JOURNAL OF BIOMOLECULAR STRUCTURE AND DYNAMICS
https://doi.org/10.1080/07391102.2020.1782265

Taylor & Francis
Taylor & Francis Group

Screening of Chloroquine, Hydroxychloroquine and its derivatives for their binding affinity to multiple SARS-CoV-2 protein drug targets

Mallikarjuna Nimgampalle[a], Vasudharani Devanathan[a] and Ambrish Saxena[b]

[a]Department of Biology, Indian Institute of Science Education and Research (IISER) Tirupati, Tirupati, India; [b]Center for Sponsored Research and Consultancy, Indian Institute of Technology (IIT) Tirupati, Tirupati, India

Communicated by Ramaswamy H. Sarma

ABSTRACT

Recently Chloroquine and its derivative Hydroxychloroquine have garnered enormous interest amongst the clinicians and health authorities' world over as a potential treatment to contain COVID-19 pandemic. The present research aims at investigating the therapeutic potential of Chloroquine and its potent derivative Hydroxychloroquine against SARS-CoV-2 viral proteins. At the same time screening was performed for some chemically synthesized derivatives of Chloroquine and compared their binding efficacy with chemically synthesized Chloroquine derivatives through *in silico* approaches. For the purpose of the study, some essential viral proteins and enzymes were selected that are implicated in SARS-CoV-2 replication and multiplication as putative drug targets. Chloroquine, Hydroxychloroquine, and some of their chemically synthesized derivatives, taken from earlier published studies were selected as drug molecules. We have conducted molecular docking and related studies between Chloroquine and its derivatives and SARS-CoV-2 viral proteins, and the findings show that both Chloroquine and Hydroxychloroquine can bind to specific structural and non-structural proteins implicated in the pathogenesis of SARS-CoV-2 infection with different efficiencies. Our current study also shows that some of the chemically synthesized Chloroquine derivatives can also potentially inhibit various SARS-CoV-2 viral proteins by binding to them and concomitantly effectively disrupting the active site of these proteins. These findings bring into light another possible mechanism of action of Chloroquine and Hydroxychloroquine and also pave the way for further drug repurposing and remodeling.

ARTICLE HISTORY
Received 18 May 2020
Accepted 9 June 2020

KEYWORDS
COVID-19; SARS-CoV-2; Hydroxychloroquine; molecular docking; Chloroquine derivatives

1. Introduction

Coronavirus disease (COVID-19) pandemic is caused by severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) (Gorbalenya et al., 2020), is a rapidly spreading disease globally and has so far claimed thousands of lives around the world and caused enormous damage to society and economy (CEBM, 2020). World Health Organization (WHO) has declared that the COVID-19 is a pandemic, and a public health emergency of international concern (Wee et al., 2020). The novel coronavirus or SARS-CoV-2 has four transmission stages in line with other infectious diseases and is generally categorized into asymptomatic, moderate, extreme, and critical. SARS-CoV-2 exhibits different symptoms depending on the severity of the disease including fever, dry cough, dyspnea, pneumonia, hypoxemia, encephalopathy, heart failure, and acute kidney injury. There is currently no defined antiviral drug or therapy available for COVID-19 treatment, and mostly the disease is managed symptomatically (Yuki et al., 2020). Several medications are being tested in clinical trials for COVID-19, including antiviral, anti-inflammatory, anti-malarial and other pharmacologically active drugs (Rabby, 2020). However, recently Chloroquine and its derivative Hydroxychloroquine are being positioned as a possible treatment for COVID-19. Presently, multi-centric global clinical trials are underway to evaluate the therapeutic potential of Chloroquine and Hydroxychloroquine as a treatment for novel coronavirus infection. The Food and Drug Administration (FDA), USA, has, however, approved both Chloroquine and Hydroxychloroquine for COVID-19 control and treatment for emergency purposes (Scholz & Derwand, 2020).

In order to address the virus infection and replication it is critical to understand proteins involved in the process. Functionally, SARS-CoV-2 consists of two different types of proteins, which include structural proteins and non-structural proteins (NSPs). The structural proteins are involved in the formation of the spherical shape of the virus, which including spike protein (trimeric), membrane protein, envelope protein, and the nucleocapsid protein. While sixteen non-structural proteins (NSPs) are formed from the proteolytic cleavage of two polyproteins (PP1a and PP1b). These NSPs are essential for the metabolic and molecular events include transcription and translation (Prajapat et al., 2020). In this context, key regulatory proteins and enzymes associated with the pathogenesis of SARS-CoV-2 were selected as drug targets for Chloroquine and its derivatives.

Chloroquine and Hydroxychloroquine are anti-malarial drugs, which are also used for the treatment of rheumatoid

CONTACT Ambrish Saxena ambrish@iittp.ac.in Indian Institute of Technology (IIT) Tirupati, Renigunta Road, Tirupati, Andhra Pradesh 517506, India
Supplemental data for this article can be accessed online at https://doi.org/10.1080/07391102.2020.1782265.

arthritis and lupus erythematosus (Touret & de Lamballerie, 2020). These drugs are believed to be relatively safe when administered within the clinically advised limits with mild side effects. Furthermore, Chloroquine derivatives have been tested on *Pneumocystis* pneumonia (PcP) for their therapeutic activity to repurposing antimalarial drugs for Pneumonia (Gomes et al., 2018; Yeo et al., 2020). Pneumonia is a life-threatening symptom for advanced stage coronavirus infected patients and clinicians are using Chloroquine and its derivative Hydroxychloroquine to treat the disease. Currently, these two drugs are being reused for the treatment of COVID-19 since the infection involves pneumonia (Devaux et al., 2020). Recently, treatment with the combination of both Hydroxychloroquine and Azithromycin has shown a significant improvement within the COVID-19 patients (Gautret et al., 2020). Similarly, patients treated with Chloroquine and Hydroxychloroquine have also shown significant recovery from COVID-19 (Singh et al., 2020). Chloroquine and Hydroxychloroquine is a cost effective drug that has long been a therapy of choice for malaria prophylaxis due to excellent results and good safety and tolerability.

Recently, world over Chloroquine and its derivative analog Hydroxychloroquine has garnered enormous attention as a possible treatment for SARS-CoV-2 infection. Although in this context, the exact mechanism of action of Chloroquine and Hydroxychloroquine is still not known. At the same time some reports have cautioned against the use of Chloroquine due to the known dose-related toxicity of Chloroquine and its derivative. Several adverse events mainly involving retinal and psychiatric symptoms are observed with Chloroquine. However, such symptoms are dose-dependent and are observed when dosage levels exceed prescribed pharmacological dosage limits.

An understanding of SARS-CoV-2 disease biology indicates that it is important to target the viral replication in order to effectively control the infection. Also, it is perceived that Chloroquine and its derivative can prevent the disease onset in COVID negative and healthy subjects and treat SARS-CoV-2 infection in healthy but asymptomatic carriers.

This can be effectively accomplished by understanding the detailed mechanism of action of Chloroquine and Hydroxychloroquine in preventing Coronavirus infection. The mechanism of action of these two drugs is not well known, but it has been demonstrated *in vitro* that these drugs inhibit SARS-CoV-2 by elevating the endosomal pH, and alter ACE-2 terminal glycosylation there by leading to the interruption of virus receptor binding (Vincent et al., 2005). However, if Chloroquine and its derivative Hydroxychloroquine acts exclusively by elevating the endosomal pH, then Chloroquine should act as a broad-spectrum anti-viral agent since modulation of endosomal pH is a common strategy utilized by viruses for internalization. This appears to be doubtful since Chloroquine is not effective against most of the viral diseases like Dengue (Tricou et al., 2010), Chickenguniya (Lamballerie et al., 2008), and HIV (Savarino & Shytaj, 2015). However, both Chloroquine and its derivative Hydroxychloroquine were shown to be of some use in countering the SARS virus (Vincent et al., 2005). It is a well-established fact that SARS Virus (SARS-CoV) and SARS-CoV-2 share almost 80% sequence similarity. With this, it can be postulated that Chloroquine might be actively binding to one or more SARS-CoV-2 proteins to inhibit viral replication. To study this, multiple Chloroquine derivatives reported earlier, were screened for their binding potential to various SARS-C0V-2 virus proteins important for its binding, internalization, replication and budding in the host cell using the molecular docking studies.

In this work, we have demonstrated the capability of Chloroquine and its derivatives, reported for their anti-PcP potential earlier (Gomes et al., 2018) for selective binding to different viral proteins. This work aims at increasing the information for anti-viral mechanism of Chloroquine and Hydroxychloroquine against the SARS-CoV-2 virus. This research can support new anti-viral drug discovery against SARS-CoV-2 virus and at the same time can support drug repurposing efforts around Chloroquine. Also these results can be used as a basis for modifying clinical dosage of Chloroquine and thereby rendering it more effective against Coronavirus infection.

