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VIRTUAL SCREENING OF DRUG LIKE INHIBITORS AGAINST ASPARTATE SEMIALDEHYDE DEHYDROGENASE OF LYSINE/ DAP PATHWAY OF *MYCOBACTERIUM TUBERCULOSIS*

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ABSTRACT

Tuberculosis (TB) is a major infectious disease killing nearly two million people, mostly in developing countries, every year. Mycobacterium tuberculosis (MTB) is the most common bacterial agent responsible for TB, however, *M. bovis, M. microti canetti*, and *M. africanum* can also result in TB. Many strains of tuberculosis resist the drugs most used to treat the disease. The increasing incidence of resistance of Mycobacterium tuberculosis strains to the most-effective (first-line) anti-TB drugs is a major factor contributing to the current TB epidemic. Drug-resistant strains have evolved mainly due to incomplete or improper treatment of TB patients. This alarming rate of antibiotic resistance to existing drugs calls for exploration of metabolic pathways for finding novel drug targets and even for prioritization of the known drug targets. The Lysine/DAP (diaminopimelate) biosynthetic pathway is a promising target due its specific role in cell wall and amino acid biosynthesis. The present study aims to evaluate the Aspartate semialdehyde dehydrogenase (ASADH; PDB id: 3LLG), an enzyme that controls the second step in the pathway for the conversion of Aspartyl phosphate to Aspartate semialdehyde as a promising drug target. Studies demonstrated with the two data sets were generated in order to screen potential inhibitors against *Mtb* drug target ASADH. The library of first data set comprised of analogues of aspartyl phosphate that is substrate for ASADH. The library of second data set comprised of antibacterial compounds obtained from Pubchem after drug like filtration. There were 76 compounds in first dataset and 9 compounds in second data set. Finally these compounds were docked to the active site of *Mtb* ASADH to select inhibitors establishing favorable interactions. The best inhibitors were docked into ASADH-NADPH complex to confirm their inhibitory activity with the complex as NADPH acts as a cofactor to the

enzyme action. These applied virtual screening procedures helped in the identification of several potent candidates that possess inhibitory activity against Mtb ASADH. Therefore, these novel scaffolds/candidates which could have the potential to inhibit *Mtb* ASADH enzyme would represent promising starting points as lead compounds and certainly aid the experimental designing of anti-tubercular in lesser time.

KEYWORDS: Mycobacterium Tuberculosis**,** Antibiotic Resistance; Lysine/ DAP Pathway.

INTRODUCTION

Tuberculosis caused by *Mycobacterium tuberculosis* has emerged as the second-most common cause of death worldwide.[1] One-third of the world's population is thought to have been infected with *M. tuberculosis*,[2] with new infections occurring in about 1% of the population each year.[3] Tuberculosis is considered as a threat in the modern era of antimicrobial warfare, which was declared a global health emergency by the WHO in 1993. The disease has always been endemic, but its severity has increased because of the global HIV pandemic and emergence of multiple drug-resistant *Mycobacterium tuberculosis (Mtb)* to isoniazid and rifampicin and extensive drug-resistant *Mtb.*[4] Tuberculosis combines one of the slowest division rates among bacteria and with a hardy cell wall defense system.[5] Both of these factors stretch treatment into a multiple month process, creating a massive window for human error in the form of incorrect or missed dosages which is the cause for emergence of resistance towards the treatment.[6] The problem is becoming ever more serious as new strains emerge which are resistant to many and sometimes all known antibiotics. The necessity for new drugs remains of utmost importance, given the fact, that this infection kills more people worldwide than any other. Therefore Increasing resistance in mycobacterium to existing drugs calls for exploration of metabolic pathways for finding novel drug targets and also for prioritization of known drug targets and to find the inhibitors besides generating analogues of existing drugs.

