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Receptor-Ligand Interactions and Simulation Studies in *S.Pneumoniae*: An in-silico approach

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ABSTRACT

Pneumonia is a lung inflammation which is often caused by the infection of bacteria, viruses or other pathogenic organisms as shown in Figure 1.1. *Streptococcus pneumoniae is* one among the most significant microbes which causes bacterial disease in humans. In developing countries pneumonia remains a leading cause of death. In this work we performed the homology modeling studies of the virulent protein which is synthesized by the genes and validated the nature of the receptor as a future drug target for the above strains of *Streptococcus pneumoniae* using modeller 9v7. The Ribosomal protein L4, Penicillin-binding Protein 3 which are coded by rpLD and Protein coding gene respectively in strain Hungary 19A-6, glycoprotein which are coded by amiD, bgaC, pyrDA, ribE in D39, hypothetical proteins coded by various coding genes in TIGR4 , Capsule Polysaccharide biosynthesis proteins CpsB, CpsC, and CpsD of G54, Polysaccharide polymerase protein and capsular polysaccharide biosynthesis protein coded by wzy and wzd respectively in CGSP14 and transposase protein and Phage encoded protein coded by protein encoding genes in TCH8431 were modelled and validated High score analogs were subjected to molecular dynamic simulation to find the stability of the protein-ligand complex.

Keywords: Pneumonia, *Streptococcus pneumoniae,* Docking,simulation and dynamics.

INTRODUCTION

Pneumonia is an inflammation of lung that is most often caused by infection with bacteria, viruses or other pathogenic organisms, *Streptococcus pneumoniae* is among the most significant causes of bacterial disease in humans (Avery, 1944; Klein, 1999; Feldman, 1991).

Streptococcus pneumoniae was described without knowingly by C.E. Aretaeus in the 2nd century, he was a Grecian physician who described the sufferings of his patient but did not know the symptoms were caused by bacteria (Avery, 1944). By the 1960s, a true pneumonia vaccine was again found (Applebaum,1996).The most development and publicity of penicillin resistance and antibiotic resistance have resulted of multiple factors, but before antibiotic use, childeren, and day care attendance were the most commonly identified risk features (Applebaum,1996). Many reports of deaths related to pneumococcal isolates with decreased susceptibility to penicillin were reported in the 1970s (Ahronheim, 1979). From 2001 to 2003, scientists found that *S. pneumoniae* bacteria were resistant to usual drug treatments. Their samples came from children attending 13 day care centers in the city of Lisbon. (Nunes, 2005). In 1980s, ceftriaxone or cefotaxime were mostly recommended for best treatment of children with suspected bacterial meningitis (McCracken, 1987). In 1990s, as penicillin-resistant *S. pneumoniae* isolates became more in number throughout the world, treatment failures associated with cefotaxime or ceftriaxone for pneumococcal meningitis were reported (Adams, 1993).

Computational biology is turned out to be an important technology applied to strengthen the biological research in the recent years. This proficient way helps to predict the structural and functional importance of macromolecules such as nucleic acids, proteins, carbohydrates, etc. Several arrays of bioinformatics tools and servers are helping us to work on different biological interests. In the present study, an attempt is made to understand the structural and functional aspects of some of the selected proteins from different strains of *S. pneumoniae* through the computational studies such as structure prediction, virtual screening, pharmacophore analysis, pharmacokinetic profiling of selected sets of ligand molecules, molecular docking, molecular dynamics and simulation studies.

The increasing prevalence of resistance to antibiotics traditionally used in the treatment of pneumococcal infections has increased remarkably during recent years which in principal demands to find novel drugs to combat pneumonia. Hence, the current work aims at identifying and characterizing novel drug targets by a detailed analysis of available genome data with bioinformatics tools. Comparative analysis of metabolic pathways will be carried out which may reveal potential targets. Antibiotic agents including natural and synthetic compounds as such will be screened

against these targets using state-of-the-art bioinformatics techniques such as molecular docking, dynamics etc. This chapter briefly explains some of the important methodologies employed to explore genome data in order to bring out few novel target structures followed by some ligand candidates.

