iJRASET
International Journal For Research in Applied Science and Engineering Technology

INTERNATIONAL JOURNAL FOR RESEARCH

IN APPLIED SCIENCE & ENGINEERING TECHNOLOGY

Volume: 9 **Issue:** VII **Month of publication:** July 2021

DOI: https://doi.org/10.22214/ijraset.2021.36198

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International Journal for Research in Applied Science & Engineering Technology (IJRASET)
ISSN: 2321-9653; IC Value: 45.98; SJ Impact Factor: 7.429
Volume 9 Issue VII July 2021- Available at www.ijraset.com

Preliminary Study on the Effect of Quinolone against Rec A Protein for the Possible Role in the Treatment of Tuberculosis through Molecular Docking

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Abstract: ***Tuberculosis is a type of ancient, chronic disease which affects humans and caused by Mycobacterium tuberculosis. They affect the lungs and other organs. The treatment is curable but in some cases it is fatal if not treated properly. The molecular docking method was used to see the interaction of the protein with the ligand. Thus, molecular docking was used to analyse the Rec A (PDB ID 1U94) target protein with their known type of ligand by using molecular docking tools. The Rec A (PDB ID 1U94) structure of protein was downloaded through online database. The best ligand after molecular docking was Quinolone, which may act as a drug after in vitro and in vivo studies.***
Keywords: AutoDock Vina, Rec A, Quinolone, Ligand, and Molecular docking,

I. INTRODUCTION

Tuberculosis (TB) is a life-threatening infectious disease which is caused by *Mycobacterium tuberculosis*. TB was affecting all types of age group which have weak immune system. According to World Health Organisation (WHO) about 8.6 million people were infected with TB and about 1.4 million people died from TB in 2011. Since, 6,000 years ago, *Mycobacterium bovis* lineage was dispersing and linked to animal domestication and early farming [1]. In 2014, a new TB DNA genome was reconstructed in Southern Peru. It has been proved that first TB infection happened about 9,000 years ago [2]. It was spread to humans, goats and cow animals.

Rec A can catalyse the hydrolysis of ATP and by the presence of single-stranded DNA. Rec A is a 38 kilo Dalton protein which repair and maintain the DNA [4, 5]. The homologous protein is called RAD51 in eukaryotes [6, 7]. Rec A is a type related protein with DNA repair. In the bacterial response was co-protease [8] function in the auto catalytic cleavage of the LexA repressor and the ƛ repressor [9].

Molecular docking is based upon computer-assisted drug design (CADD). Molecular docking will be interaction between ligand and a target. The comparison of docking molecules for proteins and drug-like molecules by initial docking molecules which allow recorded the values of docked ligands. The current study is to analyse their active sites of the Rec A protein, for perform the docking of the chemical compounds and determine the active sites and compound binding affinity against Rec A for the treatment of TB.

II. MATERIAL AND METHODS

A. Identification Of Protein By Uniprot

RecA protein and eukaryotic homolog Rad51 protein catalyses the DNA strand. It is a key reaction of homologous recombination. RecA protein is involved in SOS induction, homologous recombination, and DNA repair. In vitro, RecA protein serves as a co-protease to cleave LexA repressor of the SOS region; RecA protein in addition protein promotes homologous pairing and DNA strand exchange, steps important to homologous recombination and DNA repair. The Rec A protein was searching by Uniprot online server and clicks on the option of structure and download the RCSB PDB less resolution file in .pdb format and saved for further process. The structure of Rec A [PDB ID 1U94] was obtained by Protein Data bank (PDB) https://www.rcsb.org/.

B. Retrieval of Ligands By Pubchem

Thiophenes, Nitroimidazole, Lidamycin, and Quinolone were used for the study of docking from the literature. All Ligands were selected from different plants source and all ligand particles were retrieved by https://pubchem.ncbi.nlm.nih.gov/ (PubChem) [10]. Thiophene belongs to a class of heterocyclic compounds containing the five-membered ring made up of the Sulphur as heteroatom with the formula C4H4S. Thiophene and its derivatives exist in petroleum or coal. The structure of the Thiophene can be found in certain natural products and is also incorporated in several pharmacologically active compounds. Thiophene was discovered as a contaminant in benzene. Especially thiophene its derivatives occur in petroleum, sometimes concentrations up to 1-3%.

