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Original Article

Virtual Screening of Potential Quorum Sensing Inhibitors of *P. aeruginosa*

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ABSTRACT

Background: *Pseudomonas aeruginosa* (*P. aeruginosa*) is considered as one of the most opportunistic pathogens that may infect humans and led to increase in bacterial virulence or pathogenicity. *P. aeruginosa* exhibits variety of virulence factors and their rate of expression are associated with cell-to-cell communication process also known as quorum sensing (QS). LasR, a transcriptional factor which regulates the process of QS in *P. aeruginosa* is known as attractive drug target. **Materials and Methods:** The research work involves identification of putative inhibitors of LasR by molecular docking approach. Total 60 compounds were docked in the active site of LasR and CviR protein, followed by subsequent screening based on Lipinski's rule of five, Veber rule and molar refractivity. **Results:** Out of 60 compounds, total seven novel compounds were selected on the basis of binding energies (docking score> 10). Structures of LasR-inhibitor complexes were analyzed to have vital insights from the binding between LasR and inhibitor molecules. The selected compounds were analyzed for physico-chemical properties and drug likeness to establish correlation between oral bioavailability and pharmacokinetics of compound. **Conclusion:** The study revealed the potential of thiazole derivative as novel QSI and expedite the possibility to combat virulence of multi-drug resistant *P. aeruginosa* in more effective manner.

Keywords: Drug-resistance, *Pseudomonas aeruginosa*, LasR protein, Quorum sensing inhibitor, Molecular docking, Lipinski's rule.

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INTRODUCTION

P. aeruginosa is a rod-shaped, gram negative, opportunistic multi-drug resistant pathogen that causes severe infections in humans. The bacterium is recognized for its intrinsically advanced anti-microbial resistance mechanisms resulting in serious consequences of infections in hospitalized patients, especially those with immuno-compromised and ventilator-associated pneumonia.[1] *P. aeruginosa* exhibits variety of virulence factors and its expression is dependent on the quorum sensing mechanism which involves cell to cell communication. Quorum sensing (QS) in *P. aeruginosa* plays a crucial role in spreading of infections *via* regulation of biofilm formation, secretion of variety of virulent factors and exchange of genetic material (DNA).[2] Bacterial biofilm proliferates and fix on surfaces that are hidden by exopolymers forming multiple layers of bacterial community. Formation of these biofilm contributes to the development of secondary infections which is extremely difficult to eradicate and thus bacteria become resistance towards conventional anti-microbial agents. *P. aeruginosa* may show resistance to antibiotics via two mechanisms: first it involves the transfer of plasmids among bacterial community; second involves the targeted mutation in bacterial genome of pathogen. Both these resistant mechanisms offer a shield effect, enhances the survival of bacterial community and in turn reduces the activity of conventional antibiotics. The QS mechanism involves the production and release of auto-inducer namely N-acyl Homoserine Lactone (AHLs) which in suitable concentration bind to specific receptors and forms a receptor-AHL complex. This receptor-AHL complex acts as transcriptional modifiers of target genes in the QS region and controls the expression of virulent gene as well as induction of biofilm formation.[3-4] Two different QS circuit have been observed in *P. aeruginosa*. These circuits are genetically similar containing genes that encodes for transcriptional activator proteins (LasR and RhlR) and also genes involved in the production of AHL signaling molecules (lasI and rhlI). These QS systems produce autoinducers like N-(3-oxododecanoyl)-L-homoserine lactone (3-oxo-C12-HSL) and N-(butanoyl_-L-homoserine lactone (C4-HSL) which generates intracellular signals and are essential for the production of extracellular virulence factors and bacterial biofilms.[5] Also, another signaling molecule i.e., 2-heptyl-3-hydroxy-4-quinolone (PQS) is also involved in the QS system of *P. aeruginosa* which regulates the expression of lasB gene encoded for LasB elastases protein causing virulence. A novel approach i.e., 'anti-virulence strategy' is considered as an alternate and potential method targeting these signaling molecules and blocking expression of virulent factors thus preventing bacterial infections without interfering with the growth of pathogen.[6-7] Nowadays, virtual screening is considered as an integral part of drug discovery process. *In-silico* computational methods offer great advantage of discovery and development of novel small molecules as drugs/leads in no time and also predict the parameters that reflect the compound's behavior in synergism to its *in-vivo* performance. Several compounds have been developed and reported to have great anti-QS potential but despite of this, none of them so far is in clinical use because of pharmacokinetics and toxicity implications. The molecular docking approach is highly beneficial as the resultant lead compounds can be directly evaluated for clinical studies and drug development process.[8-10] This research work aimed at identifying novel quorum sensing inhibitors (QSIs) of target protein (LasR) of *P. aeruginosa* by using virtual screening

studies. A supported literature by Borges A. *et al*., and Chourasiya *et al*., suggested that 'thiazoles derivatives' showed stronger interaction, better than that of native ligand (OHN) with the target protein (LasR).[11-12] In the present study, initially we searched the data on reported inhibitors of gram-negative bacteria specially *P. aeruginosa* and collected total 60 compounds. Afterwards, we computationally predict a database of 60 reported inhibitors (QSIs) of LasR protein (a target responsible for virulence production) of *P. aeruginosa* and analyze the physico-chemical behaviour to identify putative LasR inhibitors against *P. aeruginosa* infections.