MATERIALS AND METHODS

Sequence of the target proteins were retrieved from UniProtKB/Swiss-Prot. Templates for the target proteins were identified and retrieved using PDB-sum based on the identity of the protein sequence. 3D structure of the template which is used for modeling the target protein was retrieved from PDB. The sequence alignment of the protein and template sequences was performed using Clustal W. Modeller 9v7 was used for homology modeling of protein three-dimensional structure. SAVS was used to visualize dihedral angles φ against ψ of amino acid residues in structure through Ramachandran plot. To view the protein structure and to perform energy minimization for making the protein stable we used Swiss PDB viewer.

Drugs which can be used as the ligands for the protein molecule were obtained from Drug port and the ligand structures were retrieved from PubChem (Wang, 2012). From the 3D structure of the proteins, structural pockets and cavities were predicted using Castp and Q-Site Finder, Argus lab is used for finding the binding energy required for each ligand to get docked with the respective protein (Joe Dundas, 2006).

Discovery Studio was used for docking and dynamic simulation study, receptor-ligand interactions were analyzed to find interactions between protein receptors and ligands. This allows to carry out structure-based design or even to examine possible interactions with theoretical structures such as homology models. The ligand fit docking was performed specifying the region of the receptor's binding. The ADMET analysis for all the ligand molecules was also studied through Discovery Studio.

RESULTS AND DISCUSSION

The best docked analog with individual protein from each strain was used for further intra strain docking,in inter strain dockin the riboflavin 7,8-dimethyl-10-(3,4,5-trihydroxypentyl) benzo[g]pteridine-2,4-dione has best dock score with D6ZS38 protein of TCH8431-A strain, B5E791 protein of G54 strain. IMIPRAMINE analog 3-(5,6-dihydrobenzo[b] [1] benza zepin-11-yl)-N,N-dimethylpropan-1-amine has best dock score with D6ZPG9 protein of TCH8431 strain and B1IB40 protein of HUNGARY 19-A strain.

The cefazolin analog ((7R)-3-[(5-methyl-1,3,4-thiadiazol-2-yl)sulfanylmethyl]-8-oxo-7-[[2-(tetrazol-1yl) acetyl] amino]-5-thia-1-azabicyclo [4.2.0] oct-2-ene-2-carboxylic acid has best dock score with Q04LD9 protein of D39 strain, B2ILP9 protein of CGSP14 strain.

The fluconazole analog 2-(2,4-difluorophenyl)-1,3-bis(1,2,4-triazol-1-yl) propanol has best dock score with Q97SI9 protein of TIGR-4 strain, B2ILP4 protein of CGSP14 strain.

From intra strain multiple docking we analyzed that few analogs had high scores with other strains of *Streptococcus pneumoniae*. The high score analogs were subjected to dynamics simulation study to find the stability of the Protein-ligand complexes. Based on our Insilco analysis we conclude that the receptors and ligands can be considered as the best drug targets and drug candidates respectively.

MOLECULAR DYNAMIC SIMULATIONS

Computer simulation is a feasible method to understand the properties of molecular assemblies in terms of their structure and the microscopic interactions between them. There are two main techniques in simulation family for understanding the nature of molecular interactions and those techniques are Molecular Dynamics (MD) simulation and Monte Carlo (MC) Simulation (Price, 2000). In addition to the above mentioned techniques there are certain hybrid techniques which combine both MD and MC simulation. The advantage of MD over MC is that it predicts a route map to the dynamical properties of a system which depends on factors like transport coefficients, time-dependent responses to perturbations, rheological properties and spectra (Lindahl, 2008). The docked complexes with a maximum dock score were subjected to Molecular Dynamics Simulation by the Protocols present in the module of Standard Dynamic Cascade in Discovery Studio. In case of MD simulation, initial step is to type the complex with CHARM force field. Then the molecule is subjected to minimization of 3000 steps by steepest descent algorithm and 500 steps by conjugate gradient

algorithm. Further, the molecules were subjected to equilibration by NVT ensemble. Finally, in production step, we have given 1, 00,000 cycles (1ns) and analyzed its trajectory.

