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THE EFFECT OF BENZIMIDAZOLE AGAINST BETA-LACTAMASE TO TREAT TUBERCULOSIS: A BIOINFORMATIC APPROACH

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ABSTRACT

Tuberculosis (TB), an old bacterial disease caused by the bacteria Mycobacterium tuberculosis that mainly effects the lungs and sometimes other parts of the body as well like- spine.This responsible for more deaths worldwide each year than any other infectious disease, including human immunodeficiency virus (HIV).The principal cause of human tuberculosis is *mycobacterium tuberculosis*.Other members of M tuberculosis that can cause tuberculosis include M *bovis ,M microti and M africanum. M microti* is not known to cause TB in humans.One in three persons across the world representing 2–3 billion individuals are known to be infected with Mycobacterium Tuberculosis (M. *Tuberculosis*) .The present study was to inhibit the expression of beta lactamase by the help of mostsuitable ligandby molecular docking for the treatment of tuberculosis.. The molecular dockingmethod was performed after the screening of molecules and selecting the ligands which can inhibit and target the molecule. Beta-lactamase(CID: 12939847) was targeted by4ligandcompounds(Quinolone, thiophenes, sulfonamides and benzimidazole)and the best ligand was selected for further molecular docking

and modeling tools. After that, interaction was visualized through PyMol. According study, among all ligands, only benzimidazole was one of the best ligand which has the best binding property with target enzyme i.e., beta lactamase, that may inhibit beta-lactamase enzyme. Benzimidazole(MW-118.14g/mol)has the highest binding properties and it's a good antibiotic compound too. Thus, it may be used further used for the treatment of tuberculosis. Benzimidazole may also be the beneficial drug for the treatments of tuberculosis in future studies after in vitro and in vivo studies.

Key words: ***mycobacterium tuberculosis, molecular docking, beta lactamase, benzimidazole.***

1.INTRODUCTION

Tuberculosis (TB) remains one of the deadliest infectious diseases responsible for millions of deaths annually across the whole world.[1](TB) is a contagious infection that usually attacks the lungs. It can also spread to other parts of the body, like your brain and spine. A type of bacteria called *Mycobacterium tuberculosis* causes it[2].Tuberculosis is an airborne infectious disease treated with combination therapeutic regimens. Adherence to long-term antituberculosis therapy is crucial for maintaining adequate blood drug level. The emergence and spread of drug-resistant Mycobacterium tuberculosis strains are mainly favored by the inadequate medical management of the patients[3].

TB is also considered as an impairing factor for economic growth and for the improvement of the general public health in many countries, because it drains human and financial resources that would otherwise be invested in the economy . Therefore, there is a pressing need to study and develop new prevention protocols and treatments for TB. Public health policy makers, national organizations and governing bodies are currently joining efforts in raising awareness in the general population regarding MTB(*mycobacterium tuberculosis*bacterium) contagion and in establishing guidelines and protocols for fighting TB [4].

Beta-lactamases are the antibiotics consisting of beta-lactam ring in the structure of their molecules.β-lactam are the most widely used group of antibiotics and they work by inhibiting the cell wall biosynthesis in the bacterial organisms .The β-lactams retain a central place in the antibacterial armamentarium .beta-lactam antibiotics are the most commercially available antibiotics in the market[5][6].The sequence information divides β-lactamases into four distinct classes, termed A, B C and D identified on the basis of specific sequence motifs but also distinguished by fundamental differences in hydrolytic mechanism. A further fundamental division is between the three classes –

- A, C and Dclasses- are of the active-site serine enzymes (seine β-lactamases; SBLs)
- B classes- comprises a heterogeneous group of zinc metalloenzymes (metallo-β-lactamases), or MBLs

The Identification of growing numbers of β-lactamases, coupled with availability of protein, and subsequently nucleotide, sequence information, established that these enzymes do not comprise a single homogeneous group but instead of that it can be subdivided into multiple classes.