2. Materials and methods

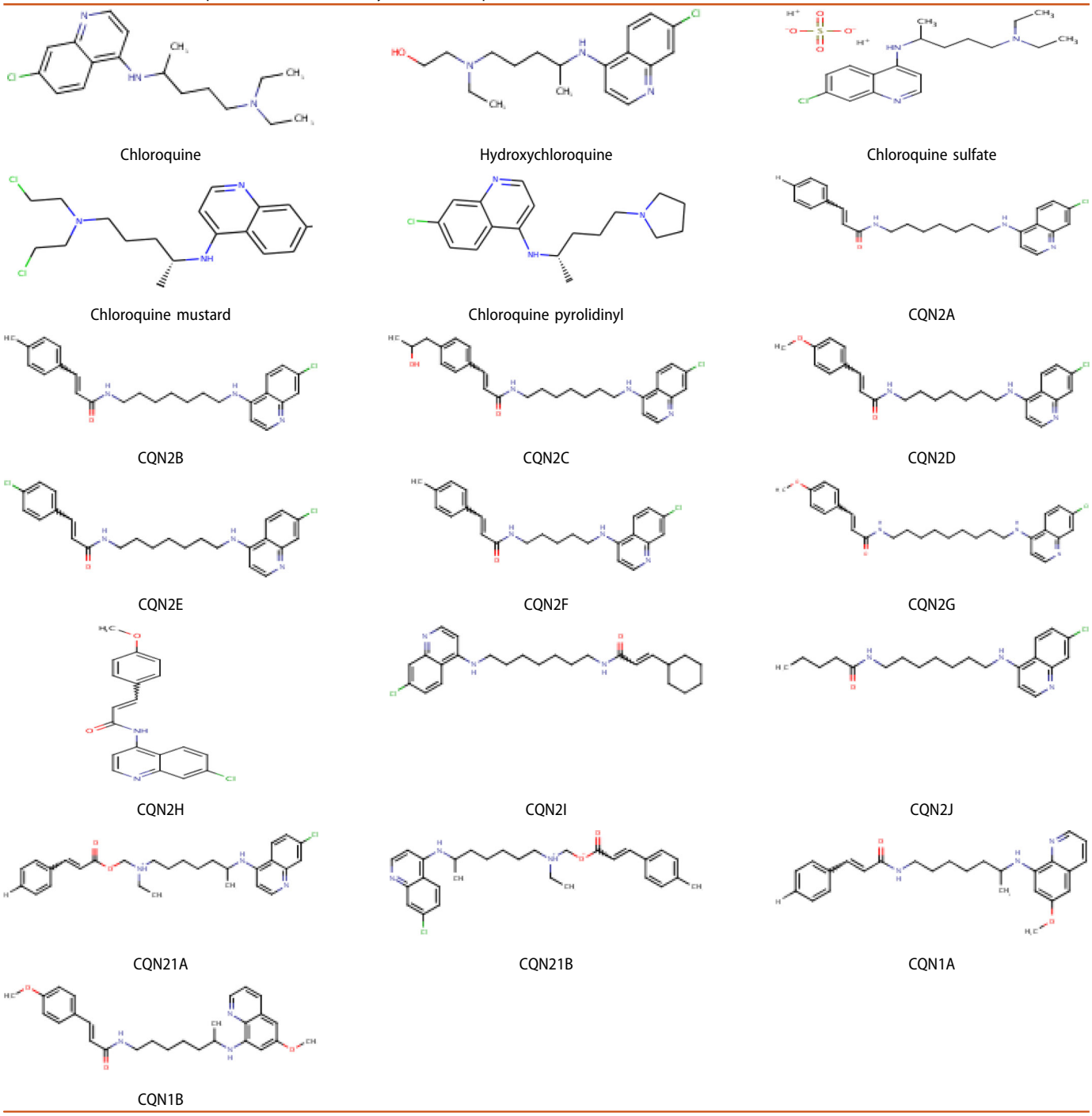
2.1. Hydroxychloroquine and chloroquine derivatives as drug molecules

Post the approval of Hydroxychloroquine by the Food and Drug Administration (FDA), USA, the same has been used as a potential drug for the treatment and management of emerging disease COVID-19. By using *in-silico* molecular docking studies, the binding potential of Chloroquine and its derivatives with different SARS-CoV-2 proteins involved in viral replication was evaluated. The 2D-structures for Chloroquine and its derivatives viz. Hydroxychloroquine, Chloroquine sulfate, Chloroquine mustard, Chloroquine pyrolidinyl, were taken from PubChem (https://pubchem.ncbi.nlm.nih.gov/) database. While, the 2D-structures of chemically synthesized Chloroquine derivatives from Gomes et al., 2018 were drawn using ChemSketch software and named as CQN2A, CQN2B, CQN2C, CQN2D, CQN2E, CQN2F, CQN2G, CQN2H, CQN2I, CQN2J, CQN21A, CQN21B, CQN1A, and CQN1B (Table 1). All the structures of Chloroquine derivatives were organized as a compound library following energy minimization using the Open Babel module in PyRx software. All the compounds in this study were also assessed for their drug likeliness based on the Lipinski's 'rule of five' using swissADME server.

2.2. Selective proteins and enzymes of SARS-CoV-2 as drug targets

The key regulatory proteins and enzymes associated with the pathogenesis of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) were selected as potential targets for Chloroquine and its analogues . Specifically, the proteins selected were Spike glycoprotein (PDB ID: 6VSB; Wrapp et al., 2020), RNA dependent RNA polymerase (PDB ID: 6M71; Gao et al., 2020), Chimeric RBD (Receptor-binding domain; PDB ID: 6VW1; Shang et al., 2020), Main protease (PDB ID: 6LU7; Jin et al., 2020), Non-structural Protein3 (PDB ID: 6W02;

Table 1. Structures of Chloroquine derivatives and its synthesized compounds from Gomes et al. (2018).



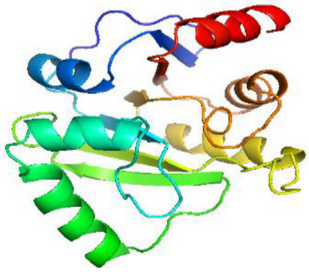
Michalska et al., 2020), Non-structural Protein 9 (Replicase protein; PDB ID: 6W4B; Littler et al., 2020), ADP-ribose-1 monophosphatase (PDB ID: 6VXS; Kim et al., 2020) (Table 2). Three-dimensional structures of the above drug targets were retrieved from the RCSB Protein Data Bank (www.rcsb.org).

Before docking, the PDB structures were observed for sequence break and their ligand association by using Pymol molecular visualization system (DeLano, 2002). Proteins (6M71 and 6SVB) with sequence break were subjected to homology modeling using the swissMODEL server (Daina et al., 2017), subsequently used for further studies. Liganded form of protein (6LU7) were identified, retrieved, and employed for validation of docking (Nimgampalle et al., 2019). While non-liganded forms of proteins (6M71, 6VSB, 6VW1, 6VXS, and 6W4B) were subjected to active site prediction using the CASTp server (Tian et al., 2018).

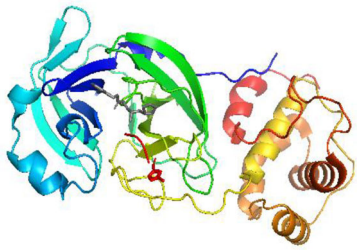
2.3. Molecular docking, analysis of binding affinities and binding interactions

In this study, molecular docking was performed at known active site of ligand form of proteins (6LU7), whereas, both blind docking and predicted active site docking (site-specific docking) was performed to the remaining non-liganded form

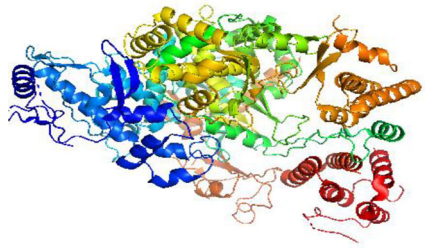
Table 2. Structures of selected therapeutic targets for SARS-CoV-2 (RCSB Protein Data Bank).



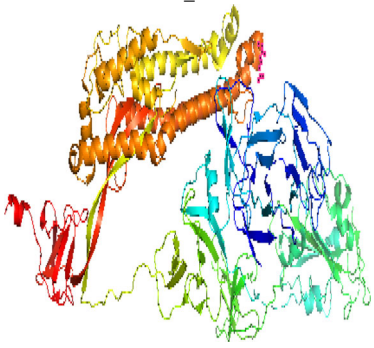
6W02_NSP3



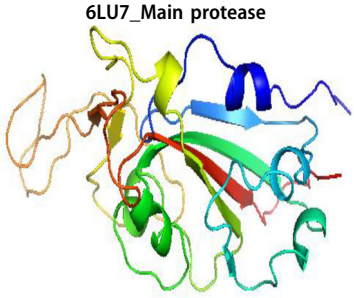
6LU7_Main protease



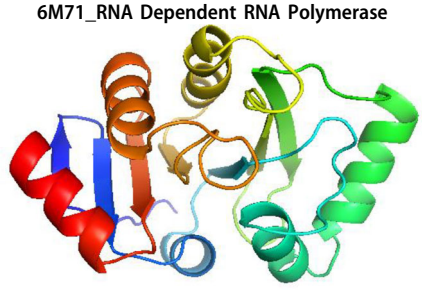
6M71_RNA Dependent RNA Polymerase



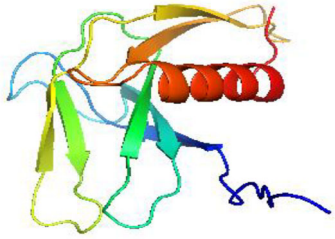
6VSB_SARS-CoV-2 spike glycoprotein



6VW1.E_2019-nCoV chimeric RBD



6VXS_ADP-ribose-1 monophosphatase



6W4B_NSP9 replicase protein

of proteins (6M71, 6VSB, 6VW1.E, 6VXS, 6W02, and 6W4B) to examine the binding affinities of Chloroquine derivatives against each drug target of SARS-CoV-2.

Docking was performed using PyRx 0.8 software consist of AutoDock 4.2 (Morris et al., 2009). The structures of chloroquine and its derivatives were organized as a compound library after subjecting them to energy minimization using the Open Babel module in the virtual screening software, PyRx. Later, the protein structures were refined for heteroatoms and water molecules to demarcate active sites of proteins. Further, the Gasteiger charges and hydrogen atoms were added to each drug target to maintain coordination between various interactions by using UCSF Chimera-1.13.1 software (Huang et al., 2014). Then, each drug target was saved in PDB format, and the Chloroquine derivative compounds under examination were also uploaded and saved in ligand.pdbqt format. The grid was set around the active site of the drug target for site-specific docking, whereas the grid was maximized to surround the entire protein surface for blind docking. To generate possible binding conformation of chloroquine derivatives, empirical free energy force fields were applied to the Lamarckian genetic algorithm (LGA) with default parameters (LGA: 2500,000 - energy evaluations) (Bommu et al., 2019). The grid spacing was generated around the active site residues of each drug target, and the grid parameters for each drug target are given in Table 2.1 as supplementary file. After completion of docking, the dock results were saved for the observation of binding affinities and binding interactions between ligand-target were analyzed by Ligplot software (Wallace et al., 1995).

3. Results

3.1. Chloroquine derivatives fall within the lipinski's rule of five

Prior to molecular docking studies, all the synthetic derivatives of Chloroquine were tested for their "Drug-likeliness" properties based on Lipinski's rule of five. As shown in Table 3, all the compounds used in the present study follow Lipinski's rule of five (Molecular weight <500, hydrogen acceptor <10, hydrogen donor <5, and LogP < 5) without any exception. These results demonstrate that all the Chloroquine derivatives studied in this study are capable of eliciting pharmacological response through their absorption, distribution, metabolism, and excretion. The details of Drug-likeness and relevant molecular properties of compounds are listed in Table 3. It is important for a chemical compound to demonstrate Drug-likeness properties since this would render

the compounds towards efficient oral absorption, aqueous solubility, and membrane permeation.

3.2. Active sites for non-liganded form of proteins

In order to completely understand the binding potential of Chloroquine and its derivatives to different SARS-CoV-2 proteins, it is important to identify their natural active sites. Active sites of proteins can be predicted by ascertaining the geometric and topological properties of protein structures. CASTp server was used to predicted active sites for the non-liganded form of proteins (6W02, 6M71, 6VSB, 6VW1, 6VXS, and 6W4B). The amino acids that form the active site of each viral protein and their exact position on the polypeptide chain are listed in Table 4. It was presumed that Chloroquine and its derivatives can bind to the active site of these viral proteins thereby inhibiting their natural function. The active site of each protein identified by this procedure was used for further molecular docking studies.

