Anti-virulence strategies to combat MDR *Pseudomonas aeruginosa*: In silico screening to target Quorum sensing as a substitution for conventional antibiotics.

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DECLARATION

I, Niharika 24/MSCBIO/54 hereby certify that the work which is being presented in the thesis entitled **"Anti-virulence strategies to combat MDR *Pseudomonas aeruginosa*: In silico screening to target Quorum sensing as a substitution for conventional antibiotics."** in partial fulfillment of the requirements for the award of the Degree of Master of Science, submitted in the Department of Biotechnology, Delhi Technological University is an authentic record of my own work carried out during the period from 2024 to 2026 under the supervision of Dr. Kriti Bhandari

The matter presented in the thesis has not been submitted by me for the award of any other degree of this or any other Institute.

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Certified that Niharika (24/MSCBIO/54) has carried out her research work presented in this thesis entitled "**Anti-virulence strategies to combat MDR *pseudomonas aeruginosa*: In silico screening to target Quorum sensing as a substitution for conventional antibiotics."** for the award of Master of Science from Department of Biotechnology, Delhi Technological University, Delhi, under my supervision. The thesis embodies results of original work, and studies are carried out by the student herself and the contents of the thesis do not form the basis for the award of any other degree to the candidate or to anybody else from this or any other University/Institution.

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"ANTI-VIRULENCE STRATEGIES TO COMBAT MDR *PSEUDOMONAS AERUGINOSA*: IN SILICO SCREENING TO TARGET QUORUM SENSING AS A SUBSTITUTION FOR CONVENTIONAL ANTIBIOTICS"

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ABSTRACT

Pseudomonas aeruginosa is a member of the world health organization's (WHO) ESKAP group and critical priority list because treating MDR (Multidrug-Resistant) *Pseudomonas aeruginosa* infections are clinically more exigent these days. Conventional antibiotics such as beta-lactams, carbapenems, and fluoroquinolones applies fatal force on bacteria that causes brisk mutations for survival producing Anti-microbial resistance (AMR). The pathogen evades host immune system by using enzymes such as elastase and protease that act as virulence factors, these factors are released by pathogen's biofilm that is formed via cell density-dependent microbial communication system called Quorum sensing.

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. 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). 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.

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NIHARIKA

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ABBREVIATIONS

AHL	Acyl Homoserine Lactones
ADME	Absorption, Distribution, Metabolism, and Excretion
3DEM	3D Electron Microscopy
AIs	Autoinducers
DTR	Difficult to Treat
HGT	Horizontal Gene Transfer
BBB	Blood Brain Barrier
CNS	Central Nervous System
ICU	Intensive Care Unit
CYP	Cytochrome P450
LBD	Ligand Binding Domain
ET	Electron Tomography
MBL	Metallo-Beta-Lactamase
GI	Gastrointestinal
IND	Investigational New Drug
MIC	Minimum Inhibitory Concentration
MDR	Multidrug Resistant
XDR	Extensively Drug Resistant
NMR	Nuclear Magnetic Resonance Spectroscopy
P-gp	P-glycoprotein
WHO	World Health Organization
NIH	U.S. National Institutes of Health
PDB	Protein Data Bank
PDBx	Protein Data Bank Exchange
PDBe	Protein Data Bank Europe
Mex	Multiple Efflux
PQS	Pseudomonas Quinolone Signal
QS	Quorum Sensing
TPSA	Topological Polar Surface Area

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. Following the guidelines of IDSA 2024, strains that turned non-susceptible to ceftazidime, meropenem, cefepime, aztreonam, piperacillin-tazobactam, imipenem-cilastatin, ciprofloxacin, and levofloxacin are considered difficult to treat resistance (DTR) [2]. This situation gives rise to "post-antibiotic era" where certain infections become incurable.

The process of acquiring resistance is not abrupt. When the fatal antibiotic force is applied on bacteria, they mutate and may acquire gene of resistance, now the resistant strain survives, multiply and transfer gene of resistance to another bacteria via horizontal gene transfer and therefore decades of antibiotics overuse, incomplete treatment courses and inappropriate prescribing results in Anti-microbial resistance [4]. 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].

Looking at WHO's 2019 report, 1.2 million deaths worldwide are due to antibiotic resistance and if, necessary initiation is not taken this data could attain 10 million per year in coming

future [5]. MDR infections have massive social and economical impact because it causes longer hospital stays, increasing cost and demands for ICU resources.

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 structural labor system is very important in the field of drug targeting. If the one at the top of this control series that is LasR is inhibited by an inhibitor, the consequences will also affect RHL and PQS systems. All three quorum sensing systems can be simultaneously quenched by a single LasR inhibitor, which is a very surprising efficiency from the pharmaceutical science point of view.

1.5 Structural basis of LasR-QsIA complex

LasR is a member of the LuxR protein family that has two distinct parts which perform different functions. It has a Ligand Binding Domain (LBD) at the N-terminal end where 3-oxo-C12-HSL, which is its natural signalling molecule sits. At C-terminal there is a helix-turn-helix DNA-binding domain where LasR binds and captures box promoter sequences [6].

