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ABSTRACT

Pseudomonas aeruginosa 's virulence and the formation of biofilms are controlled via quorum sensing (QS), a crucial intercellular interaction mechanism that greatly contributes to the bacteria's resistance to drugs. A possible anti-virulence tactic to get beyond traditional antibiotic pressure is to target the LasR receptor, a key transcriptional regulator of the QS system. In order to find effective LasR inhibitors, a thorough in silico screening of a library of 4,431 naturally occurring chemicals was carried out. PyRx was employed to perform molecular docking, and the technique was verified using two well-known inhibitors, Quercetin and Isorhamnetin, which demonstrated binding affinities of -10.5 and -10.4 kcal/mol, respectively. With an exceptionally lowest binding affinity of (-13.4 kcal/mol), virtual screening revealed Aotaphenazine as a superior lead candidate, much surpassing the clinical control, Ciprofloxacin (-7.9 kcal/mol). Aotaphenazine 's pharmacokinetic viability and drug likeness were validated by further ADME profiling, supporting its promise as a bioavailable therapeutic agent. These results establish Aotaphenazine as a potent new anti-virulence lead candidate, offering a solid basis for additional in vitro validation and the creation of innovative therapies against *P. aeruginosa* infections.

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KARTIKEN

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ABBREVIATIONS

AHL	Acyl Homoserine Lactones
ADME	Absorption, Distribution, Metabolism, and Excretion
3DEM	3D Electron Microscopy
AIs	Autoinducers
BIRD	Biologically Interesting Molecule Reference Dictionary
BMRB	BioMagResBank
BBB	Blood Brain Barrier
CNS	Central Nervous System
CCD	Chemical Component Dictionary
CYP	Cytochrome P450
EMDB	Electron Microscopy Data Bank
ET	Electron Tomography
FAIR	Findability Accessibility Interoperability Reusability
GI	Gastrointestinal
mmCIF	Macromolecular Crystallographic Information File
MX	Macromolecular Crystallography
MDR	Multidrug Resistant
NLM	National Library of Medicine
NMR	Nuclear Magnetic Resonance Spectroscopy
P-gp	P-glycoprotein
PUG-REST	Power User Gateway - REpresentational State Transfer

PUG-View	Power User Gateway – View
PDB	Protein Data Bank
PDBx	Protein Data Bank Exchange
PDBe	Protein Data Bank Europe
PDBj	Protein Data Bank Japan
PQS	Pseudomonas Quinolone Signal
QS	Quorum Sensing
TPSA	Topological Polar Surface Area
NIH	U.S. National Institutes of Health
RCSB PDB	Research Collaboratory for Structural Bioinformatics Protein Data Bank
wwPDB	Worldwide Protein Data Bank
EPS	Extracellular Polymeric Substances
GlcNAc	N-acetylglucosamine
HQNO	2-heptyl-4 hydroxyquinoline-N-oxide
SpA	Staphylococcal protein A
SiaD	diguanylate cyclase

CHAPTER 1

INTRODUCTION

Pseudomonas aeruginosa is a resistant to many drugs ESKAPE infection that particularly concerning because of the capacity to create strong biofilms that operate as a physical obstacle to antibiotics and host immune reactions, and makes traditional antibacterial therapies ineffective.[1] Individuals with AIDS, burn, cystic fibrosis, and neutropenic cancer die from *Pseudomonas aeruginosa*, through an opportunistic infection.[2] QS is a system of intercellular communication that produces, identifies, and responds to autoinducers (AIs). The activities that are QS controlled include motility, conjugation, drug resistance, biofilm formation, sporulation, antibiotic tolerance, and in addition to the generation of virulence variables. Acyl homoserine lactones (AHLs) are a very special class of AIs that had been shown to regulate Gram-negative microbial QS. Along with these two main families of AIs, it had demonstrated that microbes use an array of other signalling chemicals, including

(i) Lipids by *Ralstonia solanacearum*, *Xanthomonas*, *Xylella*, and *Burkholderia* species
(ii) Ketonic compounds by *Vibrio spp*. and *Legionella spp*.;
(iii) Steroids created by enterohemorrhagic bacteria;
(iv) (PQS) synthesized by *P. aeruginosa*;
(v) Pheromones and brief peptides generated by Gram-positive microbes, Bacilli.[3]

Antimicrobial therapies such as the poor effectiveness of medicines and increased mortality because of *P. aeruginosa*'s high innate drug resistance and flexibility.[4] The capacity of *P. aeruginosa* to shape biofilms that shield bacteria from external stimuli, prevent phagocytosis, and so provide invasion and prolonged survival makes treating infections such as these more difficult.[5] Therefore, the goal of this research is to find possible pharmaceuticals that might successfully blind bacteria by blocking LasR, which can further stop biofilm development and increase the germs' susceptibility to

immune clearance. This work uses a structure based in silico technique to test a built library of 4431 natural compounds against the LasR ligand-binding domain.

CHAPTER 2

LITERATURE REVIEW

2.1 Quorum Sensing and Biofilm Formation

Pseudomonas aeruginosa is an opportunity-driven natively gram-negative type of pathogenic microbe. About 10% for invasive microbial infections are often caused by them.[6] Particularly in immune-compromised hosts, where death rates can exceed 40%, because of its wide range of virulence indictors and naturally ability to adjust to various environments, it may cause a number of serious, frequently fatal chronic and acute medical conditions. For people with compromised immune systems, it might be fatal. Furthermore, they are contributor to pneumonia contracted in hospital and respiratory disorders, which are among the key cause of the main causes of microbial-illness and septic in neutropenic cancer undergoing chemotherapy.[7] The protein that activate transcription LasR with 175 amino acids (PDB ID: 2UV0) is necessary for the activation of several genes which are linked to its virulence nature.[2]

There are five main phases in the creation of biofilms:

- Stage I: Cells of bacteria use its cell extension to stick to surface, such as type IV pili and flagella.[8] Its restricted flagellar mobility has been associated with exopolysaccharide production and motility are necessary for surface adhesion.[9] It is possible to reverse this adherence. Proteomic studies utilizing wild-type PAO1 which had shown that the development of biofilms and bacterial reactions depend on the substance. The existence of distinct proteins in microbes and how their amounts change when *P. aeruginosa* detects and responds to particular surfaces serve as examples of this.[10]
- Stage II: The adherence of bacterial cells shift proteins of bacteria and from transient to permanent.
- Stage III: Microorganisms that are related to one another and subsequently proliferate into a more ordered structure and form microcolonies.