Most bacteria require either lysine, or its biosynthetic precursor, diaminopimelate (meso-DAP), as a component of the peptidoglycan layer of the cell wall. Bacterial biosynthesis of lysine has come under increased scrutiny as a target for novel antibacterial agents as it provides both lysine for protein synthesis and meso-diaminopimelate for construction of the bacterial peptidoglycan cell wall. Recent studies of the enzymes of the lysine biosynthetic pathway, development of inhibitors and investigations of their antibacterial properties are discussed. Consequently, the absence of DAP results in cell lysis and death, as has been

demonstrated in gene-knockout experiments with *Mycobacterium smegmatis*.[7] Therefore an inhibitor that inactivates any of the enzymes that are unique to the (S)-lysine biosynthetic pathway, preventing the synthesis of these crucial metabolites, would be a very effective antibiotic. Additionally, the absence of the (S)-lysine pathway in mammals means that inhibitors of this pathway would not be expected to have mammalian toxicity.

Aspartyl beta-semialdehyde dehydrogenase (ASADH), an important enzyme, occupying the first branch position of the biosynthetic pathway of the aspartate family of amino acids in bacteria, fungi and higher plants, is taken as drug target in this study. It catalyses reversible dephosphorylation of L: -beta-aspartyl phosphate (betaAP) to L: -aspartate-beta-semialdehyde (ASA), a key intermediate in the biosynthesis of diaminopimelic acid (DAP)-an essential component of cross linkages in bacterial cell walls. It proceeds *via* the attack of the thiol group of the active-site cysteine residue onto the γ-carbon of the substrate l-aspartyl-β-phosphate, producing the acyl-enzyme intermediate and releasing inorganic phosphate.[8] In this enzyme, Cys130 has been identified as active site nucleophile. Gln157 and Arg249 have been shown to assist in catalysis and participate in substrate recognition and binding respectively. His257 is likely the acid/base catalyst in the enzymic reaction.[9,10] The three-dimensional crystal structures of ASADH from *Escherichia coli, Mycobacterium Tuberculosis, Haemophilus influenza, Vibrio cholerae, Streptococcus pneumoniae, Methanococcus jannaschii, Thermus thermophilus HB8 and Pseudomonas aeruginosa* are available at PDB database. Various structural studies have reported the conservation of active site residues from different bacterial species but to study this conservation multiple sequence alignment was performed using ClustalW.

Though several experimental and computational methods are being applied to design inhibitors against different enzymes of DAP pathway; however the promising inhibitors have not been reported so far against *Mtb* ASADH. Considering all analysis and previous findings the attempt was made to screen inhibitors of Mtb DHDPS using virtual screening procedures. The present work of screening of ASADH inhibitors is being reported with in-silico approaches, hence, would be a great help in aiding the experimental studies and rational development of novel drugs against Mtb.

MATERIALS AND METHODS

Retrieval of protein structure

Protein structure for *Mtb* ASADH was retrieved from Protein Data Bank (PDB) which provides 3 dimensional structures for the protein. The PDB ID for *Mtb* ASADH is 3LLG. It provides information about coordinates of each residue of the protein. The structure of the Asd monomer is comprised of an N-terminal nucleotide-binding domain and a dimerization domain. The architecture of the N-terminal domain comprises an approximate Rossmann fold. The first β-strand β_1 of the N-terminal domain leads into the glycine-rich loop, which is characteristic of an NADP-binding domain. The C-terminal domain is responsible for the dimerization of the enzyme and the binding of the substrate and contains the catalytic residues.

Active Site Prediction

The three-dimensional crystal structures of ASADH from *Escherichia coli, Mycobacterium Tuberculosis, Haemophilus influenza, Vibrio cholerae, Streptococcus pneumoniae, Methanococcus jannaschii, Thermus thermophilus HB8 and Pseudomonas aeruginosa* available at PDB database. Previously, various structural studies have reported the conservation of active site residues from different bacterial species. So to study this conservation multiple sequence alignment was performed using ClustalW.

Constructing library of drug like compounds

The library of drug like compounds was constructed from PubChem. The compounds were obtained by similarity search and structure search. Two data sets were prepared for the compounds. First data set comprised of the compounds that are Aspartyl Phosphate like compounds; substrate for the enzyme. Second data set comprised of known antibacterial compounds.

Aspartyl Phosphate like molecules

Since the similarities in structures are indicative of similarities in bioactivities, therefore, structure based searching of databases/libraries has been gaining high demand nowadays. There were 247 Aspartyl Phosphate like compounds. Then, this database was subjected to Lipinski's Rule of Five constraints which reduced the size to 126 molecules. But 3D conformers were not present for all the compounds. So following the 3D search, finally, 76 molecules were retrieved from PubChem.

