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DOXYCYCLINE AS POTENT INHIBITOR OF COVID -19 MAIN PROTEASE: *IN-SILICO* DOCKING APPROACH

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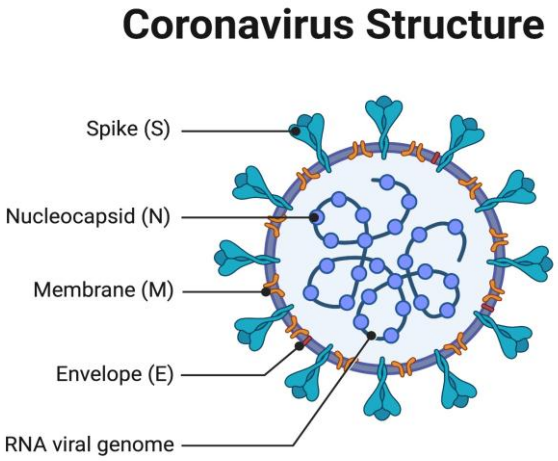
ABSTRACT
Corona virus (COVID-19) is an enveloped RNA virus that is diversely initiates in humans and wildlife. A total of 6 species have been identified to cause disease in humans. Viral infections play a critical role in human diseases, and recent outbreaks is the influx worldwide in form of novel corona .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. Doxycycline is an associate of the tetracycline class of antibiotics and has been used clinically for more than 40 years. It is a useful antibiotic for prophylaxis against and treatment of several important potential biological combat agents. Doxycycline was taken as drug which follows Lippinski's rule of five, thus having very good drug score as well as drug likeness score. The present study reveals that Doxycycline has good binding affinity with COVID-19 protease and thus can be used as prophylaxis and therapeutic treatment for corona patient.

KEYWORDS: COVID-19, Doxycycline, Molecular Docking & Prevention measures.

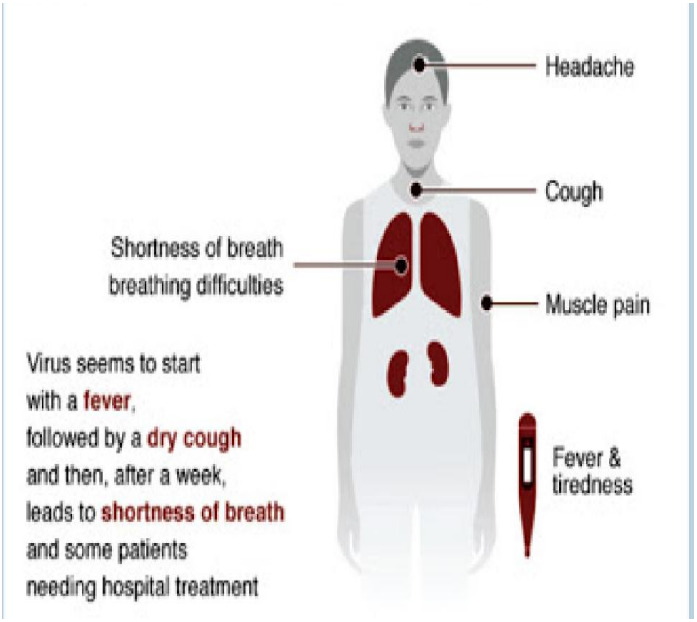
INTRODUCTION
COVID-19 also called as novel virus as coronavirus disease-2019 was announced by the World Health Organization (WHO) on February 11, 2020. The repeated appearance and outbreaks of CoVs designate a public health threat. This suggests the possibility of animal-to-human and human-to-human transmission of recently emerging CoVs. Severe acute respiratory syndrome (SARS) which had begun the previous year in Asia and secondary cases everywhere else within the world, the WHO stating this coronavirus notorious by a variety of laboratories was the contributory agent for SARS. The virus was collectively named the SARS corona virus (SARS-CoV). Coronavirus (CoV) is a huge family of + ve sense, single-stranded RNA viruses that belong to the Nidovirales order.[1]