- Stage IV: The growth of these microcolonies turns into massive, three-dimensional forms that resemble to mushroom like structure, which is a key feature associated to biofilm development.
- Stage V: The matrix cavity in the centre of the microcolony is then broken apart by cell autolysis, which cause these cells to be released and dispersed. The biofilm cycle then can be repeated when uncolonized regions are seeded via a shift from a solitary to a plankton like mode of development (Stage VI).[11]

Recent research had shown that endonuclease EndA is necessary regarding the scattering of preexisting biofilm by destroying eDNA.[12] Swarming movement affects the structure of the biofilms that are created; very mobile bacterium produce biofilms that are flat, whereas cells with in sufficient propulsion results in biofilms that resemble mushrooms, and the proportion of locomotion varied depending on the nutrition.[13]

2.1.1 Phases of *P. aeruginosa* biofilm expansion

The Synthesis cycle is consisted of six steps. Lipids, proteins, polysaccharides, and eDNA are among the extracellular polymeric substances (EPS) that the bacteria produce after adhering to the surface. Then mitosis and the transition from reversible to permanent attachment. The next stage involves the development of microcolonies and their subsequent development into structures that resembles mushrooms. The development and durability of biofilms depend on interaction between cells and the synthesis of virulence variable. Cell autolysis then produces a matrix hole in the centre of the microcolony to release the dispersed population. Over the course of around two hours, the released cells eventually get transform into planktonic morphologies and settle in uncolonized regions. The procedures via which connected forms give way to a free-swimming lifestyle that results in extremely virulent detached cells are poorly understood, despite the general belief that biofilm cells vary physiologically in contrast to their planktonic predecessors and are more resilient to antimicrobial therapies. It is believed that a specific stage of biofilm formation is connected to the shift from disengagement to planktonic expansion. (Stage VI).[14] [15]

2.1.2 Multispecies Biofilm

Instead of a single species, a diverse polymicrobial population colonizes the area. *Staphylococcus aureus*, *Burkholderia cenocepacia*, and *Streptococcus parasanguinis* are among the bacteria that *P. aeruginosa* is frequently viewed as a hybrid conqueror.[16] [17] N-acetylglucosamine (GlcNAc), a peptidoglycan created by *S. aureus*, causes *P. aeruginosa* to produce pyocyanin when these two species coexist in a dual-species colonization. Pyocyanin has toxic and antibacterial qualities that may reduce *S. aureus*'s capacity to endure inside the biofilm.[18] When *P. aeruginosa* transitioned from nonmucoid phenotypes that generated rhamnolipids, 2-heptyl-4 hydroxyquinoline-N-oxide (HQNO) including antimicrobial siderophores to mucoidal morphologies that intentionally overproduced alginate, these exoproducts reduction, leading to this cohabitant. [19] The external adhesin Staphylococcal protein A (SpA), which is released by *S. aureus*, binds to Psl and type IV pilli on the surface of microbial cells, preventing *P. aeruginosa* from forming biofilms and neutrophils from phagocytosing *P. aeruginosa*.[20]

It has been demonstrated that *P. aeruginosa* outcompetes *S. aureus* in the double species habitat by generating SiaD, a diguanylate cyclase that PsI activates during the first stages of biofilm development. [21] Despite this, *P. aeruginosa* maintains its dominance in mixed species biofilms by generating antimicrobial molecules that prevents other microbes from growing.[22]

2.2 Mechanism for Quorum Sensing in *P. aeruginosa*

According to the reports, *P. aeruginosa* possesses four connected QS signaling networks: LasI-LasR, RhlI-RhlR, PQS-MvfR, and IQS. Enzymes that are LasI and RhlI synthase turn on the Las and Rhl pathways, which generate acyl homoserine lactone (AHL) signaling molecules like 3-oxododecanoyll-homoserine lactone (3-oxo-C12-HSL) and butyryl-l-homoserine lactone (C4-HSL), correspondingly.[23] These compounds, 3-oxo-C12-HSL and C4-HSL, interact and subsequently turn on the corresponding transcription criteria, LasR and RhlR, this also contributes to the formation of biofilm and a number of factors that contribute to virulence, like elastase,

peotidases, pyocyanin, rhamnolipids, lectins and toxic substances[24] Interestingly, the Las system in *P. aeruginosa* is the main QS regulator and starts the activation of the Rhl and Pqs signaling pathways.[7] People with compromised epithelial barriers, such as those with severe burns, tracheal intubation, or mechanical breathing, *P. aeruginosa* can potentially cause acute infections.

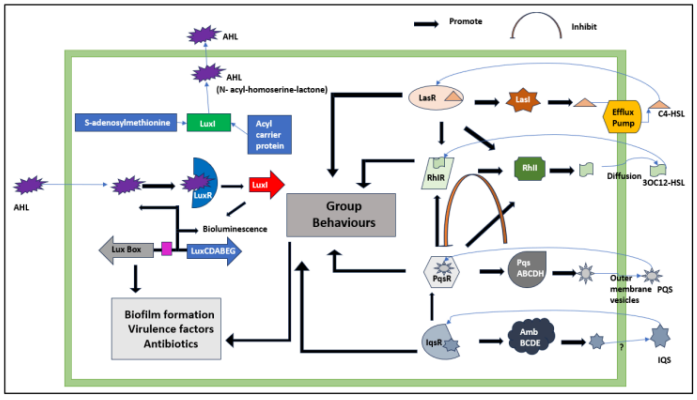


Fig I. Quorum Sensing Mechanism

Three QS systems are used by it: The PQS system, a non-LuxI/LuxR pathway, and LuxI/LuxR-type QS circuits that modulates the production of virulence compounds sequentially manner. During initial loop, LasI generates 3-oxo-C12-homoserine lactone (3OC12HSL). Furthermore, the (LasR-3OC12HSL) intricate triggers the intended genes activity, that encodes virulence agents such exotoxin, proteases, and elastase. Another luxI homolog, rhlI, is likewise regulated by (LasR-3OC12HSL). RhlI produces a second AI, butanoyl homoserine lactone (C4HSL). When the dose is high, these AI interacts to RhlR, which is another LuxR homolog. RhlR–C4HSL activated the targeted genes that encode elastase, proteases, pyocyanin, and siderophores. When appropriately folded, LasR and likely other LuxR-type receptors get dimerize, and binds with DNA, and start the process of transcription. *P. aeruginosa* makes use of an extra non-LuxI/LuxR QS mechanism to regulate pathogenicity component expression