MATERIALS AND METHODS

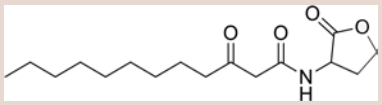
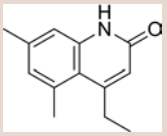
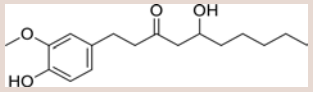
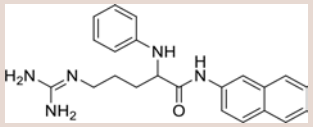
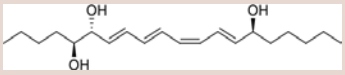
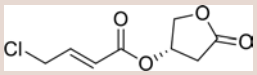
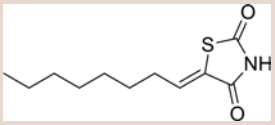
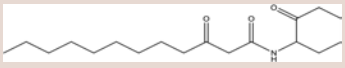
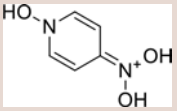
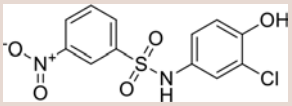
Materials: Software such as Chem Bio Draw ultra-version 12.0 2010, AutoDock Vina v. 1.1.2, and Pymol were used for docking studies.

Methods

Molecular Docking: Data Set Preparation

Two-dimensional chemical structures of all 60 reported compounds were drawn out and geometrically optimized using Chem Bio Draw ultra-version 12.0 2010, Cambridge Soft, Chem Bio office Ultra, 2010. Geometric optimization and energy minimization was done by using

Table 1: Data set of 60 reported molecules active against LasR, Rhl and PQS signals of *P. aeruginosa*.

Compound Code	IUPAC Name	Chemical Structure	Reference
OHN	3-oxo-N-(2-oxooxolan-3-yl)dodecanamide		15
C1	4-ethyl-5,7-dimethyl-1,2-dihydroquinolin-2-one		16
C2	5-hydroxy-1-(4-hydroxy-3-methylphenyl)decan-3-one		17
C3	5-[(diaminomethylidene)amino]-N-(naphthalen-2-yl)-2-(phenylamino)pentanamide		18
C4	2-(2-dodecyl-2H-1,2,3,4-tetrazol-5-yl)acetic acid		19
C5	(5S,6R,7E,9E,11Z,13E,15S)-icosa-7,9,11,13-tetraene-5,6,15-triol		20
C6	(3S)-5-oxooxolan-3-yl(2E)-4-chlorobut-2-enoate		21
C7	(5Z)-5-octylidene-1,3-thiazolidine-2,4-dione		22
C8	3-oxo-N-(2-oxocyclohexyl)dodecanamide		23
C9	N,N,1-trihydroxy-1,4-dihydropyridin-4-iminium		24
C10	N-(3-chloro-4-hydroxyphenyl)-3-nitrobenzene-1-sulfonamide		25

continued...

Table 1: Cont'd.

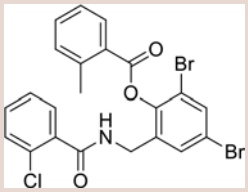
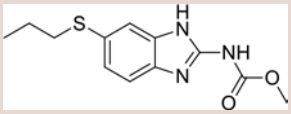
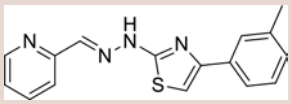
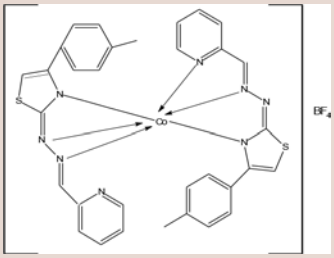
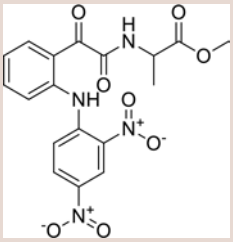
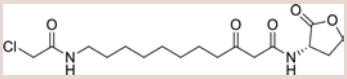
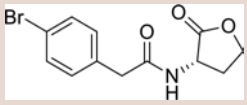
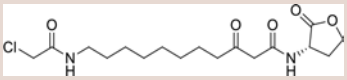
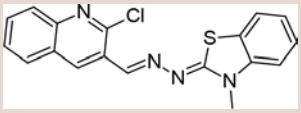
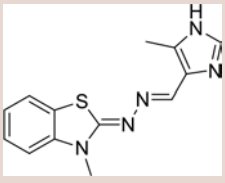
Compound Code	IUPAC Name	Chemical Structure	Reference
C11	2,4-dibromo-6-{[(2-chlorophenyl)formamido]methyl}phenyl 2-methylbenzoate		26
C12	Methyl N-[6-(propylsulfanyl)-1H-1,3-benzodiazol-2-yl]carbamate		27
C13	2-[(E)-{2-[4-(3-methylphenyl)-1,3-thiazol-2-yl]hydrazin-1-ylidene}methyl]pyridine		11
C14	2-[(E)-{2-[(2E)-4-(4-methylphenyl)-3-propyl-2,3-dihydro-1,3-thiazol-2-ylidene]hydrazin-1-ylidene}methyl]pyridine; pyridine; {3-[(2E)-2-[(2E)-2-ethylidenehydrazin-1-ylidene]-4-(4-methylphenyl)-1,3-thiazolidin-3-yl]propyl}cobalt		11
C15	methyl 2-(2-{2-[(2,4-dinitrophenyl)amino]phenyl}-2-oxoacetamido)propanoate		28
C16	N-{[(3R)-2-oxooxolan-3-yl]methyl}decanamide		29
C17	2-(4-bromophenyl)-N-[(3S)-2-oxooxolan-3-yl]acetamide		29
C18	11-(2-chloroacetamido)-3-oxo-N-[(3S)-2-oxooxolan-3-yl]undecanamide		29
C19	2-((2-Chloroquinolin-3-yl)methylene)hydrazono)-3-methyl-2,3dihydrobenzo[d]thiaz		12,30
C20	(2Z)-3-methyl-2-[(2E)-2-[(5-methyl-1H-imidazol-4-yl)methylidene]hydrazin-1-ylidene]-2,3-dihydro-1,3-benzothiazole		12

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Table 1: Cont'd.