Now the interesting thing is that when LasR does not bind its autoinducer that means it is in the unliganded state, then this protein remains quite unstable and quickly undergo proteolytic degradation. The protein's conformation changes, the structure stabilizes, and the dimerization interface is exposed. This forms an active homodimer complex that binds to DNA and activates transcription that leads expression of virulence genes.

This system has another unique feature that it contains a natural regulator called QslA. This protein is found only in P. aeruginosa and acts as an anti-activator. Two QslA molecules combine to form a homodimer and bind directly to the ligand binding domain of LasR. Upon binding, the dimerization interface of LasR is physically blocked. This means that LasR cannot activate whether the autoinducer is present or not. This is a sophisticated molecular brake that decides when and how quickly QS should be activated. QslaA teaches us an important

biological lesson. It shows that if a molecule binds to the LBD of LasR and prevents its dimerization, The entire quorum sensing system can effectively shut down. This principle is the basis of our drug discovery strategy. We also want to find a molecule that locks LasR in the "off" state like QslA, but we are using natural compounds for this purpose.

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 terpenoid compounds that are made up of 15 carbons, their molecular weight and structural characteristics makes them naturally cooperative with Lipinski drug-likeness criteria and therefore they best fit for LasR inhibition. The main constituent of Vitex agnus-castus essential oil is Beta-caryophyllene. It is a sesquiterpene that display favourable binding and acceptable ADME characteristics against the LasR-QslA complex (PDB ID: 4NG2) [7]. Beta-caryophyllene serve as 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.

Specific Targets:

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. Lead compounds were ranked based on their binding affinity to the native autoinducer and reference compound beta-caryophyllene. The interaction fingerprint of the top molecules was also extracted.
4. Check the ADME profile of the lead compounds using SwissADME to judge GI absorption, CYP liability, and Lipinski compliance to find out whether this can become an orally active drug or not.
5. Combine all data into an antiviral narrative. Binding affinity, interactions, and pharmacokinetics justifies that Glomeremophilane B is ready for experimental validation.
6. In the context of MDR P. aeruginosa, it showed why QS inhibition has an evolutionary advantage over conventional antibiotics. There is no resistance pressure, hence this approach is sustainable.

2. LITERATURE REVIEW

2.1 Infection process of P. aeruginosa

P. aeruginosa is an adaptable pathogen that uses a variety of virulence factors as its weapon to cause infection. First, it uses flagellar motility and type IV pili to adhere to the host's epithelial surface. Surface-active molecules such as rhamnolipids and lipopolysaccharide then break down the epithelial barrier, allowing the infection to reach deeper tissues [2].

The T3SS is a highly potent weapon of this bacterium. This machinery allows the four major toxic proteins, ExoS, ExoT, ExoU, and ExoY, to be directly injected into host cells. The function of ExoU is considered to be the most destructive because it acts like phospholipase and breaks down cells, causing serious injury to the lungs. ExoS and ExoT circumvent the host's defense mechanisms, specifically the process that destroys pathogens by engulfing them. ExoY disrupts the internal signaling system, preventing the cell from responding normally [1].

The effects of P. aeruginosa on the host are evident in several ways. Protein production is inhibited first. ToxA is adept at capturing the elongation factor, it ADP-ribosylates eukaryotic elongation factor EF-2 making it impossible to use it and hence production of protein stops in the tissue that is attacked [1]. The second attack is on tissue structure. An enzyme called LasB breaks down elastin and collagen, which form the basis of tissue. This disease also destroys the main weapons of the immune system. APRA is another enzyme that break down fibronectin weakens tissue joints and deactivates interferons. This breaks down the first line of defense. Pyocyanin, the characteristic pigment of this organism, not only protects the cells from the effects of oxygen but also enhances the cleaning process of the respiratory tract. It also inhibits the activation of T cells, which are the basis of specific immunity [19].

The biggest clinical problem with P. aeruginosa is its ability to form biofilms. When these bacteria are free-floating, antibiotics can easily kill them. But as soon as they stick to a surface and form a colony, their ability to fight antibiotics increases a hundred to a thousand times. This process occurs in several stages. First, bacteria adhere to the surface, then form microcolonies. They then form a slimy coating around themselves called a polysaccharide matrix, which consists of components such as Pel, Psl, and alginate. Gradually, these supporting structures forms a network through which water and nutrients flow. At this stage it is called mature biofilm and antibiotics cannot reach inside. 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.

The structural flexibility of the active site of LasR plays an important role in determining its inhibitory effectiveness. Manu et al.'s 2025 study showed that small changes within the binding cavity affect its shape and volume. As the cavity expands or contracts slightly, the damper has to be adjusted accordingly. Glomeremophilane B gives better results because it binds to multiple contact points across the entire conformational ensemble rather than sticking to a single conformation. This means that whether the cavity is slightly expanded or shrunken, these molecules always form hydrogen bonds with some residue. This dense hydrogen bond network makes its binding affinity different from other compounds.

2.4 Discovered inhibitors of Quorum sensing

In the field of QS inhibitors, many compounds including both natural and artificial, have been tested against the laser system. A 2023 study by Vetrivel et al. was an important step in this direction. They experimentally validated three LasR-blocking agents that were effective even at sub-MIC stars. These compounds inhibited biofilm formation in the PAO1 strain and also controlled the swimming and swarming motility of the bacteria [14]. The production of virulence factors was also significantly reduced. Most importantly, despite all this, bacterial growth was not affected. This made it clear that these compounds were targeting only pathogenicity and not the life of the bacteria. qRT-PCR results confirmed that genes such as lasI, lasR, rhlI, rhlR, lasB, pqsA and pqsR were all downregulated. This means that a single LasR inhibitor produces a ripple effect throughout the QS system. This is a direct validation of cascade regulation.

