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Research Article

High Throughput Screening of Quorum Sensing Inhibitors Based Lead Molecules for *Pseudomonas aeruginosa* Associated Infections

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ABSTRACT

Quorum sensing (QS) is a process of cell-cell communication in bacteria by the use of signalling molecules that bind to the receptor protein and directly or indirectly affect transcription and translation. QS proteins may be used as a drug target for the inhibition of signalling pathway to control bacterial cell population. In present study the QS protein LasR of *Pseudomonas aeruginosa* was used as druggable target. Total fifteen inhibitors of LasR in 32 conformations were used for high-throughput computational docking. On the basis of docking score and glide score suitable inhibitors were identified. These selected inhibitors can be used as lead molecules for the designing of inhibitor based drugs. Lead molecules were further characterised by ADMET analysis, which include Lipinski's rule, Jorgensen's rule, blood-brain barrier penetration, Skin permeability, Human intestinal absorption and oral absorption. [(4E)-1-hydroxy-3-methylpyridin-4(1H)-ylidene]azinic acid] was found to be best inhibitor of LasR among all the inhibitors with best ADMET properties. In this study the above lead molecule was found to be a better alternative for the inhibition of bacterial population and in prevention in associated diseases. Present study focuses on the importance of structure based *in silico* drug design which takes less time and is cost effective.

Key words: Quorum sensing, LasR, Lipinski's rule, Jorgensen's rule, ADMET.

INTRODUCTION

The term "quorum sensing" was introduced by Fuqua et al. (1994)[1] to describe cell-cell signaling in bacteria. Quorum sensing was first discovered in marine bioluminescent bacteria *vibrio fischeri* and *Vibrio harveyi*, which produce light when cells are present at high density[2]. Quorum sensing is cell to cell signaling mechanism. Different types of bacteria produce various signaling molecules; these signaling molecules are called as autoinducers or pheromones[3]. As the population density of the bacteria increases the concentration of signaling molecules also increases, these signaling molecules then bind to and activate a R protein (receptor protein) also called transcriptional activator, which then induces expression of target gene. Most of the Gram-negative bacteria contain N- acyl homoserine lactone as the signaling molecule[4].

Pseudomonas aeruginosa is an opportunistic human pathogen that infects immunodeficiency individuals and individuals with cystic fibrosis. It has two quorum sensing systems *las* and *rhl*. These systems control the expression of number of virulence genes[1]. The *las* system contains transcriptional activator protein LasR and LasI, which directs the synthesis of the signalling molecule (autoinducer) PAI-1 [*N*-(3oxododecanoyl)-L-homoserine lactone][5]. The *rhl* system consists of the transcriptional activator protein RhlR and RhlI, which directs the synthesis of the signalling molecule (autoinducer) PAI-2 (*N*-butyryl-L-homoserine lactone)[6].

Pseudomonas aeruginosa actively forms biofilms through quorum sensing. The control of biofilms has been a major interest in many areas like clinical microbiology, civil and environmental engineering, and industry. Therefore, inhibitors of these proteins that can block the interaction between signals and receptors have been developed, to control quorum sensing caused bacterial virulence and biofilm formations. Out of the two quorum sensing system of *pseudomonas aeruginosa* attention has been paid to the LasR system because it triggers the activation of other quorum sensing systems of the bacteria [7].

Antibiotics are used for the treatment of infectious diseases. But now a day most of the bacteria has become resistant to these antibiotics and this has particularly been the case in *P. aeruginosa*. Computer-aided drug design approach is used for the discovery of new inhibitors for the target protein. Virtual database screening offers a novel method to identify the chemical entities that can actively bind to the target protein[8] and can inhibit the bacterial growth as well as associated infections.

In present study, structure based drug designing (SBDD) approach is used to screen the potential inhibitors[9]. A number of modifications were made to the inhibitor to analyse the extent to which structural variations

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Table: 1: Evaluation and validation scores for predicted models.

