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Research Article

In-silico Screening of Antibiofilm Activity against *Acinetobacter baumannii*

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Abstract

Acinetobacter infections are difficult-to-treat because of their resistance patterns. Bacteria have been developing broader range of resistance against many drugs used in treating infections, the discovery of new lead compounds requires a safe strategy to combat as antimicrobials. Therefore, identification of natural compounds is highly essential for developing lead antibiotic like drugs and hence, the study is aimed at identifying the potential compound through computational screening. Computational screening for identification of potential antibiofilm agent was performed using PubChem database. The 3D structure model of Biofilm-associated protein (Bap) protein was built using Modeller 9.19 Version. Twenty five compounds were selected for screening of Antibiofilm activity against *Acinetobacter baumannii.* In computational analysis, virtual screening was performed on only one compound namely: Parecoxib. *In-silico* screening, identified Parecoxib as a potential inhibitor for (Bap) protein of *A. baumannii*. Our results suggest that Parecoxib can be developed as a lead anti-biofilm drug to treat patients infected with *Acinetobacter baumannii.*

Keywords: *A. baumannii*; Biofilm-associated protein (Bap); Parecoxib; Docking

Introduction

Bacteria release quorum sensing (QS) peptides that are detected in the cell by signaling in a process of quorum sensing. When the bacteria reached its threshold population density, several of the genes involved in metabolic functions were activated in order to release specific signaling molecules for the survival of bacteria and to acquire great antibiotic resistance. The phenomenon in which cell density of bacteria determines the genetic metabolic function in a signal dependent coordination is called quorum sensing. The expression of specific genes was hindered by quorum sensing and some such common phenotypes include virulence, antibiotic resistance and biofilm formation [1]. Biofilms are mixed or single microbial communities are immersed in an extracellular matrix composed of proteins. Microbial infections in the body are caused by pathogens that specifically develop biofilm through quorum sensing mechanism. Bacteria release signaling molecules that enable this quorum sensing mechanism. Interruption of molecular signaling network of biofilm bacteria develops drug resistance and is one of the important anti-virulence strategies used to target biofilm [2].

Quorum sensing does not affect the growth pattern but lowers the risk of resistance by the diffusion mechanism of signaling molecules. Researchers have been screening new targets from natural plant sources that can potentially be used for developing new antibiotics for effective resistance against many bacteria. Phenolic compounds provide an important strategy in drug discovery by targeting proteins and the complexes responsible for the formation of biofilm. These proteins perform very important functions and are critical for the ability to successfully infect humans. Parecoxib is a water-soluble and injectable prodrug of valdecoxib. Here, it is used as an agent that blocks the active sites on quorum-sensing receptors [3].

Materials and Methods

Homology modelling

The Biofilm-associated protein (Bap) amino acid sequence of *Acinetobacter baumannii* was retrieved from uniprot-KB (Accession no. >AKL78797.1). Blast analysis was performed on the 396 amino acid length query sequence. PDB database repository does not contain the crystal structure of Biofilm-associated protein (Bap). The protein model was built using Modeller 9.19 [4]. The model performs modelling of loops in protein structures. Based on the secondary structure data generated from RAMPAGE and Biofilm-associated protein (Bap) value, the best model would be selected for further screening. DISOPRED-3 and PSI-BLAST were used for identifying the binding residues and alignment. Ligand binding sites were predicted using Cast-P Server.

Ligand preparation and molecular docking

Anti biofilm drugs were screened from natural compound libraries. Anti-biofilm activity of selected drug was found after virtual screening analysis. Twenty five FDA approved drugs were chosen for screening by molecular docking analysis. Three dimensional structures of the drugs were retrieved from pubchem database. Stereoisomers of selected ligands were created using online web server (http://www.cheminfo.org) and ten conformations were

generated for each ligand [5]. Virtual screening was performed using MTiOpenScreen and Autodock 4.2 was used for the molecular docking analysis of Biofilm-associated protein (Bap), three dimensional homology model generated protein and the selected ligands.

Results and Discussion

Standardization of homology model

Homology models were generated by the modeller and the quality of each model was assessed using Rampage, Procheck and RMSD value. DISOPRED-3 provided the intrinsic disorder profile of protein structure which was found to be between 100 and 120 amino acid chain length, whereas PSI- Blast, PSI-PRED and MEMSAT-3 provided the information about the secondary structure (Figure 1 and 2). The model which showed highest percentage, which was about 96% of amino acids in favorable region was selected and finally the model was optimized to 98 % using SPDB viewer. Lowest value of RMSD was obtained for the same model selected after Rampage and Procheck analysis (Figure 3). Template structure was aligned with homology model and the RMSD value was found to be 0.706.