The search for natural products to inactivate LasR is an active field where different mechanisms are being explored. One pathway that leads to dimerization breakdown was worked out by Soltane et al. in 2023 with norloberidone [17]. Bakuchiol directly targeted transcriptional activators in a 2024 study by Alasiri et al. [17]. These two different methods show how the same receptor can be attacked from different angles. Our approach is also part of this broad strategy but through a new Eremophilane scaffold.

In 2023, Magri et al. identified some food-derived natural compounds whose binding affinity for the LasR receptor was greater than −13 kcal/mol [18]. It is important to look at that natural product libraries contain such strong RNA-complementary structures. In this background glomeremophilane B with −7.9 kcal/mol appears to be a biologically relevant and competitive value.

The druggability of the LasR binding pocket has also been proven through synthetic compounds. In 2024, Manson et al. developed abiotic small molecules that could activate or inhibit LasR and their potency was even greater than that of natural N-acyl homoserine lactones [17]. This shows that this pocket is accessible not only to natural ligands but also to diverse chemical scaffolds. The reference benchmark for our study is beta-caryophyllene from the 2025 study by Azzouni et al. They reported a binding affinity of −5.5 kcal/mol against the LasR-QslA complex [7]. The ADME profile was acceptable but there were some limitations which needed to be improved.

2.5 In silico application to identify anti-microbial strategy

There is no alternative to computational screening in today's drug discovery. This is even more important for anti-infective targets because it is difficult to understand the geometry of the binding site from crystal structures using experimental methods [19]. The PyRx platform bridges this gap. The integration of AutoDock Vina makes this tool reliable and therefore widely used. Screening libraries of thousands of compounds is now possible through an intuitive interface. This accessibility is what makes large-scale virtual screening campaigns feasible [20].

While choosing software for docking two things matter the most that are accuracy and accessibility. We didn't have commercial licenses, so we had to look at freely available options. Vina struck the best balance. Trott and Olson in 2010 have already shown that its performance is comparable to paid tools [21]. The logic of the scoring function is also clear. The overall

binding strength is determined by weighting different interactions [21]. To ensure protocol reliability, we redocked beta-caryophyllene and matched the published value. This gave confidence that the grid parameters were set correctly.

For ADME profiling we used the SwissADME platform. The speciality of this tool is that it provides analysis of not just one parameter but the entire pharmacokinetic profile at one place [22]. Everyone knows Lipinski's rules, but it also checks Weber, Egan and Mugge's criteria, which are found in fewer tools. Prediction of CYP inhibition is particularly important because it is the largest factor in liver metabolism. If the compound inhibits CYP, the risk of drug-drug interactions will increase, which should be detected at the preclinical stage itself. Therefore, by looking at these parameters together, it becomes clear whether a compound is suitable to become an oral drug or not.

For post docking analysis we used BIOVIA Discovery Studio visualizer. This platform is commonly used in the industry for interaction mapping. In this, different types of non-covalent interactions can be identified such as hydrogen bonds, pi-pi stacking, hydrophobic contacts and van der Waals forces. Both 2D and 3D representations can be created, making it easier to understand the binding mode in detail [20].

2.6 Plant derived compounds targeting LasR

The molecular framework of sesquiterpenes is composed of isoprene units that are synthesized via the mevalonate and methylerythritol phosphate pathways. Their C15 core is very flexible. Modifications such as cyclization, oxidation, and hydroxylation give this core different shapes. This is why sesquiterpenes possess such structural diversity that matches their naturally occurring drug-like properties. Their molecular weight ranges from 200 to 300 g/mol, which is well below the Lipinski limit of 500 g/mol. This small size helps them become suitable oral drug candidates.

Beta-caryophyllene is a bicyclic sesquiterpene that is the most studied in this class. Azzouni et al. in 2025 reported it as the main bioactive component of Vitex agnus-castus essential oil and presented its docking and ADME analysis against LasR-QslA [7]. However, this compound has some limitations. Its TPSA is zero, which limits polar interactions. Log P of 4.24 is near the lipophilicity threshold and GI absorption is poor. It also inhibits CYP2C19 and CYP2C9, increasing the risk of drug interactions. All these limitations together show that there is a lot of room for biochemical improvement in beta-caryophyllene and Glomeremophilane B can fill this space.

There is a compound found naturally in mushrooms and plants which is known as Glomeremophilane B. Its PubChem CID is 139589801. It belongs to the Eremophilane series, whose carbon framework is of the pseudo-guaiane type. This fused system of 5- and 7-membered rings is quite rigid. Because of this rigid structure, the functional groups capable of forming hydrogen bonds do not change their position even in different conformational states. This is why its compulsive behavior is easy to predict.

3. METHODOLOGY

3.1 Data Collection and Preparation of Protein and Ligand

3.1.1 Retrieving and Preparing Protein Structures:

The 3D crystal structure of the LasR-QslA complex of P. aeruginosa is taken from the RCSB Protein Data Bank. Its PDB ID is 4NG2 and it 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.

