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Research Article

# DOCKING STUDIES ON ANTIMICROBIAL PEPTIDES RELATED TO APIDAECIN-IA AND HUMAN HISTATIN AGAINST GLUTAMINE SYNTHETASE AND RNA POLYMERASE IN *MYCOBACTERIUM TUBERCULOSIS*

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**ABSTRACT**

Currently need of novel molecules can be used to treat the tuberculosis including resistance multiple drug regimen tuberculosis. Glutamine synthetase is an essential enzyme that produced the condensation reaction along with glutamate. It catalyzes the glutamate react with ammonia, leads to the formation of glutamine and hydrolysis of adenosine triphosphate. In the brain, it interacts with glutamate regulation leads to detoxification of ammonia in the brain leads to termination of neurotransmitter signals. RNA polymerase catalyzes the synthesis from a DNA template. It is a crucial enzyme for growth and survival of the mycobacteria. The compounds aspirin-intolerant asthma (AIA)-II, AIA-I were showed more potent against the glutamine synthetase with GLIDE score (G score) −8.79 and −7.97 as compared with standard amikacin and ciprofloxacin with G score −7.71 and −3.45. The compounds AIA-I, AIA-II and HH-I, were showed more potent against the RNA polymerase with G score −10.12, −9.47 and −8.74 as compared with standard rifampicin and isoniazid with G score −6.57 and −3.34.

**Keywords:** Glutamine synthetase, RNA polymerase, Amikacin, Rifampicin, Isoniazid, Tuberculosis.

## INTRODUCTION

Currently, tuberculosis is the most treacherous disease caused by *Mycobacterium tuberculosis.* The double drug resistance and multiple drug resistance (MDR) is common in the patients those who are suffered from tuberculosis. Right now the emergence of drugs to cure double, MDR and HIV-associated tuberculosis patients. Glutamine synthetase [1] produced the condensation reaction along with glutamate. It is a central part of the bacterial nitrogen mechanism. It catalyzes the glutamate that reacts with ammonia, leads to the formation of glutamine and hydrolysis of adenosine triphosphate. This enzyme is mostly present in the brain and kidney. In the brain, it interact with glutamate regulation leads to detoxification of ammonia in the brain. Finally, it produced the termination of neurotransmitter signals. Glutamine synthetase can be inhibited by using amino acid ligand like tryptophan, histidine, alanine, glycine etc.; It is an important target in a maximum number antitubercular drugs. RNA polymerase [2] is an enzyme that catalyzes the synthesis from a DNA template. It is a crucial enzyme for growth and survival of the mycobacteria. The main action of this enzyme is transcription and termination of DNA sequence or chain. It is an intracellular target in a number of antibacterial agents. It is also known as DNA-dependent RNA polymerase. At present requirement of a drug to inhibit the bacterial RNA polymerase also called as RNA polymerase inhibitors. A class of compounds of low molecular weight, which yield amino acids on hydrolysis. This group of molecules is termed as peptides. The living organisms are constantly exposed to the potentially harmful pathogens through ingestion and inhalation [3]. In contrast of acquired immune mechanism, endogenous peptides (gastrointestinal, respiratory tract, genitourinal tract), which are induced a fast and effective defense against pathogens. This group of molecules is termed as “antimicrobial peptides” (AMPs) (4]. These peptides are found in humans, animals, insects and plants [5]. AMPs have established to kill Gram-positive and Gram-negative bacteria, mycobacteria, fungus, viruses and cancer cells [6]. The AMP [7-16] are short peptides between 10 and 50 amino acids present in the sequence of the peptides. These peptides include two or more positively charged residues provided by arginine, lysine and histidine, and a bulky quantity of hydrophobic residues. The secondary structures of these peptides consist four parts including α-helical, β-stranded, β-hairpin or loop and extended. These peptides have a variety of antimicrobial activities ranging from membrane permeabilization to action on a range of cytoplasmic targets. The size of AMPs varies from 2 amino acid residues to more than 59 amino acid residues. The aim of the present study is to molecular docking studies of antimicrobial short peptides related to apidaecin intolerant asthma and human histatin against glutamine synthetase and RNA polymerase. Compare the antimycobacterial activity of the peptides with standard anti-TB drug.

## METHODS

### Protein structure preparation

The X-ray crystal structures of glutamine synthetase (protein data bank [PDB]: 2BVC) and RNA polymerase (PDB: 2RF4) retrieved from the research collaboratory for structural bioinformatics PDB (Figs. 1 and 2) was used in this study. Crystallization water molecules were detached from the composite, and the protein was optimized for docking using the protein preparation and refinement utility provided by Schrödinger LLC. Partial atomic charges were fixed agreed to the optimized potentials for liquid simulations-all atom (OPLS-AA) force field.

### Ligand structure preparation

The ligand structures (Fig. 3) were constructed using the splinter maestro 9.3 dictionary (Schrodinger, LLC) by using the OPLS-AA force field with the steepest descent followed by curtailed Newton conjugate gradient protocol. Partial atomic charges were fixed by using OPLS-AA force field.

### Docking protocol

All docking calculations were performed using the “extra precision” (XP) mode of GLIDE program. The various energy grids were calculated and stored, in terms of two concentric cubes. One is the bounding box, which contain the center of any acceptable ligand pose and the enclosing box, which contain all ligand atoms of an acceptable pose, with a root mean square deviation of <0.5 Å and maximum atomic displacement is <1.3 Å were rejected as redundant in order to increase diversity in the retained ligand poses. The van der Waals radii scale factor was applied to those atoms with absolute partial charges is equal to 0.15 and 0.25 electrons for ligand and protein, respectively. The maximum number of poses generated during the initial phase of the docking calculation were set at 5000 and keep best variable which sets the number of poses per ligand that enters the energy minimization was set at 1000.