Nitroimidazole is having molecular formula O2NC3H2N2H. It is the most common type of organic group at position 5 in their ring. The nitroimidazole was act as an antibiotic which is similar to the chemical structure. In an antibiotics complex isolated from a strain of the streptomyces and it's contained a trichomonacidal antibiotics azomycin.

Metronidazole is clinically effective in trichomoniasis, amebiasis, and giardiasis, as well as in a variety of infections caused by obligate anaerobic bacteria including Bacteroides, clostridium, and microaerophilic bacteria such as Helicobactor and Campylobactor spp. Quinolone is a family of synthetic, broad-spectrum antibiotics with bactericidal activity. The term quinolone refers to the potent synthetic chemotherapeutic antibacterial agent. Quinolones are synthetic antibacterial agents that are highly effective in the treatment of many types of infectious diseases. They are patterned after nalidixic acid, naphthyridine derivatives introduced for the treatment of urinary tract infections in 1963. The usefulness of quinolones has been largely confined to the treatment of urinary tract infections. They are used against a variety of gram-negative as well as gram-positive pathogens.These regular mixtures were recovered from PubChem online data set https://pubchem.ncbi.nlm.nih.gov/ . The mixtures were downloaded in SDF design. Every one of these compounds were changed over from .sdf organization to .pdb design by Online SMILES Translator https://cactus.nci.nih.gov/interpret/, the .pdf records of the ligands were downloaded in .pdb design

C. Protein Preparation

The protein Rec A was playing an important role in TB. The Rec A protein was searching by Uniprot online server and clicks on the option of structure and download the RCSB PDB less resolution file in .pdb format and saved for further process. The structure of Rec A [PDB ID 1U94] was obtained by Protein Data bank (PDB) https://www.rcsb.org/.

D. Drug Likeliness Property Analysis By Swiss ADME

Drug likeliness property was analyzed through an online server i.e. SwissADME. The natural compounds were used for final molecular docking studies by screening those ligands which having drug-like properties. The ligands SMILE notations were copied by PubChem and pasted on the SwissADME online web server [11]. Ligands were analyzed by Lipinski's rule of five. It had five following points:

1) Molecular mass was not more than 500 Da.
2) Hydrogen bond donors were less than 5 (< 5).
3) High lipophilicity (expressed as LogP less than 5).
4) Hydrogen bond acceptors were only having 10 (< 10).
5) 1 violated.

E. Molecular Docking Between Receptor Protein And Selected Compound By Mgl Tool

The .pdb file of protein was load into AutoDock Vina software screen. After uploading the .pdb file of protein it should be prepared due to deleting their water molecules, due to adding Kollman charges and by adding the hydrogen polar atoms, and protein file should save in ". pdbqt" format file. The ligand molecule was saved in .pdb format and then converted to the ". pdbqt" file format. Therefore, the command prompt was used, AutoDock Vina files were executed and results were analyzed (https://cactus.nci.nih.gov/translate/.) [12].

F. Structure Visualization Using Pymol

Structure visualization was done after Command Prompt software running through the tool PyMOL 2.4 which was a freely available tool on an online website. The ".pdbqt" protein file was loaded on PyMOL 2.4 graphical screen and imported the output ". pdbqt" file. PyMol software was used for better visualization of protein and ligand interaction in the molecular surface. After AutoDock Vina the output file was automatically saved in the selected folder with the name output. pdbqt file. This protein .pdbqt file with output pdbqt files were loaded on their graphical screen of PyMol. The interaction between the protein and ligand was visualized and analyzed.