The anti-tuberculosis drugs-Isoniazid (H), rifampin (R), ethambutol (E) ,pyrazinamide(Z) and streptomycin (S) are the essential first-line anti-tuberculosisclasses[7]. Aminoglycosides (kanamycin, amikacin), quinolones (ciprofloxacin, ofloxacin, levofloxacin), ethionamide or prothionamide, cycloserine, para-aminosalicylic acid (PAS) and polypeptide (capreomycin) are the second-line anti-tuberculosis drugs[8][9]. But this first line therapy often fails to cure TB for several reasons. Firstly, the treatment regimen is long, consisting of an initial 2 months of intensive phase treatment with all four drugs, followed by the continuation phase for 4 months with INH and RIF. The lengthy therapy period with multi drug treatment results in a lack of compliance causing the treatment failure[10].

Benzimidazoles are the fused heterocyclic ring systems which form an integral part of vitamin B12 and have been luring many researchers all over the world to assess their potential therapeutic significance.[11] They are remarkably effective compounds both with respect to their inhibitory activity and their favourable selectivity ratio[12].Benzimidazole and its derivatives are regarded as important heterocyclic motifs that exhibit a wide range of pharmaceutical applications including anticancers, antihypertensives, antivirals, antifungals and anti-HIVs[13].

Thiophene is monocyclic heteroarene and in most of the reactions it resembles benzene.Thiophenes are mostly used to make pharmaceuticals and dyes. There are several commercially available drugs such as Tipepidine, Tiquizium Bromides, Timepidium Bromide, Dorzolamide, Tioconazole, Citizolam, Sertaconazole Nitrate and Benocyclidine also contain thiophene nucleus[14].

Sulfonamides (sulphonamides) are a group of man-made (synthetic) medicines that contain the sulfonamide chemical group in them.. They can also be called as sulfa drugs.Sulfonamide antimicrobials are bacteriostatic (they stop bacterialreproduction but don't necessarily kill them) and work by interfering with the synthesis of folic acid in bacteria, which is essential for the nucleic acid formation and ultimately DNA and RNA.[15]These are used for conditions such as acne and urinary tract infection.

Quinolones are a type of antibiotic.Quinolones and fluoroquinolones are considered broad-spectrum antibiotics.These may also be used to treat unusual infections such as anthrax or plague.[16]The main quinolone nucleus is a nitrogen-containing, 8-membered heterocyclic aromatic quinoline ring[17].Fluoroquinolones, such as ciprofloxacin and ofloxacin, are active against both Gram-negative and Gram-positive pathogens, and they are also active against the causative agent of tuberculosis, *Mycobacterium tuberculosis.*[18]

Molecular docking can be used to model the interaction between a small molecule and a protein at the atomic level, which allow us to characterize the behavior of small molecules in the binding site of target proteins as well as to elucidate fundamental biochemical processes [19][20].Molecular docking is a method which analyses the conformation and orientation (referred together as the "pose") of molecules into the binding site of a macromolecular target.

Searching algorithms generate possible poses, which are ranked by scoring functions.[21][22].The comparison of docking molecules of the compaund was an alternative to the drug-like properties from the initial molecule, which can allow to calculate their recorded values. In the present study the antibiotic compounds from different sources such as Quinolone, thiophenes, sulfonamides and benzimidazole were selected to use them against beta-lactamases for the treatment of tuberculosis diseases with the help of molecular docking studies.

2.MATERIALS AND METHODS

Softwares required in molecular docking-

- PyRx
- SwissADME
- Autodock Vina
- PyMOL

A.Identification of Protein

The target enzyme used as Protein was obtained through a variety of sources. The RCSB Protein Data Bank (PDB) was used to derive the structure of beta- lactamase[23]. In ".pdb" format, the structure of a protein molecule was downloaded. The PDB is a worldwide database of structural data about biological macromolecules that were developed in 1971 at Brookhaven[24].