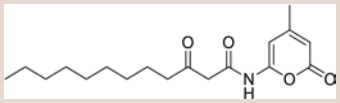
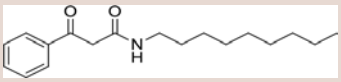
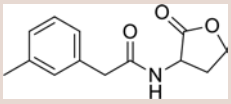
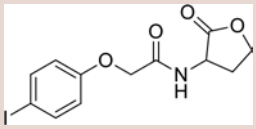
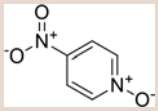
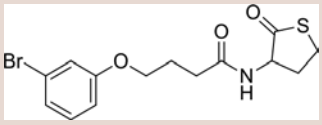
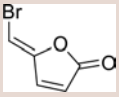
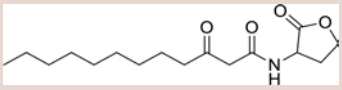
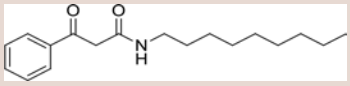
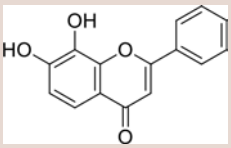
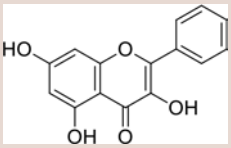
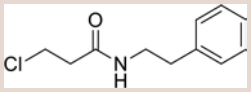
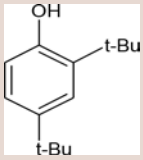
Compound Code	IUPAC Name	Chemical Structure	Reference
C21	N-(4-methyl-2-oxo-2H-pyran-6-yl)-3-oxododecanamide		31
C22	N-nonyl-3-oxo-3-phenylpropanamide		32
C23	2-(3-methylphenyl)-N-(2-oxooxolan-3-yl)acetamide		32
C24	2-(4-iodophenoxy)-N-(2-oxooxolan-3-yl)acetamide		33
C25	4-nitropyridin-1-ium-1-olate		34
C26	4-(3-bromophenoxy)-N-(2-oxothiolan-3-yl)butanamide		35
C27	(5Z)-5-(bromomethylidene)-2,5-dihydrofuran-2-one		36
C28	3-oxo-N-[(3S)-2-oxooxolan-3-yl]dodecanamide		37
C29	N-nonyl-3-oxo-3-phenylpropanamide		37
C30	7,8-dihydroxy-2-phenyl-4H-chromen-4-one		26
C31	3,5,7-trihydroxy-2-phenyl-4H-chromen-4-one		26
C32	3-chloro-N-(2-phenylethyl)propanamide		38
C33	2,4-dimethylphenol		38

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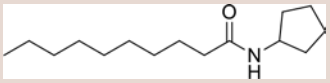
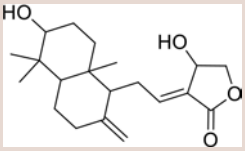
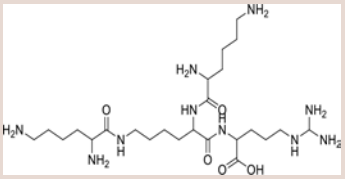
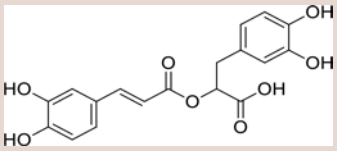
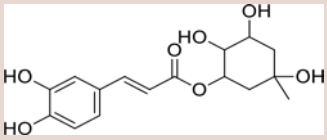
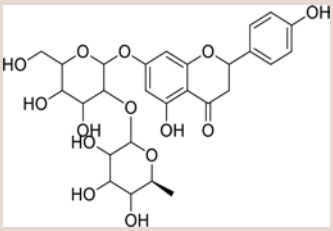
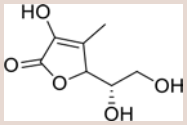
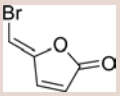
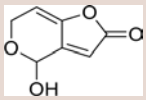
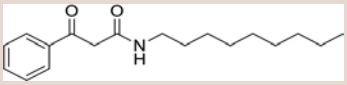
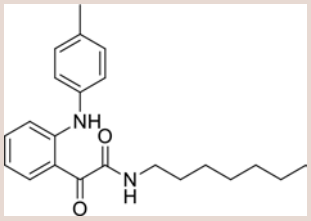
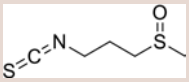
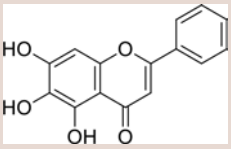
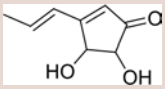
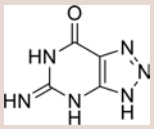
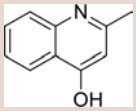
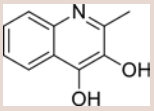
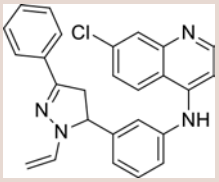
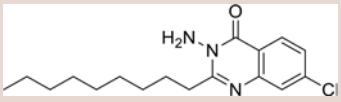
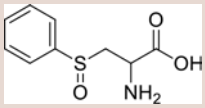
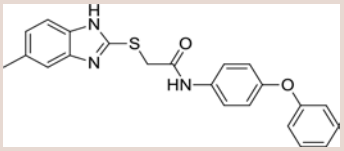
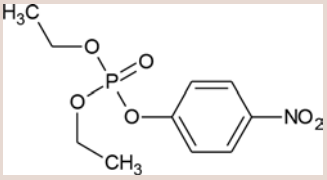
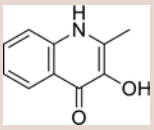
Table 1: Cont'd.