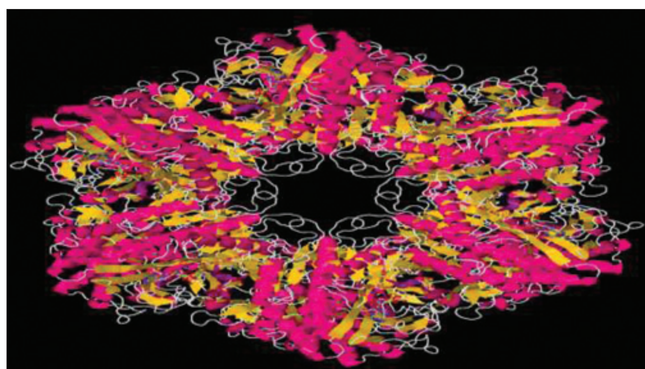

Fig. 1: X-ray crystal structure of protein glutamine synthetase (proteine data bank: 2BVC)

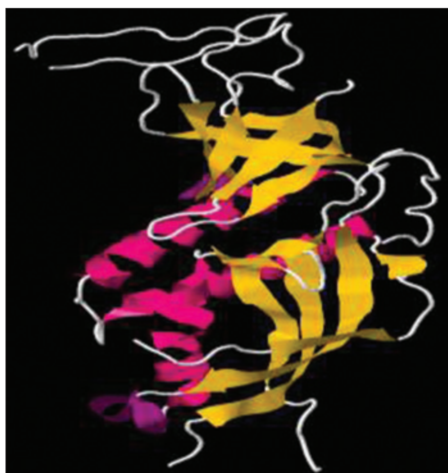

Fig. 2: X-ray crystal structure of protein RNA polymerase (proteine data bank: 2RF4)

Energy minimization protocol includes dielectric constant of 4.0 and 1000 conjugate gradient steps. Each docking calculation, the 100 poses per ligand were generated. The greatest docked structure was selected by using GLIDE score (G score) function and one more scoring function by used GLIDE E-model. This model is derived from the grouping of the G score, coulombic, van der Waals and ligand strain energy.

### Qikprop analysis

Qikprop efficiently evaluates pharmaceutically relevant properties for over half a million compounds per hour, building an indispensable lead generation and optimization tool. Correct calculation of absorption, distribution, metabolism, elimination (ADME) properties prior to expensive experimental procedures, such as high throughput screening, can eliminate unnecessary testing on compounds that will ultimately fail; ADME prediction can also be used to focus lead optimization efforts to enhance the desired properties of a given compound.

## RESULTS

### Results from Qikprop

The ADME properties of the designed ligands were predicted using Qikprop analysis. The compounds were subjected to drug-likeness filter. All the designed ligands conformed to the above mentioned criteria, and they were evaluated for docking using GLIDE software.

### Receptor grid generation

GLIDE receptor grid was generated to determine the size of the active site. The most probable orientation of the ligands in the binding pocket is identified and a scoring function is used to quantify the strength of the interaction in a molecule can make in a particular orientation. The

