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COVID-19 - In Silico Structure Prediction and Molecular Docking Studies with Doxycycline and Quinine

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Coronavirus disease (covid-19) is a pandemic of international concern. It creates serious health risk all around the globe and it has no effective treatment. Doxycycline and Quinine are the drugs used as ligands in the study as these drugs has proved in vitro antiviral activity against dengue virus and herpes simplex virus. These compounds were targeted against non structural protein (nsp 12) which plays a vital role in replication and transcription of Corona viral genome. The protein 6 NUR that showed maximal identity of target protein nsp 12 was retrieved using BLASTp. Further the protein was modelled and the compounds were docked using AUTODOCK software and to study the structure activity relationship of ligand with target protein and biochemical information of ligand receptor interaction was done. Both the compounds, Doxycycline and Quinine were well engaged into the active site of target protein nsp 12 with strong hydrogen bond interaction and non polar interaction with active site of the protein. The Docking score of Doxycycline is found to be - 7.34 kcal/mol while that of Quinine being -6.14 Kcal/mol. This indicates the potential of these drugs as a lead against the nsp target protein of Corona virus which need further analysis and Optimisation studies.

Keywords: Corona virus, In silico ,Docking, Doxycycine, Quinine.

Corona virus belongs to positive stranded RNA virus which is enveloped. Severe acute respiratory syndrome coronavirus 2 (SARS CoV -2 or COVID 2019) was reported first in Wuhan,China in December 2019.[1] Later it has spread rapidly to other countries around the world. Fever, cough, shortness of breath and dyspnea are common symptoms of a person infected with coronavirus. Covid-19 spectrum also varies from asymptomatic forms to respiratory failure that needs mechanical ventilation in ICU.[2]

Viral infection produces excessive immune reaction in the host labelled as cytokines Storm.In SARS CoV-2,viral protein structures like main protease enzyme (3CL pro),viral papain like protease,RNA dependant RNA polymerase (RdRp),helicase enzyme,spike protein could be targeted for conducting homology modelling for discovery of novel drugs against coronavirus.[3] Non structural protein nsp 12, which is RNA dependant RNA polymerase is important in replication and transcription of corona vial genome.[4]Till now no

drugs or vaccine has been approved by regulatory agencies for the treatment of covid-19. But drugs Chloroquine and its analogue hydroxychloroquine has been tried in treatment of corona virus in some countries.

Chloroquine was found to inhibit the growth of SARS-CoV 2 in vitro. Chloroquine rise the pH of endosomal vesicles inside the cells that are points of entry by virus, thereby preventing fusion and entry of virus. It also controls the cytokine storm in the late phase of critically ill covid-19 patients.[5]

Quinine which is obtained from cinchona bark also used in the treatment of malaria. It has been proved in invitro study that quinine sulphate reduces the multiplication and adsorption of herpes virus.[6] Doxycycline,which is second generation tetracycline used in the treatment of severe bacterial infection also proved its effect in invitro studies against dengue virus replication and also against vesicular stomatitis.In a dose dependant manner, Doxycycline inhibited vesicular stomatitis virue replication at concentrations of 1.0–2.0ìg ml [7] .So, as a method of drug repurposing,these drugs were chosen to analyse its effect against coronavirus by insilico method.

To speed up the drug designing process, rational drug design method was developed. Docking of drug molecule with receptor or enzyme is one such method.In this study ,effort has been made to design drugs for covid-19 using doxy cycline, quinine by analysing its structure activity relationship and also docking these drugs against Corona virus target protein which in study is non structural protein (nsp 12).

MATERIALS AND METHODS

Sequence Retrieval

The nucleotide sequence of severe acute respiratory syndrome coronavirus 2 isolate Wuhan-Hu-1, complete genome (ID: NC_045512.2) was retrieved from GenBank nucleotide database (https://www.ncbi.nlm.nih.gov/nuccore/nc_045512.2). The EMBOSS transeq was used to translate the DNA sequence to the corresponding amino acid sequence.[8]

Template Selection

The amino acid sequence was submitted to BLASTp against protein data bank (PDB) to identify the structurally homologous proteins.[9] From the BLAST result the template with maximum identity were aligned with the target sequence using MultAlin, online software for multiple sequence alignment.[10]