Order	Morphology
The order includes Roniviridae, Arteriviridae, and Coronaviridae families.[2]	Corona viruses are enveloped; non segmented, positive-sense single stranded RNA virus genomes in the size ranging from 26 to 32 kb, the largest recognized viral RNA genome. The virion has a nucleocapsid composed of genomic RNA and phosphorylated nucleocapsid (N) protein, which is buried inside phospholipid bilayers and covered by two different types of spike proteins: the spike glycoprotein trimmer (S) that can be found in all CoVs, and the hemagglutinin esterase (HE) that exists in some CoVs.[3]

Structure of Corona virus



Symptoms of SARS-CoV[4]



Doxycycline is a second-generation tetracycline. Doxycycline (alpha-6-deoxytetracycline) of the tetracycline class of antibiotics and has been used clinically since past era. Doxycycline is used to treat infective diseases because of its broad spectrum worth.[5] Doxycycline is a broad-spectrum antibiotic that ruins one of the most inexpensive antibiotic regimens for treating soft tissue and bone infection in the human body.[6]

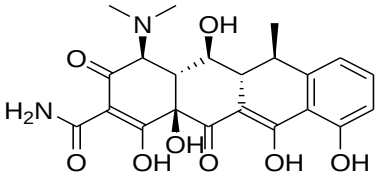
S.No.	Description of Doxycycline[7]	
1.	**IUPAC**	α-6-deoxytetracycline
2.	**Average weight**	444.4346
3.	**Chemical Formula**	$C_{22}H_{24}N_2O_8$
4.	**Category**	tetracycline antibiotics
5.	**Mechanism of action**	Inhibit translation by binding to the 16S rRNA portion of the ribosome, preventing binding of tRNA to the RNA-30S bacterial ribosomal subunit, which is necessary for the delivery of amino acids for protein synthesis.

Experimental Works

In present research work, Doxycycline binding affinity with Covid-19 main protease was accessed through Grid Based Docking studies by using Autodock4.2 as docking tool software.

Ligand Preparation

2D Structure of ligand (doxycycline) was drawn using ChemSketch[8], 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 (doxycycline) is given below.



2D structure of doxycycline

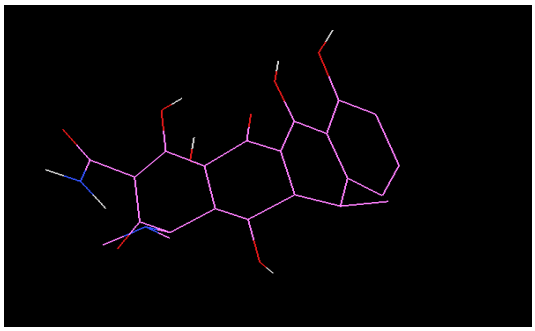


Figure 1: 2D and 3D conformer of doxycycline.

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.[9-10]

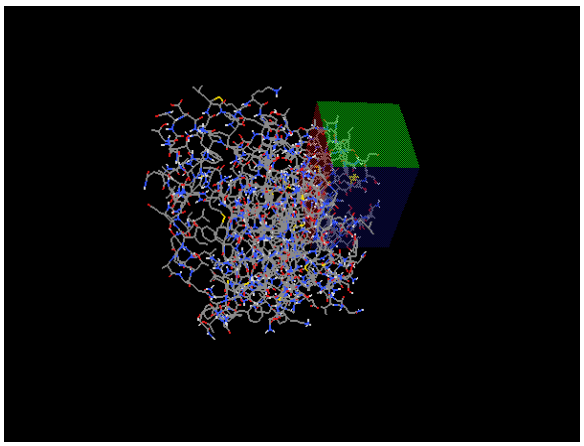


Figure 2: Grid box covering all active sites in receptor.