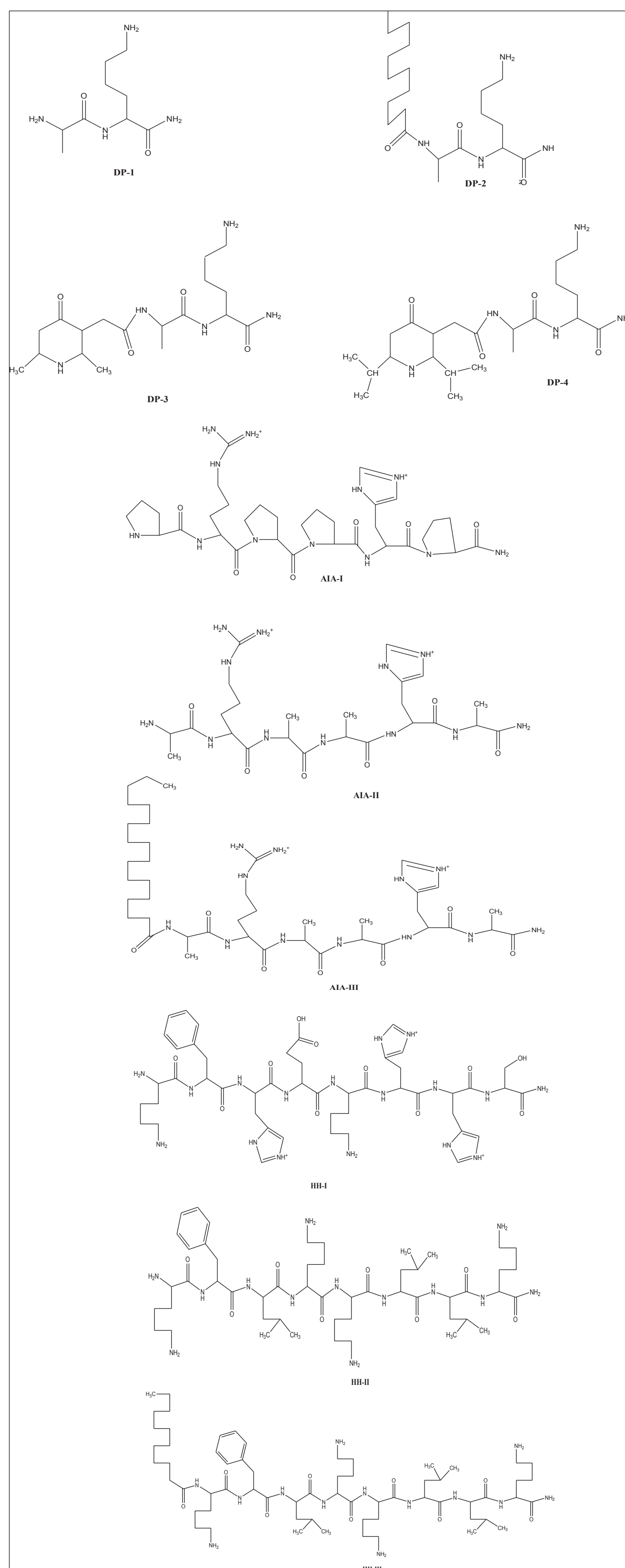


Fig. 3: Ligand structures

GLIDE XP precision was favored over the standard mode sequentially give a better correlation between good poses and good scores.

### Validation of the docking protocol

The docking analysis was done for the ligands such with the target protein glutamine synthetase and RNA polymerase using the docking

**Table 1: Docking results of the ligands against glutamine synthetase**

| Ligands code | G score | GLIDE energy |
|---|---|---|
| AIA-2 | −8.79 | −55.56 |
| AIA-1 | −7.97 | −42.11 |
| DP-1 | −6.25 | −28.29 |
| DP-4 | −5.46 | −40.60 |
| DP-3 | −4.93 | −40.95 |
| DP-2 | −2.85 | −30.99 |
| Amikacin | −7.71 | −40.67 |
| Ciprofloxacin | −3.45 | −16.42 |

AIA: Aspirin-intolerant asthma, G score: GLIDE score

**Table 2: The docking results of the ligands against RNA polymerase**

| Ligands code | G score | GLIDE energy |
|---|---|---|
| AIA-1 | −10.12 | −70.81 |
| AIA-2 | −9.47 | −72.86 |
| HH-1 | −8.74 | −84.44 |
| DP-2 | −6.85 | −50.63 |
| DP-3 | −6.76 | −50.44 |
| DP-1 | −6.20 | −39.47 |
| DP-4 | −5.19 | −51.46 |
| Amikacin | −14.39 | −72.09 |
| Streptomycin | −9.74 | −52.74 |
| Rifampicin | −6.57 | −54.38 |
| Ciprofloxacin | −4.44 | −37.07 |
| Isoniazid | −3.34 | −19.23 |