Anti-bacterial molecules

Additionally, 35 antibacterials were retrieved from PubChem database out of which only 8 anti-infectives satisfied the Lipinski's rule of five constraints. These compounds were highly diverse from the pyruvate such that none of the anti-infectives showed 2D/3D similarity with aspartyl phosphate. So total 9 anti-bacterial compounds were retrieved from PubChem. Hence, the docking of these compounds would help to screen the diverse classes of anti-tubercular agents against *Mtb* ASADH.

Receptor and ligand preparation for docking

Before docking process, several separate pre-docking steps: ligand preparation, receptor preparation and grid map calculations were performed. The ligand and receptor preparation stage involved the addition of hydrogen atoms, computing charges, merging non-polar hydrogen atoms and defining AD4 atom types to ensure that atoms conformed to the AutoDock atom types. Next, information about rotatable torsion bonds that defines the bond flexibility was acquired. The ligands and receptor molecule preparation was followed by grid construction using AutoGrid module. During grid construction, atom types of the ligand, which acted as probes in the calculation of grid maps, were identified. The grid with default volume of 40 × 40 × 40 Å with a spacing of 0.375 Å centered on the receptor was prepared. For conformational search, the docking calculations using the genetic algorithm (GA) procedure with default parameters was performed. The GA computed the fitness of a docked candidate every time by measuring the minimum values of free energy binding (ΔG) based on different types of energy evaluations.

Rigid docking

After ligand and receptor preparation, the rigid docking was performed using Autodock Vina. Receptor for docking was *Mtb* ASADH and ligands were aspartyl phosphate analogues. Results were obtained for each compound and the compounds showing good binding energy were taken into consideration for flexible docking using AutoDock 4.0.

Ligand-Receptor flexible docking

After rigid docking, flexible docking was performed for selected molecules using autodock 4.0. For validation aspartyl phosphate was docked to the active site of *Mtb* ASADH. Then, the aspartyl phosphate like compounds and anti-bacterial compounds were docked to the active site of *Mtb* ASADH as a control with the purpose of screening the compounds with docking score greater than the substrate. Then the compounds showing best inhibition were

docked to ASADH bound to NADPH. NADPH acts as cofactor for the enzyme. Again for validation aspartyl phosphate was docked to ASADH bound to NADPH. Then the results were analyzed for studying interactions.

RESULT AND DISCUSSION

The present study demonstrate, the target ligand docking using Autodock. Initially selected drug-like aspartyl phosphate like molecules were docked into the active site of *Mtb* ASADH. After which, the anti-bacterial compounds were docked into the active site of the enzyme. For the validation of docking process, the substrate aspartyl phosphate was also introduced as a control with the purpose of screening the compounds with docking score greater than the substrate. The detailed view of docking of aspartyl phosphate to the active site is shown in Fig. 1 and 2, which exhibited molecular docking with ΔG value of -5.1 (kcal/mol). The aspartyl phosphate lying near the vicinity of CYS130 observed to be forming a hydrogen bond with side chain of CYS130 and showed interactions with nearby residues.

Docking of aspartyl phosphate like molecules

The rigid docking of 76 aspartyl phosphate-like compounds (structurally similar to aspartyl phosphate) retrieved from PubChem was carried out using the same docking protocol as mentioned earlier for aspartyl phosphate. For this library, 35 pyruvate-like compounds provided free energy binding values better than aspartyl phosphate control, whereas other compounds provided free energy binding values less than aspartyl phosphate. Then these 35 molecules were subjected to flexible docking using autodock 4.0. The ΔG values in the range of -7.41 to -3.06 (kcal/mol). Then the best five inhibitors (Table 1) were selected having free binding energy in range of -7.41 to -5.67. These detailed analysis revealed that these 5 inhibitors fit very well into the binding pocket by establishing bonded and non-bonded interactions with active site residue. Hydrogen bonding was found to be dominant interactions with CYS130, LYS227, ARG99 and HIS256. The tops hits were mainly aromatic, that displayed strong binding by yielding ΔG value of -7.41 to -5.67 (kcal/mol). The interactions of these inhibitors with active site residue were mainly through hydrogen bonding and VDW.