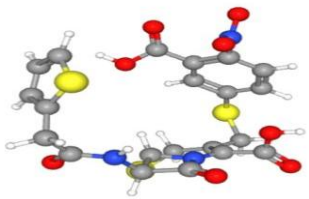


Fig.13D structure of Target enzyme- β-lactamase (pubChem)

B.Ligands retrieval

Quinolone, thiophenes, benzimidazole,sulfonamides are the compounds which were chosen from the literature for the docking investigation. PubChemwas used to find these compounds[25]. Theseligands were recovered in their 3 Dimensional structure in ".sdf" format. Using the online SMILES Translator ,all structures of ligand were translated to ".pdb"

format[26]. The files that were transformed were saved as ".pdb" files. These ".pdb" files were used to run a variety of programmes and applications[27][28].

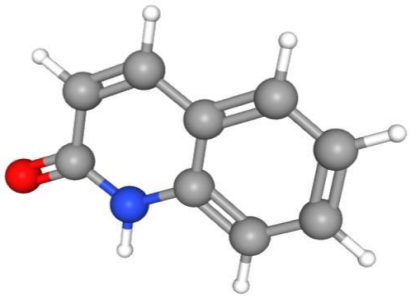
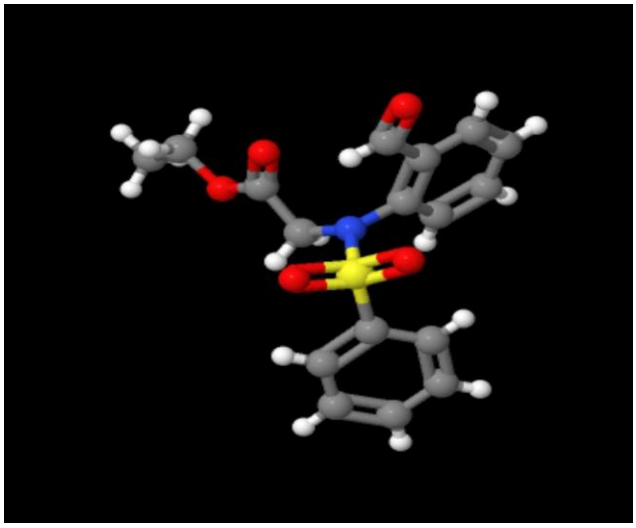
	
3D structure of Quinolone Molecular formula- C_9H_7N	3D crystalline structure of sulfonamide Molecular formula -$C_{22}H_{23}N_3O_5S$

Fig 2and 3

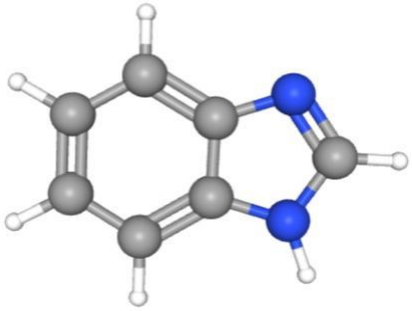
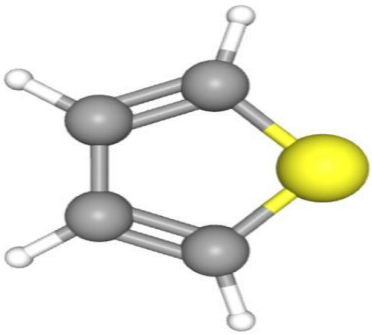
	
3D structure of Benzimidazole Molecular formula-$C_7H_6N_2$	3D structure of thiophenes Molecular formula- C_4H_4S

Fig 4 and 5

C.Preparation of Grid Parameter File

Grid maps were generated to optimise ligand binding, and spacing was increased to 1.00. The grid has been updated with 40 40 40 points. AutoDock Vina uses interaction maps for docking. Prior to the docking run, Auto Grid calculated these maps. The interaction energy between each ligand atom and the target for the full binding site, which was discretized using was computed for each ligand atom type. The interaction energy of each protein was given to each grid point, and the affinity for each ligand was computed.