AIA: Aspirin-intolerant asthma, G score: GLIDE score

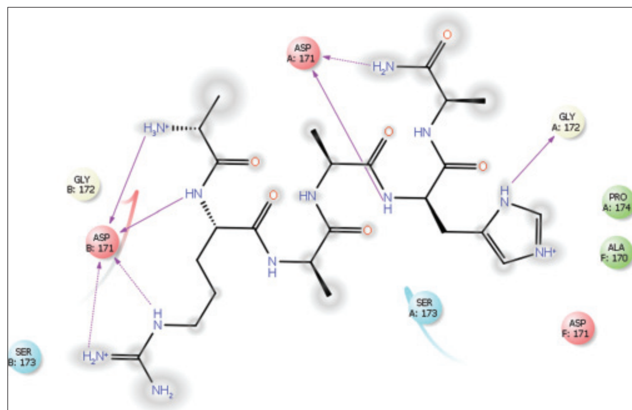

**Fig. 4: Docking structure of compound aspirin-intolerant asthma-II into the glutamine synthetase binding pocket**

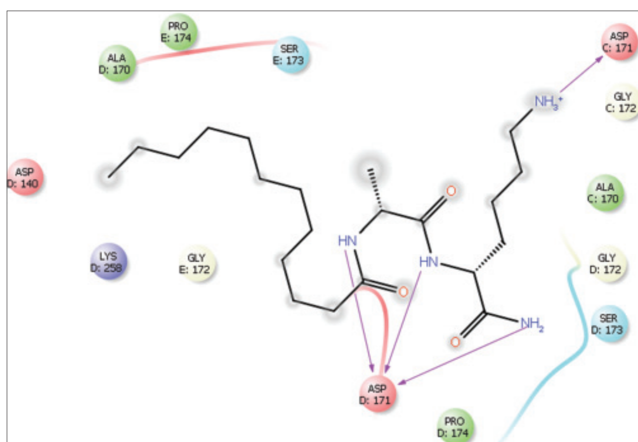

**Fig. 7: Docking structure of compound DP-II into the glutamine synthetase binding pocket**

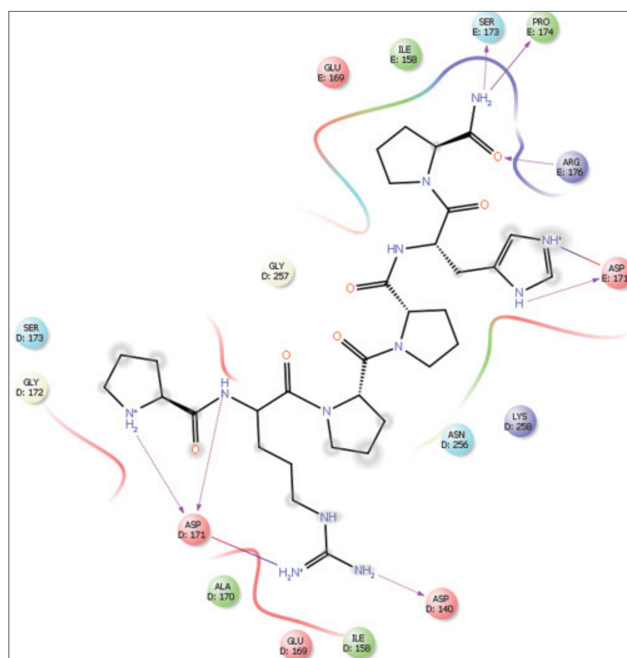

**Fig. 5: Docking structure of compound aspirin-intolerant asthma-I into the glutamine synthetase binding pocket**

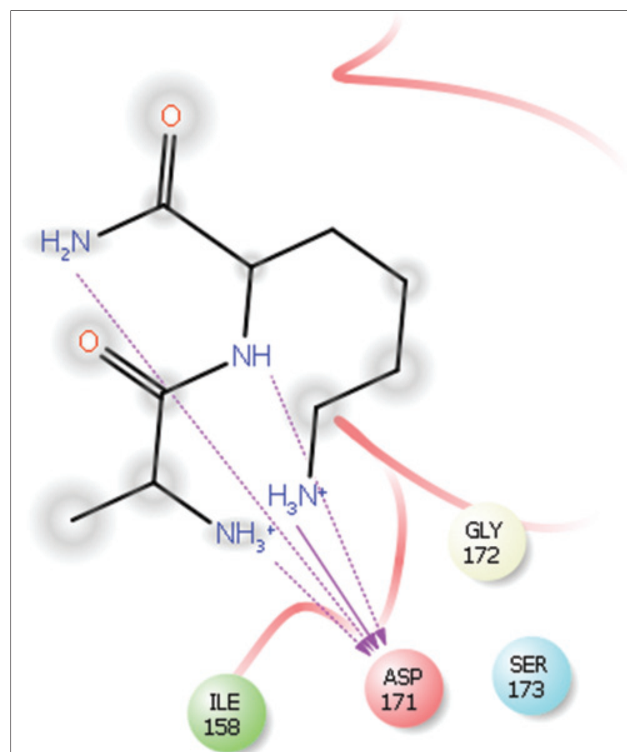


**Fig. 6: Docking structure of compound DP-I into the glutamine synthetase binding pocket**

software GLIDE and the docked images are shown. The structures docked by GLIDE are generally ranked according to the GLIDE scoring function. GLIDE docking program scoring function is presented in the G-score form. The method of evaluating the accuracy of a docking procedure is to determine how closely the lowest energy pose (binding conformation) predicted by the object scoring function. The nearby study, XP GLIDE docking procedure was validated by removing the inhibitor compound with glutamine synthetase and RNA polymerase protein has been analyzed from the G-score, GLIDE energy. To study the molecular basis of

interaction and binding affinity of the peptides to glutamine synthetase and RNA polymerase protein, all the ligands were docked into the active site of glutamine synthetase and RNA polymerase. The docking result of these ligands is given in Tables 1 and 2. The interaction energy were calculated for each complex. The docking score by using GLIDE varied from −5.19 to −10.12 against RNA polymerase and −2.85-−8.79 against glutamine synthetase. The G score for the standard amikacin, streptomycin, rifampicin, ciprofloxacin and isoniazid docked with RNA polymerase was −14.39, −9.74, −6.57, −4.44 and −3.34. The G score for the standard amikacin and ciprofloxacin docked with glutamine synthetase was −7.71 and −3.45. This result proves that the docked aspirin-intolerant asthma (AIA)-II and AIA-I could be a more potential