III. RESULT & DISCUSSION

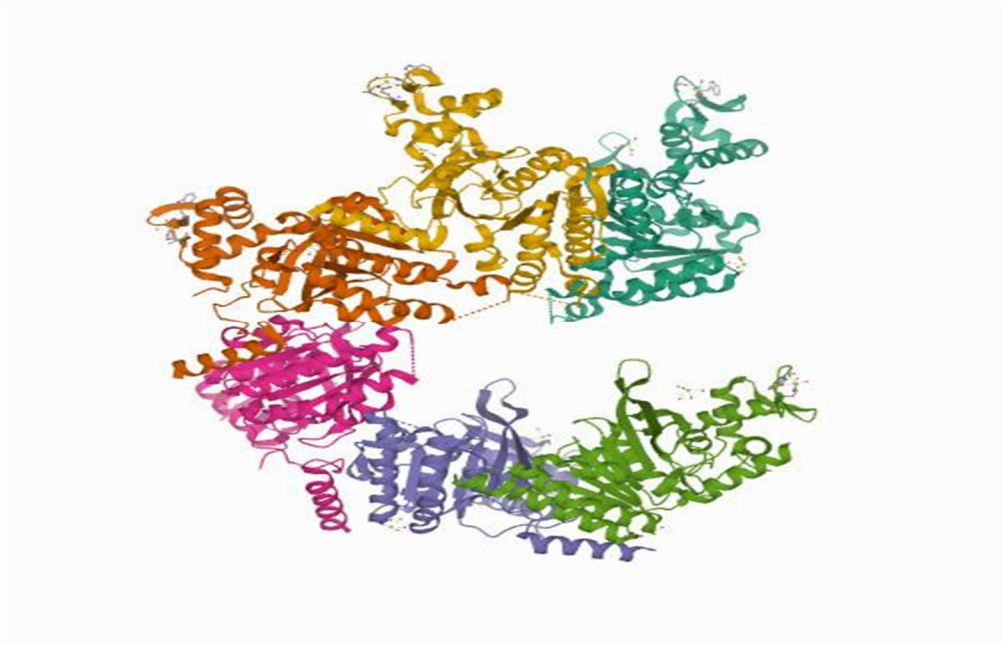
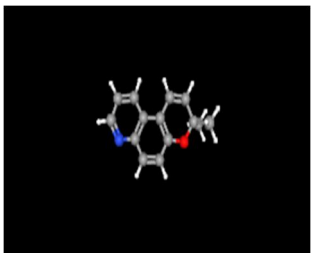


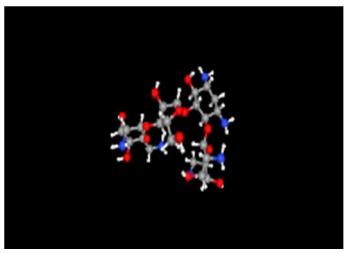
Figure 1: - Biological assembly of Protein Rec A [1U94].

PROTEIN	:	PROTEIN RECA
GENE	:	Rec A
PDB ID	:	1U94
CLASSIFICATION	:	DNA BINDING PROTEIN
EXPRESSION SYSTEM	:	ESCHERICHIA COLI
ORGANISM (s)	:	ESCHERICHIA COLI
MUTATION	:	NO
RESOLUTION	:	1.90 Å
R-VALUE FREE	:	0.222
R-VALUE WORK	:	0.187
SEQUENCE LENGTH	:	356

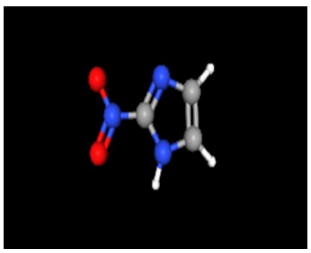
Figure 2: - 3 D Structure of Ligands



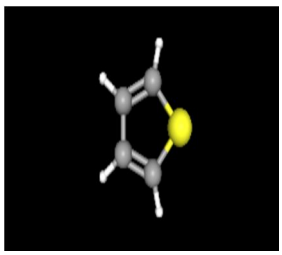
QUINOLENE CID - 6038 MF – C9H7NO MW – 145.16



LIDAMYCIN CID - 62404 MF – C23H46N6O13 MW – 614.64



NITROIMIDAZOLE CID - 10701 MF – C3H3N3O2 MW - 113.08



THIOPHENES CID - 8030 MF - C4H4S MW - 84.14

PyRx software was used for Virtual screening of the ligand molecules with their binding affinity. The binding affinity of Quinolene was -5.9, Lidamycin was - -6.1, Nitroimidazole was -4.0 and Thiophenes was -2.7 as shown in Table 1. The maximum binding affinity type of ligands was selected after the PyRx result was further analyzed for drug likeliness property analysis.

Table - 1 : Interaction Result of Compounds and Protein by PyRx

Compound	Ligand	Binding Affinity	Mode	RMSD lower Bound	RMSD upper Bound
Quinolene	RECA_6038_mmff94_E= -0.61	-5.9	0	0	0
		-5.8	1	3.242	4.375
Lidamycin	RECA_62404_mmff94_E= 381.88	-6.1	0	0	0
		-6.0	1	3.072	9.556
Nitroimidazole	RECA_10701_mmff94_E= 48.05	-4.0	0	0	0
		-3.7	1	16.396	17.117
Thiophenes	RECA_8030_mmff94_E= 4.38	-2.7	0	0	0
		-2.5	1	18.797	19.29

Table 2 : - Difference of Binding Energies in different Ligands

Sr.no.	Compound	CID	Binding Energy
1	Quinolene	6038	-5.9
2	Lidamycin	62404	-6.1
3	Nitroimidazole	10701	-4.0

Drug likeliness property analysis was completed by SwissADME and ligands were screened based upon following Lipinski's Rule of Five as shown in Table 3. Quinolene was the only molecule, which qualifying all types of Drug properties.