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.[11-12]

Docking of Main Protease with Doxycycline

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.[13]

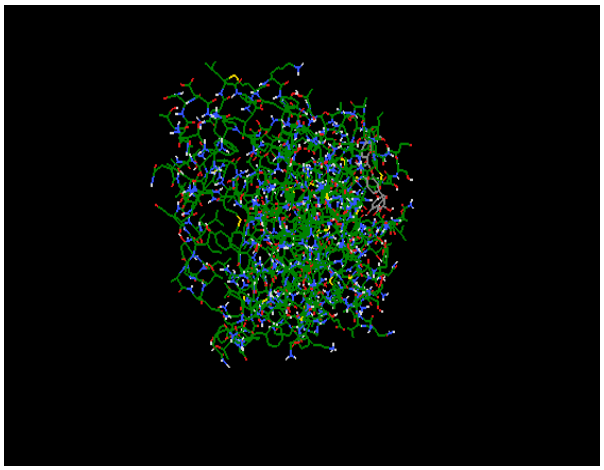


Figure 3: Crystal structure of Main Protease enzyme with bound peptide like inhibitor ligand (PDB ID-6LU7)

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.[14]

Molecular Docking Simulation Studies

Docking of doxycycline 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.[15]

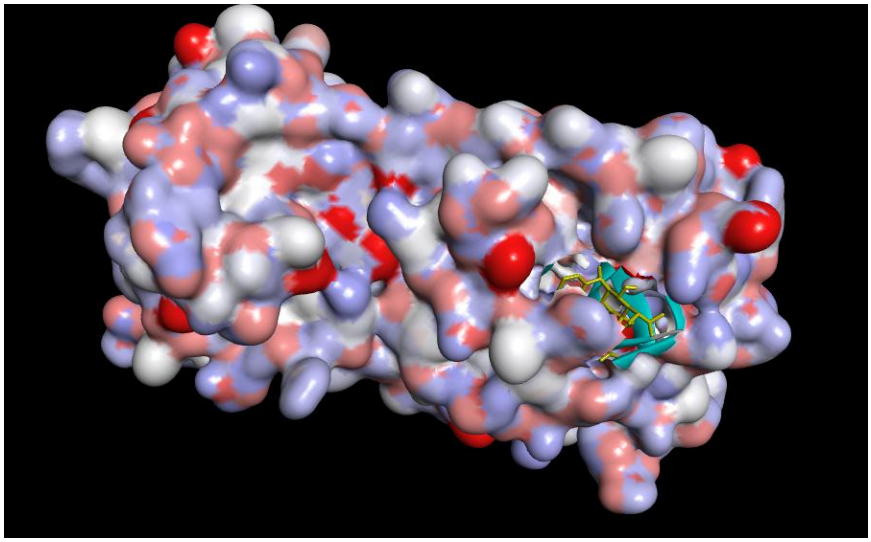


Figure 4: Binding mode of doxycycline within the active site of main protease Receptor.

Toxicity & ADME-T Studies

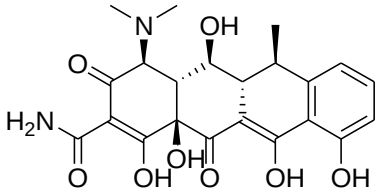
The modified lead molecules are studied by online program OSIRIS, for prediction of presence of any toxic group as well as presence of any toxic group and ADME- T properties.[16]

RESULTS AND DISCUSSION

Docking

Following result were observed in docking studies of Main Protease enzyme with doxycycline.

Table 1: Result of docking of doxycycline against Main Protease enzyme.

S. No	Compound	Structure	Binding Energy (Kcal/mole)	Ki (µM)
1	Doxycycline		-7.21	5.15

The doxycycline was docked and the binding energy was found to be -7.21 kcal/mol.

Interactions

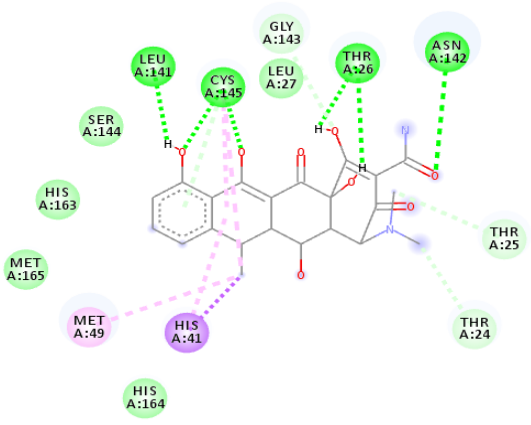


Figure 5: Binding interaction of Doxycycline with main protease.