in genes. PQS, 2-heptyl-3-hydroxi-4-quinolone, which is generated by Pqs family genes, is identified through the regulator, PqsR (also called MvfR). LasR3OC12HSL raises the phrase of pqsH and pqsR in contrast RhlR–C4HSL suppresses pqsABCD and pqsR. In addition to increasing rhlI and rhlR expression, PqsR–PQS autoinduces PQS synthesis. The PQS circuit also influences the production of virulence factors due to its tight connection with LasI/LasR and RhlI/RhlR QS systems. The QS-virulence factors that are activated of *P. aeruginosa* comprise elastase, peptidase, pyocyanin, twitching motility and rhamnolipids. Even yet, different regulons for RhlR–C4HSL and LasR–3OC12HSL have been discovered, the regulons clearly overlap. As a result, most of the genes that were first thought to be solely targeted by either RhlR or LasR can really be activated by both. Low levels of rhlI and rhlR conveying seem to enhance the C4HSL buildup and RhlI/RhlR network self-induction. Consequently, the virulence factor activation of the DlasR strain is delayed. Since many clinical isolates of *P. aeruginosa* include lasR mutations, these findings have therapeutic ramifications. It is not it is unexpected that more regulatory levels have an impact on the QS networkings because QS controls up to 10% of the *P. aeruginosa* genetic makeup. Since QS regulates the synthesis of pathogenicity compounds factors and there are no human homologs of known QS components, the *P. aeruginosa* QS networks are desirable objective for new antimicrobials. Since many *P. aeruginosa* isolates are resistant to current treatments, this is particularly important for treating recurrent infections in patients with cystic fibrosis.[24] The autoinducer, also known as an acylhomoserine lactone (acyl-HSL or AHL), is a signal molecule that starts the quorum sensing process and is constitutively produced by the bacterial synthase LuxI. AHL diffuses across the bacterial membrane attaches to LuxR, a transcriptional stimulation of the luciferase operon, and activates LuxR. When *P. aeruginosa*'s LasI synthase constitutively generates the message 3-oxo-C12-HSL (N3-oxododecanoyl-L-homoserine lactone), LasR, a transcriptional regulator similar to LuxR, is activated. Exoproteases, exotoxins, and secondary metabolites are some of the harmful pathogenic compounds whose expression is triggered by the association of targeted gene promoters by LasR dimers. Additionally, biofilms, which often result in persistent pathogenic infections, are encouraged by active LasR.[2]

2.2.1 LasR Binding Pocket and Regulation

Thirty percent of the more than 350 genes in *P. aeruginosa* that encode the virulence variables are more probably controlled by the process of quorum sensing. There is 6 intermolecular hydrogen bonds—five direct and one mediated by water—were discovered in TYR56, TRP60, ARG61, ASP73, THR75, and SER129. AHLs with comparable HSL headgroups share an activation mechanism, as evidenced by the significant conservation of TYR56, TRP60, ASP73, and SER129 in LuxR homologues. In contrast, the lengthy acyl chain penetrates a hollow encircled by hydrophobic residues, a few of these are unique to LasR and lacking in other LuxR homologues, including LEU40, TYR47, CYS-79, and THR80. These remnants that are variable, which binds to the very active methylene/methyl groups of the acyl chain, are not present from the shorter AHL ligands of most LuxR homologues. As a resut of LasR tightly get encapsulates the AHL, even very point mutation that are conservative in the AHL binding pocket can make it unusable.[2]

CHAPTER 3

METHODOLOGY

3.1 Data Collection and Preparation of Protein and Ligand

3.1.1 Retrieving and Preparing Protein Structures:

The PDB was the open-access molecular data repository for biology when it was founded in 1971. PDB is still the sole global atomic level repository, three-dimensional structure data ($making$ > 144000) which incudes of architecture established by experimentation of proteins, DNA, and RNA, as well as their sophisticated configurations with metal compounds, medications, and other small molecules. The wwPDB partners are very much committed towards maintaining the FAIR (Findability, Accessibility, Interoperability, and Reusability). To comprehend the operational roles that how macromolecules biological plays and medicine, PDB is now universally acknowledged as an essential data source. Additionally, financers of both official and nonprofit organizations studies are demanding the PDB collections for macromolecular architectural information that wasn't published. After that, all concerning this 3D structural data is saved in the case of the two wwPDB Core Archives. The PDB Core Archive also contains pertinent data from experiments and information from Macromolecular Crystallography (MX). Nuclear Magnetic Resonance spectroscopy (NMR) experimental data and metadata are stored in the BioMagResBank (BMRB; www.bmrb.wisc.edu) Core Archive. The Electron Microscopy Data Bank (EMDB; www.emdb-empiar.org), which contains relevant experimental data and metadata from 3D electron microscopy (3DEM) and electron tomography (ET), collaborates closely with the wwPDB partners. The 3D structure of *Pseudomonas aeruginosa* LasR Ligand was acquired in PDB format from the RCSB PDB database (https://www.rcsb.org/structure/ 2UV0). Formerly dispersed over multiple PDB entries, massive structures were put together under an individual PDB entries implementing the PDBx/mmCIF format. Every three-dimensional framework in the PDB Archive are currently delivered and archived in sepcific format. Wherever feasible, the wwPDB has maintained to construct structure in the archive accessible in

legacy format for the benefit of the user community. To be clear, the conventional PDB format, which was discontinued in 2012, does not conveys the broad range of sophisticated metadata present in PDBx/mmCIF files. The bulk of important structural biology data repositories and software tools have switched to the PDBx/mmCIF format, and the wwPDB strongly cautions against sticking with the antiquated PDB file format. discovered the LasR binding site pocket in Chain E by tracking the active site's amino acid chain and examining the protein structure in BIOVIA. Water molecules, heteroatoms, and all other chains except Chain E were eliminated. For docking analysis, low-confidence areas were visually examined and eliminated. After that, the protein was stored in PDBQT format for the docking process. Chemical attributes are described in each description of a chemistry component as of stereochemical assignments, chemical structure descriptors (SMILES and InChI), systematic chemical names, chemical formulae, and idealized atomic coordinates.[25]

3.1.2 Building a Ligand Library:

The National Library of Medicine (NLM), a subsection of the National Institutes of Health (NIH) in the USA, offers a chemical database that is open to the public called PubChem (https://pubchem.ncbi.nlm.nih.gov). It collects data from more than 750 data sources and allows the broader public to view it without restriction. During peak hours, PubChem has around on an annual basis in each month, about four million distinct interactive users, making it one of the most popular chemistry websites globally. PubChem is a vital source for chemical information for biomedical teams of researchers from several fields, such as medicinal chemistry, chemical biology, chem informatics, and drug development. Notably, PubChem is often utilized as a massive data source for molecule characterization, virtual evaluation, chemical toxicology prediction, medication repurposing, and adverse effect prediction in data science and machine learning research. PubChem arrange its information using three databases: Substance, Compound, and Bio Assay. Chemistry data supplied by depositors is stored in the substance database. The Compound database has distinct chemical structures that are taken from the Substance database. The Bio Assay database contains test results and biological assay descriptions. Every entry in these databases, PubChem has a committed homepage that shows its accessible data. Additionally, PubChem offers

information about a certain gene, protein, pathway, and patent through webpages that present many viewpoints. A variety of methods for accessing online services, including as PUG-REST and PUG-View, may be used to programmatically get PubChem data, or web interfaces can be used for interactive retrieval. Because PubChem is an open service that millions of people utilize from diverse backdrop, it is difficult for it to satisfy the data requirements of every user. For example, Pub Chem has to effectively manage massive volumes of diverse data gathered from hundreds of data sources across several scientific fields, while attempting to address significant differences in data from science format and reduce data mistakes. PubChem has undertaken a variety of adjustments to deal with these as well as additional issues since our latest update article published in this publication. PubChem currently has 271 million points of information on biological activity from 1.2 million biological test methods, 111 million unique chemical structures, and more than 293 million substance descriptions provided by depositors.[26]