Compound Code	IUPAC Name	Chemical Structure	Reference
C34	N-cyclopentyldecanamide		39
C35	(3E)-4-hydroxy-3-[2-(6-hydroxy-5,5,8a-trimethyl-2-methylidene-decahydronaphthalen-1-yl)ethylidene]oxolan-2-one		40
C36	2-[2,6-bis(2,6-diaminohexanamido)hexanamido]-5-[(diaminomethyl)amino] pentanoic acid		41
C37	3-(3,4-dihydroxyphenyl)-2-{[(2E)-3-(3,4-dihydroxyphenyl)prop-2-enoyl]oxy} propanoic acid		42
C38	2,3,5-trihydroxy-5-methylcyclohexyl (2E)-3-(3,4-dihydroxyphenyl)prop-2-enoate		42
C39	7-{[4,5-dihydroxy-6-(hydroxymethyl)-3-{[(6S)-3,4,5-trihydroxy-6-methyloxan-2-yl] oxy}oxan-2-yl]oxy}-5-hydroxy-2-(4-hydroxyphenyl)-3,4-dihydro-2H-1-benzopyran-4-one		42
C40	Sodium 5-[(1S)-1,2-dihydroxyethyl]-3-hydroxy-4-methyl-2,5-dihydrofuran-2-one		43
C41	(5Z)-5-(bromomethylidene)-2,5-dihydrofuran-2-one		44
C42	4-hydroxy-2H,4H,6H-furo[3,2-c]pyran-2-one		45
C43	N-nonyl-3-oxo-3-phenylpropanamide		46
C44	N-heptyl-2-{2-[(4-methylphenyl)amino]phenyl}-2-oxoacetamide		18

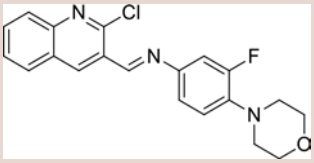
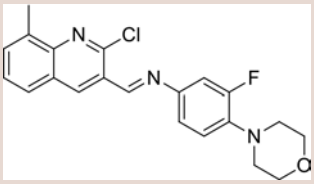
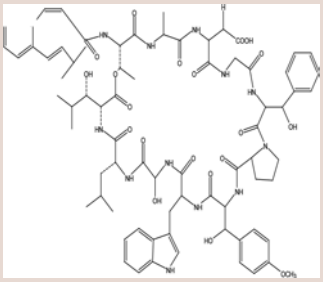
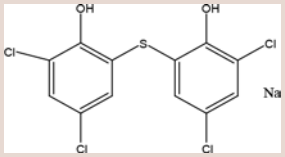
continued...

Table 1: Cont'd.

Compound Code	IUPAC Name	Chemical Structure	Reference
C45	1-isothiocyanato-3-methanesulfinylpropane		47
C46	5,6,7-trihydroxy-2-phenyl-4H-chromen-4-one		48
C47	4,5-dihydroxy-3-[(1E)-prop-1-en-1-yl]cyclopent-2-en-1-one		49
C48	5-imino-3H,4H,5H,6H,7H-[1,2,3]triazolo[4,5-d]pyrimidin-7-one		50,51
C49	2-methylquinolin-4-ol		51
C50	2-methylquinoline-3,4-diol		51
C51	7-chloro-N-[3-(1-ethenyl-3-phenyl-4,5-dihydro-1H-pyrazol-5-yl)phenyl]quinolin-4-amine		52
C52	3-amino-7-chloro-2-nonyl-3,4-dihydroquinazolin-4-one		53
C53	2-amino-3-(benzenesulfinyl)propanoic acid		54
C54	2-[(5-methyl-1H-1,3-benzodiazol-2-yl)sulfanyl]-N-(4-phenoxyphenyl)acetamide		55
C55	diethyl 4-methylphenyl phosphate		56
C56	3-hydroxy-2-methyl-1,4-dihydroquinolin-4-one		57

continued...

Table 1: Cont'd.

Compound Code	IUPAC Name	Chemical Structure	Reference
C57	(E)-1-(2-chloroquinolin-3-yl)-N-[3-fluoro-4-(morpholin-4-yl)phenyl]methanimine		58
C58	(E)-1-(2-chloro-8-methylquinolin-3-yl)-N-[3-fluoro-4-(morpholin-4-yl)phenyl] methanimine		58
C59	Benzamide- Benzamidazole derivative		59
C60	2,4-dichloro-6-(3,5-dichloro-2-oxidophenyl)sulfanylphenolate		59

Molecular Mechanics 2 (MM2) force field in order to stabilize the conformation of a molecule. After energy minimization, all files were saved in.mol2 format.[13] All 60 reference compounds (.mol2 format) were further imported in AutoDock Vina v. 1.1.2.[14] for ligand preparation. The data set of 60 reported molecules is shown in Table 1. The hit compounds were also selected in accordance with the Lipinski's rules of five.

