

“Development of potent Quorum based Anti-virulence therapeutics for targeting infections caused by *P. aeruginosa*”

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IN

PHARMACEUTICAL CHEMISTRY

By

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Statement by the candidate

This is to submit that this written submission in my thesis entitled “**Development of potent Quorum based Anti-virulence therapeutics for targeting infections caused by *P. aeruginosa***” represents original ideas in my own words and where others’ ideas or words have been included, I have adequately cited and referenced the original sources. I also declare that I have stuck to all principles of academic honesty and integrity and have not misrepresented or fabricated or falsified any idea/data/fact/source in my submission. I understand that any violation of the above will be cause for disciplinary action by the school and can also evoke penal action from the sources which have thus not been properly cited or from whom proper permission has not been taken when required.

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***DEDICATED TO
MY FAMILY AND
TEACHERS***

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LIST OF ABBREVIATIONS

Abbreviations	Full form
%	Percentage
2D	Two Dimensional
3D	Three Dimensional
AHL	Acyl-homoserine-lactone
AHLs	N-acylated-L-homoserine lactones
AI	Autoinducer
AI-1	Autoinducers sort 1
AI-2	Autoinducers sort 2
AIPS	Auto-initiating peptides
AQS	Alkyl quinolones
<i>BHL</i>	N-butanoyl-L-homoserine lactone
<i>C. elegans</i>	<i>Caenorhabditis elegans</i>
CA-MRSA	Community Acquired Methicillin Resistant <i>Staphylococcus aureus</i>
comE/ comD	two-component signal-transduction system
DKPS	2,5-diketopiperazines
DPP-IV	Dipeptidyl peptidase-4
<i>E.coli</i>	<i>Escherichia coli</i>
<i>e.g.</i>	For example
<i>et al.</i>	And others
HHL	N-hexanonyl-L-homoserine-lactone

HSL	Homoserine lactones
<i>i.e.</i>	That is
LasI/LasR/LasB	Proteins
LuxI/LuxR	Proteins
MRSA	Methicillin-Resistant <i>Staphylococcus aureus</i>
NAAA	<i>N-acylethanolamine acid amide hydrolyse</i>
NMR	Nuclear Magnetic Resonance
OdDHL	N-(3-oxododecanoyl)-L-homoserine lactone
OHHL	N-3-oxohexanoyl homoserine lactone
OHHL	Oxo-Hexanoyl Homoserine lactone
<i>P. aeruginosa</i>	<i>Pseudomonas aeruginosa</i>
PDB	Protein Data Bank structure
PMV	Python Molecular Viewer
QS	Quorum sensor
QSAR	Quantitative Structure activity relationship
QScR	Quorum Sensing Control Receptor
RMSD	Root Mean Square Deviation
<i>S. aureus</i>	<i>Staphylococcus aureus</i>
<i>V. fischeri</i>	<i>Vibrio fischeri</i>
μM	Micrometre

CHAPTER 1

1. INTRODUCTION

1.1 Quorum sensing system in bacteria

The ecological framework, as well as the populous that is controlled by the microbes by means of interchanges between the cells, possesses the reliable reaction and execution as demonstrated by various statically data and the natural conditions of their environment.

Microorganisms which are pathogenic observes the most prominent that correspond to their ability. For example, the host is defeated by them by subjecting the frame work, intrading and utilizing the group in this regard the communication exceeds in the gauge intracellular flags, utilizing and given the element of the transmitter flag convergent, in this regard as the thickness is achieved background objectives such that, the framework that acts invulnerable and find it hard to adapt, the harmfulness element discharge by the microscopic organisms. Hence, the health framework will be avoided to give a quick response.

Quorum sensing (QS) is a bacterial cell to cell correspondence preparation that includes the generation, discovery, and reaction to extracellular flagging atoms called autoinducers (AIs). AIs aggregate in nature as the bacterial populace thickness increments, and microbes screen this data to track changes in their cell numbers and by and large adjust quality expression. QS controls qualities that immediate exercises that are gainful when performed by gatherings of microscopic organisms acting in synchrony. Forms controlled by QS incorporate bioluminescence, sporulation, capability, anti-infection generation, biofilm arrangement, and destructiveness figure discharge.

In the majority detecting pathways in microbes are made out of a few principle parts, including microorganism's populaces, flag atoms, protein activators and target qualities. The capacity of majority detecting is required for some microbes like *Pseudomonas aeruginosa* cause illness and infection.¹

Majority detecting framework is a case of multicellular conduct in the unicellular universe of microorganisms, autoinducer is accepted that the adjust of concoction messages amongst life

forms and cells is a one of a kind property in eukaryotic cells. “Once the grouping of particles outside the microscopic organisms surpasses the edge, the flagging pathways are actuated and the microorganism reaction the messages by adjusting the quality expression and tweaking physiological procedures in an aggregate mode”.²

The populace thickness subordinate in the microscopic organism’s co-ordinate quality expression is finished by the Quorum detecting mechanism. Quorum detecting directly the bacterial procedures as well as it incorporates beneficial interaction, harmfulness, bioluminescence, anti-toxin creation and biofilm formation.³

1.2 Signal molecules involved in Quorum sensing

Blackwell and co-workers have done a comparison analysis of synthetic quorum sensors modulators on *Pseudomonas aeruginosa*, the beta-keto amides structure natural, non-natural and mimics were docked and these following compounds were found to be more potent. The most potent compounds as per experimental results from the varying group of compounds a shown in Table 1.

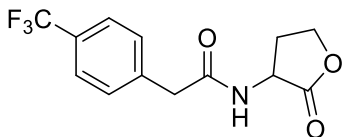
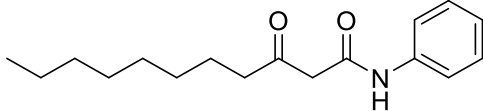
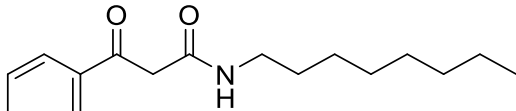
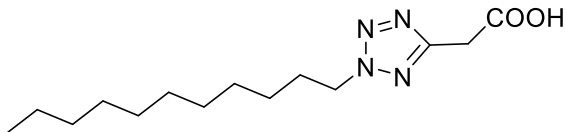
S.No	AHL	2UV0
1		-9.16
2		-9.75
3		-9.07
4		-9.21

Table1: The potent compounds from Blackwell series

1.3 Types of Quorum sensing systems

1.3.1 Quorum sensing in Gram-negative bacteria:

“Majority detecting complex has been recognized in more than 25 gram-negative microbes species over the previous decades; and among these species majority detecting framework in *Vibrio fischeri*, *Pseudomonas aeruginosa* and *Agrobacterium tumefaciens* are more considered. Discoveries demonstrate that an extensive variety of real procedures, for example, bioluminescence, swarming, swimming, twitching, antibiotics biosynthesis, the development and spread of biofilm, conjugation, sporulation, virulence markers creation, and so on in these bacterial species are directed by majority detecting, the most studies on majority detecting are performed on bioluminescence property in *Vibrio fischeri*”.²

1.3.2 Quorum sensing in Gram-positive bacteria

Gram-positive microorganisms utilize peptides, called auto-initiating peptides (AIPs), as flagging particles. Once delivered to the cell, AIPs are handled and discharged. At the point when the extracellular concentration of the AIP is high, which happens at high concentration density, it ties to a related layer bound two-segment histidine kinase receptor. Generally, restricting enacts the receptor's kinase action, it auto phosphorylates and passes phosphate to a related cytoplasmic reaction controller. The phosphorylated reaction controller actuates interpretation of the qualities in the QS region. The gram-positive microorganism's QS, AIPs are transported to the cell cytoplasm where they communicate with translation elements to balance the interpretation component's action and thus, balance quality expression changes.