3.3. Chloroquine derivatives as potential inhibitors for drug targets of SARS-COV-2

In this study, nineteen compounds including Chloroquine derivatives (Chloroquine, Hydroxychloroquine, Chloroquine sulfate, Chloroquine mustard, Chloroquine pyrolidinyl) and other chemically synthesized Chloroquine derivatives (CQN2A, CQN2B, CQN2C, CQN2D, CQN2E, CQN2F, CQN2G, CQN2H, CQN2I, CQN2J, CQN21A, CQN21B, CQN1A, and CQN1B) were studied to evaluate their binding potential against the various viral proteins and enzymes (6W02, 6LU7, 6M71, 6VSB, 6VW1.E, 6VXS, and 6W4B) through molecular docking approaches. The results clearly demonstrate that the ability of Hydroxychloroquine and Chloroquine to bind with different viral proteins albeit with different efficiencies. On the contrary some of the chemically synthesized Chloroquine derivatives exhibit the higher binding affinities with tested viral proteins than the Hydroxychloroquine (Table 5). Our results point to the fact that potential inhibitors against viral target proteins can be derived by further modifying Chloroquine structures. In the results, blind docked ligand-protein complexes are illustrated as figures and the amino acid residues involved in the site-specific docking are listed in Table 4 as predicted active sites of the non-liganded form of viral proteins.

3.3.1. Binding to non-structural protein3 (NSP3)

Our results demonstrate that Hydroxychloroquine can bind to NSP3 with a binding affinity of −5.6 kcal/mol and −7.3 kcal/mol in both blind and site-specific docking, respectively (Table 5). In parallel, even stronger binding to NSP3 when compared to Hydroxychloroquine was demonstrated by chemically synthesized Chloroquine derivative, CQN2H showing the maximum binding affinity of −8.4 kcal/mol and −8.8 kcal/mol in both blind and site-specific docking (Tables 5 and 5.1 supplementary). Hydroxychloroquine shows hydrogen bonding with the residues GLY47 and LEU126, whereas CQN2H forms hydrogen bonds with amino-acid residues ASN40, GLY46, and GLY130 (Figure 1). Overall, Chloroquine and its derivatives demonstrated a higher binding affinity for NSP3 viral protein when compared to other proteins included in this study, thereby signifying their potential as inhibitors of NSP3 function.

3.3.2. Binding to main protease

The main protease is moderately inhibited by Hydroxychloroquine with the binding affinity (−4.8 kcal/mol) (Tables 5 and 5.1 supplementary). Amino acid residues of the main protease involved in the formation of five hydrogen bonds with Hydroxychloroquine are LEU4, THR24, THR25, THR26, and THR45 (Figure 2). However, compared to Hydroxychloroquine, both CQN2H and CQN1B show considerable higher binding affinity (−6.0 kcal/mol) to the main protease (Tables 5 and 5.1 supplementary). The compound CQN2H shows hydrogen interactions with the residues of THR24, SER46 (Figure 2). Our results indicate that some derivatives of Chloroquine and its derivatives are capable of binding to the main viral protease.

3.3.3. Binding to RNA dependent RNA polymerase

The results obtained from both blind docking and site-specific docking demonstrate that Hydroxychloroquine can effectively bind to RNA polymerase with binding affinities −5.9 and −5.6 kcal/mol, respectively (Table 5). The amino acid residues (ASN52 and ASN209) of RNA polymerase are involved in formation of hydrogen bond with Hydroxychloroquine (Figure 3). Interestingly, the compound CQN2H, demonstrate strong binding with RNA polymerase with the highest binding affinity in both blind docking (−8.4 kcal/mol.) and site-specific docking (−7.0 kcal/mol) (Tables 5 and 5.1 supplementary). CQN2H shows the ability to form hydrogen interactions with the RNA polymerase residues THR319 and THR394 (Figure 3). The high binding affinity of CQN2H indicates towards its potential as an inhibitor of viral RNA polymerase.

3.3.4. Binding to SARS-CoV-2 spike glycoprotein

Hydroxychloroquine exhibits considerable binding efficiency against spike glycoprotein during both blind docking and site-specific docking with binding affinities of −6.0 and −5.4 kcal/mole, respectively (Table 5). Additionally, the compounds CQN1B, CQN2H demonstrated even stronger binding to spike glycoprotein with the binding affinities of −7.8 kcal/mol. and −7.3 kcal/mol in both blind docking and predicted active site binding, respectively (Table 5). Interestingly, only single amino acid (ILE472) of the viral spike protein is involved in the formation of hydrogen bond with Hydroxychloroquine, whereas CQN1B shows no hydrogen bonds formation rather it forms Van Dar Waal interactions with various amino acid residues (Figure 4). The obtained results demonstrate that the compounds CQN1B and CQN2H may act as potential inhibitor of viral spike glycoprotein function.

Table 3. Drug-likeness properties of Chloroquine and its derivatives predicted in swissADME web tool.

S. No.	Name of the Chloroquine derivatives	M.W (150–500g/mol)	H-acceptors (≤10)	H-donors (≤5)	LogP (0.7–5.0)	No. of violations (Rule of 5)	TPSA (20–130 Å^2)	Rotatable bonds (< 9)	LogS (> −6)	Fraction Csp^3 (>0.25)
1.	Chloroquine	319.87	2	1	3.95	0	28.16	8	−6.92	0.50
2.	Hydroxychloroquine	335.87	3	2	3.58	0	48.39	9	−6.35	0.50
3.	Chloroquine sulfate	417.95	6	1	3.14	0	116.80	8	−6.92	05.0
4.	Chloroquine pyrolidylin	317.86	2	1	3.72	0	28.16	6	−6.44	0.50
5.	Chloroquine mustard	388.76	2	1	3.76	0	28.16	10	−8.14	0.50
	Chemically synthesized chloroquine derivatives									
6.	CQN2A	421.96	2	2	4.22	0	54.02	12	−9.71	0.28
7.	CQN2B	435.99	2	2	4.55	0	54.02	12	−10.09	0.31
8.	CQN2C	480.04	3	3	4.62	0	74.25	14	−9.92	0.36
9.	CQN2D	451.99	3	2	4.51	0	63.25	13	−9.81	0.31
10.	CQN2E	456.41	2	2	4.59	0	54.02	12	−10.29	0.28
11.	CQN2F	423.98	2	2	0.0	0	54.02	10	−9.30	0.28
12.	CQN2G	480.04	3	2	5.05	0	63.25	15	−10.59	0.36
13.	CQN2H	338.79	3	1	3.16	0	51.22	5	−7.02	0.05
14.	CQN2I	428.01	2	2	4.74	0	54.02	12	−8.03	0.52
15.	CQN2J	375.94	2	2	4.02	0	54.02	13	−8.74	0.52
16.	CQN21D	495.08	3	2	5.36	0	69.49	14	−9.87	0.38
17.	CQN21A	481.05	3	2	5.13	0	69.49	14	−9.50	0.36
18.	CQN1A	417.54	3	2	4.23	0	63.25	12	−8.85	0.31
19.	CQN1B	447.57	4	2	4.58	0	72.48	13	−8.95	0.33

3.3.5. Binding to spike protein-receptor binding domain

Our studies demonstrate that Hydroxychloroquine can also bind to the receptor-binding domain with a moderate binding affinity (−5.4 and −5.3 kcal/mol) in both blind docking and site-specific docking (Table 5). The amino acid residues of the receptor-binding domain involved in the hydrogen bonding are ASP110, MET112, PHE197, and GLU198 (Figures 5 and 5.1 supplementary). On the contrary other Chloroquine derivatives like CQN1A and CQN1B show a strong binding affinity (−7.6 kcal/mol) to the Receptor binding domain of the viral spike protein in both blind docking and site-specific docking studies (Table 5). The high binding affinities exhibited by these compounds represent their potential as inhibitors of receptor-binding domain. CQN1A shows hydrogen interaction with the residues ILE154 and ASN163 (Figure 5).

3.3.6. Binding to ADP-ribose-1 monophosphatase

Molecular docking studies with Chloroquine and its derivatives with ADP-ribose-1 monophosphatase demonstrated the ability of Hydroxychloroquine to bind with the mentioned viral protein with binding affinity −6.2 kcal/mol in both blind docking and site-specific docking studies (Table 5). A detailed analysis of the dock showed that Hydroxychloroquine was able to form hydrogen bonds with LEU126, ALA129, and ALA154 residues (Figure 6). Two compounds CQN2E and CQN2I, also show effective binding with ADP-ribose-1 monophosphatase with the binding affinity of −7.4 kcal/mol in blind docking (Table 5). The amino acid residues involved in the formation of hydrogen bonding with CQN2I are VAL149 and ALA154 (Figure 6). The compound CQN2C also demonstrated a strong binding to ADP-ribose-1 monophosphatase with the binding affinity −7.6 kcal/mol in site-specific docking studies.

3.3.7. Binding to replicase protein (non-structural protein9)

Hydroxychloroquine shows moderate to minimal binding with replicase protein with binding affinities of −5.4 and −4.3 kcal/mol in blind docking and site-specific docking, respectively (Table 5). The residues of replicase protein, VAL42, and PRO58 are involved in the formation of hydrogen bonds with Hydroxychloroquine (Figure 7). However, another derivative of Chloroquine, compound CQN2H potentially inhibits replicase protein in both blind docking and site-specific docking with the binding affinities are −7.1 and −5.5 kcal/mol, respectively (Table 5) and forms hydrogen bonds with ARG40, PRO58, and THR68 residues of the replicase protein (Figure 7).

4. Discussion

In the present work, with the use of molecular docking techniques the molecular interactions between Chloroquine derivatives and selective SARS-CoV-2 viral proteins were studied. Since most of these viral proteins can be potential drug targets, this study aims at understanding the potential role of Chloroquine and its potent derivative Hydroxychloroquine in inhibition of SARS-CoV-2 viral infection and replication by assessing the binding efficiency of Chloroquine and its derivatives to various viral proteins. These results obtained in this study can also be extrapolated to evaluate the therapeutic efficiency of Chloroquine and Hydroxychloroquine in controlling SARS-CoV-2 infection. Also in this study we have attempted to screen for derivatives of Chloroquine that can bind to SARS-CoV-2 viral drug targets more efficiently than Chloroquine with the hope that this can open a case for Chloroquine remodeling for better inhibition of SARS-CoV-2 multiplication.