In order to ascertain the conformational variations of the various target-ligand complexes, molecular dynamics simulations have been carried out for the top scored receptor-ligand complexes. For each complex molecular dynamics simulations have been carried for a period of 1ns. Over all analysis of the molecular dynamics trajectories for each complex structure reveals that the complexes were well stabilized at the respective binding sites. The results of the analysis carried out on the molecular dynamics simulation trajectories of the best docked ligands after inter strain multiple docking is discussed below.

Docking and dynamics simulation study of 3-(5, 6-dihydrobenzo[b][1]benzazepin-11-yl)-N,N-dimethylpropan-1-amine with Phage encoded protein (D6ZPG9):

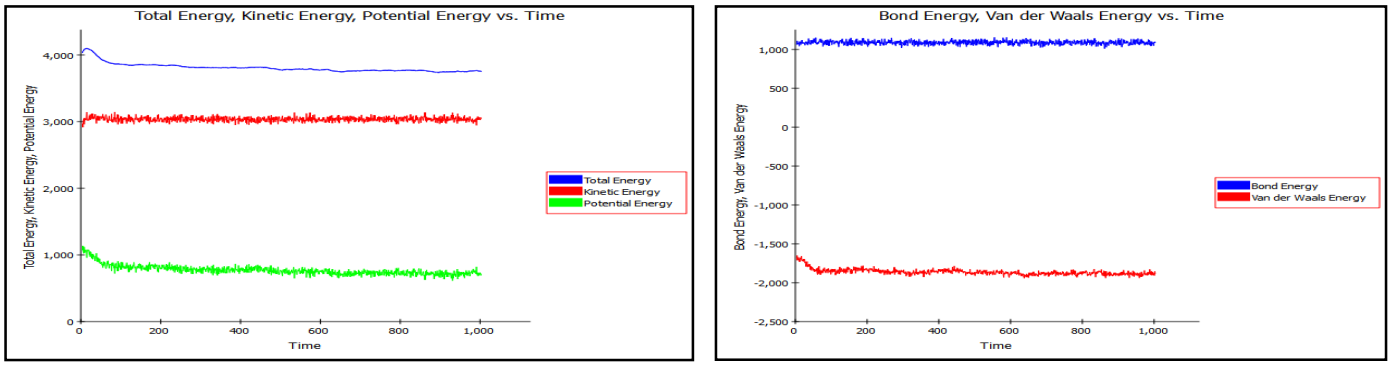

Figure.1.Energy profiles of 3-(5, 6-dihydrobenzo[b][1]benzazepin-11-yl)-N,N-dimethylpropan-1-amine with Phage encoded protein (D6ZPG9) complex

The analog of ligand imipramine had the score of 73.898 and was subjected to dynamics simulation study. Figure 1 shows the total energy, kinetic energy and potential energy profiles over the period of simulations during production run. It can be noted that there is not much deviation indicates that the compounds bind in a better position. Further analyses reveals that the binding modes of the compounds established after the MD simulation are nearly the same as that obtained of molecular docking.