Cases of correspondence in gram-positive microscopic organisms are comE/comD framework in *Streptococcus pneumonia*, the TraR/TraI destructiveness framework in phytopathogen *Agrobacterium tumefaciens* and cross breed delivery person framework in *Vibrio harveyi* and control of Ti plasmid transport in *Agrobacterium tumefaciens*.

1.3.3 Quorum sensing in *Pseudomonas aeruginosa*

“The majority detecting arrangement of *P. aeruginosa* made out of two sets of qualities as principle constitutive qualities. One set comprises of LasI and LasR qualities, encoding C12-HSL autoinducer synthase and R protein, individually. Another combine called Rhl is made out

of RhlI and RhlR qualities, encoding synthase, and R protein, separately. The synthase chemical of this match of QS framework produces C4-HSL autoinducer, however, delivers a little measure of N-hexanoyl-L-homoserine-lactone (HHL). There is likewise an administrative quality named QScR (Quorum Sensing Control Receptor) in this framework that creates an administrative protein which works as an inhibitor of LasR protein. The elements of the qualities of QS framework in *P. aeruginosa* are between related and don't work autonomously, as it were, one might say that the outflow of Rhl qualities is under control of Las qualities which is performed through the R protein of LasR gene".²

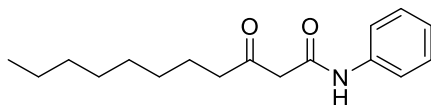


Fig1: 3-oxo-N-phenylundecanamide

1.3.4 Autoinducers

Autoinducers are little diffusible flagging atoms which are created by microbes in the majority detecting framework. These autoinducers (AI) are isolated into two classifications, autoinducers sort 1 or AI-1, which are species-particular and utilized for intraspecies correspondence. Autoinducers sort 2 or AI-2 are not particular and go about as a global dialect. Autoinducers sort 2 don't connect for interspecies correspondence, additionally empower the microscopic organisms to convey microorganisms, for example, parasites and protozoa to arrange their own particular conduct in a populace of microorganisms.⁴

1.4 MOLECULAR DOCKING

Docking is a process for predicting and analyzing the interactions between receptors and ligands. These interactions play important role in many biological processes and hence crucial for drug discovery. These studies are helpful in predicting which compound will bind well to protein's active site.

Docking involves generating optimal protein-ligand conformation and scoring. The scoring may be binding energy, free energy or some others. Docking algorithm produces the different orientations and conformations for the ligand in the active site. The different poses for a ligand

are then ranked according to the scoring function. Finally, the best-scored compounds will be then synthesized and analyzed in the laboratory.

Docking is a useful instrument in lead revelation and can screen expansive libraries in less time. It needs 3D (three-dimensional) structure of target protein. The 3D structure of a protein can be acquired from X-ray crystallography and NMR strategies. Docking does not represent harmfulness and bioavailability considers.⁶

1.5 DOCKING METHODS

There are mainly three types of docking methods:

1. Rigid docking: In rigid docking both ligand and receptor are treated as rigid.
2. Flexible ligand docking: In this ligand is flexible and the receptor is rigid.
3. Flexible docking: Receptor and ligand are flexible.

Though the docking was done with online docking (Swiss Dock) software which was used to carry out the docking of the compounds, the corresponding structures are proceeding in table 2.

1.5.1 Protein preparation:

The protein preparation is done with the help of AutoDock Suit version 4.2.6. In this the protein PDB structure (obtained from the protein data bank) is firstly prepared for the docking process.

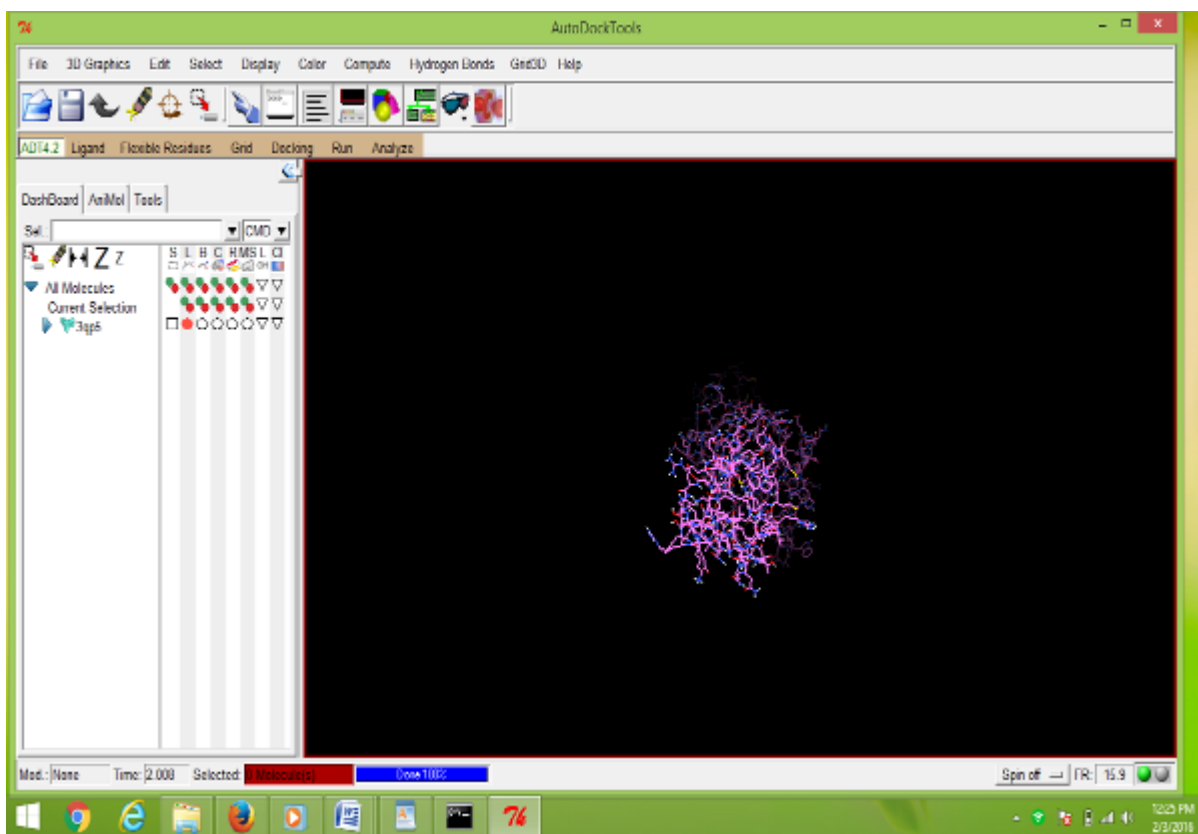


Fig 2: Showing the protein in AutoDock Suit

From the **File menu**, select **Read molecule**, highlight the PDB file for your protein and snap open or, right tap on **Python Molecular Viewer (PMV)** molecules at the base of the window and pick the protein PDB record. Likewise, settle any issues with the PDB document to realize what structures might be available. We need to keep just the protein and such cofactors as might be bound to it normally. Save it as pdbqt document. This is our protein.pdbqt file.

1.5.2 Ligand preparation:

The structures of all ligands were generated by the use of Chem BioDraw Ultra version 15.0 software and were MM2 optimized. All the structures are saved as MDL mol file (*.mol). In AutoDock Suit, go to the ligand on the menu bar, then click on input molecule and then open the ligand and choose PDB file. Then select the file containing the ligand, and click open.