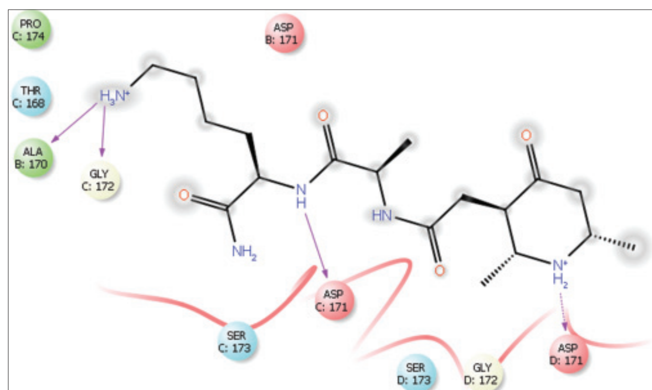


**Fig. 8: Docking structure of compound DP-III into the glutamine synthetase binding pocket**

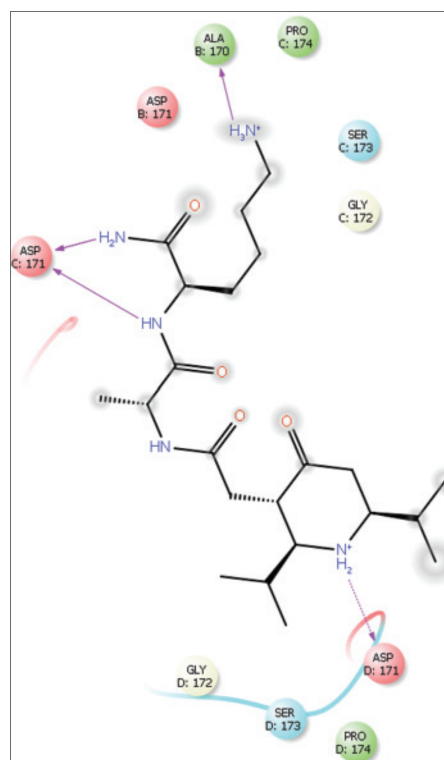


**Fig. 9: Docking structure of compound DP-IV into the glutamine synthetase binding pocket**

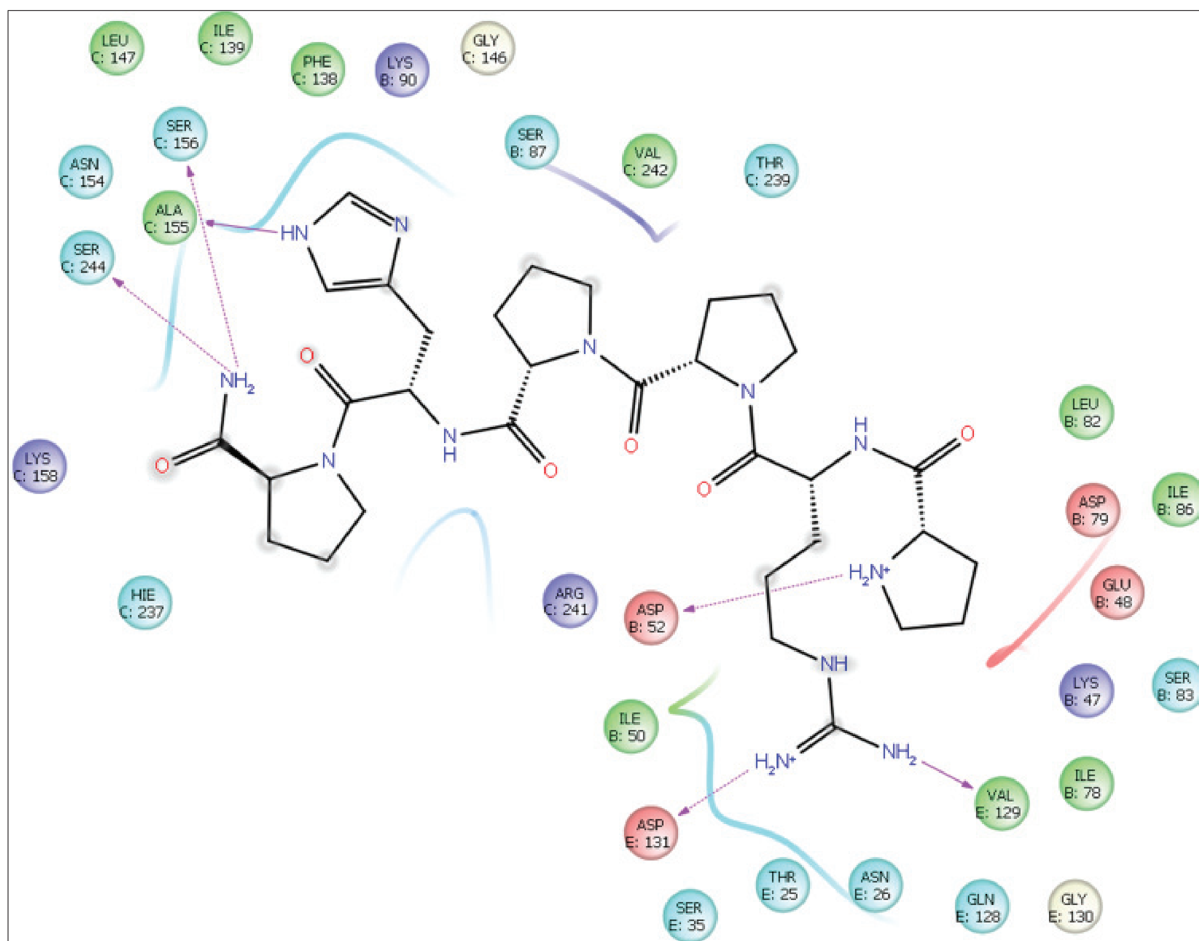