			Ramachandran Plot Statistics				
Model name	molpdf	DOPE score	Most Favoured Region (%)	Additional Allowed Region (%)	Generously Allowed Region (%)	Disallowed region (%)	Quality Factor
3IX3. 01.pdb	7204.1001	-14456.4082	79.7	10.4	7.3	2.6	75.3
3IX3. 02.pdb	7153.94287	-14744.60645	86.4	7.5	5	1.9	86.3
3IX3. 03.pdb	7231.01563	-14285.46191	75.25	10.5	9	4.75	69.8
3IX3. 04.pdb	7285.63818	-14495.15332	78.8	12.5	7.5	1.2	78.4
3IX3. 05.pdb	7148.07715	-14490.71191	78	9	12	1	73.2
3IX3. 06.pdb	7123.03271	-14494.93262	78.5	8.3	11.2	2	70.8
3IX3. 07.pdb	7135.19727	-14468.71191	77.8	9	10	3.2	69.7
3IX3. 08.pdb	7209.72168	-14763.58105	83.5	8.5	7	1	86.2
3IX3. 09.pdb	7111.57275	-14574.04102	83.7	9.5	6.5	0.3	78.5
3IX3. 10.pdb	7168.87402	-14744.13965	83.1	9	7.5	0.4	87.8
3IX3. 11.pdb	7049.98877	-14519.20313	85.3	6.6	4.1	4	79.6
3IX3. 12.pdb	7351.5332	-14280.78613	78.4	10.6	7	4	70.3
3IX3. 13.pdb	7269.58008	-14693.25781	84	8	6	2	77.4
3IX3. 14.pdb	7235.12109	-14432.41699	82.5	5.4	2.6	9.5	71.7
3IX3. 15.pdb	7013.68896	-15025.59082	90.5	8	1	0.5	94.23
3IX3. 16.pdb	7143.34668	-14796.7002	85.7	5	7	2.3	85.8
3IX3. 17.pdb	7461.48486	-14947.86035	88	5	6	1	90.5
3IX3. 18.pdb	7177.9375	-14342.56543	80.5	8.5	9.2	1.8	73.6
3IX3. 19.pdb	7213.30322	-14558.47559	81.8	8.6	7.2	2.4	75
3IX3. 20.pdb	7187.27393	-14587.67188	82.3	9	7.2	1.5	75.9

contribute to ligand-LasR binding and inhibition. The ligand molecule is designed with respect to the structure of the active site of the LasR, so that it can bind (dock) with high affinity and inhibit the function of the target. The ligand that shows best binding pose and minimum energy may be used as potential lead molecules for the development of drug against the *Pseudomonas aeruginosa* associated pathogenicity.

METHODOLOGY

Sequence retrieval and analysis: The protein sequence of LasR-A chain (gi 25858873, PDB ID 3IX3) was retrieved from National Center for Biotechnology Information (NCBI) (www.ncbi.nlm.nih.gov) in fasta format. Sequence analysis is done by Smith-Waterman algorithm by using BlastP module of Basic Local Alignment Search Tool (BLAST) (www.blast.ncbi.nlm.nih.gov). On the basis of maximum identity, minimum E-value and minimum resolution, five templates were selected for multiple template modelling.

Homology Modelling of LasR Protein: The three dimensional structure of the LasR protein was modelled with MODELLER9.11[10] tool by using Python script files with suitable modeller commands. The BLAST result was unable to find out the single template for LasR so multiple template based modelling was carried out by using five templates. The Templates with PDB ID 2Q0O, 3QP1, 3QP6, 3QP8 and 2UVO at resolution 2.00 Å, 1.55 Å, 2.00 Å, 1.60 Å and 1.40 Å were selected for the modelling and their geometrical coordinates were borrow directly as such from RCSB protein data bank (*www.rcsb.org*). Multiple-template modelling method is a novel method, which can accurately align a single protein sequence simultaneously to multiple templates. Experimental results indicate that, multiple template method can improve pairwise sequence-template alignment accuracy and generate models with better quality than single-template models[11].

Evaluation and validation of models: The final model was selected among all the predicted model by complete streochemical and geometrical analysis by DOPE score, Molpdf, Procheck (www.ebi.ac.uk/thornton-srv), What If (http://swift.cmbi.ru.nl/whatif) and ERRAT score (http://nihserver.mbi.ucla.edu/ERRATv2). The feasibility of proposed model was estimated by Ramachandran plot using an online tool SAVes (http://services.mbi.ucla.edu/SAVES). Refined model of LasR protein was submitted to protein model data base (http://mi.caspur.it/PMDB/) and protein identifier was obtained.