Table 3: - Result Analyze by SWISS ADME

Ligands	Binding Affinity	Molecular Weight	No. of H-Bond Acceptors	No. of H-Bond Donors	Log Po/w [MLOGP]	Violation
Quinolene	-5.4	229.27g/mol	3	1	1.64	Yes, 0 violation
Lidamycin	-2.8	712.72g/mol	23	15	-8.28	Yes, 3 violation
Nitroimidazole	-4.7	113.07g/mol	3	1	-1.21	Yes, 0 violation
Thiophenes	-2.8	84.14g/mol	0	0	1.12	Yes, 0 violation

The protein target RecA (PDB ID: 1U94) and Quinolene (CID: 6038) were selected for docked through AutoDock Vina software. The result was showing according 9 poses of Command Prompt with different binding affinity, by root mean square deviation (RMSD) by lower Bound and by root mean square deviation upper bound as shown in Table 4. These 9 poses are further used for interaction of visualized under PyMol as shown in Figure 3.

Table 4:- Autodock Vina Result

Mode	Affinity (Kcal/mol)	Dist. From best Mode	
		RMSD L.B	RMSD U.B
1	-5.9	0.000	0.000
2	-5.8	3.183	4.424
3	-5.6	18.04	19.09
4	-5.5	2.14	3.20
5	-5.4	18.191	18.744
6	-5.3	2.002	3.66
7	-5.3	2.751	4.075
8	-5.0	18.59	19.56
9	-4.9	26.92	27.73

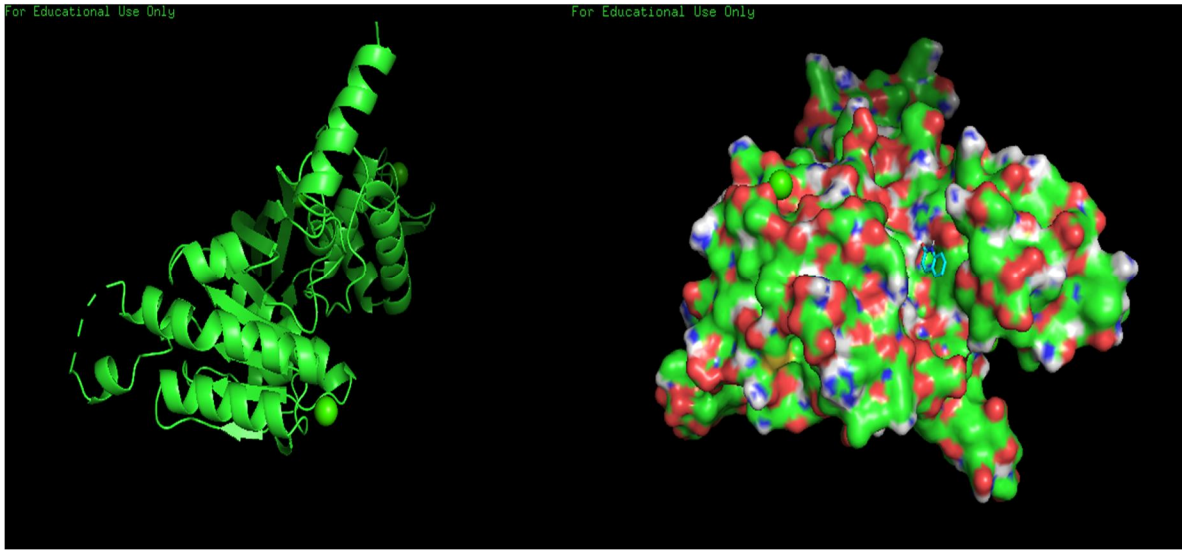


Figure 3:- : Interaction of RecA (PDB ID: 1U94) with Quinolene (CID: 6038).