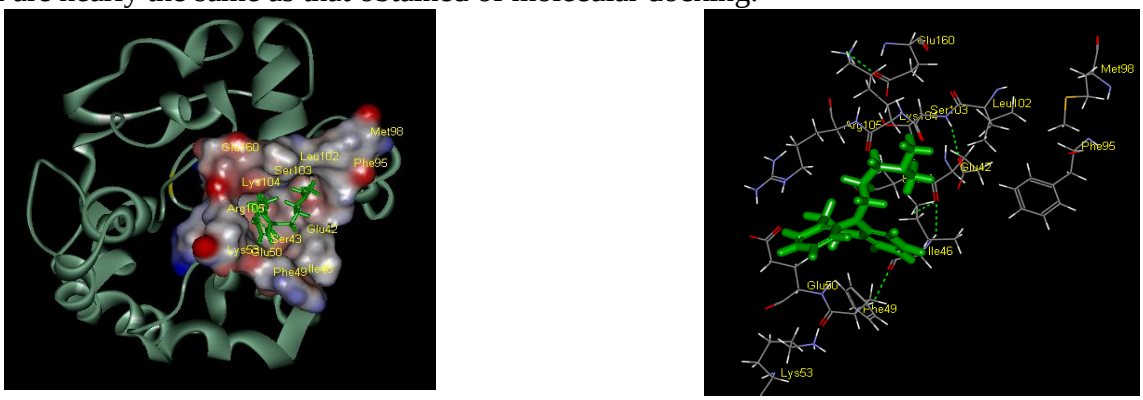

Figure 2.Molecular interactions of 3-(5, 6-dihydrobenzo[b][1] benzazepin-11-yl)-N,N-dimethylpropan-1-amine with Phage encoded protein (D6ZPG9) com

It is clear from the Figure 2 that the compound is well accommodated in the binding site cavity and is stabilized by intermolecular hydrogen bonds. It is interesting to note that in addition to the interactions with the residues there exists stacking interaction of the aromatics ring of Phe49 with the ring structure of the ligand and are observed throughout the period of dynamics simulations illustrating the importance of this interaction in ligand binding at the binding site.

The analog of ligand riboflavin had the score of 64.628 and was subjected to dynamics simulation study. Figure 3 shows the total energy, kinetic energy and potential energy profiles over the period of simulations during production run. It can be noted that there is not much deviation indicates that the compounds bind in a better position. Further analyses

reveals that the binding modes of the compounds established after the MD simulation are nearly the same as that obtained of molecular docking.

Docking and dynamics simulation study of 7, 8-dimethyl-10-(3,4,5-trihydroxypentyl) benzo[g]pteridine-2,4-dione with Capsular polysaccharide biosynthesis protein wzh (B5E791):

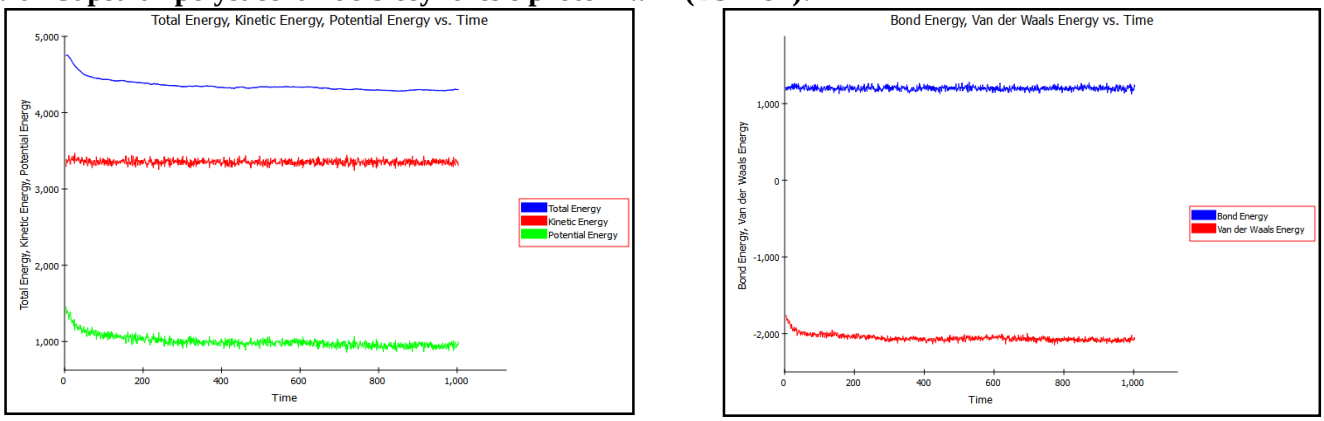

Figure.3. Energy profiles of 7, 8-dimethyl-10-(3, 4, 5-trihydroxypentyl) benzo[g]pteridine-2,4-dione with Capsular polysaccharide biosynthesis protein wzh (B5E791)

The analog of ligand riboflavin had the score of 64.628 and was subjected to dynamics simulation study. Figure 3 shows the total energy, kinetic energy and potential energy profiles over the period of simulations during production run. It can be noted that there is not much deviation indicates that the compounds bind in a better position. Further analyses reveals that the binding modes of the compounds established after the MD simulation are nearly the same as that obtained of molecular docking.