A library of 4431 Natural Compound's 3D Structures including our Control Ciprofloxacin drug were prepared by searching natural product and applied following filters criteria like molecular weight (300 to 400g/mol), H-Bond Donors(0 to 5), H-Bond Acceptors(0 to 10), Rotatable Bonds (0 to 10), LogP (ALogP) (1 to 5), TPSA (Topological Polar Surface Area) (20 to 140) and Heavy Atoms (< 40) in pubchem site (https://pubchem.ncbi.nlm.nih.gov). Ligands were obtained in SDF format from here and via Open Babel Tool convert all of them into PDBQT format.[27]

3.2 Docking and Analysis of Molecules

Molecular docking is a method of computation that the aims to accurately forecast non-covalent binding of macro-molecules, of a receptor and a its corresponding partner, beginning with their structures that are not bound, structures obtained from the modelling of homology and conventional MD simulations, etc. Among the several computational methods for simulating receptor-ligand interaction,

- Explicit solvent based molecular dynamics,
- Implicit solvent in molecular mechanics and dynamics,
- Molecular docking.

Might be considered increasingly sacrificing computational efficiency for visual depth. These strategies depend on specific assumptions, like individual molecules commitment toward a particular protonation atomic phase and a consistent charge agreement within, for instance, their confined and free-floating states. Furthermore, even if docking considers a particular group of freely rotatable covalent bonds (which is referred to simply as actively rotatable bonds below), it often presupposes that the majority or entire molecule of the aforementioned receptor is inflexible and that the covalent lengths and angles are fixed. Crucially, docking eventually has to do with replicating chemical potential, that affects the bound conformational section and the liberated energy for binding, while molecular dynamics, by contrast, deals solely with energies (also referred to as force field in chemistry). It is totally new idea that depends not only on minimum threshold of the energy profile but also on the minima of the energy profile but also on the temperature and shape of profile. Docking systems often use a scoring mechanism and may be thought of as an effort to replicate the systems conventional chemical potential. To correct for this discrepancy between energies compared to free energies, the scoring function purportedly physics-based variables, such as the 6-12 van der Waals relation and coulomb energies, must be substantially statistically weighted. It ought to come as no surprise that such purportedly physics-driven scoring systems don't always perform better than the alternatives given the previously listed factors. We think that rather of being just physics based, our methods to the scoring system are more machine learning- based. In place of theoretical issues based on some potentially overly strong approximation assumptions, it is eventually demonstrated by its performance on test situation.[28]

AutoDock Vina were used for molecular docking via the PyRx program(https://sourceforge.net/projects/pyrx/).[14] The LasR ligand-binding domain containing only saved chain E of the 2UV0 protein structure was covered in the grid box

- $(centre: X = 24.77; Y = 11.63; Z = 77.70)$
- $(Dimension: X = 25.00; Y = 25.00; Z = 25.00)$. [29]

3.3 Integration Analysis and Visualization

Investigators may examine antibody characteristics including stability, antigenicity, aggregation tendency, solubility, viscosity, and more by creating a multidimensional-models from protein sequences, which is often a crucial stage in the antibody design process. These models may also be utilized to comprehend and forecast antibody-antigen connections when combined with protein-protein docking techniques. Approval test of the BIOVIA (previously Accelrys) protein modelling package completely automated antibody structure prediction tool.[30] BIOVIA Discovery Studio Visualizer was used for interaction analysis and post-docking visualization. For molecular interaction analysis, each 2UV0 ligand complex's top-ranked binding poses (based on the lowest binding affinity) were imported into Discovery Studio. For every excipient, Van der Waals forces, hydrogen bonds, and polar/nonpolar relationship were identified and shown. In order to comprehend the nature of interaction between 2UV0 surface residues and phytochemical ligand molecules, binding postures were examined in both 2D interaction diagrams and 3D structure diagrams.

3.4 Phytochemical Safety Assessment

Whenever there is several chemicals being considered but limited the creation of drugs involves evaluating possession of physical specimens i.e. ADME. Many fast and accurate prediction for pharmacokinetics, physicochemical capabilities, drug assurity are available for free use through the Swiss ADME online service. These models include internal expert methods such as the Bioavailability Radar, iLOGP, and BOILED Egg. The website's login-free interface is easy to use (https://www.swissadme.ch/index.php) ensures easy and efficient input and interpretation. To help with drug development cheminformatics and computational chemistry specialists and novices alike may swiftly anticipate crucial characteristic for a set of compounds.

To assess drug-likeness and safety, pharmacokinetic profiling was carried out using the Swiss ADME. To estimate oral bioavailability, we compared the top-ranked ligands to Lipinski's Rule of Five. This crucial filtering stage guaranteed lead candidates with favourable ADME characteristics appropriate for additional therapeutic development. A molecular sketcher is constructed upon the top of the input data zone directly

ChemAxon's Marvin allows the consumer to transfer, alter, and convert a 2D chemical structure to a list of molecules. This list, this is the real input for computation and is shown on the delivery page's right-hand side. Since SMILES may be edited just like any other text, it can be written or pasted. The list contains each line contains one input molecule, denoted by a SMILES and, if desired, a gap between a name. If the name is left out, Swiss ADME will consequently supply an identification. The fact that the buttons for doing the computation and moving the picture to the SMILES list are dynamic—that is, they only become active when the action is possible—is remarkable. A drug-like molecule should respond in one to five seconds as of this writing.[31]

CHAPTER 4

RESULTS AND DISCUSSION

We have docked 4431 natural compounds from our prepared library against LasR i.e. (2UV0). Whereas (Table-I) summarizes the binding affinities (kcal/mol) of our top 113 compounds. Compared to the conventional antibiotic, Aotaphenazine showed the lower binding affinity (-13.4 kcal/mol).

