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Original Research Article

Rifampicin as Potent Inhibitor of COVID - 19 Main Protease: *In-Silico* Docking Approach

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Abstract

Corona virus (COVID-19) is an enveloped RNA virus that is diversely initiates in humans and wildlife. A total of 6 species have been identified to cause disease in humans. Viral infections play a critical role in human diseases, and recent outbreaks is the influx worldwide in form of novel corona .The SS-RNA virus from the enveloped corona virus family caused SARS (Severe acute respiratory syndrome) which is life threatening viral infection. The spreading of infection is quick in many countries of the world. The World Health Organization (WHO) called COVID-19 a pandemic on March 11, 2020. There are numerous drug trials going on with some positive results. Though, since no vaccine is available, the best way to fight the virus is by preventive measures. In the present research an attempt had been made to find new COVID-19 main protease inhibitor by molecular docking approach. The present study reveals that rifampicin has good binding affinity with COVID-19 protease and thus can be used as prophylaxis and therapeutic treatment for corona patient.

Keywords: COVID-19, Rifampicin, Molecular Docking & Prevention measures.

INTRODUCTION

The ongoing COVID-19 outbreak has led to global epidemics with high morbidity and mortality. Presently there are no effective therapeutics options available for the treatment of the COVID-19. At the time of this medical emergency, drug repurposing is the need of time. The repeated exterior and outbreaks of CoVs point toward a public health threat. This suggests the hazard of animal-to-human and human-to-human transmission [1, 2]. Therefore, these studies try to determine possible COVID-19 drug using docking. The study findings suggest that Rifampicin may be used as repurposed drug for the treatment of COVID-19. Further *in-vitro* and clinical trial in order to precisely confirm our docking study. Tuberculosis (TB) already exists as unprecedented pandemic worldwide over several years. It was already declared a global health emergency by the WHO in 1993. The estimated global burden of TB is 10 million, with nearly half of them having drug resistance in 2018. Out of 10 million cases, 3 million (30%) remain undiagnosed. TB remains the deadliest infectious killer as compared to the novel COVID-19. The estimated mortality was 1.2 million among HIV-negative people in 2018 and an additional 0.25 million among HIV-positive people. Around 4000 people die and 30,000 people fall ill every day despite this disease is preventable and curable. Approximately 3 out of 10 TB patients (27%) in the world belong to India. Around 1400 people die and 7500 people fall ill every day due to TB even in India [3, 4]. The introduction of rifampicin into tuberculosis treatment five decades past was serious for shortening the treatment duration for patients with pulmonary TB to 6 months when combined with pyrazinamide in the first 2 months [5]. Rifampicin proposes that it is one of the most efficient anti-tuberculosis drugs yet discovered.

S.No.	Description of Rifampicin[6]	
1.	**Synonyms**	Rifampicin, Rifampicina, Rifampicine, Rifampicinum & Rifampin
2.	**Average weight**	822.9402
3.	**Chemical Formula**	$C_{43}H_{58}N_4O_{12}$
4.	**Source**	Semi-synthetic antibiotic produced from *Streptomyces mediterranei.*
5.	**Category**	Anti-tuberculin drug (Macrocyclic antibiotic)
6.	**Mechanism of action**	Acts via the inhibition of DNA-dependent RNA polymerase, leading to a suppression of RNA synthesis and cell death.

MATERIALS AND METHODS

Molecular docking studies

Ligand Preparation

2D Structure of ligand (rifampicin) was drawn using ChemSketch [7], the two-dimensional structure of was converted into 3-D structure and optimized with 3D geometry. The optimized structure was saved in PDB format for AutoDock compatibility. The basic structure of ligand (rifampicin) is given below in fig1:

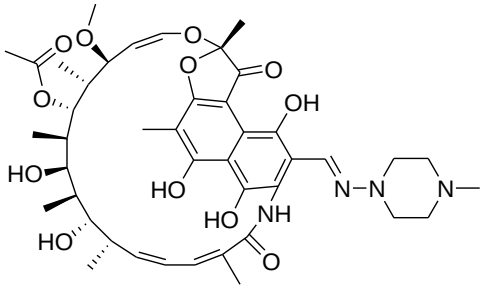


Fig-1: 2D structure of rifampicin

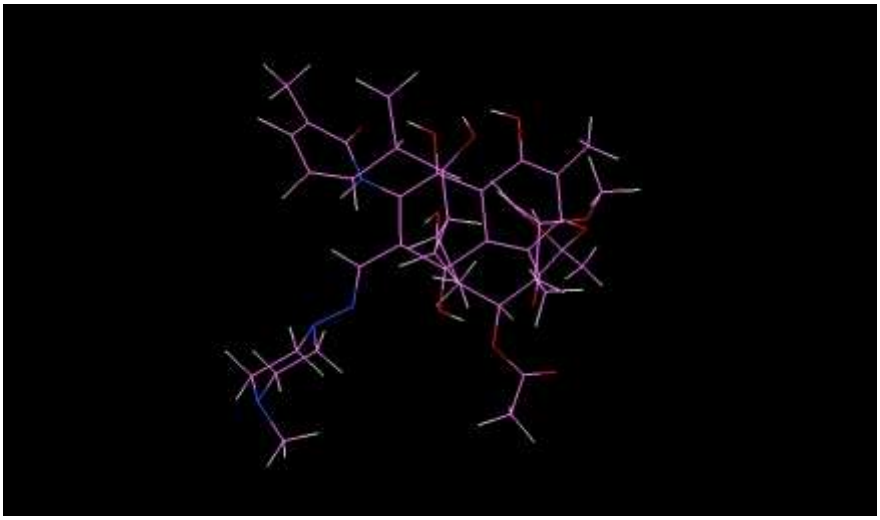


Fig-1: 2D and 3D conformer of rifampicin

Preparation of the grid file

The regions of interest used by Autodock were defined by considering grid area by making a grid box around the active sites. Grid box plays a central role in process of docking as it is made to cover all the amino acids present in active sites necessary for binding other than those present in receptor. Grid box has 3 thumbwheel widgets which let us change the number of points in the x, y and z dimensions. The spacing between grid points can be adjusted with another thumbwheel, the value in the study taken is 0.419 Å and No. of points considered are 40, 54 and 40 points in the x, y, and z dimensions and -9.732, 11.403 and68.925 as x, y, z centers [8, 9].

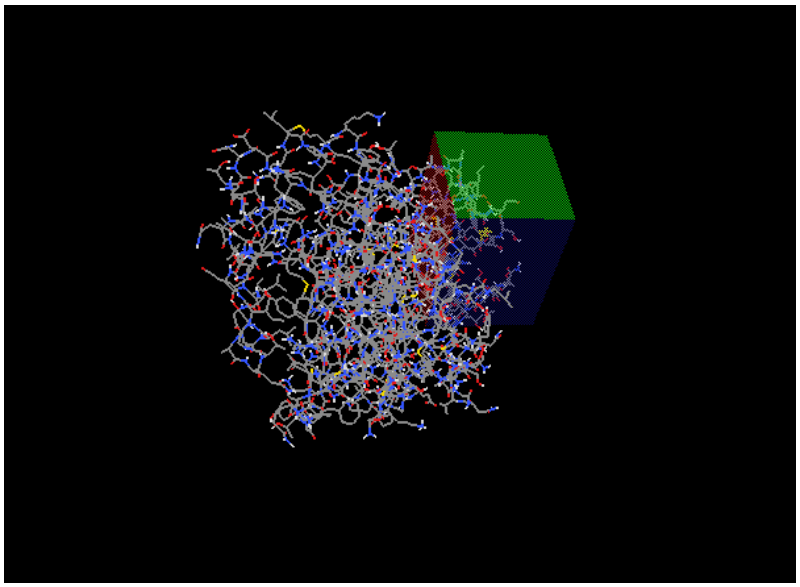


Fig-2: Grid box covering all active sites in receptor

Preparation of the docking file

All the calculations were carried out by using Autodock4.2 as docking tool. The visualization and other programs necessary for docking studies were performed out by means of Pymol, Chimera, DS visualizer, MMP Plus [10-11].

Docking of Main Protease with Rifampicin

Crystal structure

The crystal structure of the protein consisting of receptor associated with bound ligand is downloaded from the Protein Data Bank portal. All the primary information regarding receptor and structure (6LU7.pdb) registered in the Protein data bank was used. The bound ligand peptide like inhibitor is found within the receptor [12].

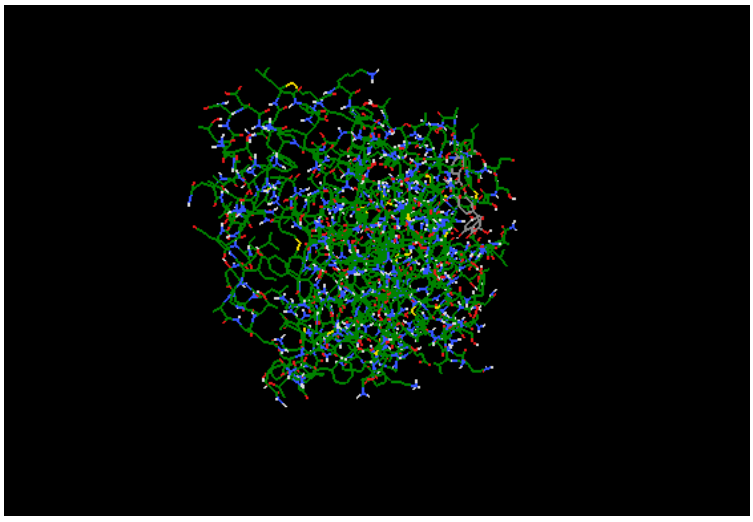


Fig-3: Crystal structure of Main Protease enzyme with bound peptide like inhibitor ligand (PDB ID-6LU7)