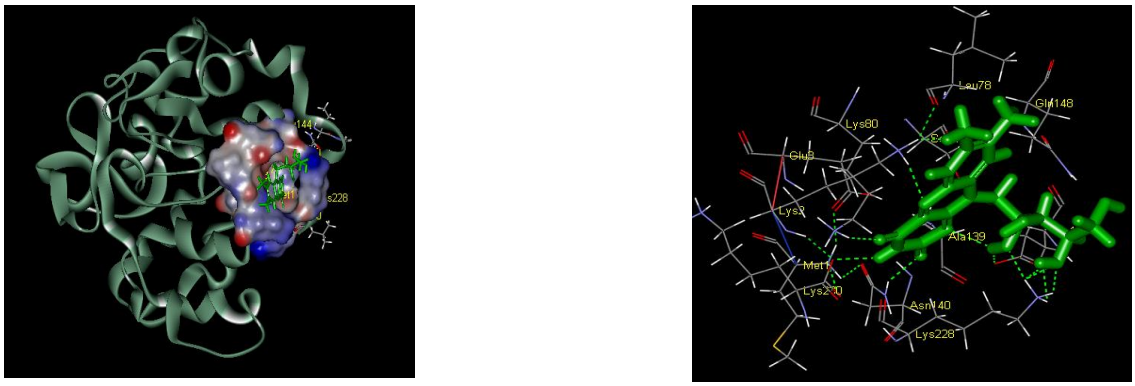

Figure.4.Molecular interactions of 7, 8-dimethyl-10-(3,4,5-trihydroxypentyl) benzo[g]pteridine-2,4-dione with Capsular polysaccharide biosynthesis protein wzh (B5E791)

It is clear from the figure 4 that the compound is well accommodated in the binding site cavity and is stabilized by intermolecular hydrogen bonds. Almost all the interactions with the residues are seen throughout the dynamics trajectory. The Lys228 plays a pivotal role in ligand binding at the active site for this complex.

Docking and dynamics simulation study of 7, 8-dimethyl-10-(3,4,5-trihydroxypentyl) benzo[g]pteridine-2,4-dione with with Transposase protein(D6ZS38):