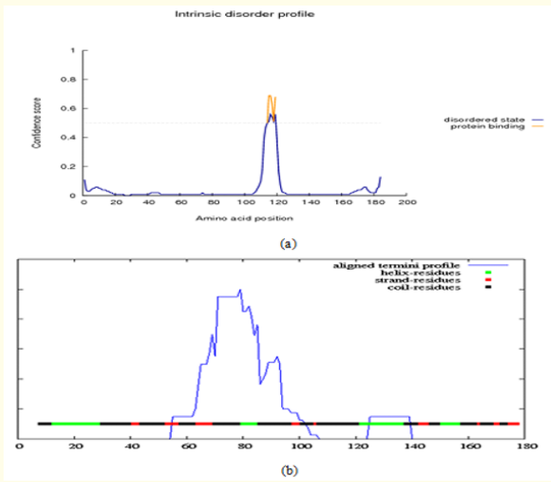

Figure 1: (a): DISOPRED-3 showed the disordered regions of the protein (b): Density of the end points of PSI-BLAST alignments in secondary structure of protein.

Ligand preparation and ADMET analysis

Twenty five ligands were prepared and 250 stereoisomers were generated. Virtual screening was performed using MTiOpenScreen which further passes only one compound, Parecoxib with pubchem accession number DB08439.

Molecular Docking using Autodock 4.2

Modeller generated homology model, Biofilm-associated protein (Bap) was docked with the selected ligand Parecoxib and their lowest energy conformations were analyzed for different stereoisomers. The residues THR 291, SER 128, GLY 177, GLU 299, ASP 190, LYS 181 and PRO 41 are positioned around ligand, whereas the residues THR 251 AND LYS 36 form a hydrogen bond (Figure 5).

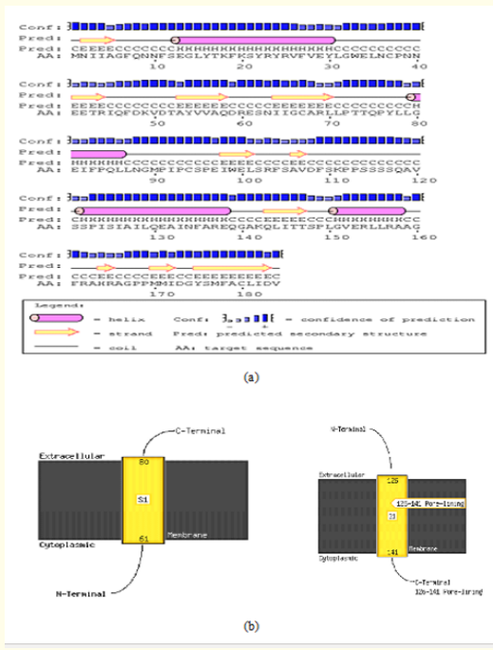

Figure 2: (a): PSIPRED shows the secondary structure information and (b): MEMSAT-3 analyzed topology of the helix integral membrane.

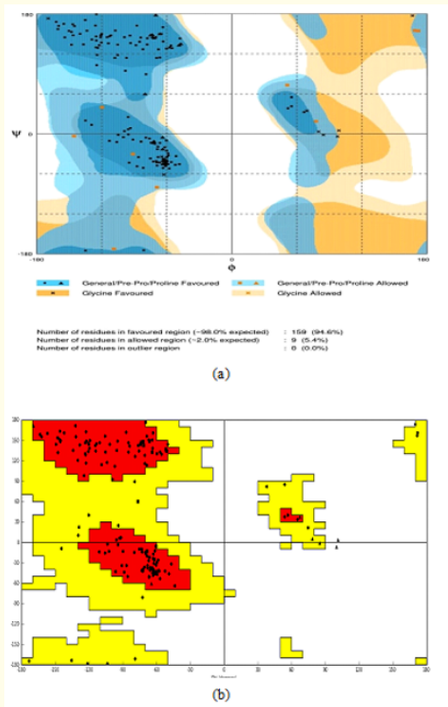

Figure 3: Ramachandran plot of Biofilm-associated protein (Bap) model of *A. baumannii* analyzed by Rampage (a) Procheck (b) where the 98% of the amino acid residues were found in favorable regions.

The selected ligand Parecoxib and the modeled receptor Biofilm-associated protein (Bap) protein were successfully docked with a docking score of -30.462 kJ/mol.

Figure 4: Homology model generated by Modeller and visualized using chimera.

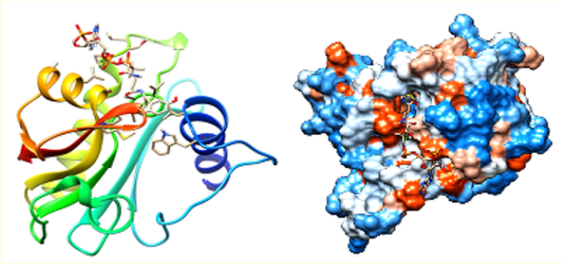

Figure 5: Parecoxib interactions and stable binding poses with Biofilm-associated protein (Bap).

Conclusion

Very limited information is currently available regarding the development of antibiofilm agents. The greatest challenge to researchers in this particular field will be the development of an antibiofilm agent which could disrupt and degrade the extracellular matrix network of biofilm secreted by the pathogenic strains of bacteria. The compound Parecoxib showed significant *in silico* anti-biofilm effects against *Acinetobacter baumannii*, hence, it can be further investigated against different resistant strains of pathogenic bacteria.

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