D.Virtual Screening Through PyRx

The ligands were screened using the PyRx programme . This software was used to find ligands with the lowest binding energy to the receptor of interest. The drug likeliness property analysis was performed on ligands that were discovered to have the lowest binding energy. PyRx uses the pdbqt file format. PyRx starts by importing a target molecule, which was transformed from ".pdb" to ".pdbqt" format before importing ligands from a specific folder in.sdf format. The ligands' energies were reduced, and the.sdf file was transformed into a ". Pdbqt" file. The target molecule and the ligands were docked, and ligands were tested on the basis of their low binding energy.

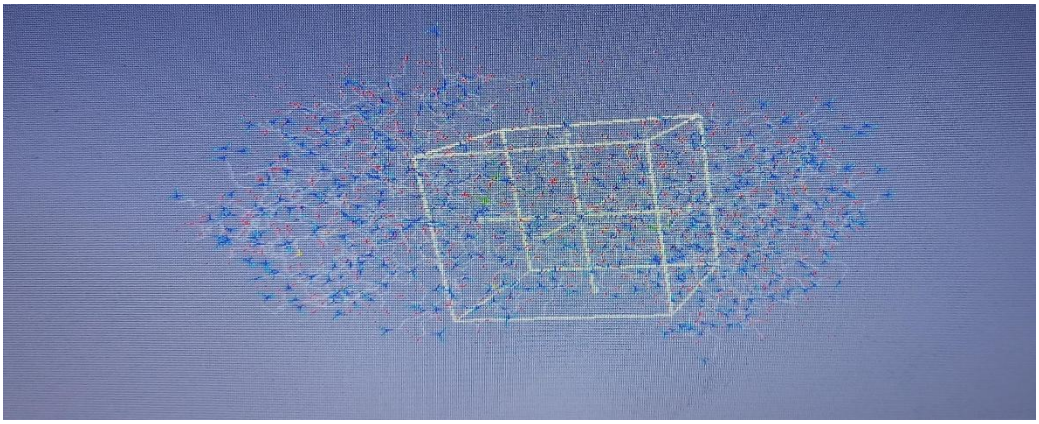


Fig.6 grid box in PyRx

E.Drug Likeliness Property Analysis

The analysis of drug likeliness properties was carried out using an internet server, i.e. SwissADME is a company based in Switzerland. The drug properties of the ligands that were tested were investigated. SwissADME an open web service, was used to download SMILE notations of screened ligands from PubChem. The Lipinski rule of five was applied to the drugs. The five points of the Lipinski rule are as follows:

1. Hydrogen bond [H-bond] acceptors less than 10.
2. Hydrogen bond [H-bond] donors less than 5.
3. Molecular mass not more than 500Da.
4. Must be less than 5 partitions co-efficient (LogP).
5. Violation of more than one rule can't be done.

AutoDock Vina was used to select ligands that followed the above Lipinski rule of five,by eliminating water molecules, adding hydrogen polar atoms, and adding Kollman For final docking.

Using AutoDock Vina to Dock the target molecule .The target was loaded into AutoDock Vina's graphical interfaces in.pdb format . Charges were applied to the protein molecule, the protein target was prepared for docking in.pdb format, and the protein was then placed in ". Pdbqt" is a pdbqt format. The ligand molecule was imported in.pdb format and then converted to ". Pdbqt" format, which is a pdbqt format. The docked region was then assigned to a grid box. AutoDock Vina was run from a command prompt, and the results were evaluated .

F. Visualization of Structure through PyMOL:

PyMOL is an open-access tool for molecular structurevisualization. Usingthis structure visualization tool PyMOL, the results of docking were visualized. On the graphical screen of PyMOL, the .pdbqt format protein molecule was loaded accompanied by the output .pdbqt file. The examination after Visualization of the interaction between the protein and ligand. Afterwards, conversion of a molecule into "molecular surface" by selecting the option "shown as" was made.[29]

3. RESULT AND DISCUSSION

Beta lactamase target molecule was retrieved in pdb format from protein data bank (PDB). [Fig-7] .Method-x raydiffraction1.73Å.