Table 1: List of five best aspartyl phosphates like molecules showing better binding energy than aspartyl phosphate.

Sr.No.	IUPAC NAME	ΔG VALUES (kcal/mol)	MOLECULAR WEIGHT
1.	(2S)-2-(phosphonoamino)butanedioic acid	-7.41	213.082581
2.	2-(phosphonomethylsulfonylamino)butanedioic acid	-6.74	291.172961
3.	2-amino-4-hydroxy-4-[hydroxy(methyl)phosphoryl]butanoic acid	-5.72	197.126241
4.	(2S)-2-amino-4-hydroxy-4-[hydroxyl(methyl)phosphoryl] butanoic acid	-5.71	197.126241
5.	(2R)-2-amino-3-deuterio-butanedioic acid	-5.67	134.108842

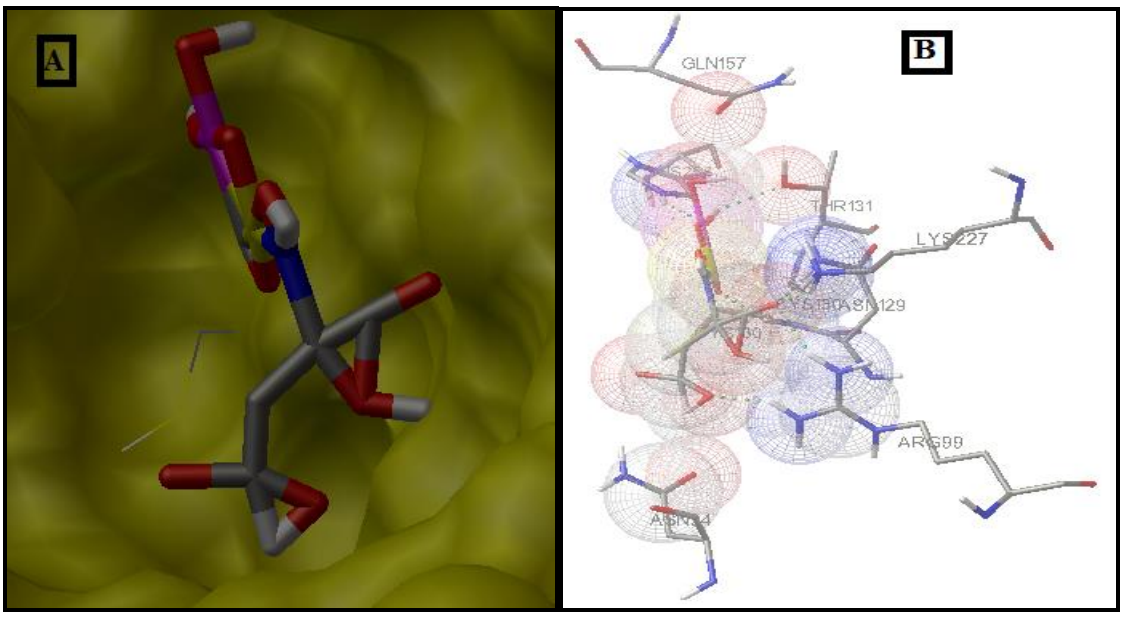

Fig. 1 Docking of aspartyl phosphate-like compounds. Top predicted inhibitor establishing interactions with active site residues of *Mtb* ASADH. (A) Molecule lying deep into the binding pocket of *Mtb* ASADH (B) bonded and non-bonded interactions between the molecule and ASADH.