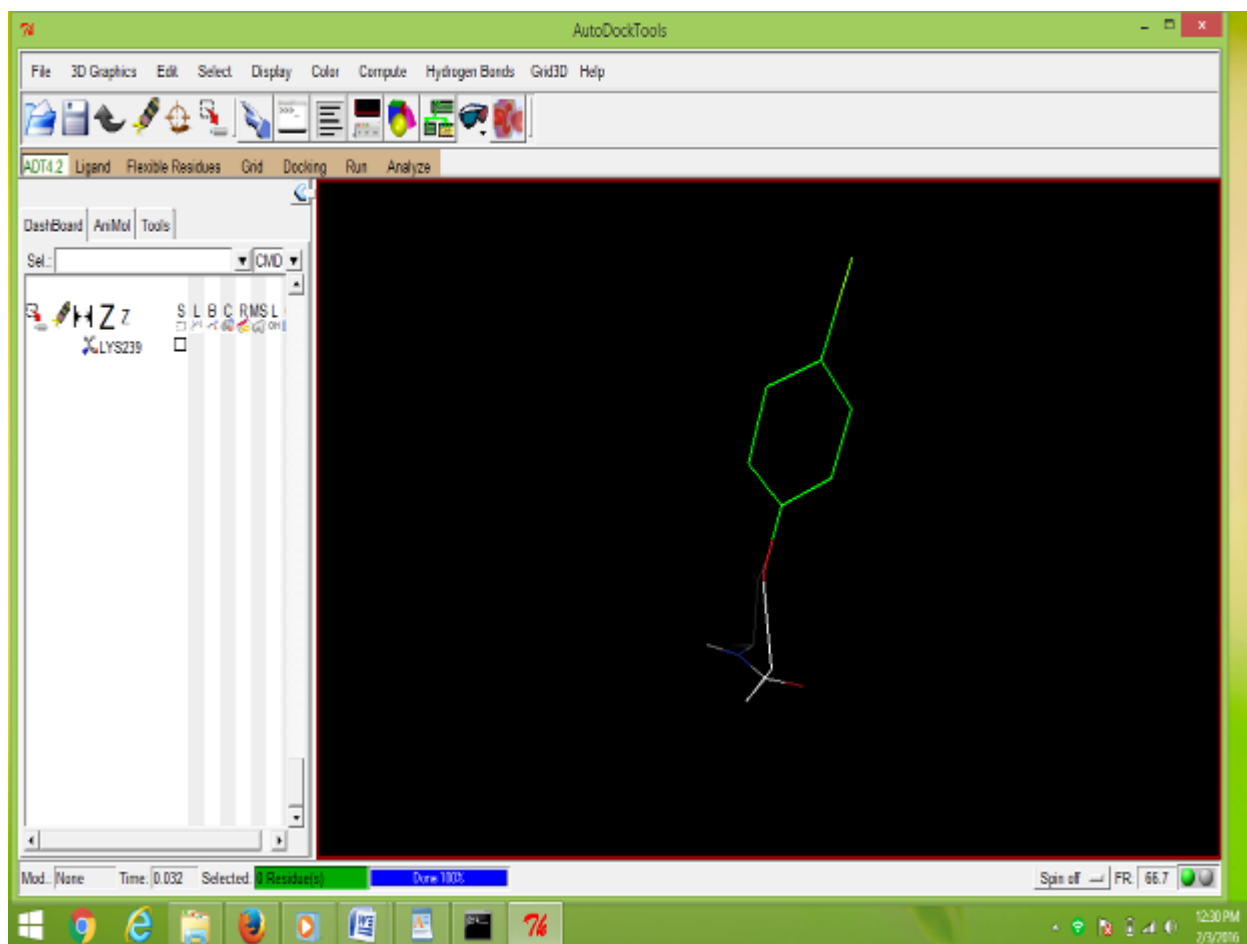


Fig 3: Showing the ligand in AutoDock Suit

Then message will be pop-up on screen as shown below:

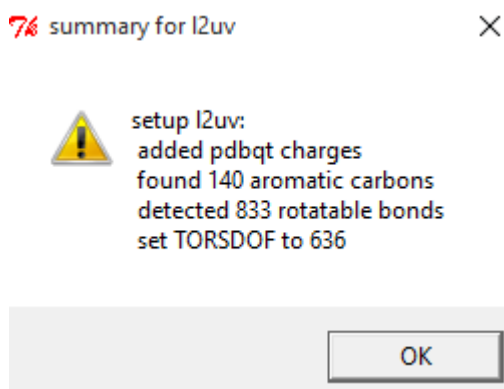


Fig 4: Protein loading message

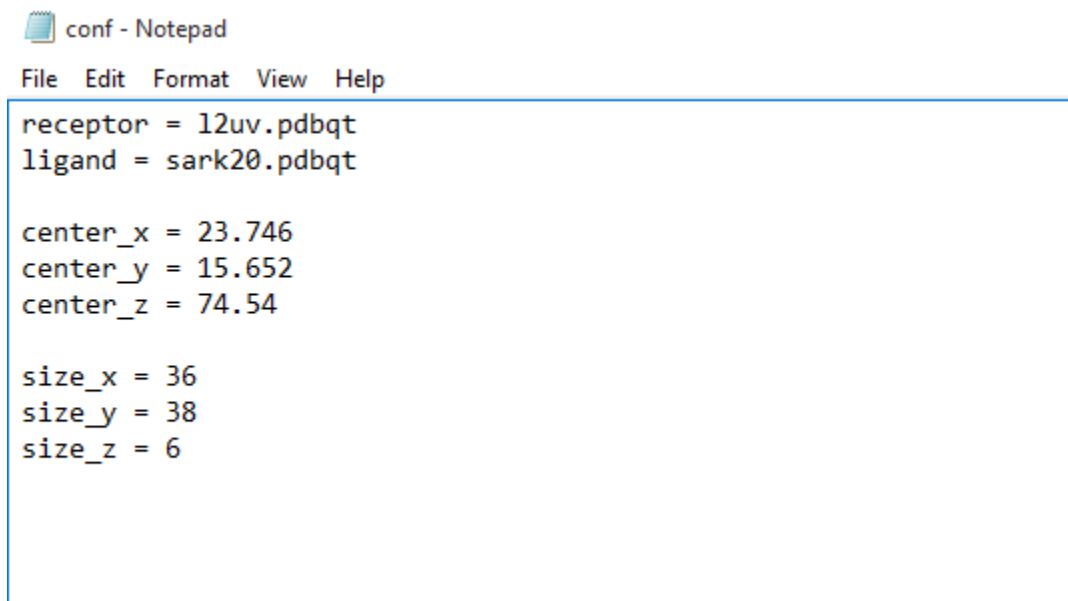
On the menu bar choose Ligand ->Torsion Tree->Detect Root. A small dot will show up, making the choice.

Then, select Ligand ->Torsion Free->Choose Torsions. The Torsion Count widget appears.

Save it as pdbqt file. This is our ligand.pdbqt file.

1.5.3 Docking and Validation of Protein:

Load ligand.pdbqt file and set it as a map type by choosing ligand. After this centralize ligand by setting grid box and then save it by close saving current. Save the protein as pdbqt file and then prepare a configuration file and save it as conf.txt. Analyse the docking results in command prompt as shown below:



```
conf - Notepad
File Edit Format View Help
receptor = 12uv.pdbqt
ligand = sark20.pdbqt

center_x = 23.746
center_y = 15.652
center_z = 74.54

size_x = 36
size_y = 38
size_z = 6
```

Fig5: Preparations of configuration file for docking

```
Select AutoDockTools-1.5.6
Copyright M.F. Sanner (March 2000)
Compilation flags
#####
# If you used AutoDock Vina in your work, please cite:      #
#                                                           #
# O. Trott, A. J. Olson,                                    #
# AutoDock Vina: improving the speed and accuracy of docking #
# with a new scoring function, efficient optimization and    #
# multithreading, Journal of Computational Chemistry 31 (2010) #
# 455-461                                                    #
#                                                           #
# DOI 10.1002/jcc.21334                                     #
#                                                           #
# Please see http://vina.scripps.edu for more information.  #
#####

Output will be sark25_out.pdbqt
Detected 4 CPUs
Reading input ... done.
Setting up the scoring function ... done.
Analyzing the binding site ... done.
Using random seed: 1871639892
Performing search ...
0% 10 20 30 40 50 60 70 80 90 100%
|----|----|----|----|----|----|----|----|----|
*****
done.
Refining results ... done.

mode |  affinity |  dist from best mode
      | (kcal/mol) | rmsd l.b. | rmsd u.b.
-----+-----+-----+-----
1      -9.2      0.000      0.000
2      -8.8      1.579      2.265
3      -8.8      1.439      1.799
4      -8.7      1.333      2.206
5      -8.6      1.998      9.153
6      -8.5      1.960      9.047
7      -8.3      1.737      3.179
8      -7.6      2.393      9.103
9      -7.5      1.644      8.714

Writing output ... done.
```

Fig6: Docking via command prompt on vina interface.