Protein Structure Prediction and Validation

The three dimensional structure of the target protein was determined using SWISS-MODEL (https://swissmodel.expasy.org/assess). SWISS-MODEL is a fully automated server that works based on the principles of homology modeling.[11] The modeled structure was energy minimized using Swiss PDB Viewer, an application that reduces the energy of the protein by repairing bond length, bond angles and other distorted geometries of the protein.[12] The quality of the predicted model was analyzed using PROCHEK Ramachandran plot and ERRAT .[13,14]

Analysis of Physico-Chemical Characteristics

The protein's molecular weight (MW), extinction coefficient, isoelectric point (pI), number of negative and positive charge amino acids (-R, +R), aliphatic index (AI), Grand Average Hydropathy (GRAVY), instability index (II) was determined using Prot Param server.[15]

N-glycosylation site Prediction

Glycosylation sites in the target viral protein were determined using the web servers GLYCOPP and N-Glycosite, online servers for prokaryotes glycosites prediction .[16,17]

Secondary Structure and Active Site Prediction

The secondary structure of the protein was determined using self-optimized prediction method (SOPMA)[18]. Active site in the protein was

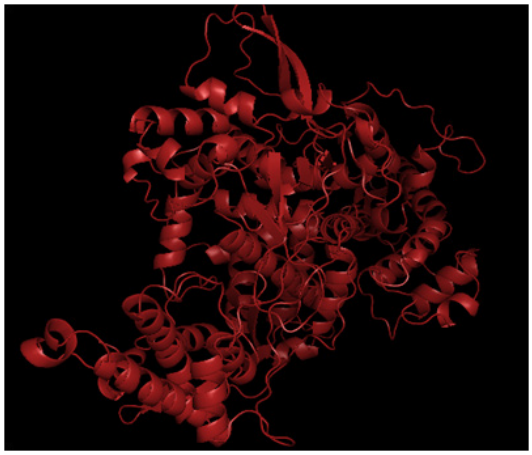


Fig. 1. Structure of severe acute respiratory syndrome coronavirus 2 target protein

determined using CASTp. The active site of the amino acids is required for the further molecular docking studies.[19] Molecular docking analysis was performed to identify the interaction nature of ligand molecules. Auto Dock is an automatic docking tool and it is designed to predict how small molecules, such as substrates, inhibitors are bind to a receptor of known 3D protein structures. A graphical user interface called Auto Dock Tools or ADT was utilized to generate grids, calculate the dock score. The software identifies the binding interactions of the reference molecule and the extracted molecule to the target protein molecule of the interest, Initially the molecule were read in the autodock software and the grid parameter file was formed and commanded to run autogrid and autodock. Once the dlg files of the molecules were performed by the software, which contains the docking score of the interaction, the file was opened in the Discovery studio for the ligand interaction.

Discovery Studio Visualizer 3.1

A widely available free software that helps in envisaging the macromolecule-ligand interactions, Discovery Studio Visualizer 3.1,