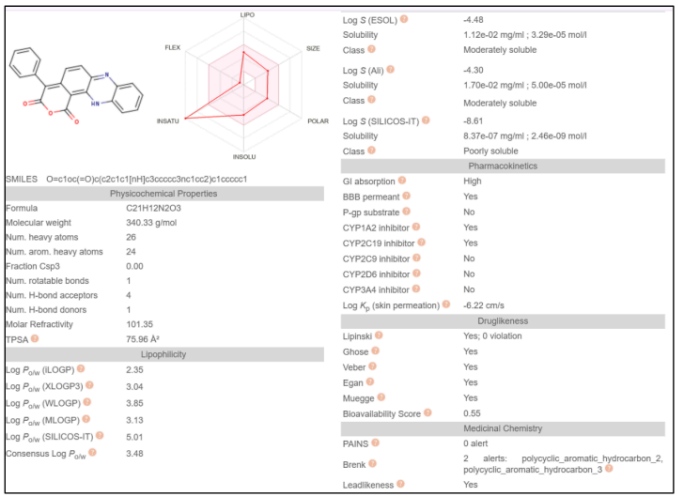
Table I: Binding energies of (2UV0) with selected Ligands

S. No.	Compound CID	Name	Binding Energy (KCal/mol)
1	132512138	Aotaphenazine	-13.4
2	169492188	Lijiquinone	-13.3
3	46223919	Dermacozine F	-13.1
4	5073	Risperidone	-12.6
5	9926867	Calothrixin A	-12.5
6	170990340	Paraphamide A	-12.4
7	16095273	Chaetominine	-12
8	10890506	Aspergione D	-11.9
9	132512161	Amethysione	-11.9
10	74574	4,4'-Oxydiphthalic anhydride	-11.8
11	115072	(-)-Merulidial	-11.8
12	135666745	Raistrickin	-11.7
13	72340	Tetrahydroalstonine	-11.6
14	221014	2-Anthraquinonesulfonyl chloride	-11.5
15	5281954	Tectochrysin	-11.4
16	24829329	Essramycin	-11.4
17	3714245	1,3-Bis(benzotriazol-1-yl)propan-2-one	-11.3
18	5281805	Pseudobaptigenin	-11.3
19	10359753	Daidzein diacetate	-11.3

20	170989581	Irpexolaceus C	-11.3
21	124337	Demethoxyviridin	-11.2
22	132576260	Paramagnetoquinone B	-11.2
23	3718	Indoprofen	-11.1
24	23648	Aflatoxin B2 alpha	-11.1
25	295934	Deoxynyboquinone	-11.1
26	5288320	Topiroxostat	-11.1
27	5335621	Pyr-41	-11.1
28	11481760	Quinolactacide	-11.1
29	71317597	Arizonin B1	-11.1
30	122218854	Cyclopiamide D	-11.1
31	139584135	Tmc-120-C	-11.1
32	139584147	Speradine H	-11.1
33	156582352	Phomopsinin C	-11.1
34	170989334	Penithochromone M	-11.1
35	65679	Droxicam	-11
36	67030	2-Anthraquinonecarboxylic acid	-11
37	95022	1,3-Indandione, 2-benzoyl	-11
38	101746	Sesamolin	-11
39	54697518	Phaeochromycin C	-11
40	122178808	Brasilamide G	-11
41	139585867	Monascodilone	-11
42	146682641	Phaseolorin G	-11
43	146682794	Fusaravenin	-11
44	146683034	Asperfuranoid C	-11
45	170989033	Asperisocoumarin G	-11
46	442021	Brucine	-10.9
47	3482905	Phytoalexin	-10.9
48	11129041	Aspergione F	-10.9
49	11277734	(Z)-Pterulinic acid	-10.9
50	132502747	Asperisocoumarin E	-10.9

51	139586323	Phomopsidone A	-10.9
52	4369359	Sitagliptin	-10.8
53	5748605	Cladrin	-10.8
54	9578005	Dexlansoprazole	-10.8
55	10756746	Clavilactone D	-10.8
56	54679633	Pinastric acid	-10.8
57	139584288	Azaphilone Multiformin B	-10.8
58	139588404	Coniochaetone	-10.8
59	156581085	Ulosporin F	-10.8
60	156581392	Asperisocoumarin B	-10.8
61	156583096	Similanpyrone A	-10.8
62	170990342	Paraphamide C	-10.8
63	68783	Nomegestrol	-10.7
64	102210	Gestonorone	-10.7
65	154279	(-)-Alpinetin	-10.7
66	4053302	Alpinetin	-10.7
67	5280445	Luteolin	-10.7
68	6914273	Dantrolene	-10.7
69	10331844	Napabucasin	-10.7
70	10445309	Pseudodeflectusin	-10.7
71	10967532	5-Deoxybostrycoidin	-10.7
72	11065168	Aspergione B	-10.7
73	15123840	4-(4-Nitrobenzoyl)benzonitrile	-10.7
74	132496757	Versicomide D	-10.7
75	139590240	Mangrovamide H	-10.7
76	139590241	Mangrovamide I	-10.7
77	146682434	Pyromyxone C	-10.7
78	146684355	Cochlearin F	-10.7
79	72307	Sesamin	-10.6
80	94672	Sesaminol	-10.6
81	5280378	Formononetin	-10.6

82	5281697	Scutellarein	-10.6
83	56598333	(-)-Trypethelone	-10.6
84	87112884	Roseobacticide G	-10.6
85	122178809	Brasilamide H	-10.6
86	122218855	Cyclopiamide E	-10.6
87	132523656	Biatriosporin K	-10.6
88	156019355	Rhodocorane F	-10.6
89	156581534	7-Methoxydehydrocyclopeptin	-10.6
90	163112036	Huanglongmycin B	-10.6
91	170989840	Amphichoterpenoid E	-10.6
92	334704	(+)-Marmesin	-10.5
93	5280343	Quercetin	-10.5
94	5280863	Kaempferol	-10.5
95	5281699	Tamarixetin	-10.5
96	10708688	Circumdatin F	-10.5
97	102146277	Orinocin	-10.5
98	132523661	Biatriosporin D	-10.5
99	139586544	Citridone D	-10.5
100	139588652	Fontizine A	-10.5
101	139591431	Asperorydine G	-10.5
102	145720952	Oxepinamide K	-10.5
103	146682900	Lansai F	-10.5
104	3883	Lansoprazole	-10.4
105	3899	Leflunomide	-10.4
106	4487	Nifenazone	-10.4
107	92201	Columbianetin	-10.4
108	161271	Salvigenin	-10.4
109	441140	Griseofulvin	-10.4
110	5281654	Isorhamnetin	-10.4
111	10015944	Bhimamycin B	-10.4
112	10802469	Sclerotigenin	-10.4



SMILES	O=c1oc(=O)c(c2c1c1[nH]c3ccccc3nc1oc2)c1ccccc1

Physicochemical Properties	
Formula	C21H12N2O3
Molecular weight	340.33 g/mol
Num. heavy atoms	26
Num. arom. heavy atoms	24
Fraction Csp3	0.00
Num. rotatable bonds	1
Num. H-bond acceptors	4
Num. H-bond donors	1
Molar Refractivity	101.35
TPSA	75.96 Å²