A specific category of compounds remaining after chemical filtering was focused on. Sesquiterpene class molecules were put forward because their good geometric fit with the LasR binding pocket was observed in earlier studies. With this thought in mind, one compound in the library was looked at with the greatest interest. This was Glomeremophilane B, with record number 139589801 in the PubChem database. 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 $\leq$ 500 g/mol, hydrogen bond donor count $\leq$ 5, hydrogen bond acceptor count $\leq$ 10, and Log P $\leq$ 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. RESULTS

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. There was nothing surprising in seeing the screening results. There were some molecules in the library that were just moving around in the binding pocket as if someone had entered the house but was not stuck to any corner. Their energy scores were so low that there was no point in taking them further. However, there were a few others whose numbers suggested they can be worked with. Table 1 lists the seven compounds that ranked highest. This list also includes our reference beta-caryophyllene and the native autoinducer 3-oxo-C12-HSL for easier comparison

Table I: Binding energies of 4NG2 with selected Ligands

Compound	PubChem CID	Binding Energy (kcal/mol)
Glomeremophilane B (Lead)	139589801	−7.9
Penicidone A	24761000	−7.6
N-formyllapatin A	156581132	−7.5
Guignardone C	50905844	−6.8
Photinide E	139586655	−6.2
Trichocladinol B	139584765	−5.9
Nemanolone C	137248626	−5.8
Beta-caryophyllene (Reference)	5281515	−5.5
3-oxo-C12-HSL (Native Autoinducer)	127864	−5.2

To ensure the reliability of the experimental protocol, beta-caryophyllene was re-docked using the same grid parameters. The redocking result was −5.5 kcal/mol, which exactly matches the published value of Azzouni et al. [7]. This match was important because it confirms that our docking setup is configured correctly. Grid parameters and scoring function both are working fine. Now it is assured that the rankings obtained in the library actually reflect the true structural fit of the compounds and are not due to any computational glitch.

Glomeremophilane B scored the best, with −7.9 kcal/mol meaning it was ahead of beta-caryophyllene. But it was binding 2.7 units more tightly than the native autoinducer. This

strong grip is essential for competitive displacement in the infection environment where the concentration of autoinducer is very high. Thermodynamically, it shows approximately a hundred times more binding capacity.

4.2 Glomeremophilane B structural interactions

The binding pose of glomeremophilane B appears to be firmly embedded in the pocket. There are direct hydrogen bonds between the nitrogen atoms of TRP75 and TRP76. OG1 of THR76 and NE2 of GLN103 also formed part of this network. Another interaction was with Leu99, which was of the carbon-hydrogen type. All these together form a dense network which locks this compound in the pocket.

The network of six hydrogen bonds was so tight that the compound was not able to move in the pocket. It doesn't sit as freely as the native ligand. This is why its grip seemed more comprehensive. 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 $Å^2$). 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. It has already been established in structural studies that the density of polar contacts is the key factor for potent LasR inhibition [6,7]. The problem with native autoinducers is that their interaction profile is very mixed. Sometimes polar, sometimes non-polar. For this reason, it does not appear to be selective. The case of glomeremophilane B is different. It has formed multipoint polar contacts that can remain stable even in complex cellular environments. This is the reason why it has a higher competitive edge.

4.4 ADME profile

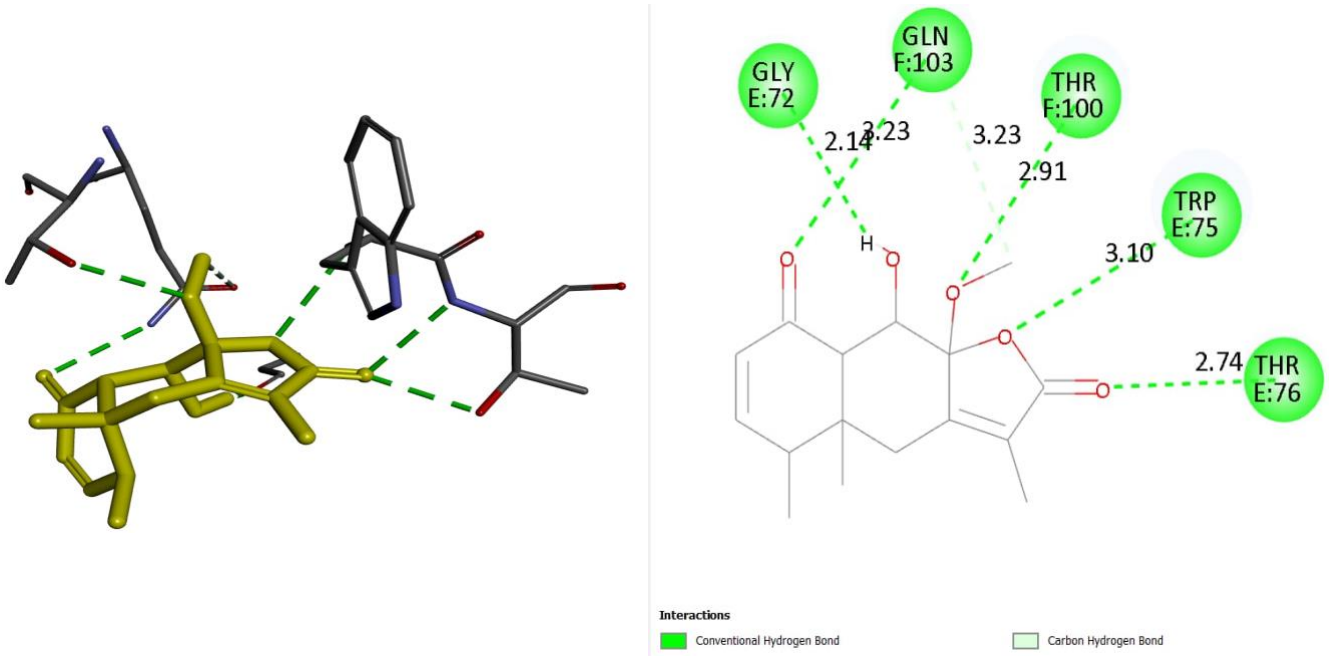
The different parameters derived from SwissADME are arranged in Table 2. In this table, glomeremophilane B is compared with beta-caryophyllene and the native autoinducer to clarify how the lead compound differs from the other two.