**Fig. 10: Docking structure of compound aspirin-intolerant asthma-I into the RNA polymerase binding pocket**

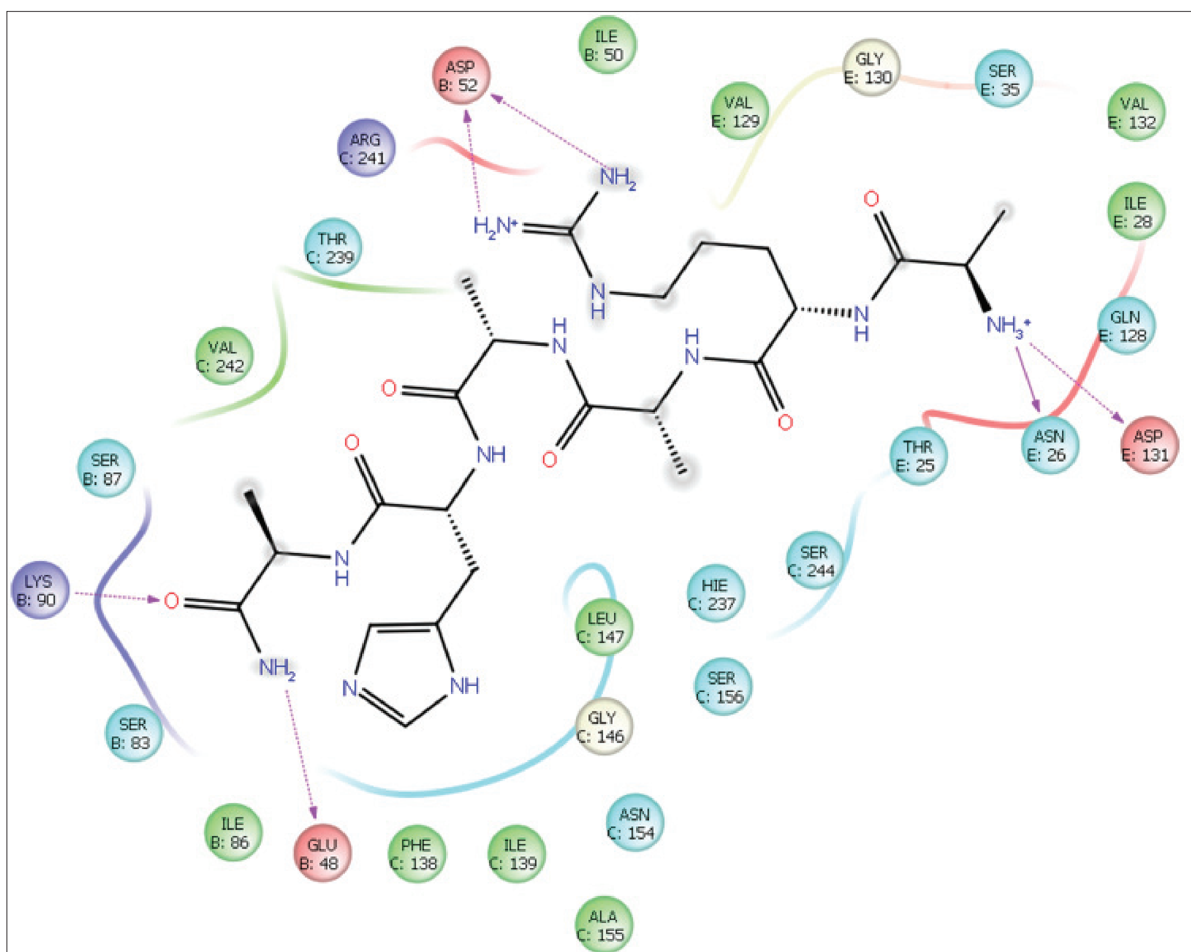


Fig. 11: Docking structure of compound aspirin-intolerant asthma-II into the RNA polymerase binding pocket

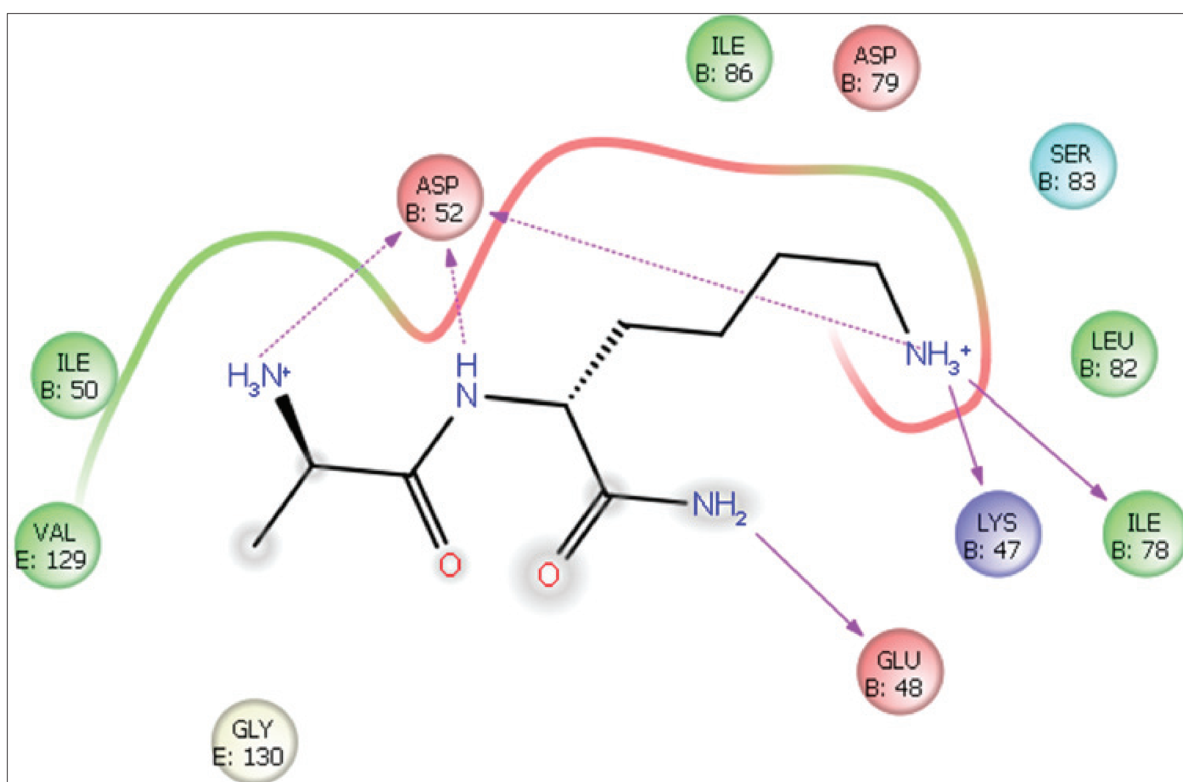


Fig. 12: Docking structure of compound DP-I into the RNA polymerase binding pocket