High Throughput Screening (HTS) and molecular docking: The ligand preparation, molecular docking and analysis of ligand-receptor interactions were carried out by using Schrodinger product suits by utilizing hierarchical search protocol based algorithms, in which the final step is minimization of a flexible ligand in the field of the Coulomb and Vander Waals potential of the protein.

Ligand preparation: The primary information about various ligands or inhibitors of LasR was collected from the literature survey and chemical databases. Initially total 15 different analogues with 32 conformations of 4-nitro-pyridine-N-oxide (4-NPO) were designed. The structures of the inhibitors were drawn in Maestro (Schrodinger. The final ligand preparation was performed by LigPrep module of Schrodinger product suits, where

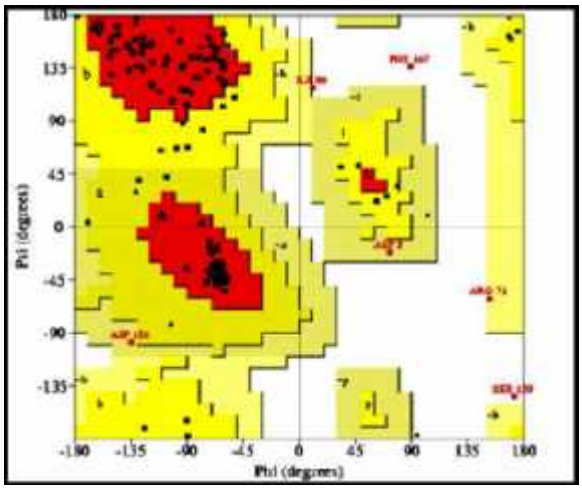


Fig: 1. Ramachandran Plot of LasR protein of P. aeruginosa

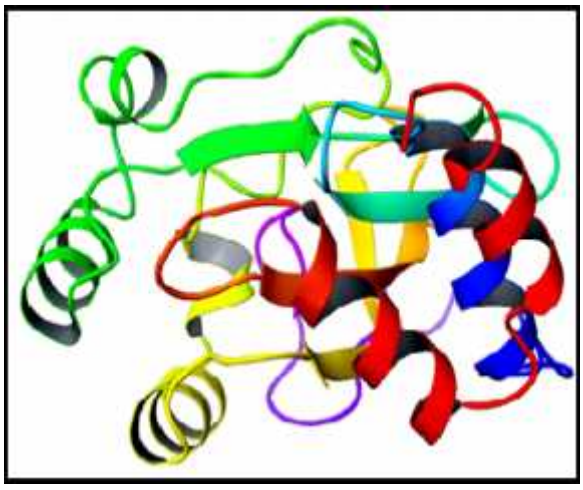


Fig: 2. Modelled structure of LasR protein of P. aeruginosa

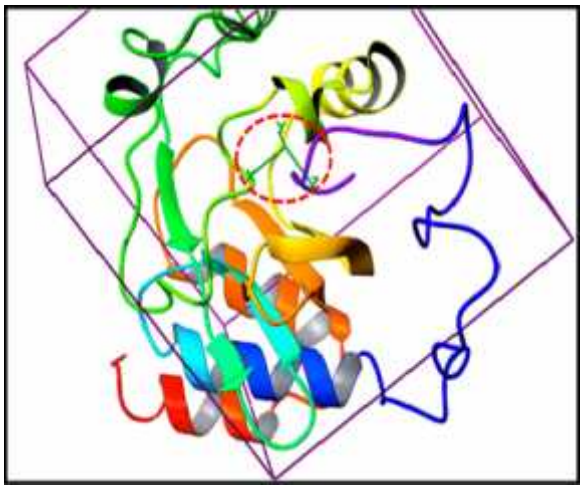


Fig: 3. Receptor-Grid generation. The binding site on LasR is indicated within circle.