CHAPTER 2

2. LITERATURE REVIEW:

Campbell *et al*, “majority detecting is the under the regulation of *N*-acylated L-homoserine lactones, and their receptors (LuxR-sort proteins) in Gram-negative microscopic organisms. Some cyclic dipeptides (2, 5-diketopiperazines) have been derived from microscopic organisms and answered to restrain the LuxR kind of proteins in AHL biosensor strains, they are clinically higher intense than local lactones. These are common dipeptides. Non natural cyclo (L-professional L-phe) subsidiaries were fit for restraining luminescence in *V. fischeri*. These DKPs don't contend with the regular lactone signals. OHHL to hinder luminescence. Together this audit proposes that DKPs and QS signs are analyzed in the microscopic organisms. This article told how the dictionary of actually happening QS utilized by against the gram negative bacteria”.⁹

Millet *al*, demonstrated that the Quorum sensor is the direction of quality expression in light of anomaly in cell-populace thickness. QS microorganisms make and discharge compound signs. These are the increment in the fixation as an element of cell thickness. The view of a negligible edge excitement convergence of an autoinducer prompts to alter in quality expression. Gram positive and negative bacteria are utilizing QS correspondence circuits to manage a different course of action of physiological action. This procedure includes advantageous interaction, destructiveness, capability, conjugation, anti-infection creation, motility, Sporulation, and biofilm development. For the most part, Gram-negative microbes utilize acylated homoserine lactones as autoinducers. Gram-positive microorganisms utilize handled oligopeptides to communicate.¹⁰

Diggle *et al*, QS is utilized to speak to the correspondence between bacterial cells. QS has not just spoken to between cells of similar species (intra-species), additionally amongst species and microorganisms and higher life forms. QS based correspondence observed to be among microorganisms is outlandish, considering that clarifying both participation and correspondence. These two are more issues in transformative science. A developmental introduction, species flagging can be clarified utilizing, for example, kinfolk's determination. This article fancied incorporating the developmental on creature motioning on QS.¹¹

Nanting *et al*, bacteria can manage group-wide practices including biofilm, harmfulness, conjugation, sporulation, swarming through a procedure called majority detecting. Inhibitors of bacterial majority detecting are critical research devices and potential helpful specialists. In this article, they have repeated late improvements in this majority sensing.¹²

Castang *et al*, the 11 likeness of N-acylhomoserine lactones in which the carboxamide bond was prevailing by a sulphonamide. These mixes actions were institutionalized for their capacity to aggressively repress the activity of 3-oxo hexamoyl-L-homoserine lactone. The enact articulation of bioluminescence in the model bacterium against *V. fischeri*. A few mixes to show foe activity.¹⁴

Run G.J. *et al*, QS directs the creation of destructiveness components and natural procedure of biofilms in a few microbes, including *P. aeruginosa*. The QS slide is initiated by the connection of flagging atoms (autoinducers). Here they have answered to characterize the stereo-chemical contributions of manufactured agonists and perform docking studies to comprehend the miniaturized scale environment of the coupling site in *P. aeruginosa*. In this article is that the ring structure and the total and relative stereochemistry's of the amide and hydroxyl bunches recommend agonist action. This article was decided the imperative auxiliary and stereo-chemical characteristics important for collaboration with the QS controller proteins of their inducer restricting site.¹⁵

Muhet *al*, *Pseudomonas aeruginosa* can detect its own populace thickness by utilizing an intercellular flagging framework. Such frameworks have been named majority detecting and reaction frameworks. In this writing, the investigation demonstrated that mixes were general inhibitors of majority detecting, i.e., the expression levels of most LasR-ward qualities were influenced. Additionally, repressed the generation of two majorities detecting subordinate harmfulness factors.¹⁶

Woodard *et al*, microbes utilize majority detecting as a method for cell-to-cell correspondence, permitting populace subordinate control of quality expression. The flag particles included are typically acyl-homoserine lactones (acyl-HSLs), consolidating unsaturated fat gatherings to give specificity by means of a progression of various flag receptors following up on subsets of qualities. In this literature, the disclosure of another minor departure from the HSL subject in the photosynthetic bacterium *Rhodopseudomonas palustris* proposes that numerous all the more

such flags may exist. The utilizations compounds by a bacterium which are like greasy acyl-HSL synthase to create p-coumaroyl-HSL from the condition. The bacterium species likewise make p-coumaroyl-HSL, raising the likelihood of intra-species correspondence in a natural context.¹⁷

Schaefer *et al*, many microbes utilize acyl-homoserine lactone (acyl-HSL) synthases to produce greasy acyl-HSL majority detecting signals, which work with flag receptors to control articulation of particular qualities in QS. The greasy acyl gathering is gotten from unsaturated fat biosynthesis and gives flag specificity, however, the assortment of signs is restricted. This survey demonstrates that the photosynthetic bacterium *Rhodopseudomonas palustris* utilizes an acyl-HSL synthase to create p-coumaroyl-HSL by utilizing ecological p-coumaric corrosive as opposed to unsaturated fats from cell pools. We likewise found that p-coumaroyl-HSL is made by other microorganisms including, what's more, *Silicibacter pomeroyi*. This disclosure amplifies the scope of conceivable outcomes for acyl-HSL majority detecting and brings up basic issues about majority detecting inside the setting of ecological signaling.¹⁸

Waters *et al*, “bacteria communicate with each other utilizing synthetic flag particles. As in developed creatures, the data provided by these particles is basic for synchronizing the exercises of huge gatherings of cells. In microscopic organisms, concoction correspondence includes delivering, discharging, distinguishing, and reacting to little hormone-like atoms named autoinducers. This procedure, named majority detecting, enables microscopic organisms to screen the earth for other microbes and to modify conduct on a populace wide scale because of changes in the number as well as species present in a group.

Along these lines, majority detecting confounds the refinement amongst prokaryotes and eukaryotes since it empowers microscopic organisms to go about as multicellular living beings. This article concentrates on the designs of bacterial concoction communication systems; how substance data is coordinated, handled, and transduced to control quality expression; how intra- and interspecies cell-cell correspondence is expert; and the interesting plausibility of prokaryote-eukaryote cross-communication”.¹⁹

Mattmann, *et al*, the reliance of QS on little atom signals has propelled natural scientific experts to outline non-local particles that can block these signs and in this manner bother bacterial gathering practices. The artful pathogen *Pseudomonas aeruginosa* has been the objective of a hefty portion of these endeavours because of its pervasiveness in human diseases. *P. aeruginosa*

utilizes no less than two N-acyl L-homoserine lactone signs and three homologous LuxR-sort receptors to start a scope of pathogenic practices at high cell densities, including biofilm development and the creation of a munitions stockpile of harmfulness variables. This survey highlights late concoction endeavours to tweak LuxR-sort receptor action in *P. aeruginosa* and offers to understand into the improvement of receptor-particular ligands as potential antivirulence strategies.²⁰

Sohaibani *et al*, this review demonstrated that *S. persica* contains bioactive against bio film operators with double functionalities of development restraint and QS controller association. This audit may offer a novel system to diminish the improvement of dental caries by repressing the underlying attachment and resulting biofilm development via cariogenic microscopic organisms.²¹