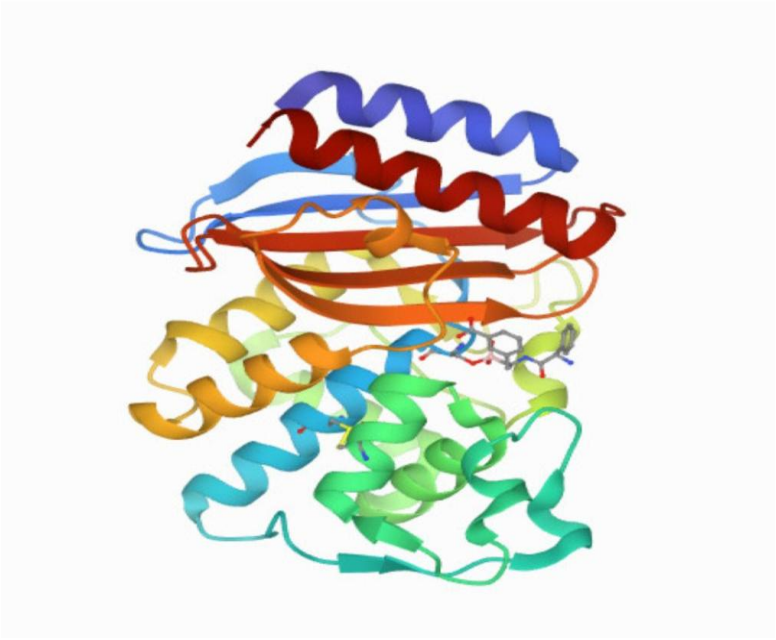


Fig.7 Crystalline 3D Structure of TEM -1 Beta-lactamase

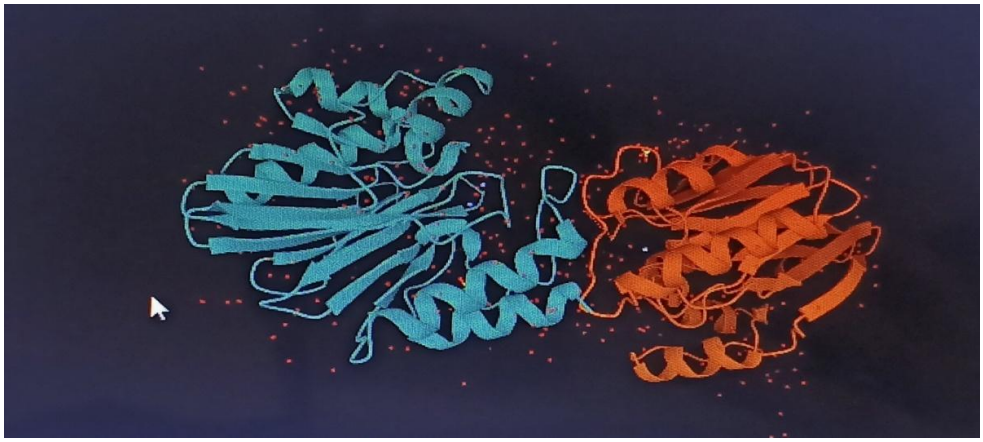


Fig 8. Structure1BC2 (DOI:10.2210/pdb1bc2/pdb)

All the 4 ligands (Benzimidazole, thiophenes, sulfonamides, quinolones) used in this study were downloaded from pubchem along with their CID in sdf format in 2 dimensional and 3 Dimensional structure.

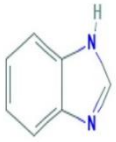
3 Dimensional structures were downloaded in .sdf format and afterward these were converted in .pdb format using online .sdf to .pdb converter.The files were saved in .pdb format so that these can be used further in many other applications.