IV. CONCLUSION

According to this study it may be concluded that the ligands (Quinolene, Lidamycin, Nitroimidazole, and Thiophenes) were interacted with the target protein Rec A (PDB ID: 1U94). Quinolene was having minimum binding energy with protein and also qualified Lipinski's rule of five. The results which were obtained were very useful for increasing the inhibitory activities and may act as a drug for the treatment of tuberculosis after pre- clinical and clinical studies.

A. Conflict Of Interest Statement

The authors declare that there is no conflict of interest.

V. ACKNOWLEDGEMENT

The authors acknowledge the help provided by the Department of Biotechnology, Faculty of Life Sciences, Institute of Applied Medicines and Research, Ghaziabad, Uttar Pradesh, India.

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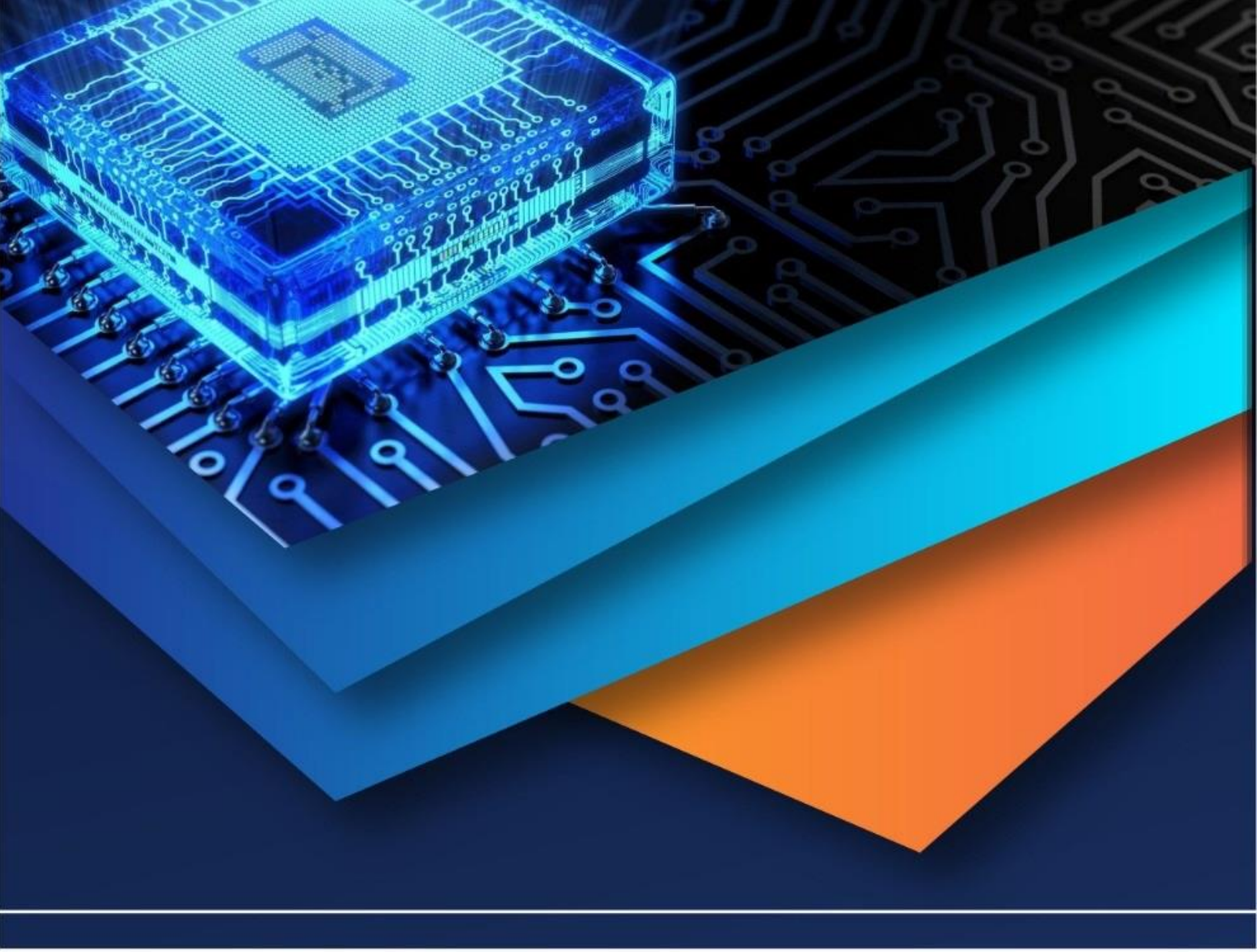

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