drugs for developing a new chemical entity against *M. tuberculosis*. The G score can be used as a semi-quantitative descriptor for the ability of ligands to bind to a specific conformation of the protein receptor. AIA-II and AIA-I were showed the best inhibition against glutamine synthetase with −8.79 and −7.97 G score as compared with standard amikacin −7.71 and Isoniazid −3.45. The compound AIA-I, AIA-II and HH-I were showed potent inhibition against RNA polymerase with 10.12, 9.47 and 8.74 as compared with standard rifampicin −6.57 and isoniazid −3.34. The compound DP-I, DP-IV and DP-III were showed the significant inhibition against glutamine synthetase with −6.26, 5.46 and 4.93 G score. The compound DP-2, DP-3, DP-1 and DP-4 were showed potent inhibition against RNA polymerase with 6.85, 6.76, 6.20 and 5.19 G score. We found a very good conformity between the localization of the inhibitor and crystal structure of the protein. The conformational analysis of different docked complexes shows the residues of ASPa171, ASPb171, GLYa172 in AIA-II and ASPd171, SERe173 ASPe174, ASPd140 and PROe174 in AIA-I of glutamine synthetase plays important role in this receptor's activity. The conformational analysis of different docked complexes shows the residues of ASPb172, ASPa171, GLY172 in AIA-I and ASPb52, LYSb90, ASPe131, ASNe26, GLUb48 in AIA-II of RNA polymerase plays an important role in the receptor site. Docking studies performed by GLIDE was confirmed that above inhibitors fit into the binding pocket of the glutamine synthetase and RNA polymerase receptors is shown in the Figs. 4-15. Finally, we may observe that the successful docking, intermolecular hydrogen bonding and lipophilic interactions between the ligand and receptor. The key cause for the increase in G score is due to the close intra-ligand contacts.