Table 1. Physico-chemical Characteristics of target protein

MW (Da)	-R	+R	pI	EC ($M^{-1}cm^{-1}$)	AI	GRAVY	II
91839.54	89	79	6.14	126990	80.35	-0.202	27.96

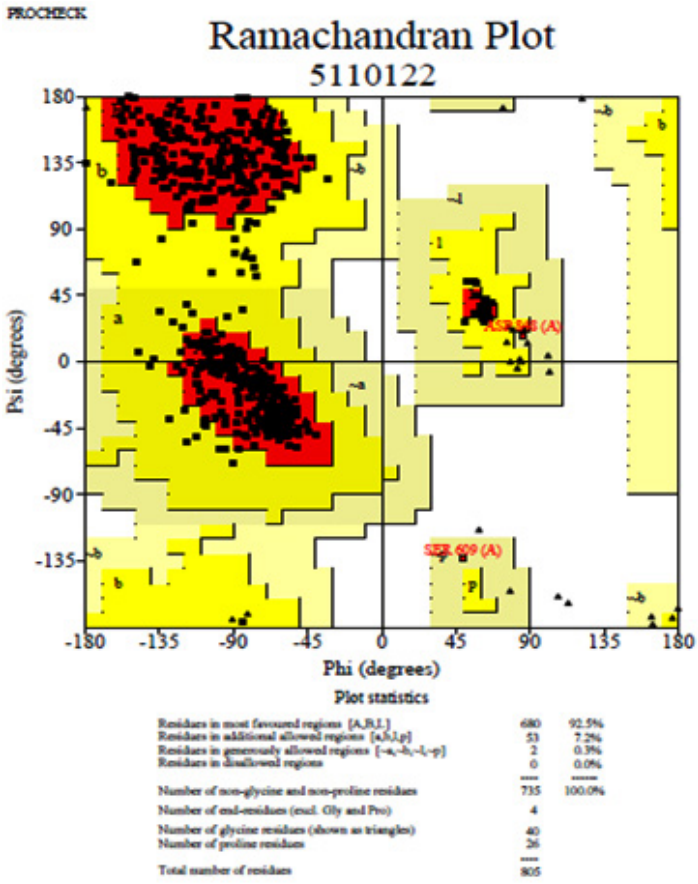


Fig. 2. Ramachandran Plot determined by PROCHECK. The regions in the red indicates favoured region, yellow shows allowed region, light yellow indicate generously allowed region of amino acid and white region indicate disallowed region.

developed and distributed by Accelrys, is used to study the simulation of small molecules and large macromolecules. This module helps in determining the structure based design, ligand interaction with the protein, validation and optimization. This also helps to analyze the structure activity relationship between protein and the ligand bind to it.

RESULTS AND DISCUSSION

Generation of 3D Structure and Validation

The experimental structure of severe acute respiratory syndrome coronavirus 2 is not determined using X-RAY crystallography and NMR methods. Hence *in silico* based techniques were used to predict the 3D structure of the target protein.The target protein,Non structural protein (nsp 12) which is RNA dependant RNA polymerase (RdRp) is important in replication and transcription of corona viral genome.The target protein's structurally homologous templates were retrieved using BLASTp. From BLAST result, two proteins (PDB ID: 6NUR and 6JYT) that showed maximum identity were chosen. Both template sequences were aligned with the target sequence using MultAlin. Finally, the protein 6NUR that showed maximum alignment was chosen as the template. SWISS MODEL, free online homology modeling software was used to predict the structure of the target protein from the structure of template protein. The predicted protein structure was energy minimized using Swiss PDB Viewer. Energy minimization is an important step in the optimization of geometry of the predicted structure. Finally the structure of the protein was visualized using Pymol molecular graphics system.[20] Fig 1 shows the energy minimized structure of target protein.

Table 2. Secondary structure elements in target protein

Secondary Structure	%
Alpha helix	41.97
3_{10} helix	0.00
Pi helix	0.00
Beta bridge	0.00
Extended strand	16.94
Beta turn	9.84
Bend region	0.00
Random Coil	31.26
Ambiguous state	0.00
Other States	0.00

The validation of the protein structure was performed using PROCHECK Ramachandran plot and ERRAT. The Ramachandran plot is the plot of phi (ö) and psi (ø) angles. The result showed that the number of amino acids in the disallowed region is zero and residues in the most favored region are 92.5%. A good quality model will have >90% amino acid in the most favored region.[21] The quality factor of the protein from ERRAT was 95.9157%. The quality factor >50% is considered to be a good quality model.[22] Fig 2 and Fig 3shows the Ramachandran plot and ERRAT result of the protein respectively.