Based on the recent reports, some of the essential regulatory proteins and enzymes associated with the pathogenesis of SARS-CoV-2 were selected as drug targets such as the Spike glycoprotein that enables virus internalization, RNA dependent RNA polymerase that supports replication of viral genetic material, Chimeric RBD (Receptor binding domain) that interacts with the ACE 2, Main protease responsible for cleaving the viral polypeptide, Non-structural Protein3, Non-structural Protein 10, Non-structural Protein 9 (Replicase

Table 4. Predicted active sites for the non-liganded form of proteins using the CASTp server.

Protein PDB ID	Active site predicted from CASTp
6W02	ALA21, ASP22, ILE23, ALA38, ALA39, ASN40, LYS44, HIS45, GLY46, GLY47, GLY48, VAL49, ALA50, ALA52, GLY97, LY97, PRO125, LEU126, LEU127, SER128, ALA129, GLY130, ILE131, PHE132, ALA154, VAL155, PHE156, ASP157, LEU160
6M71	VAL166, GLU167, HIS439, PHE441, ASP452, TYR455, TYR456, ILE494, ASN496, ASN497, LEU498, ASP499, SER501, LYS511, ARG513, THR540, MET542, ASN543, LEU544, LYS545, TYR546, ALA547, ILE548, SER549, ALA550, LYS551, ARG553, ALA554, ARG555, ARG555, THR556, THR556, VAL557, ALA558, GLY559, HIS572, LEU576, LYS577, ALA580, VAL588, ILE589, GLY590, THR591, THR591, SER592, LYS593, PHE594, TYR595, TRP598, GLY616, TRP617, ASP618, TYR619, PRO620, LYS621, CYS622, ASP623, ARG624, GLU665, VAL667, LYS676, SER681, SER682, GLY683, ALA685, THR686, THR687, ALA688, ASN691, LEU758, SER759, ASP760, ASP761, PHE793, SER795, TRP800, TRP800, GLU811, PHE812, CYS813, SER814, GLN815, PRO832, ARG836, ILE837, ALA840, VAL844, ASP845, ILE847, VAL848, THR853, LEU854, ARG858, VAL860, LEU862, ILE864, TYR903, SER904, ASN911, ARG914, TYR915.
6VSB	ALA27, TYR28, THR29, ASN30, PHE32, THR33, TYR38, PRO39, ASP40, LYS41, VAL42, PHE43, ARG44, SER45, SER46, VAL47, LEU48, HIS49, SER50, THR51, GLN52, ASP53, LEU54, LEU56, PRO57, PHE58, PHE59, SER60, ASN61, VAL62, THR63, TRP64, PHE65, PRO82, VAL83, LEU84, PRO85, PHE86, ASN87, ASP88, GLY89, LYS195, ASN196, ILE197, ASP198, GLY199, TYR200, LYS202, ILE203, TYR204, LEU212, GLN218, GLY219, PHE220, PRO225, LEU226, VAL227, ASP228, ILE233, ASN234, ILE235, THR236, ARG237, ARG246, SER247, TYR248, LEU249, THR250, PRO251, ASP253, VAL267, VAL267, GLY268, TYR269, TYR269, LEU270, GLN271, PRO272, ARG273, THR274, ASP287, ALA288, VAL289, ASP290, CYS291, ALA292, LEU293, ASP294, PRO295, LEU296, SER297, GLU298, LYS300, CYS301, THR302, LEU303, LYS304, SER305, PHE306, THR307, VAL308, GLU309, LYS310, GLY311, ILE312, TYR313, GLN314, THR315, SER316, ASN317, PHE318, ARG19, VAL320, GLN321, PRO322, THR323, GLU324, SER325, ILE326, VAL327, PHE329, GLY381, VAL382, SER383, THR385, LYS386, ASN388, ASP389, LEU390, CYS391, PHE392, THR393, PHE429, PHE515, LEU517, LEU518, ALA520, PRO521, PRO527, LYS528, LYS529, THR531, CYS538, ASN540, PHE541, ASN542, PHE543, ASN544, GLY545, LEU546, THR547, GLY548, THR549, GLY550, GLN564, PHE565, ARG567, ALA570, ASP571, THR572, THR573, ASP574, VAL576, ARG577, PRO579, ILE587, PRO589, CYS590, SER591, PHE592, GLY593, VAL595, VAL597, PRO600, GLY601, THR602, ASN603, THR604, ASN606, VAL608, VAL610, VAL620, PRO621, VAL622, ALA623, ILE624, PRO631, THR632, ARG634, SER637, THR638, GLU661, CYS662, ASP663, ILE664, PRO665, GLN675, GLN677, THR678, ASN679, SER680, PRO681, ARG682, ARG683, ALA684, ARG685, SER698, LEU699, GLY700, ALA701, ASN703, VAL705, ALA713, PRO715, THR719, ILE720, SER721, VAL722, THR723, THR724, ILE726, LYS825, VAL826, THR827, ALA829, ASP830, ILE844, ALA845, ARG847, ASP848, LEU849, ILE850, CYS851, ALA852, GLN853, LYS854, PHE855, ASN856, GLY908, ILE909, ALA930, LYS933, ILE934, SER937, LEU938, THR941, ALA942, SER943, ALA944, LEU945, GLY946, LYS947, GLN949, ASP950, ASN953, GLN954, ALA956, GLN957, ALA958, LEU959, ASN960, THR961, VAL963, GLN965, LEU966, SER967, SER968, ASN969, PHE970, GLY971, ALA972, ILE973, SER974, SER975, VAL976, ASP979, ARG983, ARG995, THR998, GLY999, ARG1000, GLN1002, GLN1010, ARG1014, GLN1036, SER1037, LYS1038, ARG1039, VAL1040, ASP1041, PHE1042, CYS1043, GLY1044, LYS1045, GLY1046, TYR1047, HIS1048, THR1066, TYR1067, VAL1068, PRO1069, ALA1070, GLN1071, GLU1072, ARG1107, ASN1108,
6VW1	PHE20, GLY21, PHE24, ASN25, VAL49, LEU50, SER53, PHE55, PHE56, TRP118,
6VXS	ALA21, ASP22, ILE23, VAL24, ALA38, ALA39, ASN40, LYS44, LY46, GLY47, GLY48, VAL49, ALA50, ALA52, VAL95, GLY97, PRO125, LEU126, LEU127, SER128, ALA129, GLY130, ILE131, PHE132, PRO136, ALA154, VAL155, PHE156, ASP157, LEU160,
6W4B	ASN3, GLU4, LEU5, SER6, VAL8, ASN34, LEU98, MET102

protein) and ADP-ribose-1 monophosphatase. In order to have a detailed understanding of interaction of Chloroquine and allied compounds with SARS-CoV-2 viral proteins the potential active site of these proteins and the amino acid residues involved in formation of these active sites were identified. With the molecular docking studies, the hydrogen bonds and Van der Waals interactions formed as a result of binding of the drugs to the SARS-CoV-2 viral proteins were identified (Table 5.1 supplementary). The normal function of SARS-CoV-2 proteins can be inhibited as a result of the binding of these drugs to the active site of these SARS-CoV-2 proteins.

Our results clearly demonstrate that Chloroquine and its derivatives can bind to SARS-CoV-2 proteins and thereby can disrupt the normal functioning of these proteins. Also it was found that some chemically synthesized derivatives of Chloroquine are more efficient in binding to viral proteins when compared with Chloroquine or Hydroxychloroquine. However, some of these derivatives can elicit toxicity and therefore better modeling of Chloroquine can lead to the identification of an effective inhibitor of SARS-CoV-2 virus (Supplementary Table 3.1).

As we write this report, extensive research is ongoing around the globe to discover specific and effective drug(s) or drug combination that can halt the spread of COVID-19 pandemic. With the help of computational methods for drug discovery and repurposing, several natural compounds, anti-viral compounds, anti-malarial drugs, antibiotics, and pharmacologically active compounds have been screened and are being investigated for their ability to inhibit SARS-CoV-2 protein function (Elfiky, 2020; Fantini et al., 2020; Wu et al., 2020). However, to the best of our knowledge not much research has been performed to probe the effect of Chloroquine, Hydroxychloroquine and their derivatives on various drug targets of SARS-CoV-2 virus.

Post internalization of the virus in the host cell, both non-structural protein 3 & 5 are involved in the formation of multi-domain M protease, which plays a key role in the replication process (Stobart et al., 2013). In this context, the binding potential of Chloroquine and its derivatives was assessed for binding to non-structural protein 3 as this interaction can disrupt SARS-CoV-2 multiplication within the host cell. Our results indicate that Chloroquine and its derivatives can effectively bind to the NSP 3. Moreover, it was found that some of the Chloroquine derivatives such as CQN1B (−8.1 kcal/mol), CQN1A (−8.5 kcal/mol) and CQN2H (−8.8 kcal/mol) can bind even strongly to NSP 3 than the binding affinity of Hydroxychloroquine (−7.3 kcal/mol) to NSP3.

The main protease is also a critical enzyme in the life cycle of SARS-CoV-2 virus involved in the cleavage of polyprotein (PP) at the C-terminal end and leads to the formation of non-structural proteins (Lindner et al., 2005). Targeting the main protease is one of the ways to prevent the replication of SARS-CoV-2. The obtained results clearly demonstrate that the Chloroquine and its derivatives CQN2H and CQN1B can bind to the main protease and thereby can potentially inhibit its activity.

Non-structural protein 12 acts as RNA dependent RNA polymerase (RdRp). It is a vital enzyme in the replication of RNA viruses. Therefore, it has been studied in various viruses

Table 5. Binding affinity (Kcal/mole) between Chloroquine derivatives and drug targets of SARS-CoV-2.

