



***In silico* Analysis of Plant Based Quorum Sensing Inhibitor against *Chromobacterium violaceum* CviR**

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Background: *Chromobacterium violaceum* (*C. violaceum*), a Gram-negative, facultative anaerobic, non-spore-forming coccobacillus has a quorum-sensing system consisting of CviI/CviR, a homologous gene. Quorum sensing (QS) is a mechanism of intercellular communication in bacteria that received substantial attention as an alternate strategy for combating bacterial resistance and the development of new anti-infective agents.

Methods: DATA SET Information of photochemical from the natural source deposited as a machine readable format in PubChem database was utilized to retrieve the compound for the study. To study ligand - receptor interactions, docking paves way to accomplish the protein ligand interaction was docked through rigid docking CviR protein (PDB ID: 3QP5) was prepared and energy minimized to evaluate the best affinity among the complex.

Results: The results showed that the Alpha.,2.Alpha.- Epoxy-1.Beta.- Methyl Cholesta-4,6- Dien-3-One had high affinity for CviR receptor protein and Alpha.,2.Alpha.- Epoxy-1.Beta.- Methyl Cholesta-4,6- Dien-3-One binds to the active site of CviR with binding energy of -9.6 kcal/mol.

Conclusion: Overall study concluded that 1. Alpha., 2. Alpha.- Epoxy-1.Beta.-Methyl Cholesta-4,6-Dien-3-One with highest binding affinity for the CviR protein possessing strong inhibitory binding

interaction. Hence, we concluded that 1.alpha.,2.alpha.-Epoxy-1.Beta.- Methyl Cholesta-4, 6-Dien-3-One good serves as potential an anti-quorum sensing molecule for treating *C. violaceum* infection.

Keywords: Quorum sensing; *Chromobacterium violaceum*; environment; docking study; ligand; receptor.

1. INTRODUCTION

Quorum sensing (QS) is a cell to cell communication system found in bacteria which coordinates the cell density and is responsible for the expression of several genes encoding virulence factor. QS depends on the release of small diffusible signaling molecules called autoinducers (AIs) [1]. The cell density proportionally increases with increase in AIs [2]. QS is not only responsible for the expression of genes encoding virulence factors but also responsible for motility, competence, sporulation, pigment production, antibiotic production, bioluminescence and biofilm formation Through this mechanism the bacterial species became resistant to wide range of antibiotics thus making it difficult to cure the disease[3]. Thus the QS system becomes the target for development of antibacterial drugs as the drugs only target the QS mechanism without interfering with the growth of bacteria [4].

In Gram-negative bacteria Acyl Homoserine Lactones (AHL) acts as the AIs. A variety of gram-negative bacterial species such as *Pseudomonas aeruginosa*, *Vibrio cholerae*, *Escherichia coli*, *Klebsiella pneumonia* and *Chromobacterium violaceum* (*C. violaceum*) etc. have been extensively studied in QS inhibition. The QS regulatory mechanism components differ from different species of bacteria. Some bacteria have a single component system and some have two or more regulatory mechanisms [5].

C. violaceum is a Gram-negative opportunistic pathogen which exhibits its virulence through QS signaling mechanisms. *C. violaceum* infections are difficult to identify because uncommon are rare infections but are fatal. Several *C. violaceum* infections caused in both humans and animals have been reported: pneumonia in buffaloes, cattle and monkeys. In humans various infections such as septicemia, multiple abscesses in the lungs, liver, spleen, lymph nodes, pneumonia, and urogenital infection have been identified as a cause of *C. violaceum* [6]. *C. violaceum* has two QS system such as Cvil and CviR (LuxI/LuxR homolog) which regulates the production of AHL.

The signalling molecule N-decanoyl-L-homoserine lactone (C10-HSL) production is mediated with the regulation of the protein synthase Cvil with the help of CviR transcriptional regulator protein. Through this signal mechanism *C. violaceum* exhibit the production of several virulence factors such as metalloproteases, exopolysaccharide, lipases, chitinase, collagenase, cytolytic toxins and also leads to the production of pigment called violacein and biofilm formation [6].

In this scientific world various drugs have been discovered for the treatment and cure of many diseases caused by microorganisms Recent studies and research shows that with continuing exposure to a broad range of drugs the microorganisms tend to develop drug-resistance which makes it difficult to cure and it has been found that this drug-resistant tendency in bacteria is regulated by the QS mechanisms [7]. Thus the QS system inhibition becomes an important part of drug discoveries. Our country is blessed with many medicinal plants which have been used as our food source. These plants contain compounds that have antimicrobial, anti-fungal, antioxidant and anti-inflammatory activities. These compounds can act as the natural inhibitors for QS inhibition. Many recent studies are focused on the identification of these QS inhibitors and their potential through various studies from naturally available plant parts and till date many plants and their QS inhibitors have been identified [8]. Our team has extensive knowledge and research experience that has translate into high quality publications [9–13]. Aim of study is to identify the plant based QS inhibitor against control strain of *C. violaceum*.

2. MATERIALS METHODS

2.1 Data Set

Information of phytochemical (Alpha.,2.alpha.-epoxy-1.beta.-methyl cholesta-4,6- dien-3-one) from the natural source deposited as a machine readable format in PubChem database was utilised to retrieve the compound for the study. To study ligand (Alpha.,2.alpha.-epoxy-1.beta.-

methyl cholesta-4,6- dien-3-one) - receptor (CviR) interactions, docking paves way to accomplish the protein ligand interaction was docked through rigid docking CviR protein (PDB ID : 3QP5) was prepared and energy minimised to evaluate the best affinity among the complex.