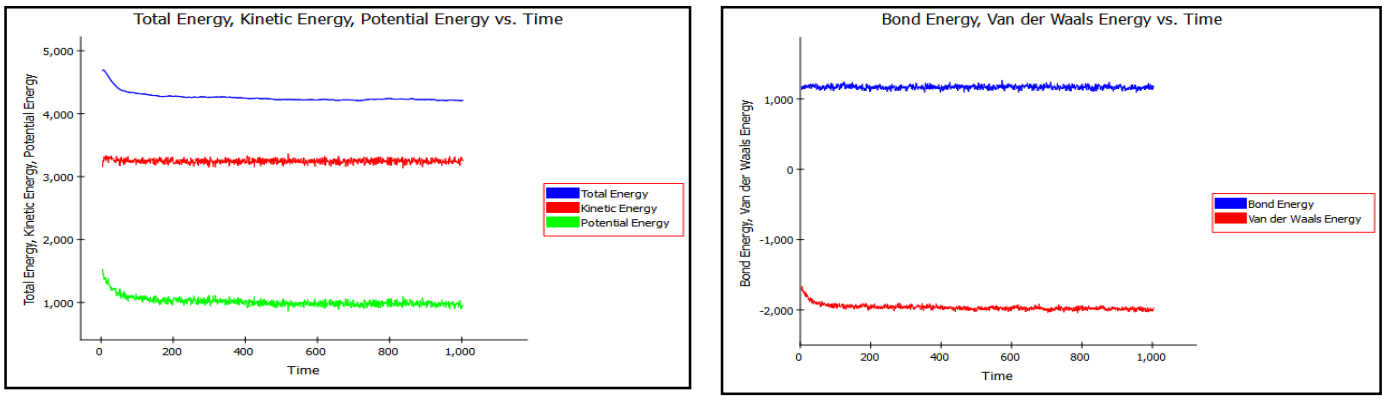

Figure.5.Energy profiles of 7, 8-dimethyl-10-(3,4,5-trihydroxypentyl) benzo[g]pteridine-2,4-dione with Transposase protein(D6ZS38)

The analog of ligand riboflavin had the score of 64.699 and was subjected to dynamics simulation study. Figure 5 shows the total energy, kinetic energy and potential energy profiles over the period of simulations during production run. It can be noted that there is not much deviation indicates that the compounds bind in a better position. Further analyses reveals that the binding modes of the compounds established after the MD simulation are nearly the same as that obtained of molecular docking.