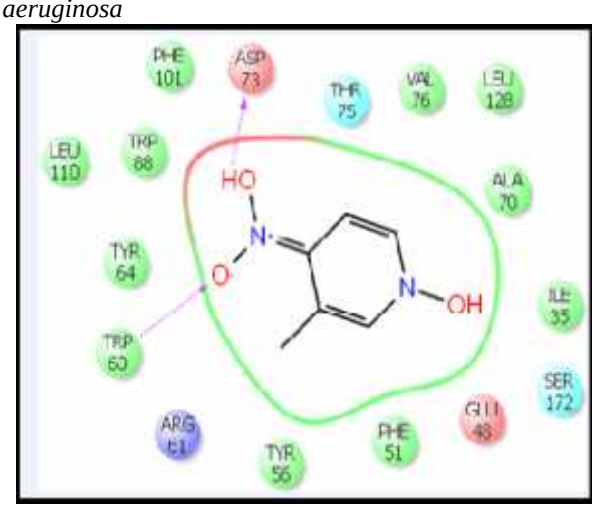


Fig: 4. Amino acid residues present in the active site of LasR protein.

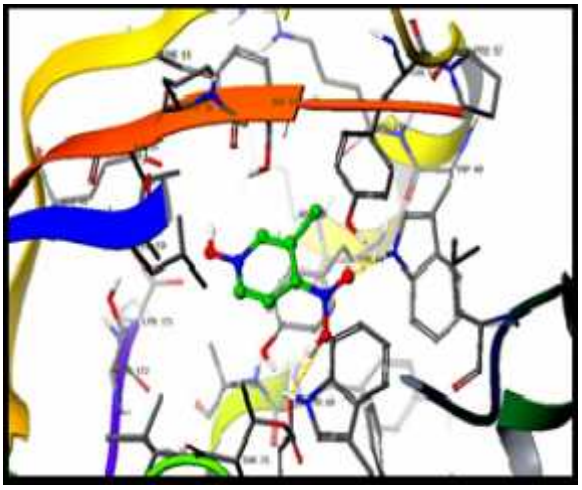


Fig: 5. Ligand-protein docking with hydrogen bond interaction

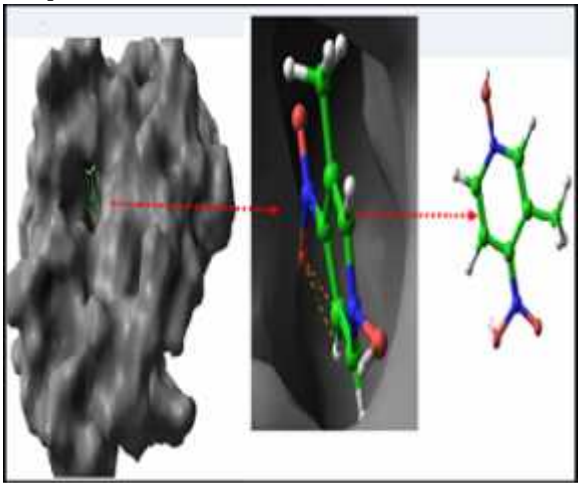


Fig: 6. Ligand bound to the protein binding pocket.

the software, clean up the structure and assigns the stable conformations of each structure in 3D space. One or two isomers of each structure are also generated. All these structures and isomers were automatically get saved in the maestro project table.

The docking was carried out by glide module and receptor grid was generated at Van der Waal radius factor

Table 2: Docking and related energies for best five inhibitors with LasR protein

S.No.	Molecular formulae of inhibitors	docking score	Potential energy	glide lipo	g evdw	g ecoul	g energy
1	$C_6H_8N_2O_3$	-7.38348	45.740887	-1.06	-17.29	-18.75	-36.03
2	$C_5H_4N_2O_3$	-7.346004	45.740887	-1.12	-18.42	-17.69	-36.11
3	$C_5H_6N_2O_3$	-7.279651	54.876144	-1.50	-19.32	-14.54	-33.86
4	$C_6H_8N_2O_2$	-7.156325	54.876144	-1.37	-18.81	-15.40	-34.22
5	$C_5H_7N_2O$	-7.133002	54.876231	-1.35	-18.56	-15.55	-34.11

scale 1.0 and charge scale factor 1.0 by selecting residues present at active sites. The information about residues at active sites were gathered from literature survey, superimposing LasR modelled structure with the template proteins having known active sites and using ligand interaction menu of Maestro.