S. No.	Chloroquine derivatives and its synthesized compounds	6W02	6LU7	6M71	6VSB	6VW1.E	6VXS	6W4B
1.	Chloroquine_2719	−5.0 (−5.6)	−4.3	−5.6 (−5.0)	−4.5 (−5.4)	−5.1 (−5.4)	−5.9 (−5.9)	−4.5 (−3.9)
2.	Hydroxychloroquine_3652	−5.6 (−7.3)	−4.8	−5.9 (−5.6)	−6.0 (−5.4)	−5.3 (−5.4)	−6.2 (−6.2)	−5.4 (−4.3)
3.	Chloroquine sulfate_ChEBI_50178	−3.9 (−4.0)	−4.5	−5.5 (−5.2)	−5.9 (−5.1)	−5.0 (−5.1)	−6.0 (−5.9)	−5.1 (−4.9)
4.	Chloroquine pyrolidylin_ZINC1666887	−7.0 (−8.0)	−5.0	−6 (−5.8)	−6.6 (−5.8)	−5.5 (6.0)	−6.3 (−6.7)	−5.7 (−3.7)
5.	Chloroquine mustard_ZINC5751278	−5.4 (−6.9)	−4.2	−5.8 (−5.2)	−5.5 (−5.5)	−5.1 (−5.5)	−5.5 (−5.6)	−5.1 (−3.9)
	Chemically synthesized chloroquine derivatives							
6.	CQN2A ($C_{25}H_{28}ClN_3O$)	−7.4 (−7.7)	−4.8	−6.8 (−6.6)	−6.0 (−5.6)	−5.6 (−5.4)	−6.7 (−7.2)	−6.2 (−3.7)
7.	CQN2B ($C_{26}H_{30}ClN_3O$)	−6.4 (−7.6)	−4.9	−6.9 (−6.9)	−5.3 (−6.7)	−5.2 (−6.6)	−7.0 (−7.3)	−6.1 (−4.2)
8.	CQN2C ($C_{28}H_{34}ClN_3O_2$)	−6.2 (−7.7)	−4.7	−6.7 (−5.4)	−6.9 (−6.3)	−5.5 (−6.2)	−6.2 (−7.6)	−5.7 (−4.3)
9.	CQN2D ($C_{26}H_{30}ClN_3O_2$)	−5.9 (−7.7)	−5.0	−6.5 (−6.2)	−6.6 (−5.3)	−6.3 (−6.6)	−7.1 (−7.2)	−5.6 (−4.1)
10.	CQN2E ($C_{25}H_{27}Cl_2N_3O$)	−5.6 (−6.9)	−4.7	−5.9 (−6.7)	−5.7 (−5.6)	−5.5 (−6.4)	−7.4 (−6.7)	−5.5 (−4.8)
11.	CQN2F ($C_{25}H_{30}ClN_3O$)	−1.0 (−1.1)	−0.9	−1.0 (−1.0)	−1.0 (−1.2)	−1.3 (−1.3)	−1.0 (−1.0)	−0.9 (−0.6)
12.	CQN2G ($C_{28}H_{34}ClN_3O_2$)	−6.2 (−7.7)	−5.3	−6.4 (−6.4)	−6.1 (−6.1)	−5.9 (−7.1)	−7.0 (−7.2)	−5.0 (−4.9)
13.	CQN2H ($C_{19}H_{15}ClN_2O_2$)	−8.4 (−8.8)	−6.0	−8.4 (−7.0)	−7.6 (−7.3)	−6.9 (−6.5)	−7.0 (−7.1)	−7.1 (−5.5)
14.	CQN2I ($C_{25}H_{34}ClN_3O$)	−5.6 (−6.5)	−4.8	−5.3 (−5.6)	−6.2 (−5.3)	−6.6 (−6.2)	−7.4 (−7.5)	−6.1 (−3.9)
15.	CQN2J ($C_{21}H_{30}ClN_3O$)	−7.0 (−7.0)	−5.3	−6.5 (−5.7)	−6.2 (−5.8)	−4.7 (−5.4)	−6.4 (−5.8)	−5.8 (−4.2)
16.	CQN21D ($C_{28}H_{35}ClN_3O_2$)	−6.6 (−6.8)	−4.7	−6.1 (−6.0)	−5.9 (−5.6)	−5.7 (−6.3)	−6.2 (−7.3)	−5.8 (−3.7)
17.	CQN21A ($C_{29}H_{37}ClN_3O_2$)	−7.1 (−7.7)	−4.7	−6.0 (−6.2)	−5.9 (−5.7)	−4.7 (−6.5)	−6.0 (−7.4)	−5.7 (−3.9)
18.	CQN1A ($C_{26}H_{31}N_3O_2$)	−7.1 (−8.5)	−5.1	−6.5 (−6.0)	−6.8 (−7.0)	−7.6 (−6.9)	−6.6 (−6.9)	−6.8 (−3.8)
19.	CQN1B ($C_{27}H_{33}N_3O_3$)	−7.8 (−8.1)	−6.0	−5.9 (−6.3)	−7.8 (−6.0)	−5.5 (−7.6)	−6.8 (−7.0)	−6.5 (−4.4)

Note: Binding affinities in the parenthesis mentioned form site-specific docking.

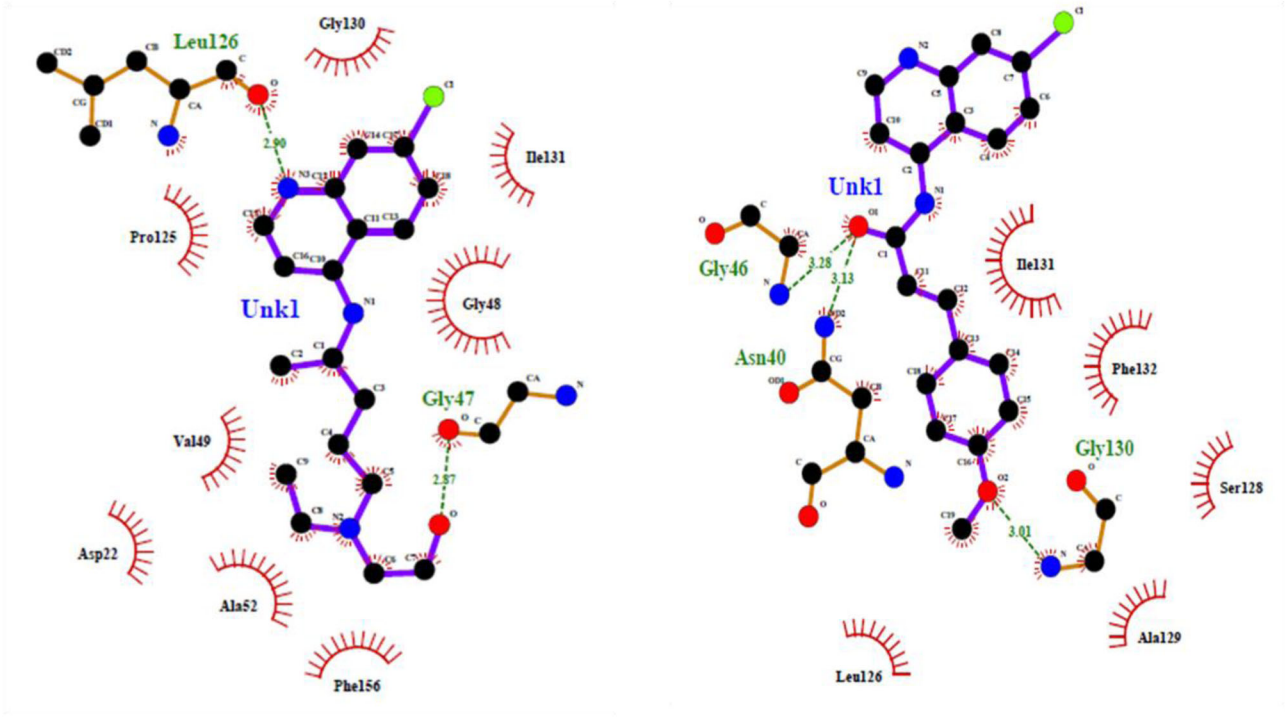


Figure 1. Hydroxychloroquine and CQN2H showing various interactions with Non-structural protein-3.

including the hepatitis C virus and the Zika virus (Elfiky, 2016; Ganesan & Barakat, 2017). Recently, the FDA approved anti-RdRp drugs (Ribavirin, Remdesivir, Sofosbuvir, Galidesivir, and Tenofovir) have shown potential inhibitory activity against the RdRp of SARS-CoV-2 (Elfiky, 2020). In our study, Chloroquine derivative CQN2H demonstrated strong binding affinity to RdRp (−8.2 kcal/mol) which is even stronger that that of Hydroxychloroquine with RdRp protein. These results can be valuable in repurposing and redesigning CQN2H as a potential inhibitor of RdRp function.

Spike glycoprotein plays an essential role in the attachment of coronavirus with the ACE2 on the host cell surface. Hence, this protein is considered as an important drug target for drug discovery (Prajapat et al., 2020). Our results demonstrate that Hydroxychloroquine can effectively bind to both spike glycoprotein and chimeric receptor binding domain along with compounds CQN1B, CQN2H that can effectively inhibit spike glycoprotein. Besides, the compounds CQN1A, CQN1B can also potentially inhibit receptor binding domain. These results point to the fact that the Chloroquine

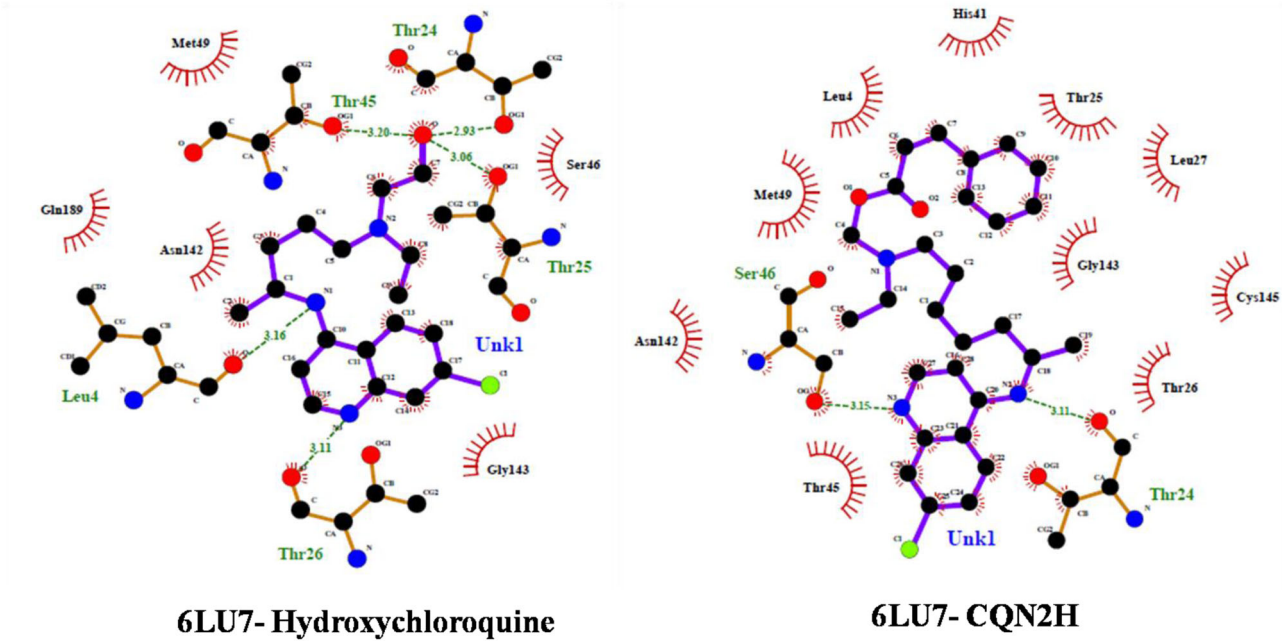


Figure 2. Hydroxychloroquine and CQN21D showing various interactions with Main protease.