Kim *et al*, the inhibitory exercises of the mixes which were explored in this audit demonstrated resistance against QS flagging the mixes were measured utilizing correspondent frameworks and contrasted and the assessed restricting energies from the displaying study. This examination demonstrated the genuinely great connection, proposing that the in-silico translation of ligand-receptor structures can be an important apparatus for the pre-outline of better-focused inhibitors. Likewise, these inhibitors additionally indicated hostile to biofilm exercises against *Pseudomonas aeruginosa*.²²

Al-masri *et al*, docking tests in this audit upheld the coupling modes proposed by QSAR/pharmacophore examinations. The legitimacy of the QSAR condition and the related pharmacophore models were built up by the recognizable proof of amazing failure micro molar hostile to DPP-IV leads recovered by in-silico screening.²³

Souleret *et al*, docking based virtual screening of synthetic mixes library focusing on the coupling locales of LuxR-sort proteins. The screening groupings of docking performed with LuxR, TraR and LasR, in a request to explicitly focus on the monitored build-ups. Strikingly, a few mixes as potential QS modulators of a conceivable cross talk amongst microscopic organisms and host. An organic assessment of the LuxR subordinate QS framework prompted the disclosure of new QS inhibitors.²⁴

Sabbah *et al*, the QS agonists and antagonist checked on in this article have an incentive as unthinking tests to study QS, they have planned and blended two centered libraries of non-lactone AHL imitates and assessed their exercises as agonists and rivals of LasR, LuxR, and TraR. This review was roused by their enthusiasm for improving the hydrolytic strength of such autoinducer copies keeping in mind the end goal to conceivably expand their stabilities.²⁵

Taha *et al*, the hormone sensitive lipase (HSL) has been as of late involved in diabetes and obesity, provoking endeavours to find new HSL inhibitors. Toward this article they investigated the pharmacophoric space of HSL inhibitors utilizing four differing sets of mixes. Along these lines, hereditary calculation and different straight relapse investigation were utilized to choose ideal blend of pharmacophoric models and 2D physicochemical descriptors equipped for yielding a self-reliable and prescient quantitative structure–activity relationship (QSAR).²⁶

Tan *et al*, the centralization of these flag particles increments locally accordingly of expanding cell thickness, and after achieving a limit level (when the populace is "quorate"), the populace enacts a planned cell reaction, for example, the generation of harmfulness elements and development as a biofilm group

In this review, they have demonstrated that structure-based virtual screening is a suitable and successful means for the disclosure of novel QS inhibitors. From a library of 3,040 normal mixes, 22 mixes met choice criteria and were tried for organic movement. Five of these mixes were found to have measurement subordinate hindrance of the las QS framework. Notwithstanding, they appeared to have measurement subordinate hindrance of LasB in both the *P. aeruginosa* and *E. coli* strains, demonstrating its specificity for the LasR protein.²⁷

Ping-hua *et al*, microbes can survey their surrounding populace densities utilizing low-atomic weight particles (autoinducers) and modify quality expression at high cell number to carry on as a gathering. This procedure, named majority detecting, is generally utilized by microscopic organisms to start amass practices that have coordinate and frequently obliterating impacts on human wellbeing and there environment.

New techniques are expected to grow the present arrangement of majority detecting modulators dynamic in Gram-negative microorganisms. Shockingly, the structures of known foes and agonists shift broadly, and their components of activity are hazy; in this way, no undeniable

methods of reasoning have developed for new ligand plan. To address this issue, they have made up a plan for centered combinatorial libraries of ligands for the balance of majority detecting. Here, they have reported the disclosure of a group of non-local AHLs able to do either hindering or, at times, firmly inciting majority detecting in the marine symbiont *Vibrio fischeri*. What's more, they have reported the main super agonist of majority detecting in *V. fischeri*. These ligands give another plan to the outline of both majority detecting agonists and adversaries and speak to intense new substance tests to examine the components of bacterial correspondence.²⁸

Solorzano *et al*, in this review, they used the structures of the α -acylamino- β -lactones 1 and 7a23 for mixes having a place with the substance class of N-(2-oxo-3-oxetanyl) amides with the goal of finding NAAA inhibitors with enhanced strength. Specifically, they investigated the part of the β -lactone ring and amide side chain, first by surveying stereo electronic prerequisites in closeness to the lactone ring, and next by advancing the size and state of the lipophilic tail of the amide moiety.²⁹

Johari *et al*, *Staphylococcus aureus* is a gram-positive bacterium that is responsible for nosocomial infections as well as skin diseases in humans and is characterized by its thick peptidoglycan cell wall layer common to gram-positive bacteria. The case of diseases caused by community-acquired methicillin-resistant *Staphylococcus aureus* (MRSA) (CA-MRSA) strains in healthy individuals has been a concern worldwide. Computer-aided drug design approaches like molecular docking studies and QSAR studies can be used in drug discovery in several ways. Molecular docking has been carried out on *Staphylococcus aureus* with Dehydrosqualene synthase inhibitors. This review reflects the alternative inhibitors designed for combating MRSA infections.³⁰

O'Loughlin *et al*, in article they investigated the blockage with major detectors that showed mBTL, a simple of the local *P. aeruginosa* autoinducers, quells articulation of the qualities encoding the destructiveness consider pyocyanin, averts biofilm arrangement, and secures *C. elegans* and human lung epithelial cells from attack by *P. aeruginosa*. Both the LasR and RhIR majority detecting receptors are incompletely repressed by mBTL; nonetheless, in the settings that we they have inspected, RhIR, not LasR, is the pertinent *in vivo* target. Much accentuation has been set on the revelation of LasR inhibitors in light of the fact that LasR is arranged at the highest point of the *P. aeruginosa* majority detecting course. There outcome recommends that

the basic in vivo focus for regulation is RhlR. Analyses in *Drosophila melanogaster* additionally exhibit that the *P. Aeruginosa*.³¹

Souleré *et al*, this quorum sensor (QS) enables microscopic organisms to adjust their conduct in light of their populace thickness. This cell to cell correspondence is broadly examined for the comprehension of atomic instruments required in transcriptional direction. QS is additionally focused to grow new treatments for bacterial contaminations which regularly require anti-toxin treatment prompting the rise of safe bacteria. This article proposes that carboxamide AHL analogues showing an agonist action embrace a comparable restricting mode as common ligands while carboxamide AHL analogues with a terminal aryl substituent showing foe action create appealing co-operations with Tyr61.³²

Nath *et al*, a few microbes utilize moieties of indole for intercellular flagging as controllers of different bacterial phenotypes essential for avoiding the inborn host safe reaction and antimicrobial resistance. A scope of regular and engineered indole subordinators have been found to go about as inhibitors of QS-ward bacterial phenotypes, supplementing the bactericidal capacity of conventional anti-toxins. In this article, different indole-based AHL copies were outlined and integrated by means of the 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride and N,N'-dicyclohexylcarbodiimide intervened coupling responses of an assortment of substituted or unsubstituted aminoindoles with various alkanolic acids. All blended mixes were tried for QS restraint utilizing a *P. aeruginosa* QS strain by measuring the green fluorescent protein production. Docking studies were performed to look at their capability to attach and hindering QS receptor protein.³³

Sumit *et al*, the combined compounds were tried for their ability to obstruct CviR receptor based QS motions in *Chromobacterium violaceum*. The bioassay screening comes about proposed that two compounds displayed powerful QS hindrance movement rivalry with CviR receptor, indicating violacein restraint (>50%) at 200 µM. Promote, the positive hits were evaluated for their ability to repress LasR receptor-based QS utilizing the PlasB-gfp bio monitor strain of *Pseudomonas aeruginosa*.³⁴

CHAPTER 3

3. Scope of study

This study covers studies of potential quorum sensing inhibitors. QS bacteria produces and release chemical signaling molecules. That increases in concentrations as a function of cell density. This mechanism leads to a generation of virulence factors which causes bacterial density to grow and propagate. Inhibiting this mechanism to approach to target the protein of resistance of antibiotics and different proteins such as, LasR (a protein) screening.