Name of the ligand- Benzimidazole

MW- 118.14 g/mol

MF- $C_7H_6N_2$

CID- 5798

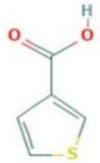


Name of the ligand- thiophenes

MW- 84.14g/mol

MF- C_4H_4S

CID- 8030

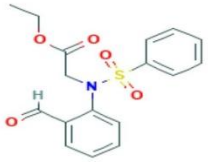


Name of the ligand-. Sulfonamides

MW- 347.4g/mol

MF-$C_{22}H_{23}N_3O_5S$

CID- 91392493

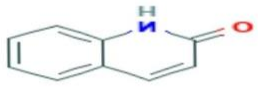


Name of the Ligand- Quinolone

MW- 145.16g/mol

MF- C9H7N

CID- 6038



All the above ligands were retrieved from pubchem with their MW,MF and CID.

Name of the compound	**Ligands**	**Binding affinity (Kcal/mol)**	**Mode**	**RMSD lower bound**	**RMSD upper bound**
Quinolone	Beta_lactamase_quinolone_uff_E=92.88	-5.3	0	0.0	0.0
Thiophenes	Beta_lactamase_thiophenes_uff_E=212.66	-4.8	0	0.0	0.0
Sulfonamides	Beta_lactamase_sulfonamides_uff_E=694.26	-6.1	0	0.0	0.0
Benzimidazole	Beta_lactamase_benzimidazole_uff_E=292.36	-4.2	0	0.0	0.0

Table1. PyRx and SwissADME result showing binding affinities of the ligands

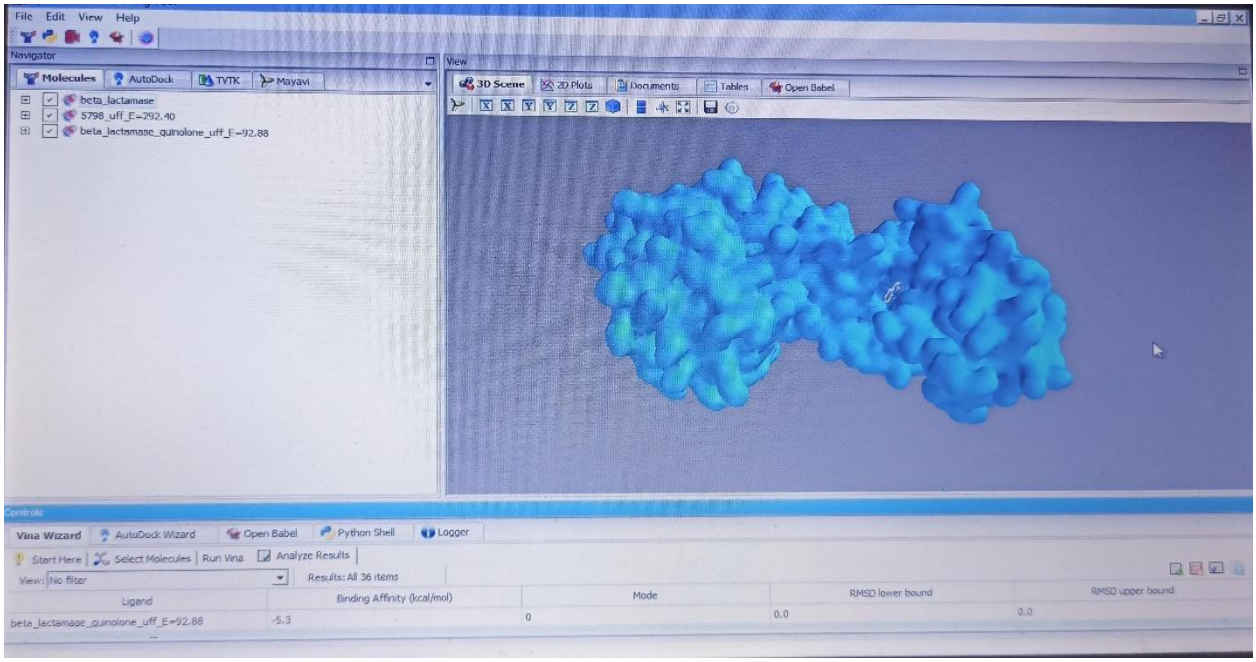


Fig.9 virtual screening tool PyRx showing 3D Structure of the molecule

Autodock vina result-

Mode	Mode Binding Affinity(Kcal/mol)	Distance from best mode RMSD lower bound	Distance from best mode RMSD upper bound
1	-6.8	0	0
2	-6.6	22.455	24.619
3	-6.5	19.850	23.056
4	-6.4	17.971	18.915
5	-6.3	20.011	19.415
6	-5.9	13.336	18.806
7	-5.8	13.642	16.037
8	-5.6	24.020	29.023
9	-5.4	14.589	19.511