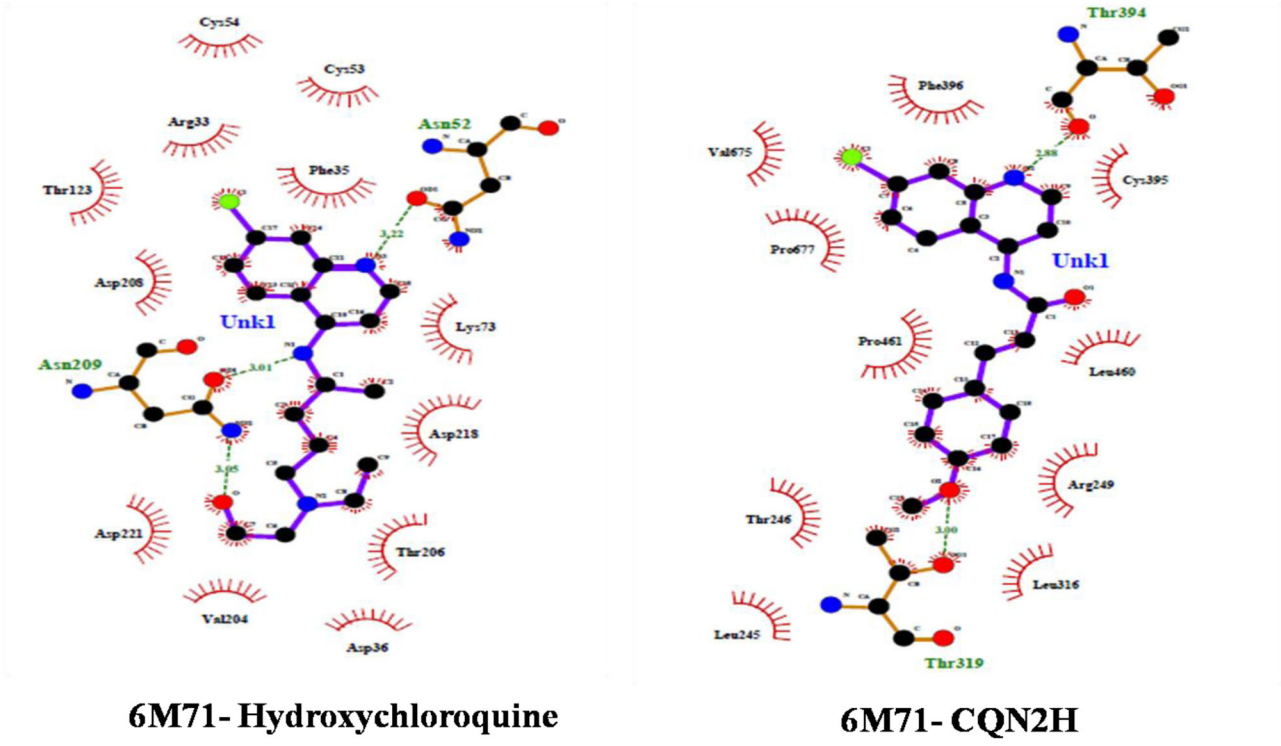


Figure 3. Hydroxychloroquine and CQN2H showing various interactions with RNA dependent RNA polymerase.

derivatives can bind to Spike glycoprotein, which can potentially lead to disruption of Spike protein interaction with the ACE-2 on host cell surface. However, in another recent study is was demonstrated that both Chloroquine and Hydroxychloroquine can prevent the interaction of spike protein with sialic acids and gangliosides present on the host cell surface (Fantini et al., 2020 Matrosovich et al., 2013).

Non-structural protein 9 (nsp9) acts as a replicase protein (RNA-binding protein) and its dimerization is essential for viral propagation (Prajapat et al., 2020). Therefore, targeting the dimerization of NSP9 can be an effective strategy to control virus multiplication (Egloff et al., 2004, Hu et al., 2017). Our results show that Chloroquine derivative CQN2H can bind to the replicase protein with higher efficiency than the Hydroxychloroquine. Further, the derivatives of Chloroquine such as CQN2E, CQN2I, and CQN2C can also effectively inhibit ADP-ribose-1 monophosphatase than the Hydroxychloroquine. From these results, it is apparent that these Chloroquine derivatives possess therapeutic activity against SARS-CoV-2 virus.

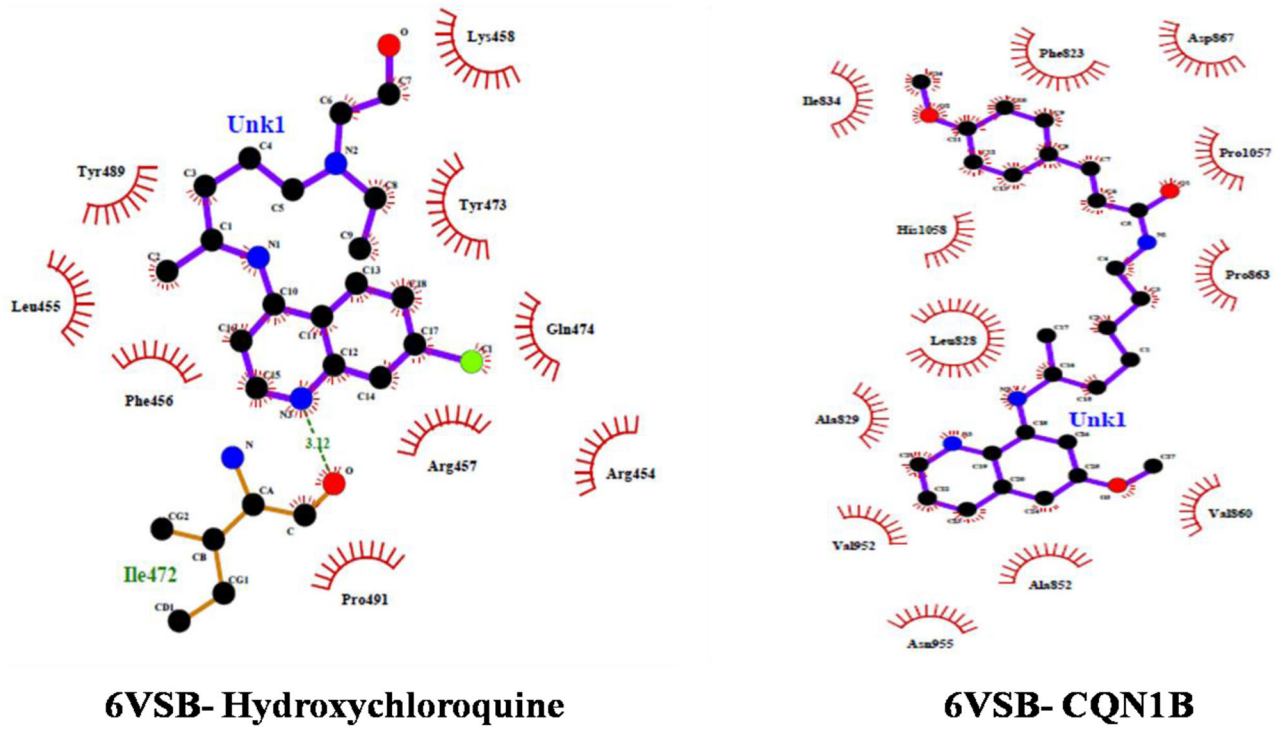


Figure 4. Hydroxychloroquine and CQN1B showing various interactions with SARS-CoV-2 spike glycoprotein.

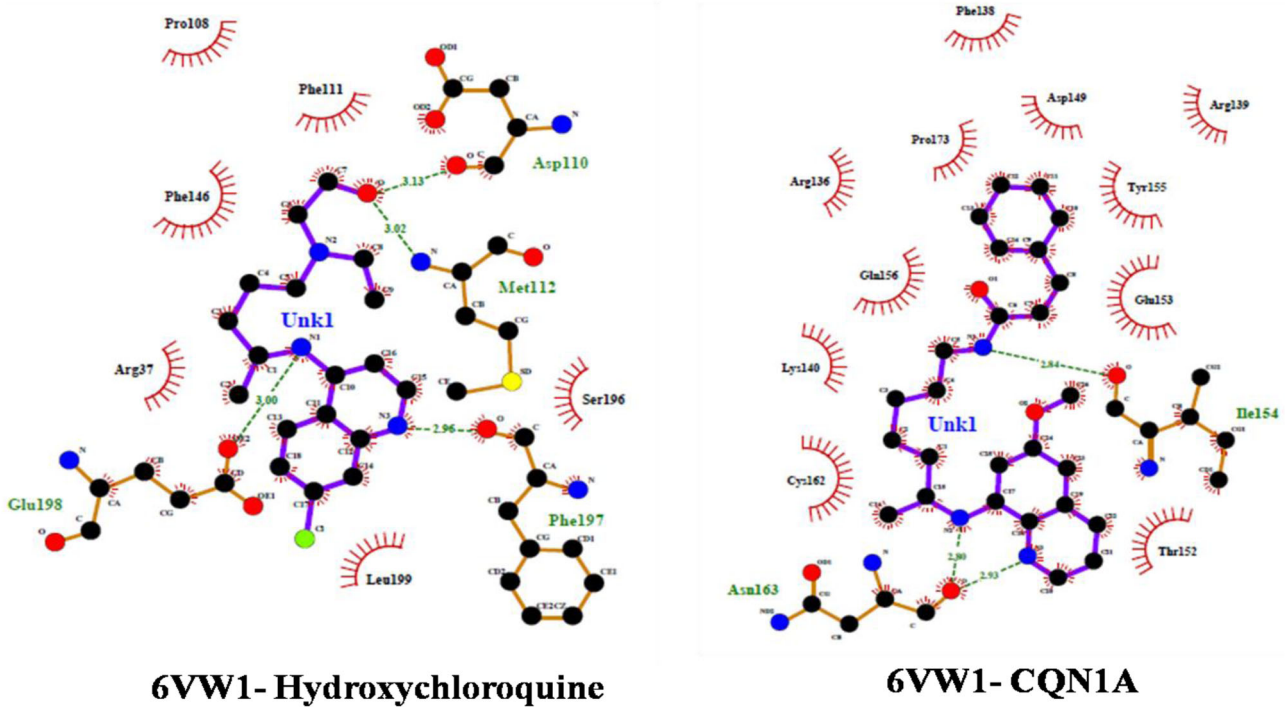


Figure 5. Hydroxychloroquine and CQN1A showing various interactions with spike protein – Receptor Binding Domain.