## CONCLUSION

Based on the results, the current study will be helpful for the progress

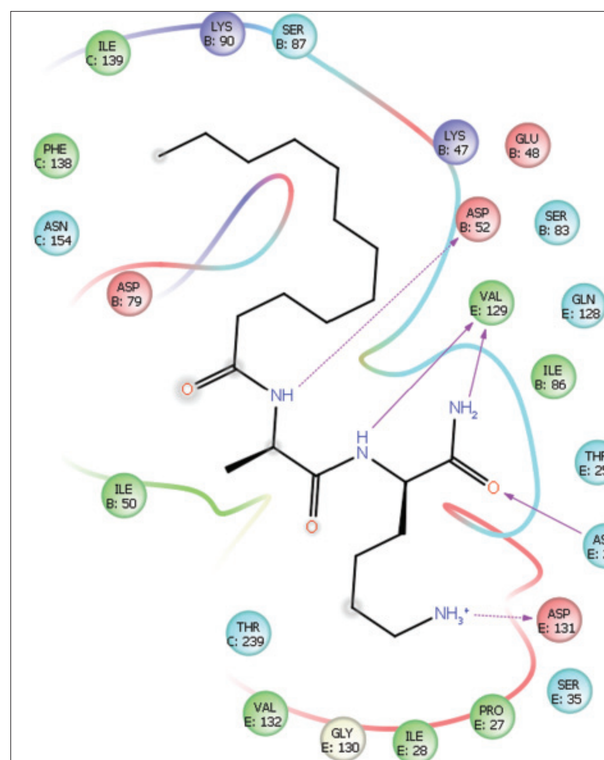


**Fig. 13: Docking structure of compound DP-II into the RNA polymerase binding pocket**

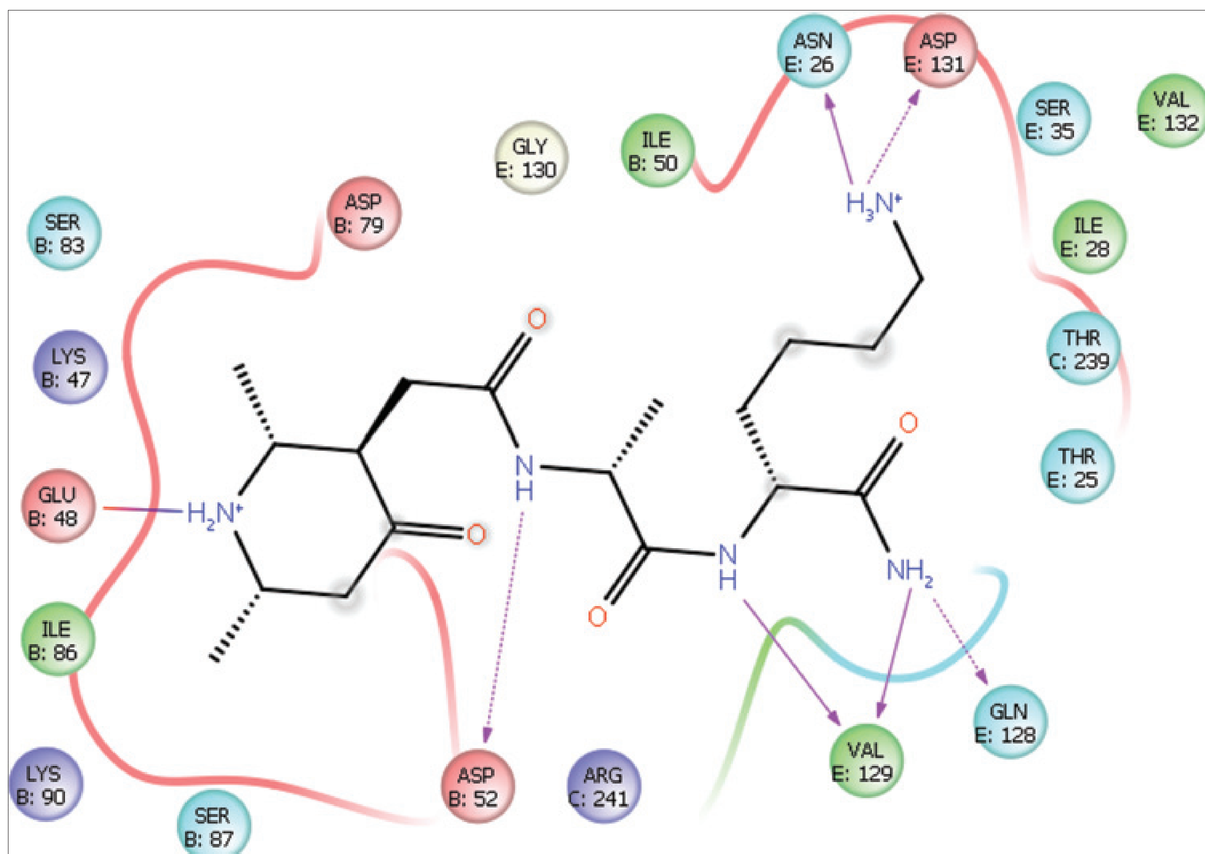


**Fig. 14: Docking structure of compound DP-III into the RNA polymerase binding pocket**

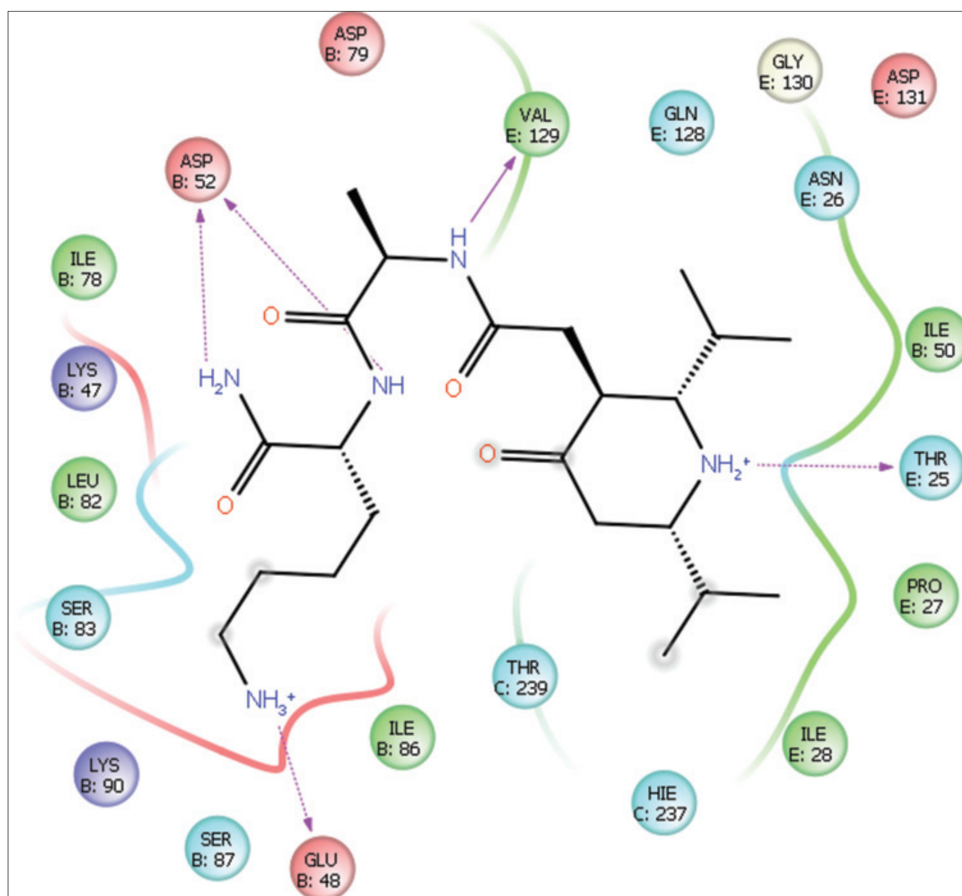


**Fig. 15: Docking structure of compound DP-IV into the RNA polymerase binding pocket**

of the novel peptides against the *M. tuberculosis*. The compounds AIA-II and AIA-I having more potent and tight binding against the glutamine synthetase and the compounds AIA-I, AIA-II, HH-I, DP-2 and DP-3 having more potent and tight binding against the RNA polymerase in the *M. tuberculosis* as compared with standard anti-tubercular drug. These potential drugs will be analyze in the wet lab based on the results obtained by molecular docking.

## ACKNOWLEDGMENTS

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