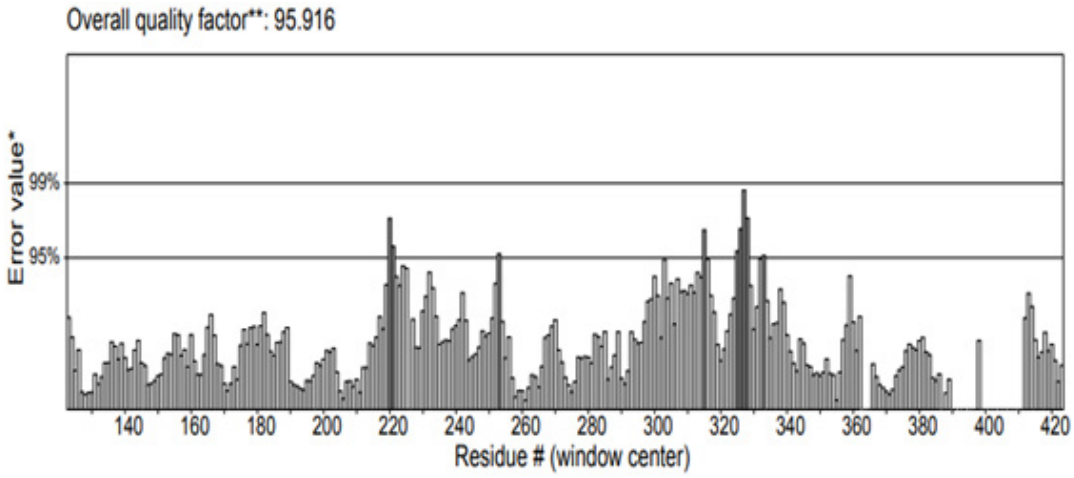


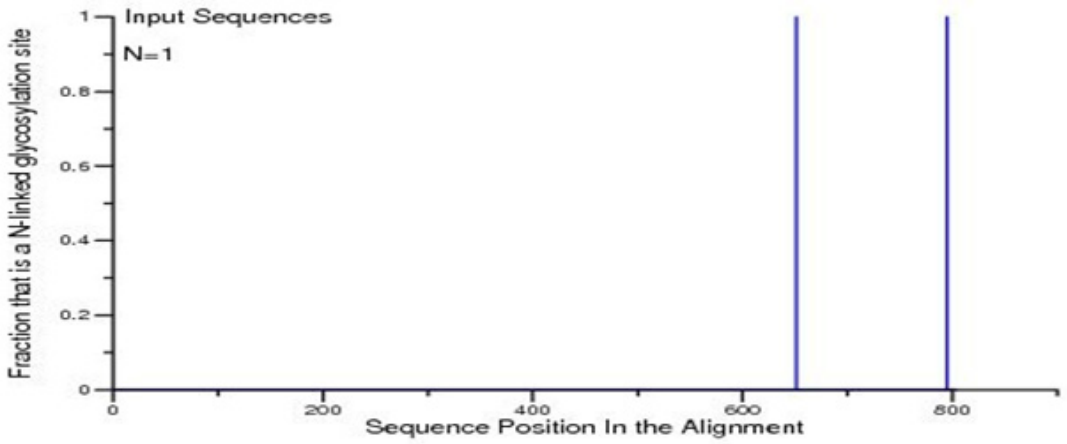
Fig. 3. Structure Validation result of ERRAT Tool.

Physico-chemical characterization

The physical and chemical properties of the protein was analyzed using Prot Param server, an online server for analysis of molecular weight, isoelectric point and many other properties of the protein. The half life of the protein *in vitro* mammalian reticulocytes is 0.8 hours. As proteins absorb well at 280nM, the extinction coefficient is calculated at this wavelength. The aliphatic index determines the thermostability of the protein. The results suggest that the protein is heat stable. The Grand Average hydropathy (GRAVY) indices are used to determine the hydrophobicity of the protein. It is measured by dividing the sum of hydropathy

Table 3. CASTp result of active site amino acids

ASP	166	ASN	509	ALA	560	GLY	618	ASP	686
VAL	168	LYS	513	GLY	561	TRP	619	ALA	687
GLU	169	THR	542	VAL	562	ASP	620	THR	689
LYS	413	GLN	543	THR	567	TYR	621	ALA	690
LYS	440	MET	544	ARG	571	PRO	622	TYR	691
HIS	441	ASN	545	GLN	575	LYS	623	ASN	693
PHE	443	LEU	546	LEU	578	LYS	623	LEU	760
ASP	454	LYS	547	LYS	579	CYS	624	SER	761
TYR	457	TYR	548	ALA	582	ASP	625	ASP	762
TYR	458	ALA	549	VAL	590	ARG	626	ASP	763
ILE	496	ILE	550	VAL	590	MET	628	ALA	764
VAL	497	SER	551	ILE	591	GLU	667	PHE	795
ASN	498	ALA	552	GLY	592	VAL	669	ALA	799
ASN	499	LYS	553	THR	593	LYS	678	LYS	800
LEU	500	ARG	555	SER	594	THR	682	CYS	801
ASP	501	ALA	556	LYS	595	THR	682	TRP	802
LYS	502	ARG	557	PHE	596	SER	683		
SER	503	THR	558	TRP	600	SER	684		
GLY	505	VAL	559	MET	603	GLY	685		



Sequence position(s) of High N-glycosylation Site (>60%)

Pos	Top seq	Num of N-glycosylation	Fraction
651	N	1	1.000
795	N	1	1.000

Fig. 4. N-Glycosylation sites in target protein analyzed using N-Glycosite web server

values of all the amino acids to the total number of residues in the protein. A GRAVY score below 0 is considered as hydrophilic globular protein.[23] Instability index value determines the stability of the protein. A protein with instability index less than 40 is considered stable .[24] Table 1 shows the results of physico-chemical properties of the protein.