Processing of Protein

The downloaded receptor protein is having two chains A and C, and both the chains have been used for experimental purpose. The bound ligand peptide like inhibitor was separated from the macromolecular complex by using software Chimera [13].

Molecular Docking Simulation Studies

Docking of rifampicin ligand on Main Protease enzyme was performed by Autodock. All the bonds of ligand were kept flexible, while no residues in receptor were made flexible [14].

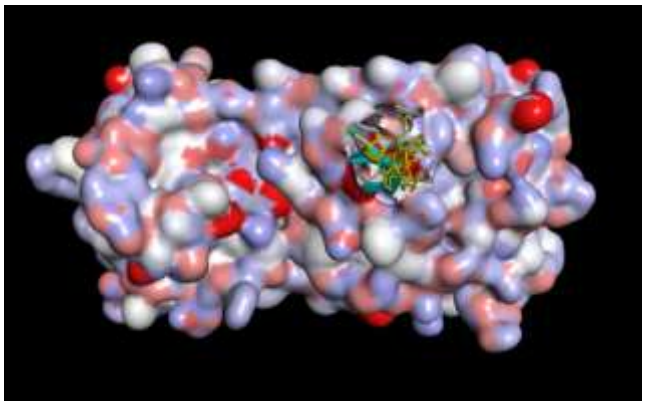


Fig-4: Binding mode of rifampicin within the active site of main protease receptor

Toxicity & ADME-T Studies

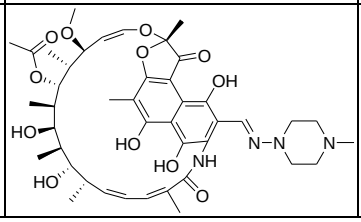
The modified lead molecules are studied by online program OSIRIS, for prediction of presence of any toxic group as well as presence of any toxic group and ADME- T properties [15].

RESULTS AND DISCUSSION

Docking

Following result were observed in docking studies of main Protease enzyme with rifampicin.

Table-1: Result of docking of rifampicin against main protease enzyme

S. No	Compound Name	Structure	Binding Energy (Kcal/mole)	Ki (µM)
1	Rifampicin		-7.24	4.96

The rifampicin was docked and the binding energy was found to be -7.24 kcal/mol.

Toxicity & ADME-T Studies

The pharmacokinetic profile of rifampicin reveals that it is having good pharmacokinetic profile but having presence of some mutagenic and tumorogenic toxic effects. The pharmacokinetic and toxicity profiling results of rifampicin were shown in figure 5.

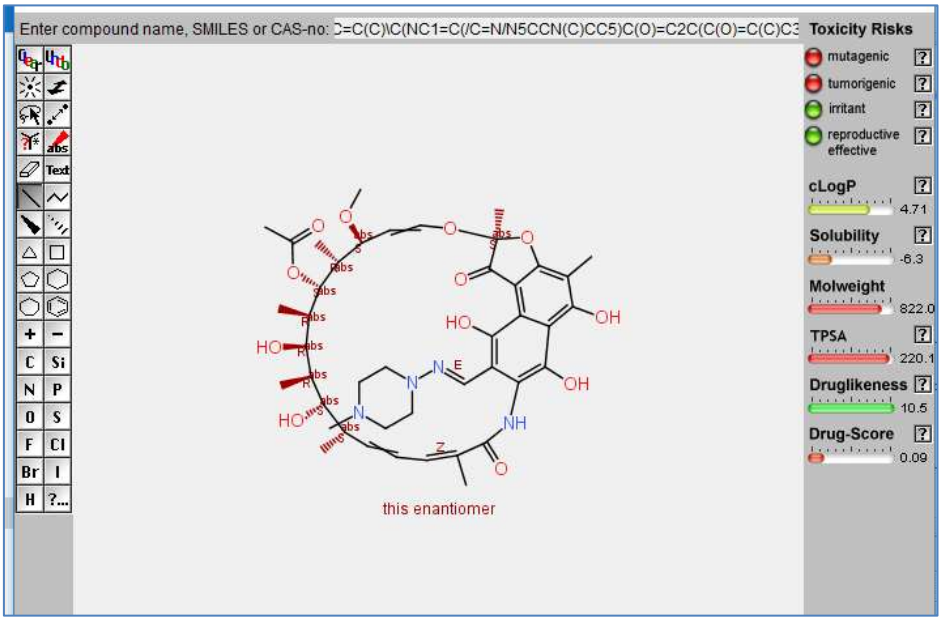


Fig-5: Pharmacokinetic and toxicity profiling of rifampicin.