Table II: Comparative Analysis of Swiss ADME Result

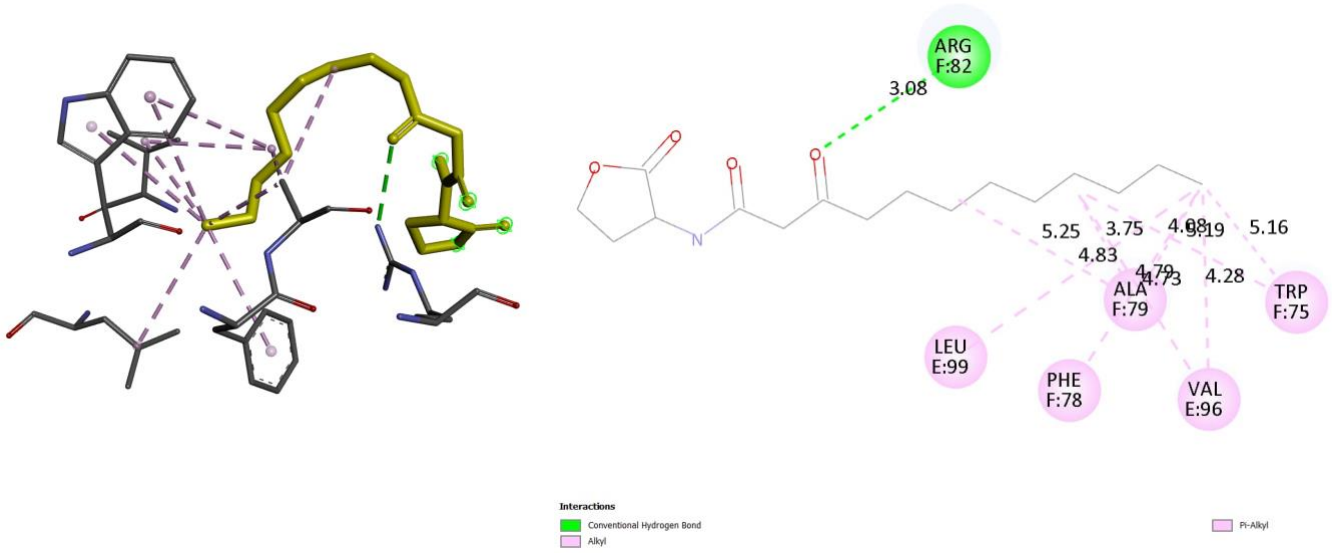
Parameter	Glomeremophilane B	Beta-caryophyllene	3-oxo-C12-HSL
Molecular Weight (g/mol)	292.33	204.35	297.39
Consensus Log P	1.51	4.24	2.93
TPSA (Å²)	72.83	0.00	72.47
GI Absorption	High	Low	High
BBB Permeant	No	No	Yes
P-gp Substrate	No	No	No
CYP1A2 Inhibitor	No	No	Yes
CYP2C19 Inhibitor	No	Yes	Yes
CYP2C9 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

- At first glance at its molecular weight, it appears that this compound is small. 292 g/mol means it can easily cross the epithelial barrier. Lipinski's limit of 500 is far from this.
- Log P is a different matter. A value of 1.51 suggests it is neither very lipophilic nor very hydrophilic. This is the sweet spot for oral drugs. Aqueous solubility is also good and can cross membranes.
- TPSA was measured at 72.83, which is in the high GI absorption range. Also, the hydrogen bonding capacity is good which will be useful with the polar residues of the active site.

- Most importantly, GI absorption was high. This means the oral route is possible. In chronic infections like cystic fibrosis, it can be given in tablet form to the patients instead of giving it IV repeatedly. This is very practical in outpatient management.
- Property of BBB impermeability is particularly beneficial because P. aeruginosa primarily targets lung tissue, infects wounds, and can also enter the systemic circulation. There is no need to go into the brain, so there is no benefit in crossing the BBB. On the contrary, it is good that the compound remains concentrated at peripheral sites and the risk of CNS side effects is reduced.
- 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.