CHAPTER 4

4. AIM AND OBJECTIVES

4.1. Aim

Development of potent Quorum based Anti-virulence therapeutics for targeting infections caused by *P. aeruginosa*

4.2. Objectives

1. Preparation of the ligand/protein using PDB files
2. Docking of the ligand inside the target protein
3. Energy minimization and geometric optimization
4. Analysis of the results for quorum sensing inhibitors

CHAPTER 5

5. RESEARCH/COMPUTATIONAL METHODOLOGY

Swiss Dock (online) and Autodock Vina software will be used for molecular docking studies. The study will use computational tools like Swiss Dock, AutoDock vina, ChemBio Draw 2D and ChemBio 3D for the preparation of structures to be docked.

CHAPTER 6

RESULT AND DISCUSSION

Molecular docking was done using MGLtools version 1.5.6 along with AutoDock Vina version 1.1.2, AutoDock Suite version is 4.2.6 and Swiss Dock online server into the structures of all ligand binding site to find compare the high binding affinity of all the structures. Genetic algorithm in Autodock was used to dock inhibitors and correlate the obtained binding free energies with their root mean square deviation (RMSD).

The structures of all ligands were generated by the use of ChemBioDraw Ultra version 15.0 software and were MM2 optimized. From the ligands, all hydrogen's were added and Gasteiger charges were assigned, after which the non-polar hydrogen's were removed. The search was conducted in a grid of 60 points per dimension and a step size of 0.375 Å centred on the binding site. The binding energy scores of all ligands with their reference RMSD value.

Relative RMSD values and binding energies of compound no. 12 were compared between computer model and manual model using two different receptors (2uv0 and 3ix3) an increase in binding energy value was high in computer model (both receptors) compared to manual model as shown in table 2.

Relative RMSD values and binding energies of compound no. 13 were compared between computer model and manual model using two different receptors (2uv0 and 3ix3) an increase in binding energy value was high in computer model (both receptors) compared to manual model which had high binding energy in one receptor (3ix3) as shown in table 2.

Relative RMSD values and binding energies of compound no. 14 were compared between computer model and manual model using two different receptors (2uv0 and 3ix3) an increase in binding energy value was high in computer model (both receptors) compared to manual model which had high binding energy in one receptor (3ix3) as shown in table 2.

Relative RMSD values and binding energies of compound no. 15 were compared between computer model and manual model using two different receptors (2uv0 and 3ix3) an increase in binding energy value was high in computer model (both receptors) compared to manual model which had high binding energy in one receptor (3ix3) as shown in table 2.

Relative RMSD values and binding energies of compound no. 16 were compared between computer model and manual model using two different receptors (2uv0 and 3ix3) an increase in binding energy value was high in computer model (both receptors) compared to manual model which had high binding energy in one receptor (3ix3) as shown in table 2.

Relative RMSD values and binding energies of compound no. 17 were compared between computer model and manual model using two different receptors (2uv0 and 3ix3) an increase in binding energy value was high in computer model (both receptors) compared to manual model which had high binding energy in one receptor (3ix3) as shown in table 2.

Relative RMSD values and binding energies of compound no. 18 were compared between computer model and manual model using two different receptors (2uv0 and 3ix3) an increase in binding energy value was high in computer model (both receptors) compared to manual model which had high binding energy in one receptor (3ix3) as shown in table 2.

Relative RMSD values and binding energies of compound no. 19 were compared between computer model and manual model using two different receptors (2uv0 and 3ix3) an increase in binding energy value was high in computer model (both receptors) compared to manual model which had high binding energy in one receptor (3ix3) as shown in table 2.

Relative RMSD values and binding energies of compound no. 20 were compared between computer model and manual model using two different receptors (2uv0 and 3ix3) an increase in binding energy value was high in computer model (both receptors) compared to manual model which had high binding energy in one receptor (3ix3) as shown in table 2.

Relative RMSD values and binding energies of compound no. 21 were compared between computer model and manual model using two different receptors (2uv0 and 3ix3) an increase in binding energy value was high in computer model (both receptors) compared to manual model which had high binding energy in one receptor (3ix3) as shown in table 2.

Relative RMSD values and binding energies of compound no. 22 were compared between computer model and manual model using two different receptors (2uv0 and 3ix3) an increase in

binding energy value was high in computer model (both receptors) compared to manual model which had high binding energy in one receptor (3ix3) as shown in table 2.

Relative RMSD values and binding energies of compound no. 23 were compared between computer model and manual model using two different receptors (2uv0 and 3ix3) an increase in binding energy value was high in computer model (both receptors) compared to manual model which had high binding energy in one receptor (3ix3) as shown in table 2.

Relative RMSD values and binding energies of compound no. 24 were compared between computer model and manual model using two different receptors (2uv0 and 3ix3) an increase in binding energy value was high in computer model (both receptors) compared to manual model which had high binding energy in one receptor (3ix3) as shown in table 2.

Relative RMSD values and binding energies of compound no. 25 were compared between computer model and manual model using two different receptors (2uv0 and 3ix3) an increase in binding energy value was high in computer model (both receptors) compared to manual model which had high binding energy in one receptor (3ix3) as shown in table 2.

Relative RMSD values and binding energies of compound no. 26 were compared between computer model and manual model using two different receptors (2uv0 and 3ix3) an increase in binding energy value was high in computer model (both receptors) compared to manual model which had high binding energy in one receptor (3ix3) as shown in table 2.

Relative RMSD values and binding energies of compound no. 27 were compared between computer model and manual model using two different receptors (2uv0 and 3ix3) an increase in binding energy value was high in computer model (both receptors) compared to manual model which had high binding energy in one receptor (3ix3) as shown in table 2.

Relative RMSD values and binding energies of compound no. 28 were compared between computer model and manual model using two different receptors (2uv0 and 3ix3) an increase in binding energy value was high in computer model (both receptors) compared to manual model which had high binding energy in one receptor (3ix3) as shown in table 2.

Relative RMSD values and binding energies of compound no. 29 were compared between computer model and manual model using two different receptors (2uv0 and 3ix3) an increase in

binding energy value was high in computer model (both receptors) compared to manual model which had high binding energy in one receptor (3ix3) as shown in table 2.

Relative RMSD values and binding energies of compound no. 30 were compared between computer model and manual model using two different receptors (2uv0 and 3ix3) an increase in binding energy value was high in computer model (both receptors) compared to manual model which had high binding energy in one receptor (3ix3) as shown in table 2.

Relative RMSD values and binding energies of compound no. 31 were compared between computer model and manual model using two different receptors (2uv0 and 3ix3) an increase in binding energy value was high in computer model (both receptors) compared to manual model which had high binding energy in one receptor (3ix3) as shown in table 2.

Relative RMSD values and binding energies of compound no. 32 were compared between computer model and manual model using two different receptors (2uv0 and 3ix3) an increase in binding energy value was high in computer model (both receptors) compared to manual model which had high binding energy in one receptor (3ix3) as shown in table 2.

Relative RMSD values and binding energies of compound no. 33 were compared between computer model and manual model using two different receptors (2uv0 and 3ix3) an increase in binding energy value was high in computer model (both receptors) compared to manual model which had high binding energy in one receptor (3ix3) as shown in table 2.

Relative RMSD values and binding energies of compound no. 34 were compared between computer model and manual model using two different receptors (2uv0 and 3ix3) an increase in binding energy value was high in computer model (both receptors) compared to manual model which had high binding energy in one receptor (3ix3) as shown in table 2.