Our studies clearly demonstrate binding of Chloroquine, Hydroxychloroquine and their derivatives to various SARS-CoV-2 proteins. However, these results need to be substantiated with binding experiments to ascertain other drug-protein interaction parameters. At the same time, the specificity of these studies can be ascertained from the fact that one of the derivatives of chloroquine, CQN2F did not show binding to any viral protein included in the study. Although Chloroquine and Hydroxychloroquine are losing their popularity due to possible mutagenic activity our literature survey points to the fact that Chloroquine and Hydroxychloroquine have well established toxicokinetic properties and might only elicit dosage dependent toxicity. Also Chloroquine and Hydroxychloroquine represent an economical option to prevent the spread of COVID-19 pandemic. We believe that from our study informed approach can be taken to understand the drug-protein interaction and then further modify the drug structure which will have a minimal toxic

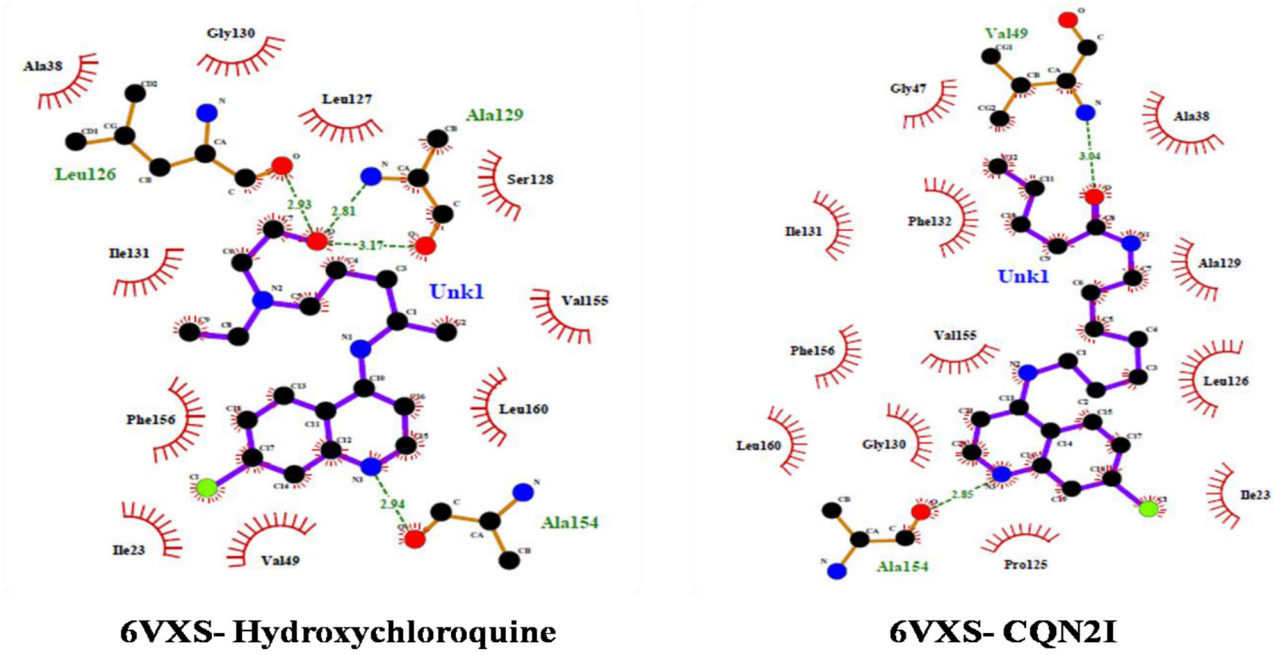


Figure 6. Hydroxychloroquine and CQN2I showing various interactions with ADP-ribose-1 monophosphatase.

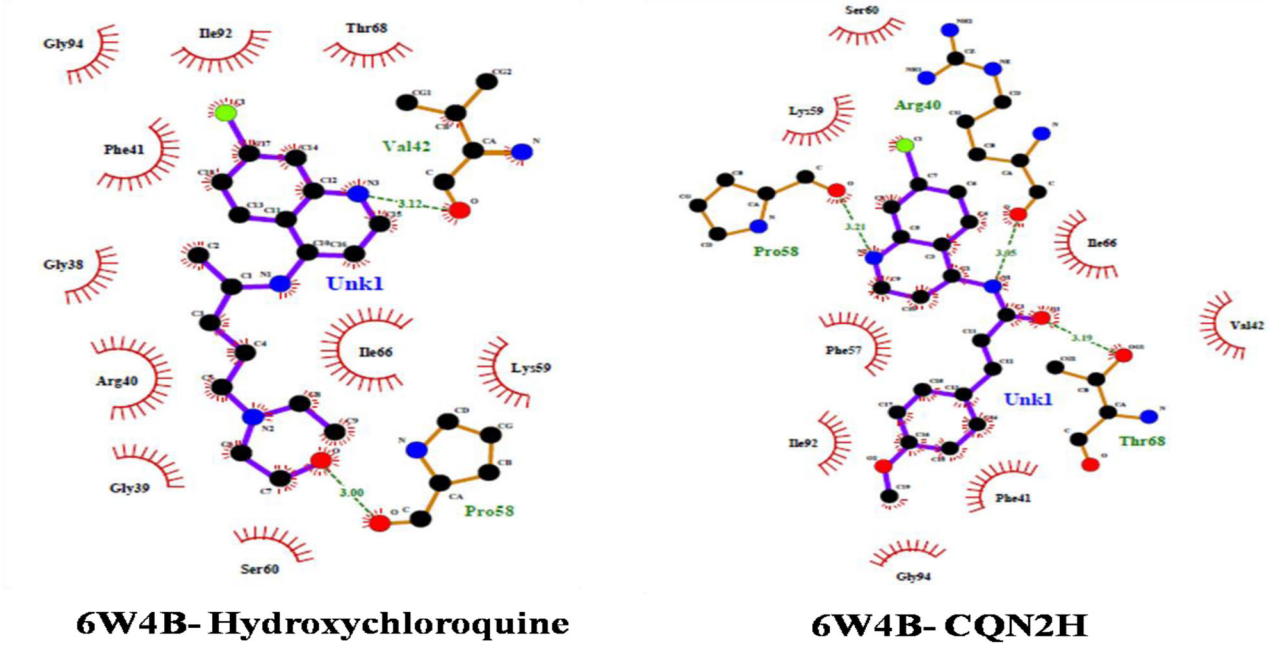


Figure 7. Hydroxychloroquine and CQN2H showing various interactions with Non-structural protein-9 (Replicase protein).

burden and maximal inhibitory potential against SARS-CoV-2 viral proteins. Our results also enhance the basic knowledge of the interaction of Chloroquine derivatives with the various drug targets of SARS-CoV-2 and point to a possible novel mechanism of action of the century old drug.

5. Conclusion

From our results, it can be infered that Hydroxychloroquine considerably inhibits all the drug targets of SARS-CoV-2. Further, it was found that Hydroxychloroquine might not only enhance the endosomal pH, but it could also interact with various proteins of SARS-CoV-2. Chemically synthesized Chloroquine derivatives reveal more effective inhibitory activity against all drug targets of SARS-CoV-2 than Hydroxychloroquine. Among the various derivatives of Chloroquine, CQN2H and CQN1B show potential inhibition against all the drug targets. Our results could also be used for further constructive research on chemically synthesized Chloroquine derivatives to identify successful drugs for the treatment of COVID-19.

Acknowledgements

The authors would like to thank IISER Tirupati and IIT Tirupati for their support.

Disclosure statement

There is no conflict of interest.

Funding

The current project being an *in-silico* work did not require any additional financial support. This is purely a working arising from "work from situation". Otherwise, the authors are thankful for the financial support from their respective institutes.

ORCID

Vasudharani Devanathan http://orcid.org/0000-0002-2052-400X
Ambrish Saxena http://orcid.org/0000-0001-8620-7683

References

Bommu, U. D., Konidala, K. K., Pamanji, R., & Yeguvapalli, S. (2019). Structural probing, screening and structure-based drug repositioning insights into the identification of potential Cox-2 inhibitors from selective coxibs. *Interdisciplinary Sciences: Computational Life Sciences*, *11*(2), 153–169. https://doi.org/10.1007/s12539-017-0244-5

CEBM. (2020). *Global Covid-19 case fatality rates*. Retrieved May 7, 2020, from https://www.cebm.net/covid-19/global-covid-19-case-fatality-rates/.

Daina, A., Michielin, O., & Zoete, V. (2017). SwissADME: A free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules. *Scientific Reports*, *7*, 42717. https://doi.org/10.1038/srep42717

DeLano, W. L. (2002). Pymol: An open-source molecular graphics tool. *CCP4 Newsletter on Protein Crystallography*, *40*(1), 82–92.

Devaux, C. A., Rolain, J. M., Colson, P., & Raoult, D. (2020). New insights on the antiviral effects of chloroquine against coronavirus: What to expect for COVID-19? *International Journal of Antimicrobial Agents*, *55*(5), 105938. https://doi.org/10.1016/j.ijantimicag.2020

Egloff, M.-P., Ferron, F., Campanacci, V., Longhi, S., Rancurel, C., Dutartre, H., Snijder, E. J., Gorbalenya, A. E., Cambillau, C., & Canard, B. (2004). The severe acute respiratory syndrome-coronavirus replicative protein nsp9 is a single-stranded RNA-binding subunit unique in the RNA virus world. *Proceedings of the National Academy of Sciences*, *101*(11), 3792–3796. https://doi.org/10.1073/pnas.0307877101

Elfiky, A. A. (2020). Ribavirin, Remdesivir, Sofosbuvir, Galidesivir, and Tenofovir against SARS-CoV-2 RNA dependent RNA polymerase (RdRp): A molecular docking study. *Life Sciences*, *253*, 117592. https://doi.org/10.1016/j.lfs.2020.117592

Elfiky, A. A. (2016). Zika viral polymerase inhibition using anti-HCV drugs both in market and under clinical trials. *Journal of Medical Virology*, *88*(12), 2044–2051. https://doi.org/10.1002/jmv.24678

Fantini, J., Di Scala, C., Chahinian, H., & Yahi, N. (2020). Structural and molecular modeling studies reveal a new mechanism of action of chloroquine and hydroxychloroquine against SARS-CoV-2 infection. *International Journal of Antimicrobial Agents*, *55*(5), 105960. https://doi.org/10.1016/j.ijantimicag.2020.105960