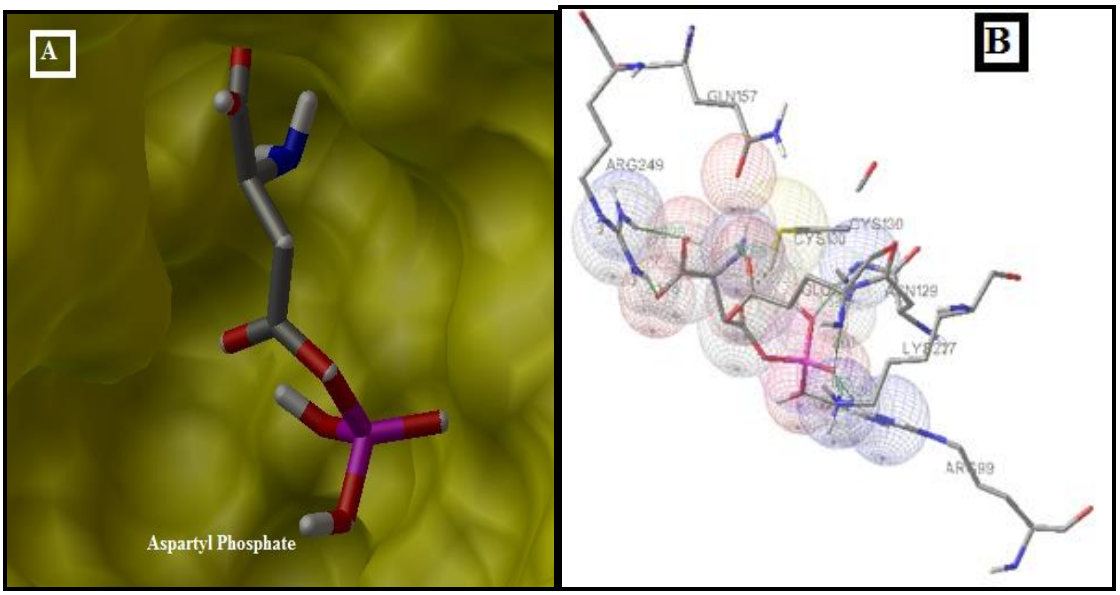

Fig.2 Docking of aspartyl phosphate. (A) Aspartyl phosphate lying deep into the binding pocket of *Mtb* ASADH (B) bonded and non-bonded interactions between aspartyl phosphate and ASADH.

Docking of anti-bacterial molecules

Then the anti-bacterial molecules from PubChem were docked to the active site of ASADH. Only few anti-bacterial molecules(Table 2) showed better free binding energy than aspartyl phosphate. Most of the molecules showed negative binding energy but it was less than aspartyl phosphate. Only four molecules showed binding energy better than that of aspartyl phosphate in the range -5.73 to -5.20 (kcal/mol). However, the best two compounds with ΔG values -5.73 and -5.69 (kcal/mol) were assumed to be the best and potent inhibitors of ASADH screened out. The tops hits were mainly aromatic, that displayed strong binding by yielding ΔG value of -5.73 and -5.69 (kcal/mol). The interactions of these inhibitors with active site residue were mainly through hydrogen bonding and VDW as. These detailed analysis revealed that these 2 inhibitors fit very well into the binding pocket by establishing bonded and non-bonded interactions with active site residue. Hydrogen bonding was found to be dominant interactions with CYS130, ASN129, ARG99 and HIS256.