Absorption Distribution Metabolism Excretion Toxicity (ADMET): Computational characterizations of selected ligands were carried out using the QikProp module of Maestro (Schrodinger software) in normal mode. QikProp is a quick, accurat prediction program for absorption, distribution, metabolism, excretion and toxicity (ADMET). QikProp predicts physically significant descriptors and pharmaceutically relevant properties of organic molecules, either individually or in groups. QikProp can be run either from the Maestro GUI or from the command line.

The characterized ligands were considered as potential lead molecules. The qualities of lead molecules were assessed according to Lipinski's rule of five and Jorgensen's rule of three.

RESULT AND DISCUSSION

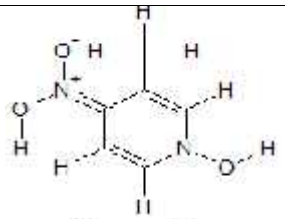
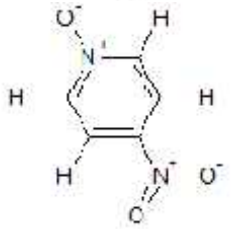
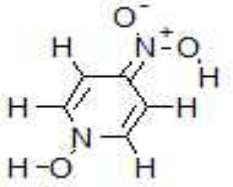
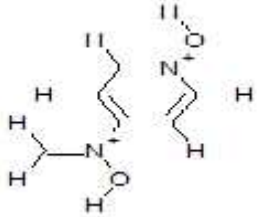
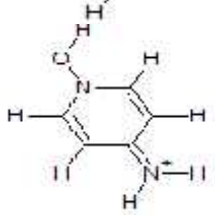
Modelling: Multiple template based homology modelling is the most accurate structure prediction method. The quality of the homology model is dependent on the level of sequence identity between target and template and resolution of the templates. Total 20 models of LasR protein were generated by Modeller9.11 (Table: 1). The best model (3IX3. 15.pdb) was selected by considering minimum molpdf (7013.68896), minimum DOPE score (-15025.59082) and GA341 scores (1). All other stereochemical parameters were in acceptable limit. The RMS Z-Score for bond length (0.98), bond angle (1.235), omega angle restaints (1.431), side chain planarity (1.420), improper dihedral distribution (0.782) and outside distribution values (1.064) were in good agreement with good modelled proteins. The overall quality factor of modelled LasR protein was 94.23 which reflect the good quality of model and acceptable physiochemical environments of protein. Analysis of backbone phi and psi dihedral angles by Ramachandran plot indicates the good percentage of residues in most favoured region i.e. 90.5%. Residues in additional allowed and generously allowed regions are 8.0% and 1.0% respectively. Residues in disallowed region were 0.5% only (Table-1). Allowed regions in the plot show low energy regions. The red color region in the plot represents most favoured region. Yellow color region represent additional allowed region. Light yellow color region represent generously allowed region (Fig-1). Fig - 2 shows the three dimensional structure of modeled LasR protein of *P. aeruginosa*.

Ligand Docking: The 3D structure of the receptor obtained from modelling was used in the docking program. The docking was carried out with all the 32 selected ligands after energy minimization and generating the grid. The area under the circle is the binding site of ligands (Fig-3).

The better ligands were primarily screened on the basis of dock score. Screened ligands were further analysed at the level of their hydrophobicity, columbic interaction, polar interactions, rotable bonds and internal torsional energy (Table-2). Potential energy of a molecule is due to the position of the molecule or arrangement of the particles in the molecule. Molecule with low potential energy forms stable complex. Docking score predicts the binding affinity of two molecules after they have been docked. Among all the designed ligands [(4E)-1-hydroxy-3-methylpyridin-4(1H)-ylidene]azinic acid], 4-nitropyridine-1-oxide, (1-hydroxypyridin-4(1H)-ylidene)azinic acid, 1-hydroxy-4-[hydroxyl (methylidene) ammonio]pyridinium and 1-hydroxypyridin-4(1H)-iminium having comparatively similar docking score (Table-2). [(4E)-1-hydroxy-3-methylpyridin-4(1H)-ylidene]azinic acid] shows minimum docking score of -7.38348. On the basis of docking score, [(4E)-1-hydroxy-3-methylpyridin-4(1H)-ylidene]azinic acid] shows better binding affinity than other inhibitors. Therefore newly designed molecule [(4E)-1-hydroxy-3-methylpyridin-4(1H)-ylidene]azinic acid] can be more potent and selective towards Las-R inhibition. 4-nitropyridine-1-oxide shows docking score of -7.346004, so it may have similar efficacy as previous one. The IUPAC name, molecular formulae, molecular weight and chemical structure of best five inhibitors are mention in Table-3. The molecular weight cut-off of all screened inhibitors were within acceptable limit (<500).