Virtual Screening by Molecular docking approach

Molecular docking technique is useful in predicting ligand orientation within the key region of protein for finding putative hits for the specific target.[60] It helps to screen out the large databases of molecules against specific protein/receptor in order to analyze the potential drug molecule based on their binding energies and protein-ligand interactions.[61] The following steps were performed to conduct this study.

Protein Preparation

X-ray, three-dimensional crystal structure of CviR (PDB ID:3QP5) protein of *Chromobacterium violaceum* and LasR (PDB ID: 2UV0) of *P. aeruginosa*, co-complexed with natural ligand Chlorolactone (HLC, MF: C14H16CINO4) and 3-Oxo-N-[(3S)-tetrahydro-2-oxo-3-furanyl]-dodecanamide (OHN, MF: C16H27NO4) respectively, retrieved from Protein Data Bank depository and used for the grid preparation.[62] The protein preparation was done by using wizard of Autodock Vina v. 1.1.2.[14] Various steps were done prior to docking procedure like exclusion of water molecule as it may hinders with the surface exposure of protein to the ligand. Only chain E and its binding site were used for the docking procedures, deleting other homologous chains present in the protein. This software tools facilitates addition of polar hydrogens, assigning partial atomic charges (Kollman charges) and adding AD4 atoms type to all atoms present in molecules.[63]

Ligand Preparation

In ligand preparation, the various module of Autodock Vina like hydrogen's and Gasteiger charges were added followed by assigning AD4 atom type to all atoms of a ligand. The torsion angles of ligands across the rotatable bonds were fixed by detecting the roots in order to generate different conformations of ligand followed by fixation of aromaticity criteria to 7.5. Afterwards, the predictive reference molecules were subjected to molecular docking studies.[64]

Ligand Docking

For validation, only the E monomer of Ligand Binding Domain (LBD) complexed with OHN ligand in LasR (PDB ID: 2UV0) was imported into AutoDock Vina v. 1.1.2.[14] The water atoms were removed and default parameters were employed at import stage. The docking grid was set by choosing the OHN ligand as center of grid box dimensions. For initial molecular docking, the prepared sets of 60 reference ligand (known QSIs) were used. Neither tautomeric nor alternative protonation states were used for the ligand for the docking. Each compound was docked to get energy scores based on protein-ligand conformation and one docking pose was achieved after each run.[51,65]

Visualization

The results observed from AutoDock Vina v. 1.1.2 were projected through Pymol Software, academic version.[66-67] The 3-D images of interaction

shown by protein-ligand complex were also prepared using the same software.

RESULTS

Molecular Docking

A user-friendly interface to the molecular docking program AutoDock Vina was used to carry out molecular docking simulations initially on 60 reference molecules. The x-ray crystal structure of CviR protein (PDB ID:3QP5) of *C. violaceum* complexed with antagonist, Chlorolactone (HLC, MF: C14H16CINO4) as reported by Chen *et al*., (2011).[68] is shown as Figure 1 and 3D structure LasR-LBDs (Ligand-binding domain)[18] is shown as Figure 2. The molecular docking studies of all 60 reference compounds against QS LasR protein (PDB ID: 2UV0) of *P. aeruginosa* was performed in order to elucidate the binding affinity, docking pose and molecular interaction with LasR receptor. Based on the molecular docking results for LasR (2UV0), it is observed that all 60 reference molecules showed the docking scores in a range between -4.3 to -12.4 Kcal/mol. Among all ligands (C1-C60), compound C19 showed the highest docking score for both CviR and LasR proteins and also exhibited strong ligand protein interactions. The interactions studies of compound C19 with CviR (3QP5) and LasR (2UV0) demonstrated similar key amino acid interaction as of natural ligands HCL and OHN respectively (Figure 3 and Figure 4). The molecular docking results yielded pertinent information about the binding energy, binding affinity and orientation of ligand-receptor interactions and details are provided in Table 2.

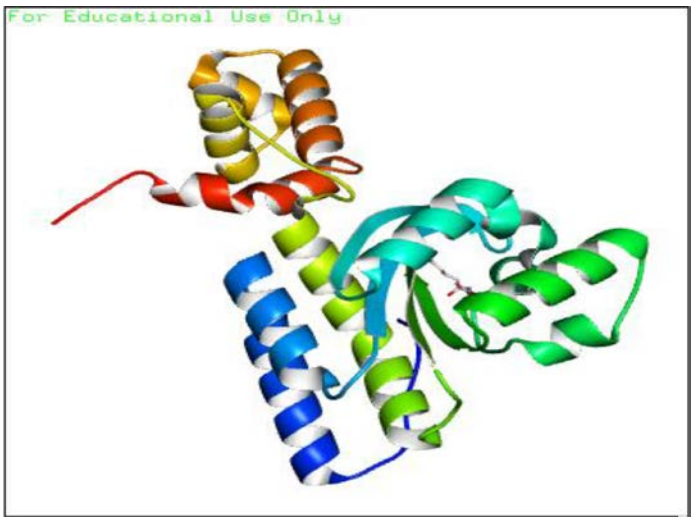


Figure 1: The 3D cartoon representation of CviR (PDB ID:3QP5), co-crystallized with natural ligand Chlorolactone (HLC, MF: $C_{14}H_{16}CINO_4$).