Table 2. Autodock vina

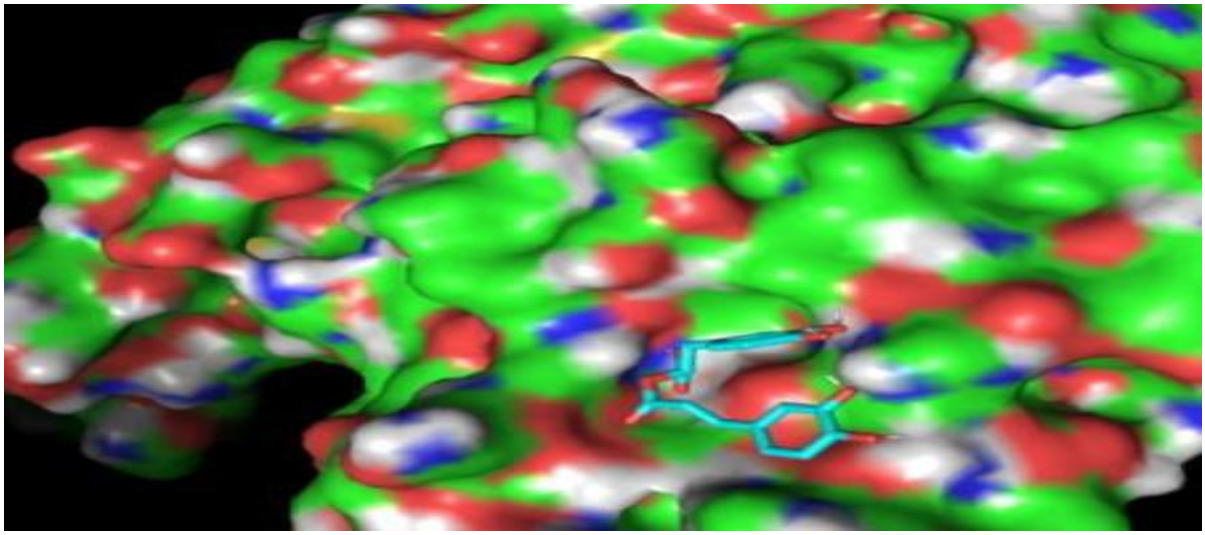


Fig.10 image showing interaction between target beta lactamase and the ligand through PyMol visualiser

4. CONCLUSION

Molecular docking predicts the interaction between a target molecule i.e. protein and a drug candidate i.e. ligand. In this in study, molecular docking was performed to examine the interaction of 4 ligand molecules with target enzyme taken as Protein . The study predicted that the ligand molecule i.e. benzimidazole (CID: 5798) has a strong binding affinity with the receptor or target protein i.e. beta- lactamase(CID: 12939847) and its potential as a drug against tuberculosis is conformed. After PyRx and SwissADME analysis, the only compound with the best minimum binding energy was benzimidazole. Also, it was following all the five rules of Lipinski along with 0 violations. Hence, based on this in docking study, beta lactamase which is produced by Mycobacterium tuberculosis can be inhibited by ligand benzimidazole and for the treatment of tuberculosis, benzimidazole might act as a potential drug. And also can be used for the treatment of tuberculosis disease.

5.FUTURE PROSPECTS

The Benzimidazole showed the inhibition of beta lactamase which is responsible for causing tuberculosis in Human Beings. Therefore, in future Benzimidazole can be used to analyse whether it would be successful for in vitro and in vivo studies to treat tuberculosis.

6.CONFLICT OF INTEREST

The authors declare there is no conflict of interest.

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