Relative RMSD values and binding energies of compound no. 34 were compared between computer model and manual model using two different receptors (2uv0 and 3ix3) an increase in binding energy value was high in computer model (both receptors) compared to manual model which had high binding energy in one receptor (3ix3) as shown in table 2.

Relative RMSD values and binding energies of compound no. 35 were compared between computer model and manual model using two different receptors (2uv0 and 3ix3) an increase in

binding energy value was high in computer model (both receptors) compared to manual model which had high binding energy in one receptor (3ix3) as shown in table 2.

Relative RMSD values and binding energies of compound no. 36 were compared between computer model and manual model using two different receptors (2uv0 and 3ix3) an increase in binding energy value was high in computer model (both receptors) compared to manual model which had high binding energy in one receptor (3ix3) as shown in table 2.

Relative RMSD values and binding energies of compound no. 37 were compared between computer model and manual model using two different receptors (2uv0 and 3ix3) an increase in binding energy value was high in computer model (both receptors) compared to manual model which had high binding energy in one receptor (3ix3) as shown in table 2.

Relative RMSD values and binding energies of compound no. 38 were compared between computer model and manual model using two different receptors (2uv0 and 3ix3) an increase in binding energy value was high in computer model (both receptors) compared to manual model which had high binding energy in one receptor (3ix3) as shown in table 2.

Relative RMSD values and binding energies of compound no. 39 were compared between computer model and manual model using two different receptors (2uv0 and 3ix3) an increase in binding energy value was high in computer model (both receptors) compared to manual model which had high binding energy in one receptor (3ix3) as shown in table 2.

Relative RMSD values and binding energies of compound no. 40 were compared between computer model and manual model using two different receptors (2uv0 and 3ix3) an increase in binding energy value was high in computer model (both receptors) compared to manual model which had high binding energy in one receptor (3ix3) as shown in table 2.

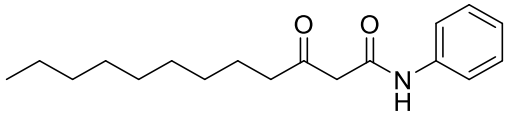
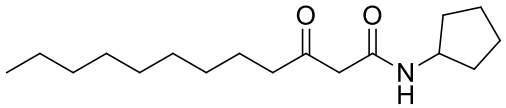
Relative RMSD values and binding energies of compound no. 41 were compared between computer model and manual model using two different receptors (2uv0 and 3ix3) an increase in binding energy value was high in computer model (both receptors) compared to manual model which had high binding energy in one receptor (3ix3) as shown in table 2.

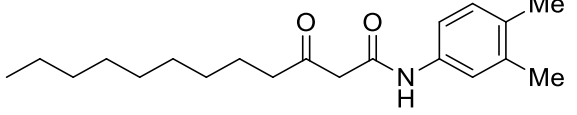
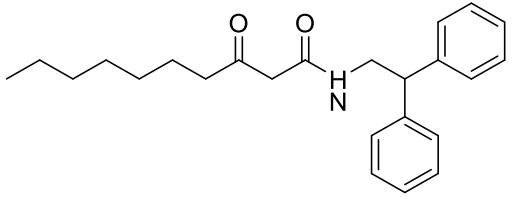
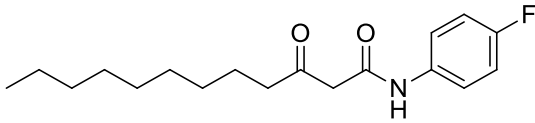
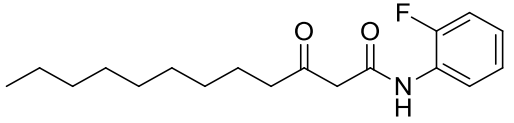
Relative RMSD values and binding energies of compound no. 42 were compared between computer model and manual model using two different receptors (2uv0 and 3ix3) an increase in

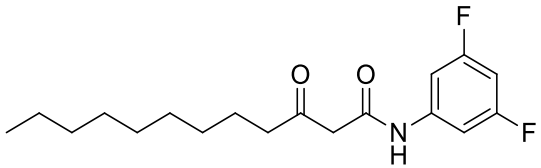
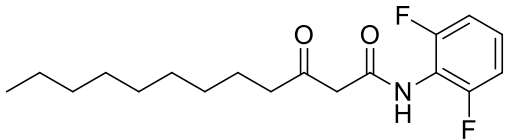
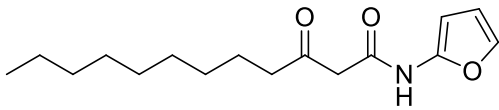
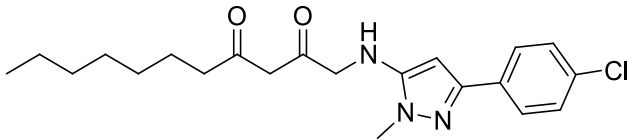
binding energy value was high in computer model (both receptors) compared to manual model which had high binding energy in one receptor (3ix3) as shown in table 2.

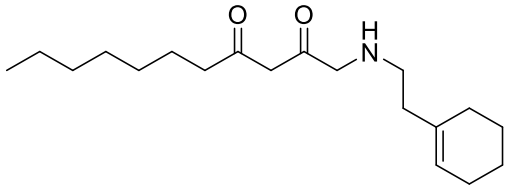
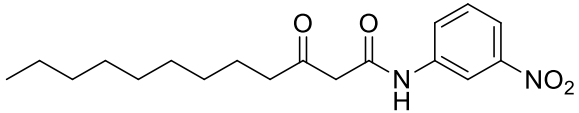
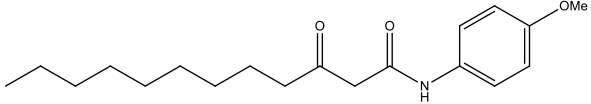
Relative RMSD values and binding energies of compound no. 43 were compared between computer model and manual model using two different receptors (2uv0 and 3ix3) an increase in binding energy value was high in computer model (both receptors) compared to manual model which had high binding energy in one receptor (3ix3) as shown in table 2.

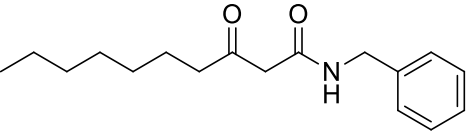
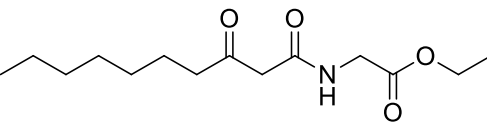
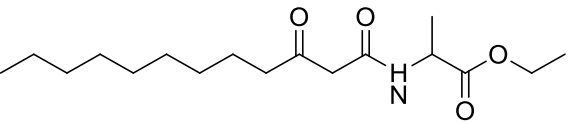
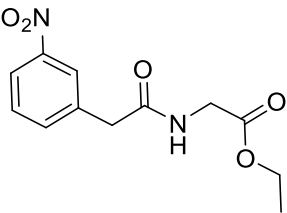
Table 2: List of compounds having antagonism and binding energies (Both from Swiss Dock and AutoDock Vine)

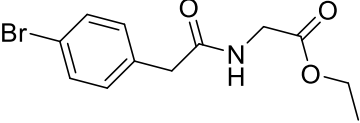
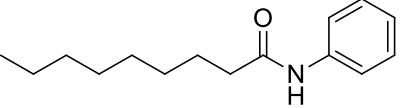
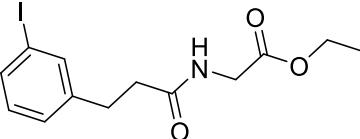
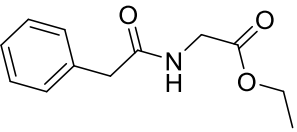
S. No.	Compounds	Antagonism (%)	Antagonism b (%)	Swiss Docking Computer Server (Estimated energy (Kcal/mol))		Manual Docking (AutoDock Vina)	
				2uv0	3ix3	2uv0	3ix3
1.	 3-oxo-N-phenyldodecanamide	54	29	-9.79	-9.79	-4.6	-9.0
2.	 N-cyclopentyl-3-oxododecanamide	-75	-9	-9.98	-9.59	-4.4	-8.8