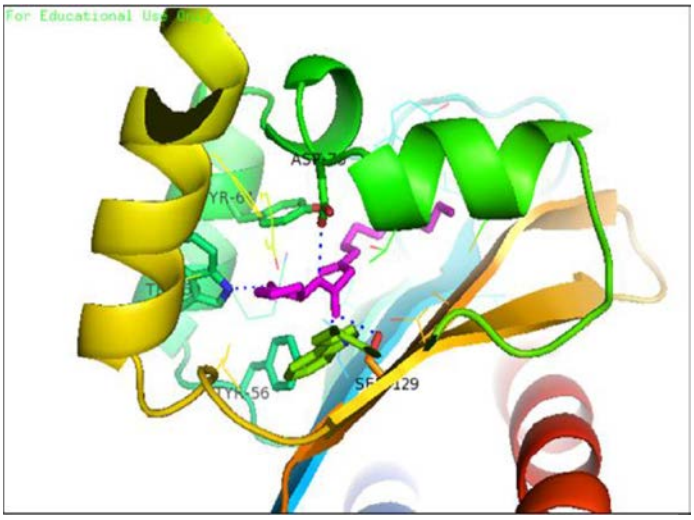


Figure 2: The docking pose of co-crystallized standard ligand (OHN) with LasR (PDB ID: 2UV0).

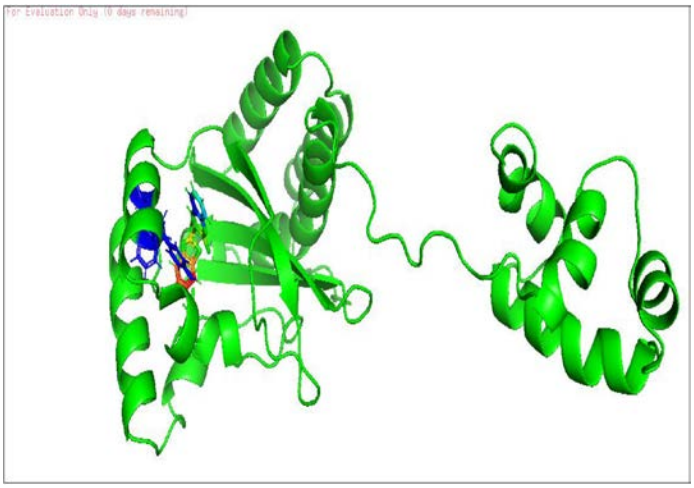


Figure 3: Docking pose of compound C19 inside active site pockets of CviR protein (3QP5).

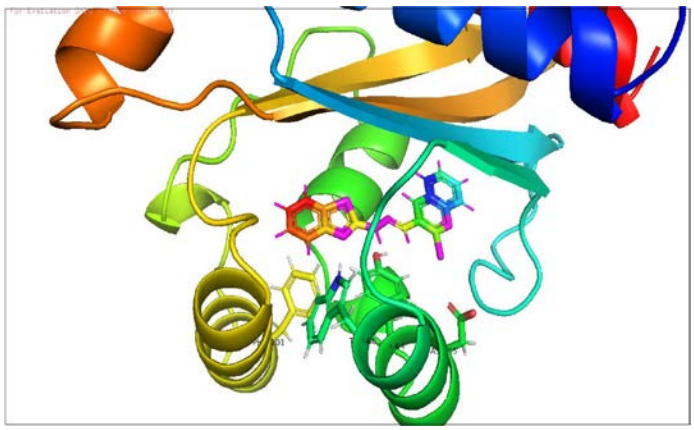


Figure 4: Docking pose of compound C19 inside active site pockets of LasR (2UV0).

Lipinski's Rule of Five

The Lipinski's "Rule of Five" is a set of *in-silico* guidelines applied to drug discovery to prioritize compounds with an increased likelihood of high oral absorption. After molecular docking, the drug likeliness of all the ligands was assessed by the Lipinski's rule of five parameters. The rule of 5 was used to correlate oral bioavailability on the basis of four important physicochemical properties such as molecular weight (MW), partition coefficient (Log P), Hydrogen bond donors, Hydrogen bond acceptors and each relate to the number of 5. The molecules are more likely to have good membrane permeation and easy absorption by the body if the values lie within the criteria such as MW less than 500 mg/mol, Log P less than 5, hydrogen bond donors (HD) less than 5, hydrogen bond acceptor (HA) less than 10. The rule describes those molecular properties representing oral bioavailability considered important for analyzing

Table 2: Docking scores (in Kcal/mol) of all 60 reference antagonists of *P. aeruginosa* along with molecular interactions with LasR (PDB ID: 2UV0).