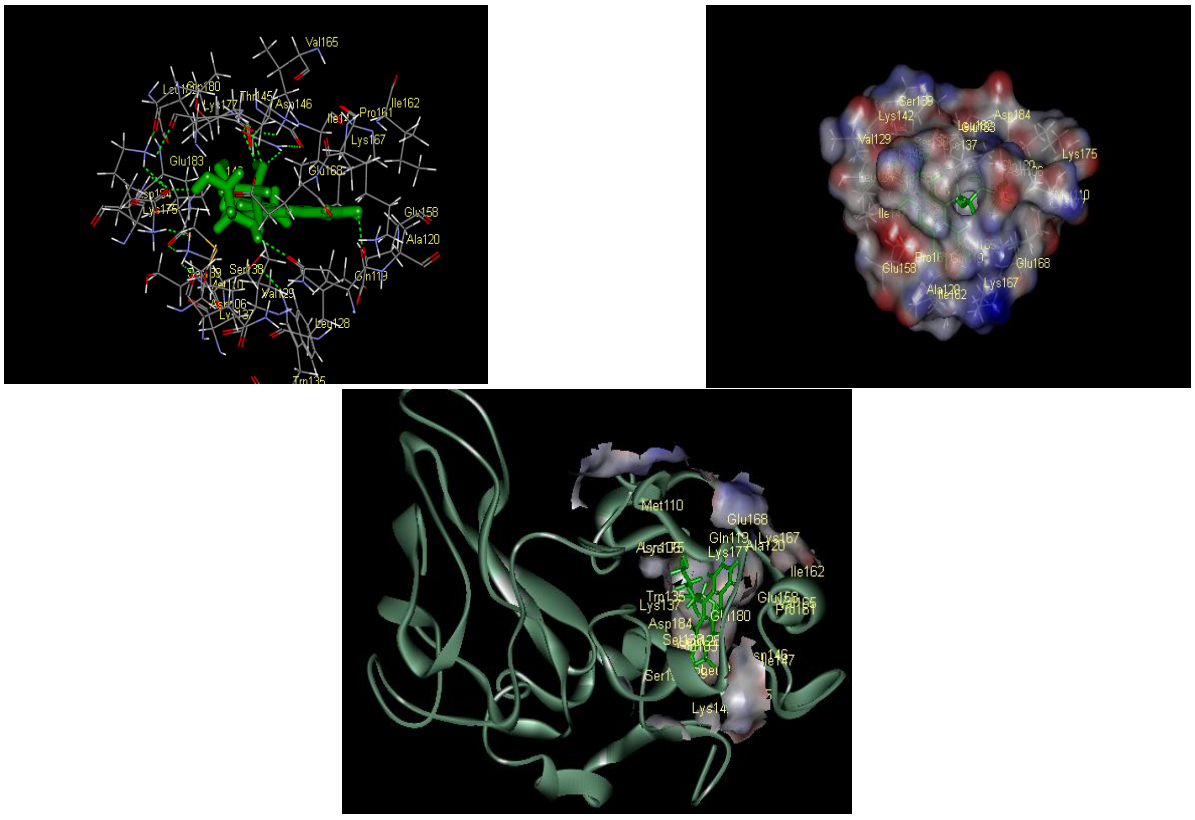

Figure.6.Molecular interactions of 7, 8-dimethyl-10-(3,4,5-trihydroxypentyl) benzo[g]pteridine-2,4-dione with with Transposase protein(D6ZS38).

It is clear from the Figure 6 that the compound is deeply buried in the binding site cavity and is stabilized by number of intermolecular hydrogen bonding interactions. Further analysis of the molecular dynamics trajectory reveals that the flexibility of the walls of the binding site plays a major role in ligand binding. In addition it is worth to mention that there exist local domain movements which may contribute significantly to push the ligand deep into the binding site.

The interactions with the residues Asp184, Gln119 and Lys177 are some of the important residues involved in the interactions of the complex at the ligand binding site.

Docking and dynamics simulation study of 5-[(3aS,4S,6aR)-2-oxo-1,3,3a,4,6,6a-hexahydrothieno [3,4-d]imidazol-4-yl] pentanoic acid with tyrosine-protein phosphatase wzh(B5E790):

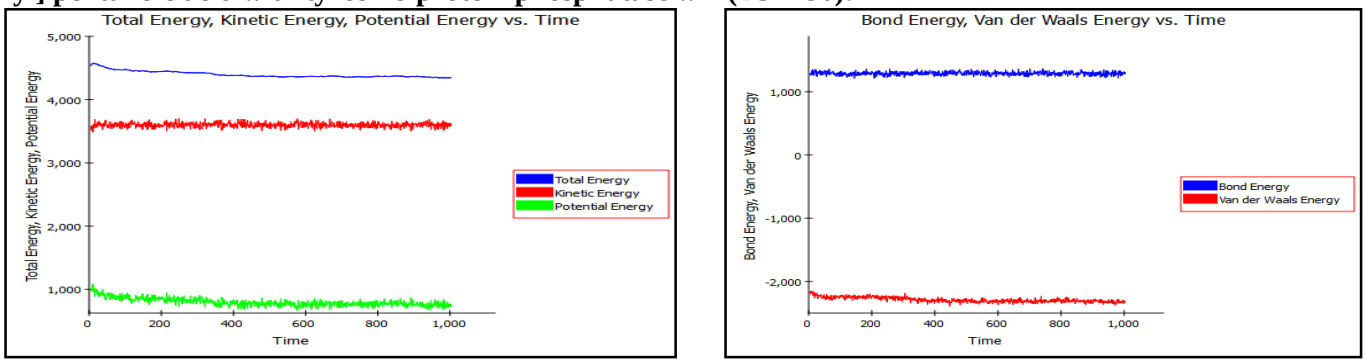

Figure.7.Energy profiles of 5-[(3aS,4S,6aR)-2-oxo-1,3,3a,4,6,6a-hexahydrothieno [3,4-d]imidazol-4-yl] pentanoic acid with tyrosine-protein phosphatase wzh(B5E790)

The analog of ligand biotin had the score of 48.207 and was subjected to dynamics simulation study. Figure 7 shows the total energy, kinetic energy and potential energy profiles over the period of simulations during production run. It can be noted that there is not much deviation indicates that the compounds bind in a better position. Further analyses reveals that the binding modes of the compounds established after the MD simulation are nearly the same as that obtained of molecular docking.