N-glycosylation site Prediction

Glycosylation is the important post translational modification in prokaryotic protein, and in many cases it is essential for its binding to the host cell .[25] The results from N-Glycosite showed that the protein has two potential N-glycosylation sites. The result was further confirmed using GLYCOPP that also showed two glycosylation sites.

Secondary Structure Prediction

Secondary structure is the folded structure of protein that is formed due to the backbone atoms interaction with each other. The common secondary structures are alpha helix, beta sheets and coils. The secondary structure prediction is important to study the function and activity of the protein.[26] The secondary structure of the protein was determined using SOPMA, default parameters of window width 17, similarity threshold 8, and number of states 4 were used for the study. The result of secondary structure in the target sequence is shown in Table 2.

Table 4. Docking score and the interaction for the ligand compounds

Compound	Binding energy kcal/mol	Atoms forming Hydrogen bond	Other interactions
Quinine	-6.14	ASP 625, ASN 693	SER 684, THR 682, ASP 762, CYS 624, LYS 623, TYR 621, ASP 620, PRO 622, LYS 800
Doxycycline	-7.34	ARG 555, ASP 620, CYS 624, ASP 625	ARG 557, THR 558, PRO 622, TYR 621, LYS 623, ARG 626, ASP 762, LYS 800

Fig. 5. CASTp result of active site amino acid residues

Active Site Prediction

For setting the grid in docking studies, the determination of active site amino acids of the target protein is important.[27]The active site residues of the protein were determined using CASTp Server. Table 3 and Fig 5 show CASTp results of the amino acids that are in the active site region.

Structure activity relationship and Docking result of Doxycycline against corona target protein (nsp 12)

The structure activity relationship for the Doxycycline compound and the modelled protein revealed that the Doxycycline is well engaged into the active site of the target protein.