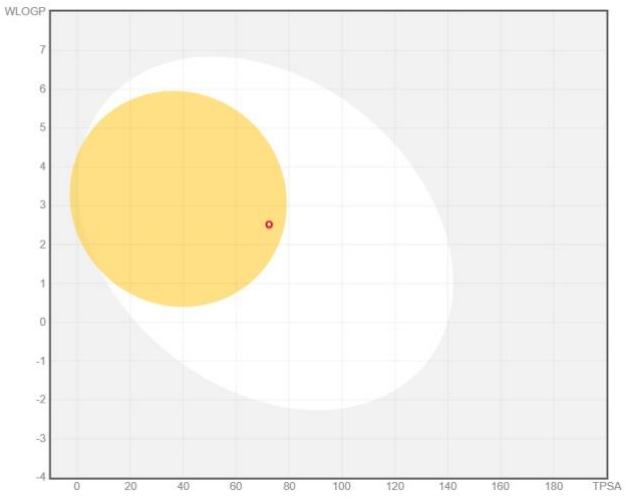
(A) . 3D and 2D representation of 4NG2- Glomeremophilane B complex

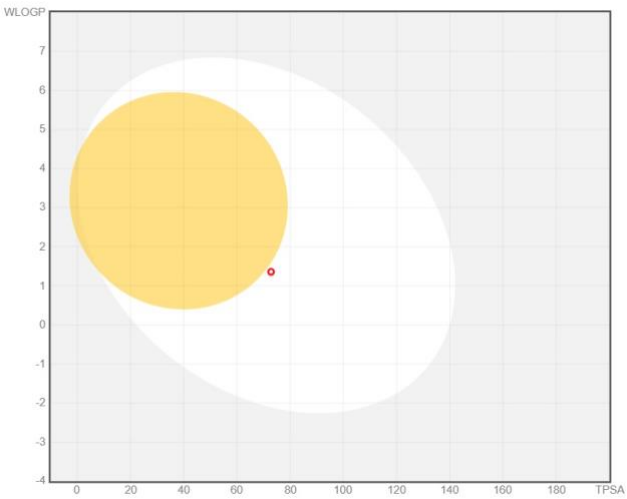


(B) 3D and 2D representation of 4NG2- N-3-Oxo-Dodecanoyl-L-Homoserine Lactone complex

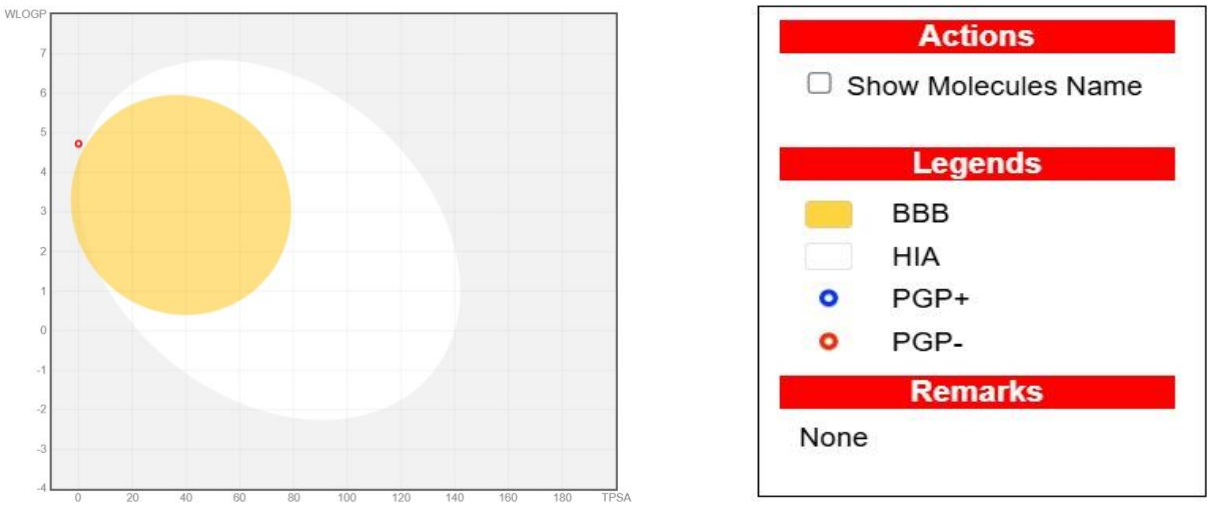
Fig. 1 Showing 3D-2D docking binding results (A) Glomeremophilane B's binding to 4NG2 through conventional hydrogen bonds with (TRP75:N, TRP76:N, THR76:OG1, GLN103:NE2,and LEU99) and Carbon hydrogen interactions with (LEU99).

(B) N-3-oxo-dodecanoyl-L-Homoserine lactone forms strong surface interactions with 4NG2 through conventional hydrogen bonds with (ARG82:NH2) and Pi donor hydrogen bonds with (TYR56, TYR64, SER129). Additionally, there are Pi anion electrostatic connections with (TRP75) and hydrophobic interactions with (TRP88, LEU36, VAL76, ALA127, LEU110, LEU40, ALA50).