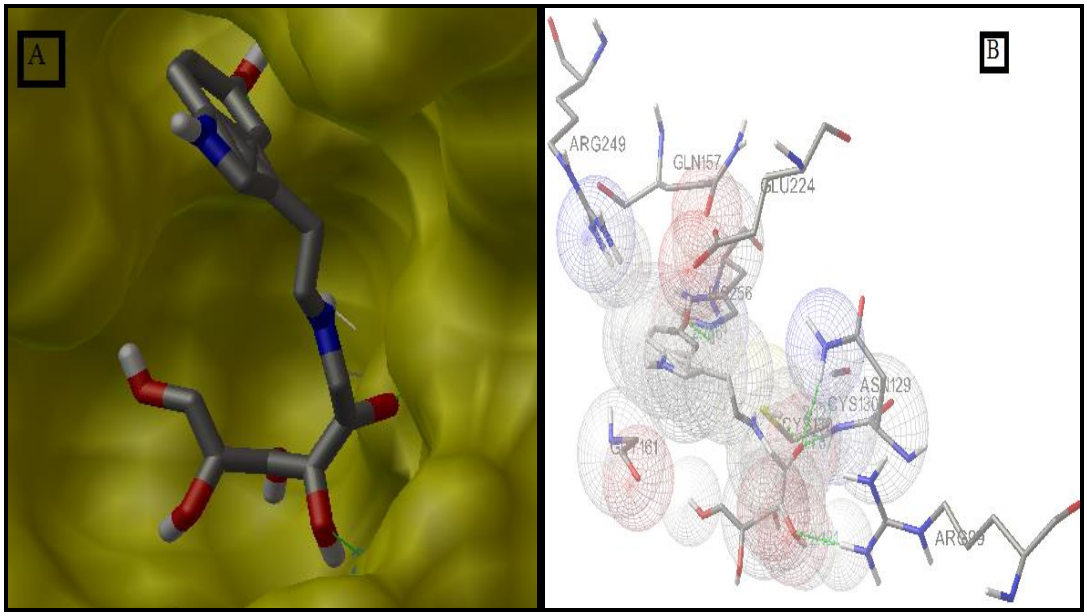

Fig. 3 Docking of anti-bacterial compounds. Top predicted inhibitor establishing interactions with active site residues of *Mtb* ASADH. (A) Molecule lying deep into the binding pocket of *Mtb* ASADH (B) bonded and non-bonded interactions between the molecule and ASADH.

Table 2: List of best anti-bacterial molecules showing better binding energy than aspartyl phosphate.

Sr.No.	IUPAC NAME	ΔG VALUES (kcal/mol)	MOLECULAR WEIGHT
1.	N-[4-(4-acetamidophenyl) sulfonylphenyl] acetamide	-5.73	332.37424
2.	(3S,4R,5R)-3,4,5,6-tetrahydroxy-1-[2-(5-hydroxy-1H-indol-3-	-5.69	338.35568
3.	yl)ethylamino]hexan-2-one 2-(2,6-dioxopiperidin-3-yl)isoindole-1,3-dione	-5.22	258.2295
4.	N,5-bis(4-chlorophenyl)-3-propan-2-yliminophenazin-2-amine	-5.20	473.39638

Docking of best inhibitors into ASADH bound to NADPH

NADPH acts as a cofactor for ASADH in *Mycobacterium tuberculosis*. So it was important to study the interactions of selected best inhibitors with ASADH bound to NADPH. For this initially NADPH was docked to the active site of ASADH at the position of NADPH binding. Then aspartyl phosphate was docked to this complex which provided binding energy of -2.38.

So to act as inhibitors the selected molecules must show better binding energy than aspartyl phosphate. Then selected best inhibitors were docked to ASADH-NADPH complex that showed binding energy in the range of -6.28 to -2.38(kcal/mol). The best inhibitor (Table 3) that showed free binding energy of -6.28 is aspartyl phosphate like molecule and the second best inhibitor that showed binding energy of -6.19 is anti-bacterial molecule.