Lipophilicity	
Log $P_{o/w}$ (iLOGP)	2.35
Log $P_{o/w}$ (XLOGP3)	3.04
Log $P_{o/w}$ (WLOGP)	3.85
Log $P_{o/w}$ (MLOGP)	3.13
Log $P_{o/w}$ (SILICOS-IT)	5.01
Consensus Log $P_{o/w}$	3.48

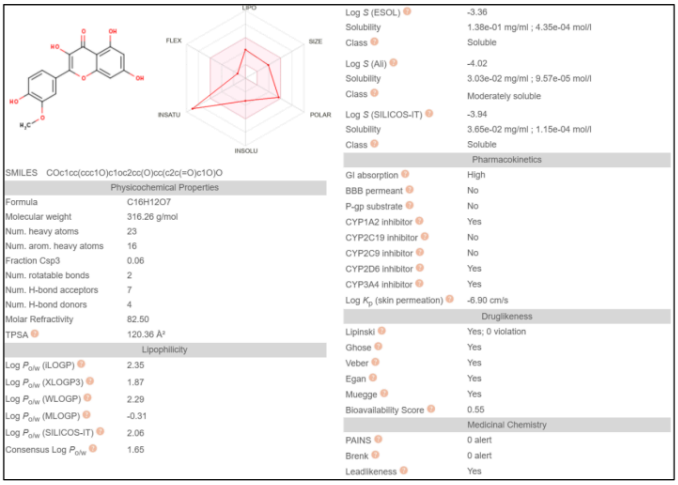
Log *S* (ESOL)	-4.48
Solubility	1.12e-02 mg/ml ; 3.29e-05 mol/l
Class	Moderately soluble
Log *S* (Ali)	-4.30
Solubility	1.70e-02 mg/ml ; 5.00e-05 mol/l
Class	Moderately soluble
Log *S* (SILICOS-IT)	-8.61
Solubility	8.37e-07 mg/ml ; 2.46e-09 mol/l
Class	Poorly soluble

Pharmacokinetics	
GI absorption	High
BBB permeant	Yes
P-gp substrate	No
CYP1A2 inhibitor	Yes
CYP2C19 inhibitor	Yes
CYP2C9 inhibitor	No
CYP2D6 inhibitor	No
CYP3A4 inhibitor	No
Log K_p (skin permeation)	-6.22 cm/s

Druglikeness	
Lipinski	Yes; 0 violation
Ghose	Yes
Veber	Yes
Egan	Yes
Muegge	Yes
Bioavailability Score	0.55

Medicinal Chemistry	
PAINS	0 alert
Brenk	2 alerts: polycyclic_aromatic_hydrocarbon_2, polycyclic_aromatic_hydrocarbon_3
Leadlikeness	Yes

(A) Aotophenazine result



SMILES	COc1cc(ccc1O)c1oc2cc(O)cc(c2c(=O)c1O)O

Physicochemical Properties	
Formula	C16H12O7
Molecular weight	316.26 g/mol
Num. heavy atoms	23
Num. arom. heavy atoms	16
Fraction Csp3	0.06
Num. rotatable bonds	2
Num. H-bond acceptors	7
Num. H-bond donors	4
Molar Refractivity	82.50
TPSA	120.36 Å²

Lipophilicity	
Log $P_{o/w}$ (iLOGP)	2.35
Log $P_{o/w}$ (XLOGP3)	1.87
Log $P_{o/w}$ (WLOGP)	2.29
Log $P_{o/w}$ (MLOGP)	-0.31
Log $P_{o/w}$ (SILICOS-IT)	2.06
Consensus Log $P_{o/w}$	1.65

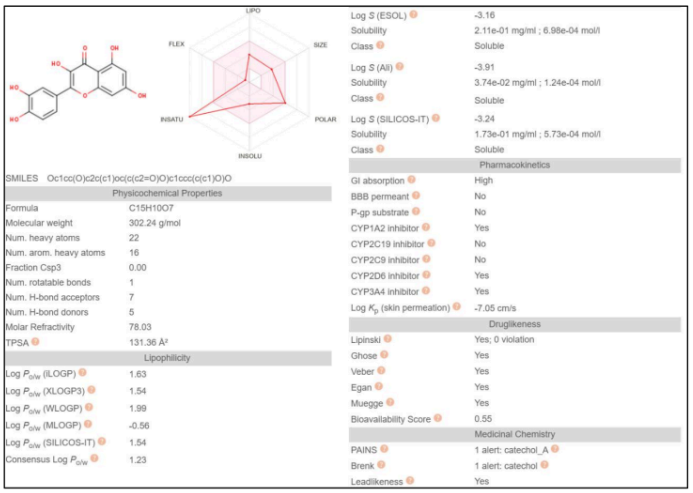
Log *S* (ESOL)	-3.36
Solubility	1.38e-01 mg/ml ; 4.35e-04 mol/l
Class	Soluble
Log *S* (Ali)	-4.02
Solubility	3.03e-02 mg/ml ; 9.57e-05 mol/l
Class	Moderately soluble
Log *S* (SILICOS-IT)	-3.94
Solubility	3.65e-02 mg/ml ; 1.15e-04 mol/l
Class	Soluble

Pharmacokinetics	
GI absorption	High
BBB permeant	No
P-gp substrate	No
CYP1A2 inhibitor	Yes
CYP2C19 inhibitor	No
CYP2C9 inhibitor	No
CYP2D6 inhibitor	Yes
CYP3A4 inhibitor	Yes
Log K_p (skin permeation)	-6.90 cm/s

Druglikeness	
Lipinski	Yes; 0 violation
Ghose	Yes
Veber	Yes
Egan	Yes
Muegge	Yes
Bioavailability Score	0.55

Medicinal Chemistry	
PAINS	0 alert
Brenk	0 alert
Leadlikeness	Yes

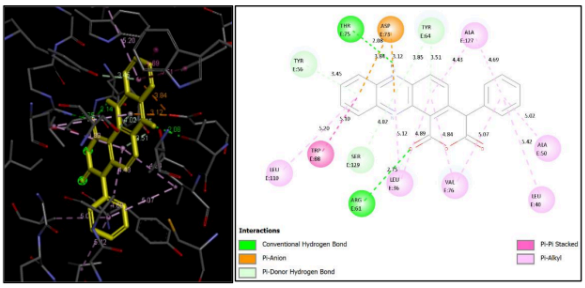
(B) Isorhamnetin result



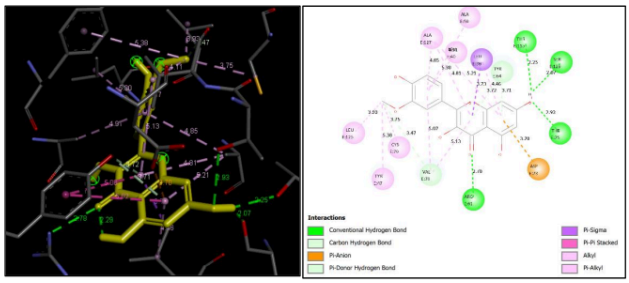
(C) Quercetin result

Fig. II: Showing SwissADME Comparative analysis result: (A) Aotaphenazine, (B) Quercetin and (C) Isorhamnetin.