N-3-Oxo-Dodecanoyl-L-Homoserine Lactone Glomeremophilane B



Beta-caryophyllene

Fig. II: Boiled Egg Graphs of N-3-Oxo-Dodecanoyl-L-Homoserine Lactone, Glomeremophilane B and Beta-caryophyllene

4.5 Result Interpretation

There are clear ADME impairments in 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, CYP1A2 and CYP2C19 inhibition is the clinical concern, and despite an improved synthesis ease score of 3.05 its metabolic liability profile remains clinically problematic. After all these limitations, Glomeremophilane B therefore proves to be a better alternative as it has high GI absorption, no BBB permeability, no CYP inhibition and zero Lipinski Violation with TPSA of 72.83.

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 edge matters a lot. At the infection site, there is so much autoinducer that the molecule with less binding capacity will simply be left outside. The grip of Glomeremophilane B is so strong that it makes its place even in the crowd.

Now understanding why is this compound so tightly packed in the pocket. It looked as if it was stuck at six places. It is directly bound to the nitrogen of TRP75 and TRP76. The oxygen of Thr76 and the nitrogen of Gln103 also form part of this network. Another contact is with Leu99, which together form a strong grip. Each hydrogen bond has its own direction and enthalpy contribution. Some bonds contribute −1 kcal/mol and some can go up to −5 kcal/mol. When these six different contributions are combined, the overall binding energy is significantly higher. Therefore, molecules that form fewer polar contacts have no chance in comparison.

Observing contacts with TRP75 and THR76 brought to mind an earlier study. Manu et al. had also shown that these residues are crucial for active site stability [7b]. After observing their action, it seemed that our compound was, by chance, targeting these residues. This did not seem like a coincidence. Even when the active site changes its shape, these contacts do not break. Therefore, in addition to static docking, kinetic stability can also be expected. Under physiological conditions, this compound will remain bound for a longer time. This matters a lot for clinical efficacy.

When we talked about scaffold, it seemed that the structure of Glomeremophilane B was saying something different. Flexible molecules can move freely, but its C15 skeleton is so rigid that functional groups can only point in one place. This rigidity may seem like a limitation at first glance, but it actually turns out to be an advantage. When a molecule enters the pocket, its oxygenated parts automatically face the polar residues. No adjustments are needed. The stereochemistry of the Eremophilane ring has already fixed this arrangement. Therefore, binding behavior is more predictable and there are fewer clashes with the cavity topography.

5.2 Scientific justification for targeting virulence mechanism.

After looking at the docking numbers, it dawned on me that this wasn't just a computational exercise. Glomeremophilane B's binding score is actually part of a larger therapeutic argument. LasR-QslA, which controls virulence genes, is not essential for bacterial survival. Genes such as lasB, aprA, toxA, and biofilm matrix operons are all required to infect the host. But if there is no risk of infection, then the bacteria can survive comfortably even without these genes. This is very important from an evolutionary perspective because it means that by blocking this target

the bacteria is not being killed but is simply being rendered helpless. There is no benefit in developing resistance because survival is unaffected.

Bacteria have two options to avoid LasR inhibitors that is either modify LasR so that the inhibitor doesn't detect it, or change the structure of LasR to avoid the inhibitor. Neither approach is easy. With traditional antibiotics, bacteria can survive simply by changing a pump or enzyme. But here, the QS regulatory mechanism itself would be compromised. Defoirdt in 2018 had already understood that targeting QS reduces the chance of resistance [4]. Therefore, this approach is different from conventional antibiotics.

One thing was noticed in mixed communities where there is no antibiotic pressure, QSI-resistant bacteria do not have any significant advantage. These mutants do not grow selectively in normal colonization or environmental survival. The situation is different in the case of traditional antibiotics. There, resistant strains easily outcompete sensitive ones. This advantage is also available at sub-MIC levels. Therefore, the spread of resistance is inherently slow in antiviral approaches. This is a major plus point for long-term treatment.

Inhibiting LasR doesn't just shut down the Las system. It's at the top of the hierarchy, so its effects radiate downward.The RHL is activated and PQS is also regulated through the LAS. Therefore, the effect of competitive inhibition is not limited to the LAS region but spreads throughout the QS network. 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 $Å^2$, well below the 140 $Å^2$ 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. This thing especially matters because P. aeruginosa patients are often already taking multiple medications. Antifungals, bronchodilators, immunosuppressants, and antibiotics are all commonly prescribed. Many of these are metabolized via CYP3A4, CYP2D6, or CYP2C9. If any QSIs inhibit these CYP enzymes, plasma levels of concomitant medications may increase and there may be a risk of toxicity. Glomeremophilane B has a completely clean CYP profile, so this concern is completely eliminated. Its use as an adjunct component in complex therapeutic regimens is 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. It is easy to perform in normal lab conditions without specialized setup [14].

It remains to be seen whether the pose created by static docking will remain stable over time. This requires running an MD (molecular dynamics) simulation for 100 to 500 nanoseconds. This run will also reveal some contacts that were not visible during static docking. The MM-PBSA or MM-GBSA methods will also give a better estimate of the binding free energy because they also take solvation and conformational changes into account. Manu et al.

previously showed that the LasR active site is quite flexible, so MD validation is particularly important 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.

CONCLUSION AND FUTURE PERSPECTIVE

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.

FUTURE PERSPECTIVE

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.
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.