Ganesan, A., & Barakat, K. (2017). Applications of computer-aided approaches in the development of hepatitis C antiviral agents. *Expert Opinion on Drug Discovery*, *12*(4), 407–425. https://doi.org/10.1080/17460441.2017.1291628

Gao, Y., Yan, L., Huang, Y., Liu, F., Zhao, Y., Cao, L., Wang, T., Sun, Q., Ming, Z., Zhang, L., Ge, J., Zheng, L., Zhang, Y., Wang, H., Zhu, Y., Zhu, C., Hu, T., Hua, T., Zhang, B., … Rao, Z. (2020). Structure of the RNA-dependent RNA polymerase from COVID-19 virus. *Science (New York, N.Y.)*, *368*(6492), 779–782. https://doi.org/10.1126/science.abb7498

Gautret, P., Lagier, J.-C., Parola, P., Hoang, V. T., Meddeb, L., Mailhe, M., Doudier, B., Courjon, J., Giordanengo, V., Vieira, V. E., Dupont, H. T., Honoré, S., Colson, P., Chabrière, E., La Scola, B., Rolain, J.-M., Brouqui, P., & Raoult, D. (2020). Hydroxychloroquine and azithromycin as a treatment of COVID-19: Results of an open-label non-randomized clinical trial. *International Journal of Antimicrobial Agents*, 105949. https://doi.org/10.1016/j.ijantimicag.2020.105949

Gomes, A., Ferraz, R., Ficker, L., Collins, M. S., Prudêncio, C., Cushion, M. T., Teixeira, C., & Gomes, P. (2018). Chloroquine analogues as leads against Pneumocystis lung pathogens. *Antimicrobial Agents and Chemotherapy*, *62*(11), e00983. https://doi.org/10.1128/AAC.00983-18

Gorbalenya, A. E., Baker, S. C., Baric, R. S., de Groot, R. J., Drosten, C., & Gulyaeva, A. A. (2020). The species severe acute respiratory syndrome-related coronavirus: Classifying 2019-nCoV and naming it SARS-CoV-2. *Nature Microbiology*, *5*(4), 536–544. https://doi.org/10.1038/s41564-020-0695-z

Hu, T., Chen, C., Li, H., Dou, Y., Zhou, M., Lu, D., Zong, Q., Li, Y., Yang, C., Zhong, Z., Singh, N., Hu, H., Zhang, R., Yang, H., & Su, D. (2017). Structural basis for dimerization and RNA binding of avian infectious bronchitis virus nsp9. *Protein Science: A Publication of the Protein Society*, *26*(5), 1037–1048. https://doi.org/10.1002/pro.3150

Huang, C. C., Meng, E. C., Morris, J. H., Pettersen, E. F., & Ferrin, T. E. (2014). Enhancing UCSF chimera through web services. *Nucleic Acids Research*, *42*(Web Server issue), W478–W484. https://doi.org/10.1093/nar/gku377

Jin, Z., Du, X., Xu, Y., Deng, Y., Liu, M., Zhao, Y., & Duan, Y. (2020). Structure of Mpro from COVID-19 virus and discovery of its inhibitors. *bioRxiv*, 1–33.

Kim, Y., Jedrzejczak, R., Maltseva, N., Endres, M., Mececar, A., Michalska, K., & Joachimiak, A. (2020). Crystal structure of ADP ribose phosphatase of NSP3 from SARS CoV-2. *bioRxiv*, 1–24. https://doi.org/10.2210/pdb6vxs/pdb

Lamballerie, X. D., Boisson, V., Reynier, J. C., Enault, S., Charrel, R. N., Flahault, A., Roques, P., & Grand, R. L. (2008). On chikungunya acute infection and chloroquine treatment. *Vector-Borne and Zoonotic Diseases*, *8*(6), 837–839. https://doi.org/10.1089/vbz.2008.0049

Lindner, H. A., Fotouhi-Ardakani, N., Lytvyn, V., Lachance, P., Sulea, T., & Ménard, R. (2005). The papain-like protease from the severe acute respiratory syndrome coronavirus is a deubiquitinating enzyme. *Journal of Virology*, *79*(24), 15199–15208. https://doi.org/10.1128/JVI.79.24.15199-15208.2005

Littler, D., Gully, B., Colson, R. N., & Rossjohn, J. (2020). Crystal structure of the SARS-CoV-2 non-structural protein 9, Nsp9. *bioRxiv*, 1–6.

Matrosovich, M., Herrler, G., & Klenk, H. D. (2013). Sialic acid receptors of viruses. In *SialoGlyco chemistry and biology II* (pp. 1–28). Springer.

Michalska, K., Kim, Y., Jedrzejczak, R., Maltseva, N., Endres, M., Mececar, A., & Joachimiak, A. (2020). Crystal Structure of ADP ribose phosphatase of NSP3 from SARS CoV-2 in the complex with ADP ribose. *bioRxiv*, 1–24. https://doi.org/10.2210/pdb6W02/pdb

Morris, G. M., Huey, R., Lindstrom, W., Sanner, M. F., Belew, R. K., Goodsell, D. S., & Olson, A. J. (2009). Autodock4 and AutoDockTools4: Automated docking with selective receptor flexiblity. *Journal of Computational Chemistry*, *30*(16), 2785–2791. https://doi.org/10.1002/jcc.21256

Nimgampalle, M., Banavath, H. N., Chakravarthy, H., Saxena, A., & Devanathan, V. (2019). Novel inhibitors of Rho-kinase mediated neuroinflammatory pathways and their potential application in recovery of injured spinal cord. *Journal of Biomolecular Structure and Dynamics*, 1–18. https://doi.org/10.1080/07391102.2019.1686066

Prajapat, M., Sarma, P., Shekhar, N., Avti, P., Sinha, S., Kaur, H., Kumar, S., Bhattacharyya, A., Kumar, H., Bansal, S., & Medhi, B. (2020). Drug targets for corona virus: A systematic review. *Indian Journal of Pharmacology*, *52*(1), 56–65. https://doi.org/10.4103/ijp.IJP_115_20

Rabby, M. I. I. (2020). Current drugs with potential for treatment of COVID-19: A literature review. *Journal of Pharmacy & Pharmaceutical Sciences*, *23*(1), 58–64.

Savarino, A., & Shytaj, I. L. (2015). Chloroquine and beyond: Exploring anti-rheumatic drugs to reduce immune hyperactivation in HIV/AIDS. *Retrovirology*, *12*, 51. https://doi.org/10.1186/s12977-015-0178-0

Scholz, M., & Derwand, R. (2020). Does zinc supplementation enhance the clinical efficacy of chloroquine/hydroxychloroquine to win todays battle against COVID-19? *Medical Hypotheses*, 109815.

Shang, J., Ye, G., Shi, K., Wan, Y., Luo, C., Aihara, H., & Li, F. (2020). Structural basis of receptor recognition by SARS-CoV-2. *Nature*, 1–8.

Singh, A. K., Singh, A., Shaikh, A., Singh, R., & Misra, A. (2020). Chloroquine and hydroxychloroquine in the treatment of COVID-19 with or without diabetes: A systematic search and a narrative review with a special reference to India and other developing countries. *Diabetes & Metabolic Syndrome: Clinical Research & Reviews*, *14*(3), 241–246. https://doi.org/10.1016/j.dsx.2020.03.011

Stobart, C. C., Sexton, N. R., Munjal, H., Lu, X., Molland, K. L., Tomar, S., Mesecar, A. D., & Denison, M. R. (2013). Chimeric exchange of coronavirus nsp5 proteases (3CLpro) identifies common and divergent regulatory determinants of protease activity. *Journal of Virology*, *87*(23), 12611–12618. https://doi.org/10.1128/JVI.02050-13

Tian, W., Chen, C., Lei, X., Zhao, J., & Liang, J. (2018). CASTp 3.0: Computed atlas of surface topography of proteins. *Nucleic Acids Research*, *46*(W1), W363–W367. https://doi.org/10.1093/nar/gky473

Touret, F., & de Lamballerie, X. (2020). Of chloroquine and COVID-19. *Antiviral Research*, *177*, 104762. https://doi.org/10.1016/j.antiviral.2020.104762

Tricou, V., Minh, N. N., Van, T. P., Lee, S. J., Farrar, J., Wills, B., Tran, H. T., & Cameron, P. S. (2010). A randomized controlled trial of chloroquine for the treatment of dengue in Vietnamese adults. *PLoS Neglected Tropical Diseases*, *4*(8), e785. https://doi.org/10.1371/journal.pntd.0000785

Vincent, M. J., Bergeron, E., Benjannet, S., Erickson, B. R., Rollin, P. E., Ksiazek, T. G., Seidah, N. G., & Nichol, S. T. (2005). Chloroquine is a potent inhibitor of SARS coronavirus infection and spread. *Virology Journal*, *2*, 69. https://doi.org/10.1186/1743-422X-2-69

Wallace, A. C., Laskowski, R. A., & Thornton, J. M. (1995). LIGPLOT: A program to generate schematic diagrams of protein-ligand interactions. *Protein Engineering*, *8*(2), 127–134. https://doi.org/10.1093/protein/8.2.127

Wee, S. L., McNeil, D. G., Hernandez, J. C. (2020). WHO declares global emergency as Wuhan coronavirus spreads. *New York Times*.

Wrapp, D., Wang, N., Corbett, K. S., Goldsmith, J. A., Hsieh, C.-L., Abiona, O., Graham, B. S., & McLellan, J. S. (2020). Cryo-EM structure of the 2019-nCoV spike in the prefusion conformation. *Science (New York, N.Y.)*, *367*(6483), 1260–1263. https://doi.org/10.1126/science.abb2507

Wu, C., Liu, Y., Yang, Y., Zhang, P., Zhong, W., Wang, Y., Wang, Q., Xu, Y., Li, M., Li, X., Zheng, M., Chen, L., & Li, H. (2020). Analysis of therapeutic targets for SARS-CoV-2 and discovery of potential drugs by computational methods. *Acta Pharmaceutica Sinica B*, *10*(5), 766–788. https://doi.org/10.1016/j.apsb.2020.02.008

Yeo, K. J., Chen, H. H., Chen, Y. M., Lin, C. H., Chen, D. Y., Lai, C. M., & Chao, W. C. (2020). Hydroxychloroquine may reduce risk of Pneumocystis pneumonia in lupus patients: A Nationwide, population-based case-control study. *BMC Infectious Diseases*, *20*(1), 112. https://doi.org/10.1186/s12879-020-4826-1

Yuki, K., Fujiogi, M., & Koutsogiannaki, S. (2020). COVID-19 pathophysiology: A review. *Clinical Immunology (Orlando, Fla.)*, *215*, 108427. https://doi.org/10.1016/j.clim.2020.108427