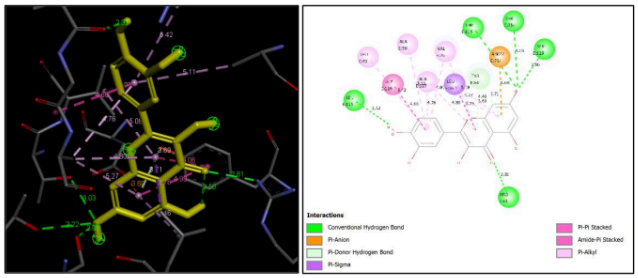
Compounds like Aotaphenazine, Isohamnetin and Quercetin are shown in (Fig III) with their best possible binding interaction in LasR's chain E active binding pocket site. (Fig III) shows both 3D and 2D interactions to suggest binding nature. Which strongly suggest Aotophenazine with good binding interaction nature among them.



(A) Aotophenazine - 2UV0 binding



(B) Isorhamnetin - 2UV0 binding



(C) Quercetin – 2UV0 binding

Fig. III: Showing 3D-2D docking binding results (A) Aotaphenazine forms strong surface interactions with 2UV0 through conventional hydrogen bonds with (THR75, ARG61) and Pi donor hydrogen bonds with (TYR56, TYR64, SER129). Additionally, there are Pi anion electrostatic connections with (ASP73) and hydrophobic interactions with (TRP88, LEU36, VAL76, ALA127, LEU110, LEU40, ALA50). (B) Isorhamnetin binding to 2UV0 through conventional hydrogen bonds with (THR115, SER129, ARG61, THR75); Pi donor hydrogen bonds with (TYR64); and carbon-hydrogen bonds with (VAL76). Additionally, it exhibits hydrophobic interaction with (LEU36, TYR64, VAL76, CYS79, LEU125, ALA127, LEU40, ALA50, and TYR47.) (C) Quercetin binding to 2UV0 through conventional hydrogen bonds with (LEU125, ARG61, THR75, THR115, SER129) and Pi donor hydrogen interactions with (TYR64). It exhibits an electrostatic binding with (ASP73) and hydrophobic contact with (LEU36, TYR64, GLY126, VAL76, ALA127, LEU40, ALA50).

Fig II and Fig IV suggest that among them Aotaphenazine is the only substance that can effectively increase its therapeutic potential to encompass Central Nervous System (CNS) targets by passing across the blood-brain barrier (BBB). Additionally, Aotaphenazine high intracellular retention is further ensured by its resistance to P-glycoprotein (P-gp) efflux. All of these findings point to Aotaphenazine having the ideal ratio of lipophilicity to metabolic stability needed to be a potent oral medication.an cross Blood Brain Barrier.

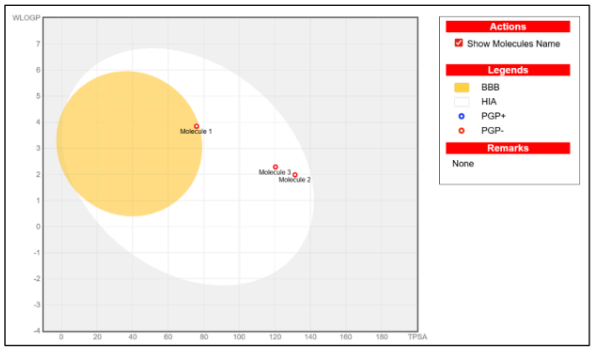


Fig. IV: Boiled Egg Graph: where molecule 1 (Aotaphenazine), molecule 2 (Quercetin) and molecule 3 (Isorhamnetin).

4.1 Result Interpretation

This study provides in-silico evidence that Aotaphenazine is a potent LasR inhibitor. The molecular docking tool PyRx was employed to evaluate the binding affinity of 4431 natural compounds against *Pseudomonas aeruginosa*. In comparison to our chosen control Ciprofloxacin, the binding energies ranged from -13.4 kcal/mol to -7.9 kcal/mol, indicating that over 2318 molecules exhibited a thermodynamically favourable interaction with the receptor (2UV0). However, the top 113 natural compounds significantly outperformed the control drug, ciprofloxacin (-7.9 kcal/mol). Aotaphenazine had the lowest binding affinity (-13.4 kcal/mol), suggesting binding more strongly to the LasR active site than the traditional antibiotic. To ensure the correctness of the in-silico process, the docking data were validated against known

LasR inhibitors. The high binding affinities of quercetin (-10.5 kcal/mol) and isorhamnetin (-10.4 kcal/mol) were effectively replicated by the procedure, which is consistent with the experimental results of Sawi et al. [29] Accuracy of the grid box dimensions and AutoDock Vina parameters used in this study is confirmed by this validation. According to our findings, ciprofloxacin (-7.9 kcal/mol) has higher binding affinity than Aotaphenazine (-13.4 kcal/mol). The communication network necessary for the production of biofilms is disrupted by LasR inhibitors. These natural phytochemicals are able to successfully compete with the natural autoinducer for the LasR binding pocket at far lower doses than conventional antibiotics, according to the good docking scores. Moreover, the Swiss ADME the outcome of conventional forecasting indicated that the molecular weight of Aotaphenazine is 340.33 g/mol and a consensus Log *Po/w* of 3.48. By preventing CYP1A2 and CYP2C19 isoforms while staying dormant against CYP2C9, CYP2D6, and CYP3A4, the possibe biochemical connections are suggested by the pharmokinetics profile. Furthermore, it predicts high BBB permeability and GI absorption. Using a synthetic accessibility score of 3.27 and a bioavailability score of 0.55, the substance exhibits notable drug-likeness and satisfies Lipinski's Rule of Five with no violations. Whereas Quercetin has a molecular weight of 302.24 g/mol and a consensus Log *Po/w* of 1.23. The pharmacokinetic profile predicts significant gastrointestinal (GI) absorption but shows that the drug is not permeable to the blood-brain barrier (BBB). Potential metabolic interactions are indicated by the inhibition of CYP1A2, CYP2D6, and CYP3A4 isoforms but not CYP2C19 or CYP2C9. Strong drug-likeness and zero violations of Lipinski's Rule of Five are demonstrated by the molecule, which has a bioavailability score of 0.55 and a synthetic accessibility score of 3.23. The Isorhamnetin has a molecular weight of 316.26 g/mol and a consensus Log *Po/w* of 1.65. The pharmacokinetic profile predicts significant gastrointestinal (GI) absorption but shows that the drug is not permeable to the BBB. While it is inactive against CYP2C19 and CYP2C9, the reduction of CYP1A2, CYP2D6, and CYP3A4 isoforms suggests potential metabolic interactions. The molecule has a synthetic accessibility score of 3.26 and a bioavailability score of 0.55, indicating significant drug-likeness and zero violations of Lipinski's Rule of Five. Aotaphenazine's SwissADME results recommend that, it possesses a molecular size of 340.33 grams per mole. That's according to the optimal drug-like size gap