Compound Code	Docking Scores (Affinity (Kcal/mol)	Interaction with Amino acids of LasR (2UV0)	
		Hydrogen Bonding	Hydrophobic Residues
OHN	-8.6	Tyr56,Ser129, Asp73, Trp60	Thr-80, Tyr-47, Cys-79, Leu-40
C1	-8.4	Ser129, Tyr56, Thr75	Tyr64
C2	-5	Gly54	Arg61, Ile52
C3	-8.2	Phe87	Leu84, Pro85, Asn141, Glu145
C4	-5.1	-	Gly6, Leu154
C5	-4.7	Gly6	Leu154, Tyr157, Leu23
C6	-4.3	-	Ile86, Hln78
C7	-7.5	Tyr56	Tyr64, Val75, Thr75, Ser129
C8	-5.3	Gly6	Lue10, Leu154
C9	-7	Tyr56, Ser129,Thr75	Trp88, Phe101, Leu110
C10	-6.4	Phe7	Ala27, Gly6, Asp29
C11	-6.7	Arg61	Ile52, Lys16, Val53
C12	-8.7	-	Asp73, Thr75, Tyr47, Val76
C13	-11.4	Ser129	Thr75, Tyr93, Val76
C14	-11.1	HOH 222 and HOH 453	Leu110, Trp88, Ser129, Leu36, Asp73, Thr75
C15	-6.2	Phe7, Lys25, Ile22	Gly6, Leu154
C16	-5.1	-	Phe7, Leu154,Tyr157
C17	-9	-	Asp73, Tyr64, Tyr56
C18	-4.8	-	Gly6, Phe7, Leu154
C19	-12.4	Ser129	Thr175, Trp60, Tyr64
C20	-8.4	Tyr47	Asp73, Thr75, Ser129
C21	-5.1	-	Ile22, Leu154,Tyr157
C22	-9.5	Tyr56, Ser129, Asp73	Trp88, Thr75
C23	-10.2	Ser129, Asp73	Tyr64, Gly38, Ala127
C24	-9	Ser129, Asp73	Ala127, Tyr56, Thr75, Trp88
C25	-6.3	Tyr56, Ser129	Trp88, Thr115, Phe101
C26	-8.9	Asp73	Ser129, Trp88, Cys79, Leu125
C27	-5.8	Thr75, Ser129	Tyr56, Tyr64, Leu36
C28	-4.8	-	Gly6, Asp29, Leu30, Ile22
C29	-9.5	Ser129	Trp88, Leu36, Asp73, Tyr93, Tyr56
C30	-11.5	Ser129	Val76, Tyr56, Tyr64, Thr115, Gly126
C31	-6.4	-	Ser44, Asp43, His119
C32	-8.3	-	Trp88, Phe101, Trp60, Tyr56, Ser129, Tyr64
C33	-5.5	-	Thr150, Leu154, Ala127
C34	-8.4	Ser129, Asp73	Tyr47, Trp88, Tyr56
C35	-6.7	Gly6	Ile22, Leu154, Leu30
C36	-4.9	Ala50, Gly54	Ser20, Gln24, Asp65, Glu48
C37	-6.1	Phe7, Ile22	Thr150, Tyr157, Asp29
C38	-6.3	His78, Phe87, Ser77, Gln81	Pro75, Ile86, Ile92
C39	-7.4	Glu48, Tyr56	Gly54, Ile52, Ala50, Arg61
C40	-6	Ser129	Asp73, Thr75, Tyr47, Tyr64
C41	-5.8	-	Asp73, Tyr93, Trp88
C42	-7.1	Tyr56, Ser129, Asp73	Tyr93, Trp88, Phe101
C43	-5.7	Leu23	Ile122, Leu154, Thr150
C44	-5.3	Gly6	Thr150, Leu154, Leu30
C45	-4.7	Ser129	Asp73, Tyr56, Leu36
C46	-10.6	Trp60	Thr115, Thr75, Tyr64, Ser129
C47	-6.5	Ala140	Gly113, Leu114, Gly113, Glu145, Val147
C48	-7.5	Trp60, Thr75	Tyr56, Ser129, Asp73, Tyr93
C49	-7.7	Ser129, Thr75	Trp60, Tyr56, Tyr64
C50	-7.4	Ser129	Tyr64, Thr75, Leu36
C51	-8.1	-	Pro74, Glu89, Phe87, His78, Gln94
C52	-9.4	-	Thr75, Tyr64, Ala127, Ser129, Trp60, Tyr56
C53	-8	Ser129, Asp73	Tyr93, Tyr64, leu110, Tyr56
C54	-7.4	-	Thr150, Pro149, Tyr157, Leu23
C55	-8	Ser129	Ala50, Tyr47, Tyr64, Val76
C56	-7.9	-	Tyr64, Ile52, Tyr64, Arg61, Leu36
C57	-10.3	Leu110	Asp73, Thr75, Gly126, Leu36, Trp88
C58	-7.1	-	Ile92, Pro74, Phe87, Ile86
C59	-5.2	-	Phe87, His78, Gln94
C60	-5.7	-	Leu114, Gly113, Val147

Table 3: The physicochemical properties of high docked compounds.

Compound Code	*M W[2] (g/mol)	logP[1]#	*X logP[2]#	*HD[2]	*HA[2]	*RB[2]	*(PSA)2A°	*MR[3]
Criteria	500<	5<		5<	10<	=10<	**140<=**	**40-130**
C13	298.23	2.31	2.28	3	6	1	114	71.25
C14	288.26	1.98	1.70	4	7	2	120	84.76
C19	246.31	1.64	1.87	3	5	1	97	68.59
C23	311.53	2.49	2.63	4	6	2	107	97.28
C30	302.74	1.82	1.72	2	4	1	111	103.45
C46	354.36	2.72	2.24	2	5	2	94	74.67
C57	292.11	1.93	1.86	3	7	1	85	79.23

1. Calculated by ALOGPS 2.1 program (http://www.vcclab.org/lab/alogps/start.html)

2 Calculated by zinc database (https://zinc.docking.org/)

3-Calculated by ACD (Available Chemical Directory)

#Octanol/Water partition coefficient

drug's pharmacokinetics such as adsorption, distribution, metabolism and excretion (ADME). This helps in identification of pharmacologically active compound which can be optimized step-wise for enhanced activity, selectivity and drug likeness as per Lipinski's rule. The molecular docking studies and Lipinski's rule helps in drug development process by limiting post-clinical experimentation expenses.[69] Veber rule describes that the compound which meets the criteria and have rotatable bond count less than10 and polar surface area (PSA) less than or equals to 104 Å are considered to have good oral bioavailability. Molar refractivity (MR) is a measure (from 40-130) the overall polarity of molecule and also related to the forces which govern the ligand-receptor interactions.[70] Based on Lipinski's Rule of five, Veber rule, molar refractivity and drug likeness, total seven high docked lead compounds (docking score >10 Kcal/mol) were prioritized are listed in Table 3.