The amino acid residues present around the ligands in the receptor protein were – ASP(73), THR(75), VAL(76), LEU(128), ALA(70), ILE(35), SER(172), GLU(48), PHE(51), TYR(56), ARG(61), TRP(60), TYR(64), TRP(88), PHE(101) (Fig. 4). The ligand protein interaction reveal that the nature of association was main Vander waal and Columbic with energy value of 17.285938 and 18.748469 respectively for best docked ligand [(4E)-1-hydroxy-3-methylpyridin-4(1H)-ylidene]azinic acid]. All the five ligands having capacity to form the Hydrogen bonds. It is a stronger interaction

Table: 3. Specification of best five inhibitors

S.No	IUPAC name of inhibitors	Molecular formulae	Molecular Weight	Structure
1	(4E)-1-hydroxy-3-methylpyridin-4(1H)-ylidene]azinic acid	$C_6H_8N_2O_3$	156.139	
2	4-nitropyridine-1-oxide	$C_5H_4N_2O_3$	140.096	
3	(1-hydroxypyridin-4(1H)-ylidene)azinic acid	$C_5H_6N_2O_3$	142.112	
4	1-hydroxy-4-[hydroxyl(methylidene) ammonio]pyridinium	$C_6H_8N_2O_2$	140.138	
5	1-hydroxypyridin-4(1H)-imin ium	$C_5H_7N_2O$	111.121	

than above mention interactions. The amino acids ASP (73) and TRP (60) are mainly involved in the formation of Hydrogen bonds with these ligands. TRP (60) acts as H-bond donor and ASP (73) as acceptor with ligands (Fig-5). The presence of ligand in the hydrophobic pocket at the time of interaction (Fig-6) gives the proof of some extent of hydrophobic interaction (glide lipo) with the hydrocarbon moiety of the ligands. Hydrophobic interaction energy was lowest (1.05991) for [(4E)-1-hydroxy-3-methylpyridin-4(1H)-ylidene]azinic acid] and highest (1.50029) for (1-hydroxypyridin-4(1H)-ylidene)azinic acid.).

[(4E)-1-hydroxy-3-methylpyridin-4(1H)-ylidene]azinic acid] having overall good quality parameters so it may be a better lead molecule for the development of protein inhibitor based drug against the *Pseudomonas aeruginosa*. The inhibitor 4-nitropyridine-1-oxide differs only in the attachment of nitro group in ortho position of both the ring. So may also be considered as a good lead molecule.

ADMET Analysis: All the five screened ligands were further subjected to ADMET analysis before considering them as potential lead molecules. Before designing a drug it is important to keep in mind, that the drug must satisfy Lipinski's Rule of five and Jorgensen's rule of three (Table-4). According to the rule the number of hydrogen bond donor atom in ligand molecule should not be more then 5 as well as the number of hydrogen bond acceptor from the solvent (water) to the ligands should not be greater than 10. All the five ligands satisfied these conditions. The calculated molecular weights of all the five ligands were below the 500, which further satisfied the second criterion. The predicted aqueous solubility (QPlogS) of all the five ligands were in acceptable range (>-5.7). The Caco-2 based computational models were used to estimate the gut-blood barrier permeability efficacy of the lead molecules. The QPPCaco (>22 nm/sec) reflects the nature drug like nature of all five molecules. It is a measure for the non-active transport.