The molecular docking of rifampicin with Main Protease enzyme revealed that (Table 1), it has exhibited the chemical interaction with the amino acids in the active pockets which is showed in Figure.3. Theoretically, the ligand molecule has shown encouraging docking score. The docking result of rifampicin revealed that their docking scores was-7.24 kcal mol–1, and it can predicted as a very good inhibitor of main Protease enzyme.

CONCLUSION

The World Health Organization declared COVID-19 a global pandemic on 11 March 2020. India confirmed its first case of COVID-19 on 30 January and within 8 weeks the total number of cases had crossed the 1000 mark. Much of India's 1.3 billion populations resides in densely populated, resource constrained environments, which puts them at risk for emerging outbreaks. In addition, the burden of chronic diseases such as tuberculosis (TB), HIV and malaria puts further pressure on the health system. The limited number of hospital beds and ventilators is a serious concern for India. According to the National Health Profile 2019, there are approximately 714 000 government hospital beds available, amounting to 0.55 beds per 1000 population. This ratio is extremely low by international standards, but the problem is compounded by the fact that a large proportion is already occupied by patients suffering from chronic diseases such as TB. The Indian Government has begun to mobilize beds for isolation and management of COVID-19 patients. However, this may lead to the reduced availability of beds for TB patients who are critically ill or require hospitalization for management of adverse drug reactions. Based on preliminary data from China and Italy, an estimated 5–10% of all COVID-19 patients will require critical care in the form of ventilator support. According to one estimate (based on a worst-case scenario without intervention), India could have 2.2 million COVID-19 cases by the end of May 2020, which implies a need for 200 000 ventilators. India currently has an estimated 18 000 to 25 000 ventilators. The need for ventilator support for TB patients who are co-infected with COVID-19 is difficult to estimate at this moment, but the demand for ventilators could quickly outstrip this limited supply. To contain the spread of COVID-19, we need to educate people on infection control for vulnerable populations and how to care for the sick. This can be done with the help of local volunteers via the public health system. However, according to World Bank data, 24% of the urban population of India lives in slums. In these crowded environments, self-isolation in often poorly ventilated dwellings can itself pose a risk for the spread of COVID-19 (and for TB). India accounts for approximately one quarter of the world's TB burden, with the estimated number of new TB cases in 2018 at 2 800 000. To curb the spread of COVID-19, the Indian Government announced a nationwide lockdown for 21 days beginning 25 March 2020.2 The lockdown is a major hurdle to TB patients seeking health care and may result in a delay in diagnosis, treatment interruptions and disease transmission in household contacts. Lockdown has also forced many migrant workers to return to their homes, leading to treatment interruption. Many health facilities are turning to the use of digital platforms in an attempt to make medical care accessible in difficult to reach areas. Although India aims to eliminate TB by 2025, the present political and economic focus on COVID-19 could result in a shift in priority. The current scenario may lead to a loss of earnings and malnutrition, and also lead to an increased incidence of TB. However, measures to prevent airborne infection and general cleanliness for COVID-19 may mitigate this and reduce the transmission of TB. In the longer term, a renewed focus on lung health may allow us to achieve the goal of making India TB-free. As India braces for a wave of patients with COVID-19, we need to ensure the availability of key equipment to care for patients and to ensure the safety of health care workers. This will require a coordinated approach from all sectors, from state and national governments through to the private sector and health care providers. The present study concludes that, Rifampicin clinically approved drugs are identified as potent inhibitors against SARS-CoV-2 protease activity. The result of study showed that rifampicin is best hit molecules which interfere the life cycle of the virus by interacting with the covid main protease. Therefore the outcome of this study signifies that Rifampicin, a well-established medicine for the treatment of tuberculosis has a stronger binding affinity for COVID-19 main protease. These outcomes afford a strong foundation for the use of these drugs is for CORONA management. Moreover, the dynamic ligand inhibited the catalytic response of protease by blocking the residues of amino acids intricate in the processing and strand transmission reactions. The interactions by the structural model at the protease active site can afford a valuable guide for additional strategies for structure based medicines and development of new operative inhibitors of SARS-CoV-2 protease. Therefore, the effect of these inhibitors can be further revealed through in vitro and in vivo analysis in the termination of intracellular replication of corona virus, prior to the use as drugs in humans.

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