(<500 gram per mole), that encourages receptive movement and diffusion kinetics. Fraction Csp^3 value 0.00 shows that it is completely flat, stiff and highly twisted and with no saturated carbons. It drastically lowers core 3D complexity even though this rigid shape might enhance binding affinity by reducing entropic costs upon binding. This increases likelihood of non-specific hydrophobic interaction like DNA intercalation and poor formulation solubility. Using single rotatable bond build it very stiff structure. Veber's criteria said in order to improve oral-available; molecule should have ≤10 rotatable bond. Highly successfully passively membrane movement is predicted since TPSA is much below 140$A^{\circ 2}$ threshold for cell permeability and below the 90 $A^{\circ 2}$ threshold for normally needed for CNS crossing. A comparatively powerful lipophilic nature is indicated by LogP of 3.48. it is on the upper end, that can lead to undesirable binding and raise hepatic enzyme metabolism clearance rate, regardless of that it's contained in a acceptable drug like bound. The compound's entirely flat aromatic framework is the cause of the substantial disparity between (SILICOS-IT class recommend that molecule is poorly aqueous soluble) and (ESOL/Ali class suggest molecule is moderately aqueous soluble) approach. The procedure for dissolution is frequent impeded by the elevated crystal lattice anticipated from a totally planar molecule. The perfect combination of moderate lipophilicity suggests very high Gi absorption which make it highly viable oral delivery. It crosses with ease the BBB. This is fantastic if the objective is situated in CNS. Yet, the profile suggested a potential risk of CNS based off-target toxicity if it is intended for a peripheral target. The chemical is not vulnerable to P-gp-mediated efflux active transport. This aids in maintaining intracellular concentrations by preventing the substance from being aggressively pushed out of intestinal lumen or brain parenchyma. Drug-Drug Interaction risk is evident when CYP1A2 an CYP2C19 are selectively inhibited. Co-administration of therapeutic pharmaceuticals that are mostly metabolized by these two isoforms may result in hazardous plasma buildup of such co-medication. Zero violation of Lipinski, Ghose, Veber, Egan, Muegge model and with bioavailability score of 0.55 suggested that compound has good drug-like characteristics and high likelihood of therapeutic plasma exposure when taken orally. The scaffold does not naturally function as a covalent modifier, chelator, or redox-cycler in standard

biochemical tests, as confirmed by the absence of Pan-Assay Interference Compounds warnings. Rather than being artifactually screening hits, the reported in vitro target activities are probably specific. The molecular mass and lipophilicity are sufficiently low to allow upcoming alternations to structure or metabolite synthesis, regardless the stiffness. The extended, continuous polycyclic aromatic ring structure is flagged by the Brenk filter. Heavy polycyclic aromatic systems are intensively examined in medicinal chemistry because potential for toxicity, mutagenicity, and poor metabolic stability.

CHAPTER 5

CONCLUSION AND FUTURE PERSPECTIVE

This work successfully employed a structure-based in silico technique to look into the anti-virulence capability of 4431 natural compounds against the LasR quorum sensing regulator of *Pseudomonas aeruginosa*. It has been demonstrated that Aotaphenazine works well against LasR quorum sensing. As far as we are aware, this research is the first to, discuss the use of a specific LasR inhibitor, Aotaphenazine, in the management of bacterial infections. Aotaphenazine is an encouraging lead scaffold for the advancement of novel Quorum Quenching medicines that can disarm drug-resistant *Pseudomonas aeruginosa* without using selective pressure. Future research ought to concentrate on biofilm inhibition testing and in vitro validation to bolster these computational findings. When compared to Quercetin, Isorhamnetin, and Aotaphenazine, all three compounds show good gastrointestinal absorption, excellent drug-likeness, and complete adherence to Lipinski's Rule of Five while maintaining acceptable Synthetic Accessibility scores. The only drug that can successfully expand its therapeutic potential to include CNS targets by crossing the BBB is Aotaphenazine. Furthermore, by inhibiting fewer isoforms of Cytochrome P450 (especially avoiding the primary CYP3A4 and CYP2D6 pathways), Aotaphenazine has a far better metabolic profile and reduces the likelihood of adverse drug-drug interactions. Its resistance to P-glycoprotein (P-gp) efflux further guarantees high intracellular retention. Beside few red flags in Aotaphenazine our main structural optimization objectives for this scaffold should be to disrupt the planarity by including heteroatoms linkers, cyclohexyl bioisosteres, or saturated alkyl decorations to improve water solubility and disrupt crystal packing. And minimize DDI risks by mitigation CYP inhibition by adding steric hindrance or changing electronic densities around the particular aromatic rings engaging with the CYP1A2/CYP2C19 binding sites. According to all of these results, Aotaphenazine possesses the optimal lipophilicity to metabolic stability ratio required to be a powerful oral drug.

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Kartiken

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EXPERIENCE

Anandi Institute Apr. 2025 – Present

Chemistry Teaching Faculty Sadar Bazar, Delhi Cantt-110010

- A dedicated Chemistry teacher with a proven track record at Anandi Institute, effectively taught and mentored students in classes XI, XII NEET and JEE. Job role involved designing comprehensive lesson plans, delivering engaging lectures, and providing personalized guidance to help students excel in their academic pursuits.

Nishant Tanwar Classes Dec. 2022 – Apr. 2024

Biology Teaching Faculty Janakpuri, Delhi Cantt-110010

- A dedicated Biology teacher with a proven track record effectively taught and mentored students in classes XI, XII, and NEET. Job role involved designing comprehensive lesson plans, delivering engaging lectures, and providing personalized guidance to help students excel in their academic pursuits.

EDUCATION

Delhi Technological University Jun. 2024 – Jun 2026

Master of Sciences in Biotechnology Delhi, India

Lab Expertise: Biocomputational, Molecular Docking

Sri Venkateswara College (University of Delhi) Dec. 2020 – Jul 2023

Bachelor of Sciences in Life Sciences New Delhi, India

Coursework: Zoology, Botany, Chemistry

Research Work

Unpublished (Conference Paper)

- **Topic**-In-Silico Identification of Natural compound as High-Affinity Quorum Sensing Inhibitors against *Pseudomonas aeruginosa.*
- **Abstract** —Molecular Docking and ADME profiling to examine a prepared library of 4431 naturally occurring compounds as LasR (2UV0) inhibitors.

SKILLS

- **Tools**: TeX Maker, PyRx, AutoDock Vina, BIOVIA Discovery Studio, SwissADME

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