The doxycycline interacts with the Leu141, Cys145, Thr26, Asn142, His41, and Met49 residues of main protease to form a complex structure.

Toxicity & ADME-T Studies

The pharmacokinetic profile of doxycycline reveals that it is having good pharmacokinetic profile but having associated reproductive effects. The pharmacokinetic and toxicity profiling results of doxycycline were shown in figure 6.

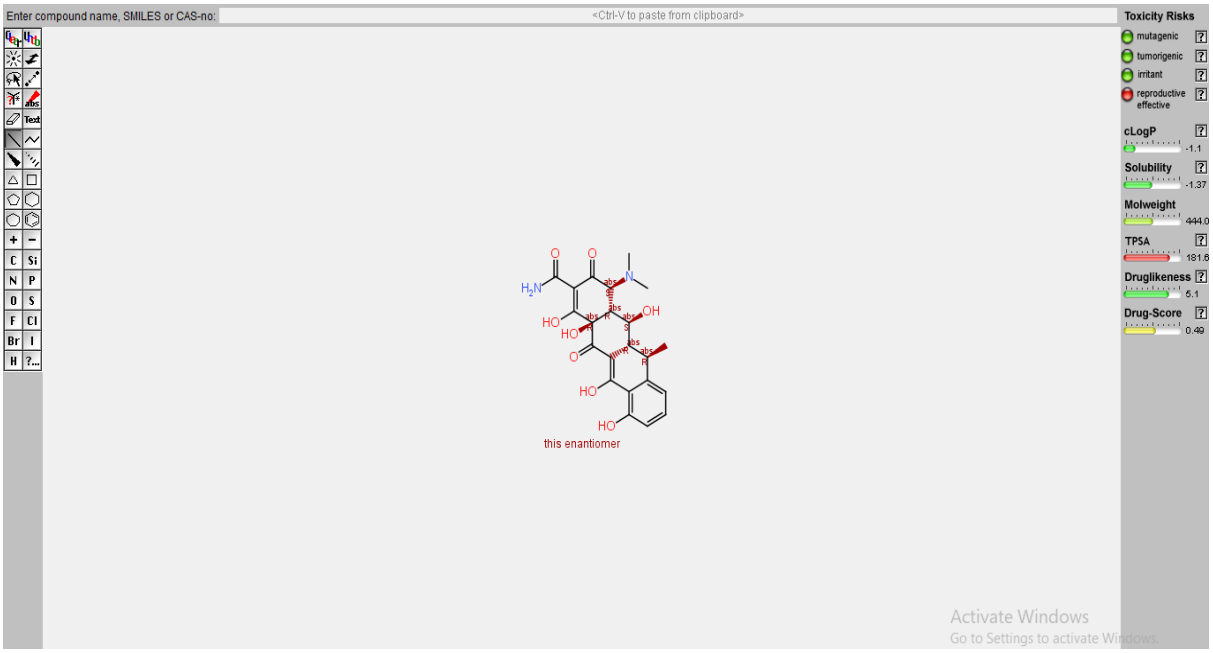


Figure 6: Pharmacokinetic and toxicity profiling of doxycycline.

The molecular docking of doxycycline 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 doxycycline revealed that their docking scores was -7.21 kcal mol^{-1}, and it can predict as a very good inhibitor of Main Protease enzyme.

CONCLUSION

In current era, multiple drug resistance has urbanized due to random use of existing antimicrobial drugs in the treatment of infectious diseases. The resultant showed the outbreak of worldwide COVID-19(novel corona) having an exponential growth rate of a 16% increase in cases per day is shown. Approximately at present in India 3,546,705 active cases was identified and an above 63,690 deaths occurred. However preventive measures need to be done for spreading the SARs. From the present molecular docking investigation, it is concluded that doxycycline acts as a potent inhibitor of Covid-19 main protease and may act as a preventive drug for the treatment of SARS as it shows good binding affinity with the macromolecule with extremely good dock score and various binding interactions.

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