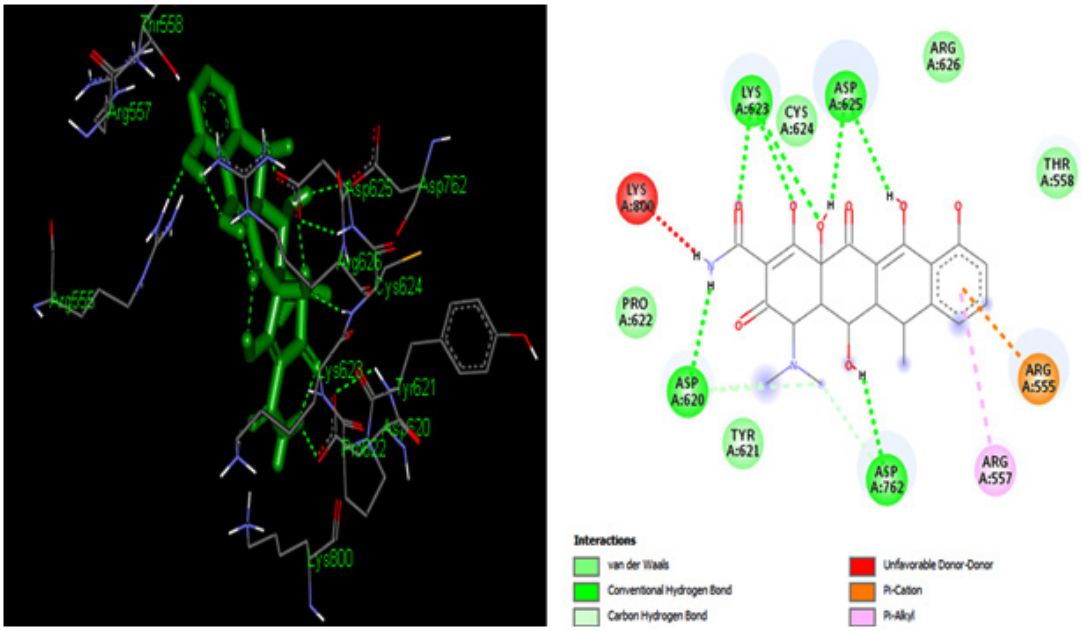


Fig. 6. Binding analysis and the ligand interaction for the compound Doxycycline

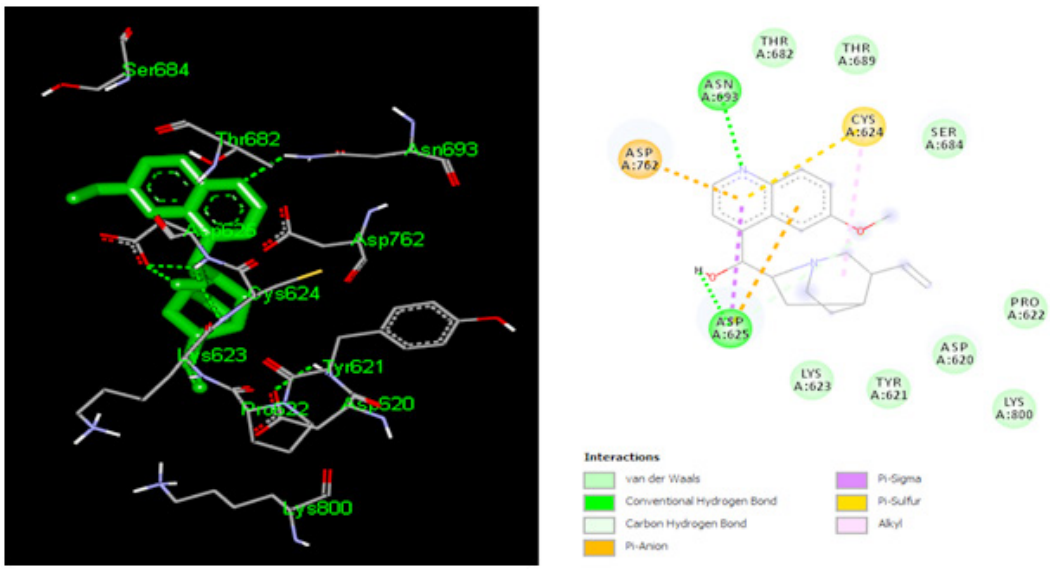


Fig. 7. Binding analysis and the ligand interaction for the compound Quinine

The compound has strong hydrogen bonding interaction with the non-polar amino acids. The compound(Doxycycline) also has positively charged interaction with the amino acid Arg557, Arg626 and Lys800. The bulkier phenolic group present in the compound makes the compound engage into the active site of the protein more effectively. This also leads to stacking interaction with positively charged aminoacid Arg555 and Arg557. The docking score of the compound(Doxycycline) is found to -7.34 kcal/mol as shown in Table 4. The compound also shows vander wals force interaction with the aminoacid Asp620 and Asp762. The non-structural protein have stacked the compound inside its active site which helps in targeting the polymerases effectively by the compound doxycycline. This will terminate the replication of RNA which further stops the transcription and production of viral proteins. Further targeting the non-structural protein with the drug shows competitive inhibition towards the drug doxycycline. The binding analysis and the ligand interaction is depicted in the Figure.6

Structure activity relationship and Docking result of Quinine against corona target protein (nsp 12)

The binding analysis and the ligand interaction for the compound (Quinine) revealed that the compound is well interacted with hydrogen bonding interaction with Asn693 and Asp635, negatively charged amino acid Asp762 and the Pi-Pi interaction with Cys624. The compound Quinine is slightly away from the active site due to anionic interaction with Asp762. The carbonyl group in the benzene ring is away from the active site of the Polymerase protein. The compound is also less surrounded by the non-polar aminoacids like Pro622, Tyr621 etc. This makes this compound less potent than the Doxycline. The compound Quinine also doesn't possess any vander wals interaction with the aminoacids present in the modelled protein Polymerases. Vander wals forces plays an important role in Protein-Ligand binding. Thus failing of Vanderwals force may lead this compound energy level lesser than doxycycline. Thus the docking score of the compound Quinine was found to be -6.14 kcal/mol as shown in Table 4.The binding analysis and the ligand interaction for the compound quinine is depicted in the figure. 7.

CONCLUSION

The Compounds Doxycycline and Quinine found to have good binding affinity with corona viral non structural protein. Predicting the structure activity relationship of both drugs has also shown that,they have well engaged in the active site of the protein with hydrogen bond interaction. This can lead to novel drug identification of anti-viral therapy towards Covid-19. As this is the current pandemic, this can be used as a initial lead identification. Further, the current results need to be validated by in-vitro and in-vivo anti-viral method for further medicinal usage in corona virus treatment.

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