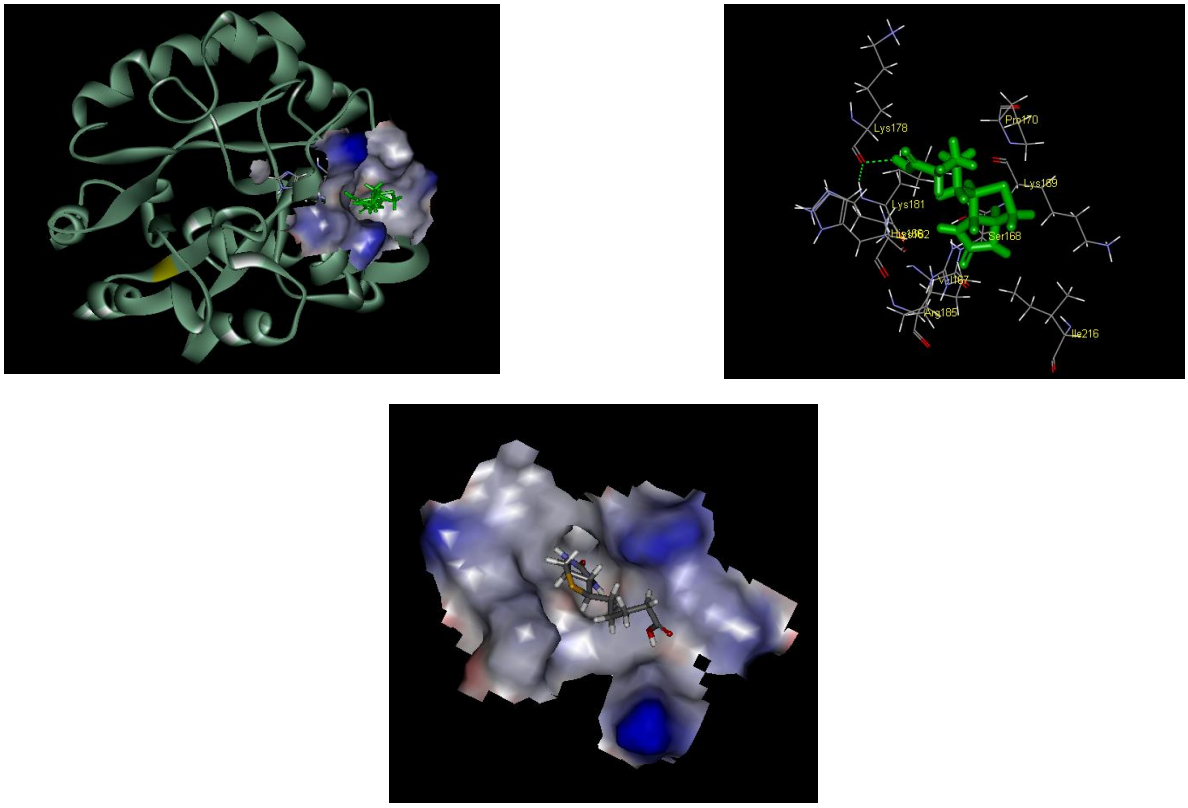

Figure.8.Molecular interactions of 5-[(3aS,4S,6aR)-2-oxo-1,3,3a,4,6,6a-hexahydrothieno [3,4-d]imidazol-4-yl] pentanoic acid with tyrosine-protein phosphatase wzh(B5E790)

It is clear from the Figure 8 that the compound is good interaction in the binding site cavity and is stabilized by number of intermolecular hydrogen bonding interactions. Further analysis of the molecular dynamics trajectory reveals that the flexibility of the walls of the binding site plays a major role in ligand binding. In addition it is worth to mention that there exist local domain movements which may contribute significantly to push the ligand deep into the binding site.

Invariably in all the complexes, the interaction with the key residues are well coincided with the molecular docking studies and may provide guidance for the rational design of more potent inhibitors against *S.pneumoniae*.

CONCLUSION

From intra strain multiple docking we analyzed that few analogs had high scores with other strains of *Streptococcus pneumoniae.* The high score analogs were subjected to dynamics simulation study to find the stability of the Protein-ligand complexes. Based on our Insilco analysis we conclude that the receptors and ligands can be considered as the best drug targets and drug candidates respectively.

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