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Defense Institute of Physiology and Allied Science (DIPAS) - DRDO June 2025 – July. 2025
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Worked in a controlled laboratory environment, adhering to ethical and confidentiality protocols. Performed experiments by using the following techniques – Protein Analysis (Bradford's assay, Western Blotting), ELISA and SDS- PAGE, DNA extraction from human blood using Qiagen kit, UV-spectrophotometer and Nanodrop techniques

Delhi Milk Scheme, Government of India July 2023 – Aug. 2023
Intern, Quality Control Lab New Delhi, India

Gained hands on experience in quality assessment of dairy products in accordance with FSSAI standard. Performed routine microbiological and chemical tests on milk products to ensure product safety and compliance. Documented tests results and observations accurately for quality assurance records. Worked alongside food technologists and lab technicians in maintaining quality control protocols.

EDUCATION

Delhi Technological University Aug. 2024 – Jun 2026
Master of Sciences in Biotechnology Delhi, India

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Conference paper

- **Topic**- In-Silico Screening of Natural Compounds as Potential LasR-QsiA complex Inhibitors Against Pseudomonas aeruginosa LasR.
  Presented at the 3rd International Conference on Advances in Engineering and Medical Sciences (ICAEMIS 2026).

Abstract —Molecular Docking and ADME profiling to examine a prepared library of 2500 naturally occurring compounds as LasR- QsiA complex inhibitors.

SKILLS

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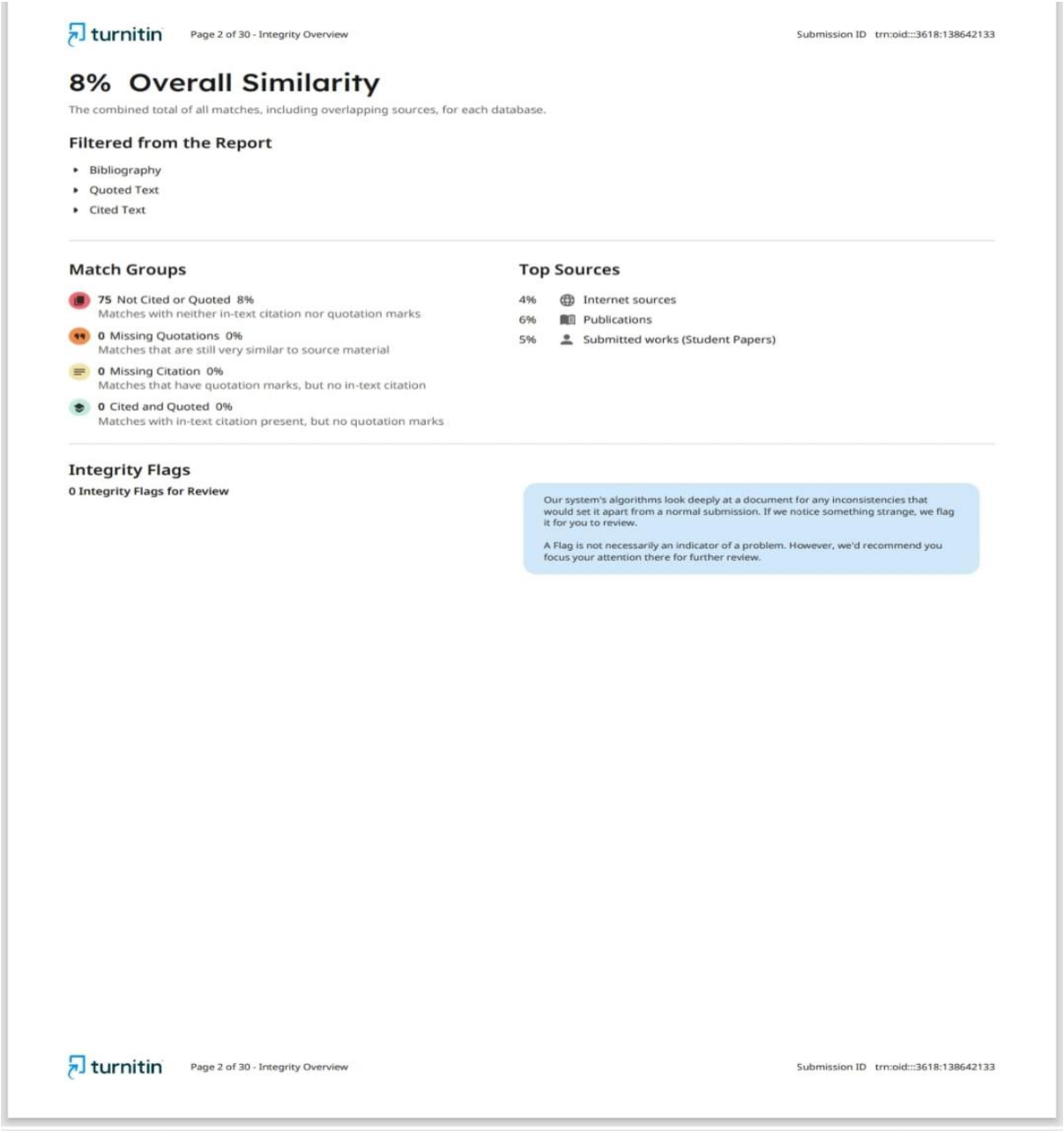

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