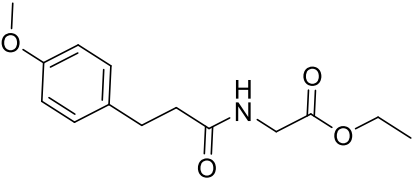
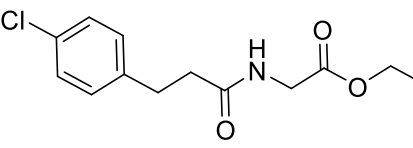
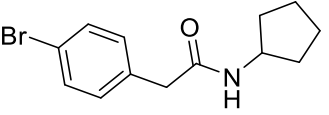
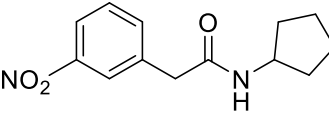
3.	 <p>N-(3,4-dimethylphenyl)-3-oxododecanamide</p>	13	9	-8.88	-9.27	-4.0	-9.5
4.	 <p>N-(2,2-diphenylethyl)-3-oxododecanamide</p>	17	8	-7.53	-7.70	-4.7	-10.0
5.	 <p>N-(4-fluorophenyl)-3-oxododecanamide</p>	13	13	-9.76	-9.75	-4.7	-9.3
6.	 <p>N-(2-fluorophenyl)-3-oxododecanamide</p>	38	13	-10.20	-9.57	-5.1	-9.2

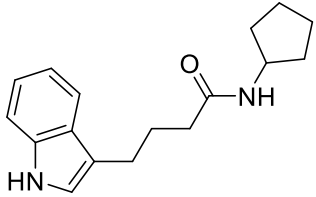
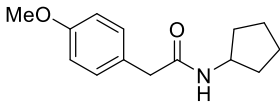
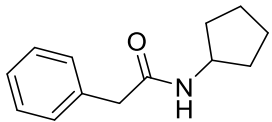
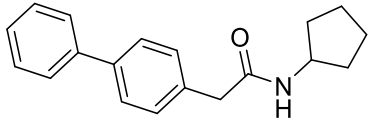
7.	 <p>N-(3,5-difluorophenyl)-3-oxododecanamide</p>	22	16	-9.79	-9.93	-5.2	-9.6
8.	 <p>N-(2,6-difluorophenyl)-3-oxododecanamide</p>	13	5	-9.97	-9.01	-4.3	-9.4
9.	 <p>N-(furan-2-yl)-3-oxododecanamide</p>	0	26	-9.40	-9.38	-4.7	-8.5
10.	 <p>1-((3-(4-chlorophenyl)-1-methyl-1H-pyrazol-5-yl)propyl)-3-oxododecanamide</p>	0	0	-7.32	-9.16	-4.2	-7.4

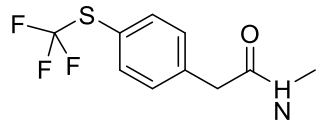
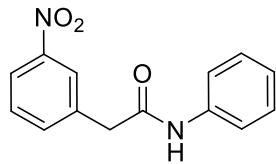
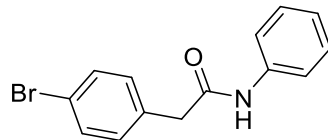
	yl)amino)undecane-2,4-dione						
11.	 <p>1-((2-(cyclohex-1-en-1-yl)ethyl)amino)undecane-2,4-dione</p>	10	-7	-8.78	-9.49	-4.1	-9.0
12.	 <p>1-((2-(4-nitrophenyl)ethyl)amino)undecane-2,4-dione</p>	41	17	-8.92	-9.25	-4.3	-9.5
13.	 <p>N-(4-methoxyphenyl)-3-oxododecanamide</p>	-68	38	-9.53	-9.56	-3.4	-8.1

14.	 <p>N-benzyl-3-oxodecanamide</p>	-4	5	-9.09	-9.30	-5.0	-9.0
15.	 <p>ethyl (3-oxodecanoyl)glycinate</p>	-136	11 -	-9.44	-8.87	-3.9	-7.9
16.	 <p>ethyl (3-oxododecanoyl)alaninate</p>	-4	23	-9.66	-9.34	-3.9	-8.2
17.	 <p>ethyl (2-(3-nitrophenyl)acetyl)glycinate</p>	41	-21	-8.49	-8.28	-4.9	-8.4

18.	 <p>ethyl (2-(4-bromophenyl)acetyl)glycinate</p>	33	-15	-8.68	-8.13	-4.5	-8.0
19.	 <p>N-phenylnonanamide</p>	33	-15	-8.77	-8.66	-4.5	-8.9
20.	 <p>ethyl (3-(3-iodophenyl)propanoyl)glycinate</p>	38	-27	-9.07	-9.12	-5.2	-8.9
21.	 <p>ethyl (2-phenylacetyl)glycinate</p>	38	-17	-8.01	-8.05	-4.5	-8.3

22.	 <p>ethyl (3-(4-methoxyphenyl)propanoyl)glycinate</p>	36	-10	-8.91	-8.73	-5.2	-8.4
23.	 <p>ethyl (3-(4-chlorophenyl)propanoyl)glycinate</p>	38	-6	-8.42	-8.94	-5.2	-8.4
24.	 <p>2-(4-bromophenyl)-N-cyclopentylacetamide</p>	11	-11	-8.42	-8.42	-5.2	-9.3
25.	 <p>N-cyclopentyl-2-(3-nitrophenyl)acetamide</p>	29	-5	-8.59	-8.45	-5.3	-9.3

26.	 <p>N-cyclopentyl-4-(1H-indol-3-yl)butanamide</p>	20	-10	-8.27	-8.77	-5.7	-10.2
27.	 <p>N-cyclopentyl-2-(4-methoxyphenyl)acetamide</p>	24	-1	-8.41	-8.35	-5.2	-9.5
28.	 <p>N-cyclopentyl-2-phenylacetamide</p>	11	-26	-8.02	-7.98	-5.0	-9.2
29.	 <p>2-([1,1'-biphenyl]-4-yl)-N-cyclopentylacetamide</p>	4	-26	-8.22	-8.52	-5.0	-11.7

30.	 <p>N-methyl-2-(4- ((trifluoromethyl)thio)phenyl)acetamide</p>	20	5	-8.03	-7.94	-4.9	-8.3
31.	 <p>2-(3-nitrophenyl)-N-phenylacetamide</p>	12	0	-8.37	-8.15	-5.9	-10.5
32.	 <p>2-(4-bromophenyl)-N-phenylacetamide</p>	20	12	-7.92	-4.98	-5.5	-10.0

According to the docking studies done on the compounds above, the Swiss Dock online server in respect with the receptor 3ix3 compounds 7,1,5,2,6 showed high binding affinity but inhibition properties were low, while receptor 2uv0 showed high binding affinity and low inhibition properties with compounds 6,2,8,1,7.

The AutoDock manual docking in respect with the receptor 2uv0 showed high binding affinity as well as inhibition with the compound 31,26,32,25,7 while the receptor 3ix3 showed high binding affinity and inhibition with compound 29,31,26,32,4.

From the above study we can also conclude that manual docking is more reliable than computer model and among all the docked compounds in respect with both the receptors compound 29 with receptor 3ix3 showed to be more potent molecule which showed high binding affinity as well as inhibition properties, this molecule has opened up a novel to the discovery of more drugs which can be used in the development of potent Quorum based anti-virulence therapeutics for targeting infections caused by *Pseudomonas aeruginosa*.

CHAPTER 7

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