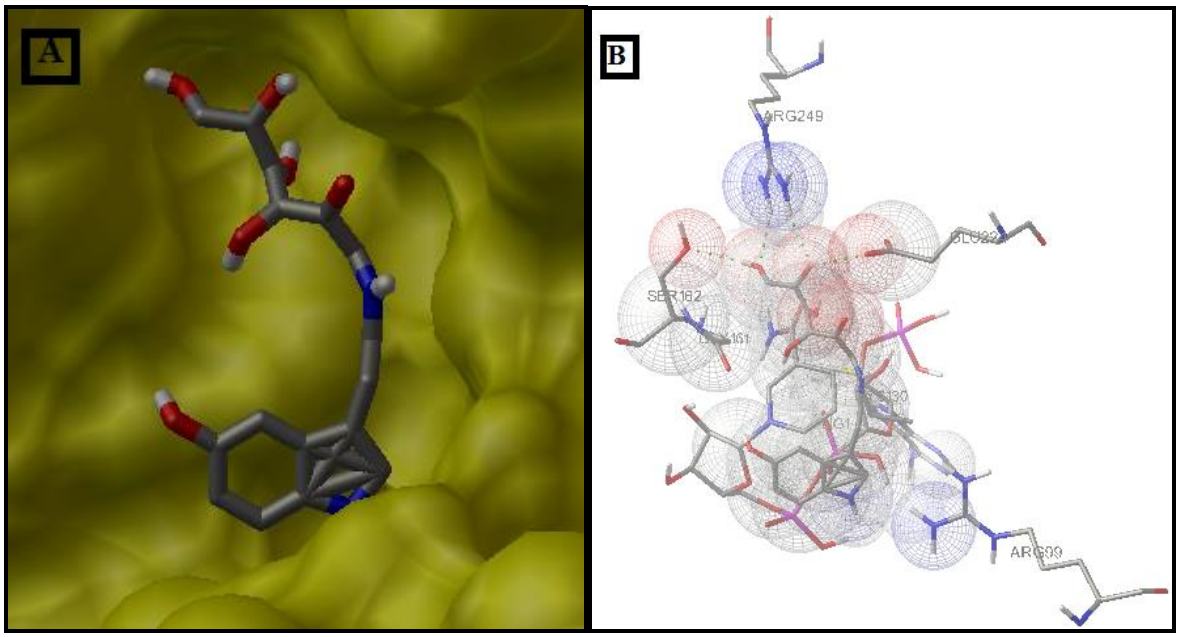

Fig. 4 Docking of best inhibitor compounds into ASADH-NADPH complex. (A) Inhibitor lying deep into the cavity and (B) top predicted inhibitor establishing interactions with active site residues of *Mtb* ASADH.

Table 3: List of best inhibitors showing better binding energy than aspartyl phosphate to ASADH-NADPH complex.

Sr.No.	IUPAC NAME	ΔG VALUES (kcal/mol)	MOLECULAR WEIGHT
	Aspartyl phosphate like molecules		
1.	(2S)-2-(phosphonoamino)butanedioic acid	-6.28	213.082581
2.	2-amino-4-hydroxy-4-[hydroxy(methyl)phosphoryl]butanoic acid	-4.87	197.126241
3.	(2R)-2-amino-3-deuterio-butanedioic acid	-4.43	134.108842
4.	N-[4-(4-acetamidophenyl) sulfonylphenyl] acetamide	-4.29	291.172961
5.	(2S)-2-amino-4-hydroxy-4-[hydroxyl(methyl)phosphoryl]butanoic acid	-3.96	197.126241
	Anti-Bacterial molecules		
6.	(3S,4R,5R)-3,4,5,6-tetrahydroxy-1-[2-(5-hydroxy-1H-indol-3-yl)ethylamino]hexan-2-one	-6.19	258.2295
7.	2-(2,6-dioxopiperidin-3-yl)isoindole-1,3-dione	-5.92	338.35568
8.	N-[4-(4-acetamidophenyl) sulfonylphenyl] acetamide	-3.85	332.37424
9.	N,5-bis(4-chlorophenyl)-3-propan-2-yliminophenazin-2-amine	-2.38	473.39638

CONCLUSION

Tuberculosis caused by *Mycobacterium tuberculosis* has emerged as the biggest curse of our time causing significant morbidity and mortality. In a population of *Mycobacterium tuberculosis*, random chromosomal mutation that results in genetic resistance to anti-tuberculosis (TB) drugs occurs at a relatively low frequency. Lysine/DAP biosynthetic pathway is a promising target due its specific role in cell wall and amino acid biosynthesis. Mycobacterial cell walls are characterized by an unusually high DAP (Diaminopimelic acid) content. Consequently, the absence of DAP results in cell lysis and death, as has been demonstrated in gene-knockout experiments with *Mycobacterium Tuberculosis*. So in present study Aspartate semialdehyde dehydrogenase was taken as drug target.

To conclude, several virtual screening protocols such as generation of combinatorial library, and molecular docking to identify potential inhibitors against *Mtb* ASADH have been employed. About 76 potential drug-like inhibitors have been screened out; among them several showed strong binding affinity to *Mtb* ASADH. But top 5 inhibitors were discussed that showed binding energy in the range of -7.41 to -5.67 (kcal/mol) that is stronger than Aspartyl Phosphate that showed binding energy of -5.1 kcal/mol. Additionally, four out of 9 anti-infective with highly diverse topology from the aspartyl phosphate also displayed strong binding than substrate (-5.1 kcal/mol) in the range of -5.73 to -5.20 (kcal/mol). Then these selected inhibitors were docked to ASADH-NADPH complex. With ASADH-NADPH complex also, these inhibitors showed strong binding energy than substrate (-2.38 kcal/mol) in the range of -6.28 to -2.38(kcal/mol). Though experimental studies are indispensable to mark them as lead compound for the development of novel drugs against *Mtb*, however, screened out inhibitors would certainly aid the experimental designing of anti-tubercular agents.

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