2.2 Docking Study

C. violaceum (CviR protein) was prepared and energy minimization was done to evaluate affinity among the ligand receptor complex. The proposed binding mode of the eleven derived compounds was obtained through docking calculations between the derived compounds (Alpha.,2.alpha.-epoxy-1.beta.-methyl cholesta-4,6- dien-3-one). CviR protein - Alpha.,2.alpha.-epoxy-1.beta.-methyl cholesta-4,6- dien-3-one complex was imported to the system. The autodock was selected for docking study because the algorithm maintains a rigid macromolecule while allowing ligand flexibility; the docking simulation was conducted using the Veena docking algorithm. The binding affinity of identified compounds of the bioactive compound from NIST library and the natural autoinducers to the transcriptional receptor, CviR protein was retrieved from Pubchem and data bank. In crystal structure, missing residues are added and water

molecules around the receptor were removed using the protein preparation wizard. The crystal structure active site grinds generation was adjusted with co crystallization compounds. The chemical compounds energy minimizing, rotatable bonds and hydrogen bond optimization was executed with OPLS force field .Interaction of receptor and ligands were prepared using the maestro schrodinger suite. Docking of bioactive compound and receptor was consequently performed using a glide module to analyse the confirmation of protein ligand complex.

3. RESULTS

The bioactive compound (Alpha.,2.alpha.-epoxy-1.beta.-methyl cholesta-4,6- dien-3-one) from database and the selected compound was docked against the ligand binding domain of CviR using the docking programme (Glide 5.5). The docking score of this bioactive compound (natural sources) was compared with the docking score of CviR protein. The bioactive compound Alpha.,2.alpha.-epoxy-1.beta.-methyl cholesta-4,6- dien-3-one was docked against CviR the binding affinity was scored in (Table 1) .Alpha.,2.alpha.-epoxy-1.beta.-methyl cholesta-4,6- dien-3-one scored the highest binding affinity is -9.6. Kcal/mol (Table 1).

Table 1. The table represents the binding affinity of the compound (Alpha.,2.alpha.-epoxy-1.beta.-methyl cholesta-4,6- dien-3-one) and the ligand CviR (PDB ID, 3QP5) is -9.6

S. No	Ligand	Name of the Compound	Binding affinity
1.	CviR_3034666	1 Alpha.,2.Alpha-Epoxy-1Beta-Methylcholesta-4,6-Dien-3-One	-9.6

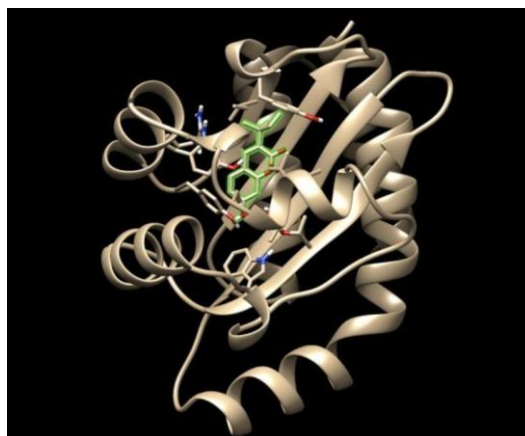


Fig. 1. The figure represents the interaction between the compound (Alpha.,2.alpha.-epoxy-1.beta.-methyl cholesta-4,6- dien-3-one) and the ligand CviR (PDB ID, 3QP5)

4. DISCUSSION

Plant releases various phytochemical constituents which are mainly used to prevent bacterial and fungal infection. Similarly the phytochemical compounds inhibit the QS system and biofilm formation of Gram positive and Gram negative bacteria. Only limited compounds have been identified as potential QS inhibitor. Docking study revealed that Alpha.,2.alpha.-epoxy-1.beta.-methyl cholesta-4,6- dien-3-one scored the highest binding affinity is -9.6. Kcal/mol. The interaction of Alpha.,2.alpha.-epoxy-1.beta.-methyl cholesta-4,6- dien-3-one and CviR ligand complex was presented as 3-D image (Fig. 1). Venkatramanan et al 2020 reported that the bioactive compounds were docked and had the highest binding affinity of CviR receptor. In similar study aromatic aldehyde 5-hydroxymethylfurfural at subinhibitory concentration was capable of modulating quorum sensing regulated phenotypes and inhibited biofilm formation in the *C. violaceum* as determined by in silico analysis. Similarly, Annapoorani et,al reported that *Chromobacterium violaceum* were docked with lasR receptors. The bioactive compound inhibited biofilm formation and docking analysis showed that selected compounds bind with CviR protein [14]. Our team has extensive knowledge and research experience that has translate into high quality publications [15–34].

5. CONCLUSION

Overall study concluded that 1.Alpha.,2.Alpha.-Epoxy-1.Beta.-Methyl Cholesta-4,6-Dien-3-One with highest binding affinity for the CviR protein possessing strong inhibitory binding interaction. Further, the study revealed that molecular docking analysis can provide focused finding of QS and biofilm inhibitors to combat Gram-negative bacterial infections. Hence, we concluded 1.Alpha.,2.Alpha.-Epoxy-1.Beta.-Methyl Cholesta-4, 6-Dien-3-One good serves as an anti-quorum sensing agent.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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