DISCUSSION

The structure based molecular docking was performed to observe putative interactions formed by test compounds with CviR protein of *C. violaceum and* LasR receptor protein of *P. aeruginosa. Chromobacterium violaceum (*CviR,/LuxR homologue) has been massively used as a model bacterium in primary screening for quorum sensing inhibitors for other gram-negative microbes like *P. aeruginosa.*[71] Therefore, the selected compounds were subjected to molecular docking analysis to elucidate putative interaction with CviR receptor as well. CviR is a homodimer with two binding regions namely ligand binding domain (LBD) and DNA-binding domain (DBD) situated in a "crossed-region" conformation. In this crossed conformation of protein, two DBD units are apart and have reduce binding affinity with DNA. The HLC balance this closed assembly of CviR, thereby preventing QS activity (Figure 1). For LasR receptor, LasR-LBDs contain symmetrical dimer conformations containing deeply immersed ligand with each monomer (Figure 2). The monomer of LasR is folded as α-β-α clubbed orientation in which three α-helices remains on both sides of a five stranded β-sheet anti-parallel. The OHN ligand makes parallel orientation to β-sheet and α-helices (α3, α4, α5). The α6- helix makes the major intermolecular hydrogen bond, on the other side of β-sheet, makes mainly the intermolecular hydrogen and hydrophobic bonds give rise to the formation of large dimer interface surface area (1900Å^2).[18] The native ligand (OHN) re-docking study recommended that the software made reliable speculations (similarly as reported literature). The prediction of the AHL binding site in LasR-ligand binding domain (LBD) structure confirms the molecular interactivity of native ligand i.e., OHN (AI) with LasR protein. The OHN ligand exhibited hydrogen bonding of all polar groups present with LasR-LBD, except the lactone ring oxygen. The interaction study showed total four intermolecular hydrogen bonding involving Tyr-56, Trp-60, Ser-129 and Asp-73 (Figure 2). In particular, these amino acids are specific and show characteristics interaction in LuxR homologues suggesting that AHL shared a similar activation mechanism with HSL due to similar functional groups. The compact alliance of OHN ligand within LasR protein suggested high ligand specificity and its negative effect on quorum sensing among bacterial species.[72-73] Compound C19 showed the highest binding affinity (-9.4 Kcal/mol) with CviR protein and indicate a strong interaction with key amino acids (Tyr88 and Trp84). The docking poses of compound C19 with CviR protein (Figure 3). The key amino acid residues found to interact with C19 ligand are Ser129 makes hydrogen bond while Thr175, Trp60, Tyr64 showed hydrophobic interaction (Table 2). Compound C19 (A azine derivative, thiazole compound) is not analogues of natural ligand (OHN) but despite this the docking pose of C19 showed exquisite overlapping with the OHN (Figure 4). The fact here is that azines do bear pharmacophoric feature similar to natural ligand by two ways, first benzothiazole nucleus in C19 covered by the hydrophobic acyl chain in OHN resulting in forming hydrophobic interactions and secondly, the azine spacer (-C=N-N=C-) in C19 overlap with the polar region i.e., amide group (–CONH). The aryl moiety of C19 overspread the aromatic lactone ring in OHN and binds to the same site where OHN lactone ring binds. Hence, compound C19 adjust well in the active region of protein thus inhibits quorum sensing operation. Highest docked compound C19 showed similar hydrogen bond and hydrophobic interactions with amino acids almost similar as of reference ligands (HLC and OHN) for CviR and 2UV0 proteins respectively.[18,69-71] Many reported literatures suggested that 'thiazoles derivatives' showed stronger interaction, better than that of native ligand (OHN) with the target protein (LasR).[11,12,30,42] It is also worth to mention that C19 do contains thiazole substitution. The hydrogen bonding mainly contributes to the stability of ligand-receptor complex within active region of the target LasR protein. Another report highlighted the importance of hydrogen bond and hydrophobic residues in stabilizing a docked pose of azoles compounds highlighted the significance of hydrogen bonding in the ligand recognition at receptor site.[74] Therefore, molecular docking studies revealed that both compounds C19 fit well in the active region of LasR protein and inhibit quorum sensing in *P. aeruginosa*. In addition, the amino acid interaction plots of C19 with LasR and CviR are almost identical highlighting its inhibitory potential to suppress bacterial virulence. In addition, the physicochemical properties are also found within the criteria indicating the compound C19 is pharmacologically active and showed strong ligand-receptor interactions and safe pharmacokinetic profiling

suggesting its candidature for product development and field utilization to treat pseudomonal resistant infections.

CONCLUSION

The molecular docking study revealed strong pieces of evidence in support of C19, a thiazole compound with highest binding affinity (-12.4 K/cal) and potential interaction with receptors among all compounds suggesting its great effectivity to target specific LasR receptor in virulence suppression of *P. aeruginosa*. The findings suggests that among all reported compounds, compound (C19), a thiazole (non-lactone moiety) is found as promising ligand in terms of ligand-protein interaction with amino acids mainly contributes to its virulency and safe physicochemical profiling. Also, the study highlights a novel discovery of non-lactones (thiazole compounds) over lactones in blocking LasR gene suggesting it as a potent quorum sensing inhibitor of *P. aeruginosa*. However, the study also suggests that further investigation (*in-vitro* and *in-vivo*) needs to be implemented to confirm its real effectiveness in a way to establish this potential thiazole compound as a novel treatment option to combat serious infections caused by *P. aeruginosa*.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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