After ADMET calculations, it is concluded that [(4E)-1-hydroxy-3-methylpyridin-4(1H)-ylidene]azinic acid] was not violated the rule of five and rule of three. The human intestinal absorption rate of all these five lead molecules were above 80%, within the acceptable range and the human oral absorption value is 2-3 (medium to high) for all the inhibitors.

Table 4: Comparison of ADMET properties of selected ligands

ADMET Properties	Name Of Inhibitors				
	$C_6H_8N_2O_3$	$C_5H_4N_2O_3$	$C_5H_6N_2O_3$	$C_6H_8N_2O_2$	$C_5H_7N_2O$
CNS	+2	+2	+2	+1	+1
Donor HB	2	2	2	0	2
Accpt HB	2.7	2.7	3.7	3.2	1.5
QPP Caco (nm/s)	652.002	652.132	18.89	300.059	98.165
QPlog BB	-2.722	-2.721	-2.011	-2.872	-2.710
QPP MDCK	51.193	51.193	43.156	23.874	18.392
QPlogKp	-3.556	-3.546	-3.631	-2.908	-5.103
QPlogS	-1.116	-1.105	-0.643	-0.478	-1.406
QPlogPo/w	0.942	0.932	1.4	0.063	0.430
SASA	342.44	341.733	316.798	290.72	275.289
Human Oral Absorption	3	2	2	2	3
Percent Human-Oral Absorption	91.888	87.754	80.45	79.248	68.953
Mol_MW	156.139	140.096	142.112	140.138	111.121

The predicted values of Blood-Brain barrier (BBB) penetration for these lead molecules were highly significant. BBB penetration index indicates the ease to cross the blood-brain barrier by the drug molecules. The good quality of drug should have capacity to cross the barrier, generally the CNS-active compounds must pass across it and CNS-inactive compounds mustn't pass across it in order to avoid CNS side effects. QPlogBB denotes blood partition co-efficient, the value ranges between -3.0 to -2. The normal QPPMDCK score indicates the ease to cross the blood brain barrier.

It is also important to predict the skin permeability rate of the drugs that come into contact with the skin either accidentally or by design. Denoted by QPlogKp, the value ranges from -8.0 to -1.0. The skin permeability of the [(4E)-1-hydroxy-3-methylpyridin-4(1H)-ylidene]azinic acid] was -3.556 which was found in good agreement with other available drugs . All other ligands also having the $QPlogK_P$ value within the acceptable range.

In this study [(4E)-1-hydroxy-3-methylpyridin-4(1H)-ylidene]azinic acid] inhibitor has satisfied all the above ADMET properties. The summarized ADMET properties of best five ligands are mentioned in table-4.

CONCLUSION

The increase in antibiotic resistance in bacteria increases the wide variety of bacterial infection and biofilm formation in human population. QS in bacteria plays a vital role in regulation of virulence gene expression. Many researches have been carried out by the scientist all around the world to study the QS system and find a way to inhibit the signalling pathway.

High-throughput ligand docking or structure based drug design has become a powerful technique in the area of computational drug design. It gives an easy way for designing of suitable inhibitors which can block the QS pathway. Among large number of network of QS proteins LasR having vital role in sensing mechanism in *P. aeruginosa*. The multiple template based homology modelling was carried out to generate the better quality of 3D structure of LasR protein. The screening and docking studies with 32 different ligand conformations only five were found to be suitable for further studies. ADMET analysis of these proposed lead molecules were highly promising. All these lead molecules were not violating the Lipinski's Rule of five and Jorgensen's rule of three. Their all ADMET properties were within the acceptable limit.

Yet all the five lead molecules having comparable features, but [(4E)-1-hydroxy-3-methylpyridin-4(1H)-ylidene]azinic acid] was found to be the best qualities. 4-nitropyridine-1-oxide having almost similar ADMET properties. It only differ in bonding orientation, so may have same efficacy as former one. Now we may conclude that the [(4E)-1-hydroxy-3-methylpyridin-4(1H)-ylidene]azinic acid] along with all four lead molecules may have drug like properties and may be effective in *P. aeruginosa* associated infections. It may be considered as a better drug candidate for LasR inhibition in comparison to other available inhibitors in market. The laboratory and clinical trial on the proposed lead molecule may further confirm their potency.

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