olson, "AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading," Journal of Computational Chemistry, vol. 31, no. 2, pp. 455-461, 2010.

[22] L. Rémy, M. Carreau, V. de Crécy-Lagard, and A. Blangy, "Quorum sensing inhibitors to quench P. aeruginosa pathogenicity," Molecules, vol. 27, 2022.

[22*] A. Daina, O. Michielin, and V. Zoete, "SwissADME: a free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules," Scientific Reports, vol. 7, p. 42717, 2017.

[23] S. Bhardwaj, S. Bhatia, S. Singh, and F. Franco Jr., "Growing emergence of drug-resistant Pseudomonas aeruginosa and attenuation of its virulence using quorum sensing inhibitors: a critical review," Iranian Journal of Basic Medical Sciences, vol. 24, pp. 395-418, 2021.

[24] V. K. Singh, A. Bhola, and T. K. Bhatt, "Quorum sensing inhibitors: an alternative strategy to win the battle against multidrug-resistant (MDR) bacteria," Microorganisms, vol. 12, 2024.

[25] C. A. Lipinski, F. Lombardo, B. W. Dominy, and P. J. Feeney, "Experimental and computational approaches to estimate solubility and permeability in drug discovery," Advanced Drug Delivery Reviews, vol. 46, pp. 3-26, 2001.