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

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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 we have screened 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, we have selected some essential viral proteins and enzymes 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 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.

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), 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 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 possible treatment. 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 and 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, we have selected key regulatory proteins and enzymes associated with the pathogenesis of SARS-CoV-2 as drug targets for Chloroquine and its derivatives.

Chloroquine and Hydroxychloroquine are anti-malarial drugs, which are also used for the treatment of rheumatoid arthritis and lupus erythematosus (Touret and 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) to know their therapeutic activity for 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 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 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 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. 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 and Shytaj, 2015). However both Chloroquine and its derivative Hydroxychloroquine were shown to be of some use in countering 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 we postulated that Chloroquine might be actively binding to one or more SARS-CoV-2 protein to inhibit viral replication. To study this we screened multiple Chloroquine derivatives reported earlier for binding to various viral 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. We believe that this information 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.

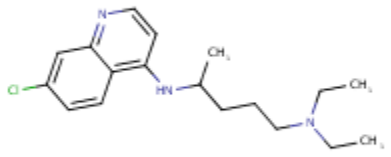
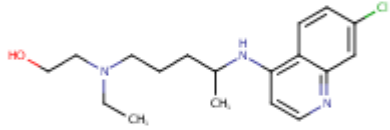
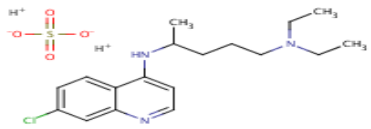
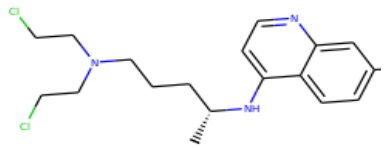
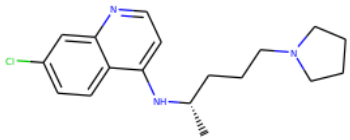
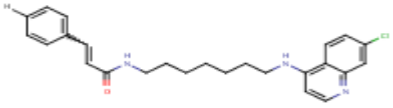
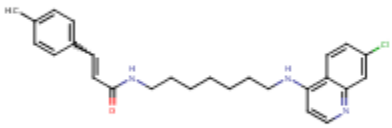
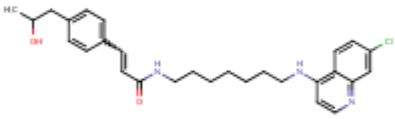
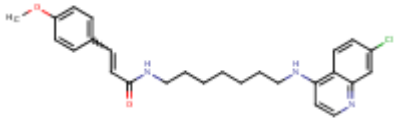
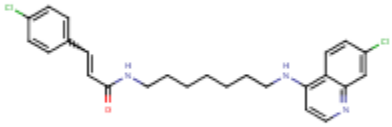
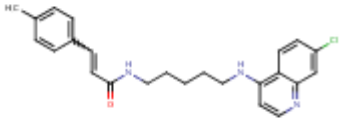
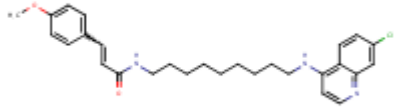
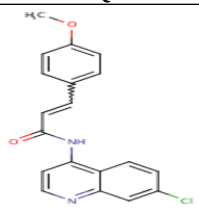
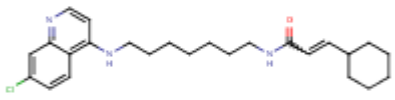
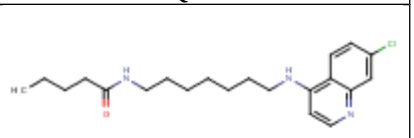
2. Materials and Methods:

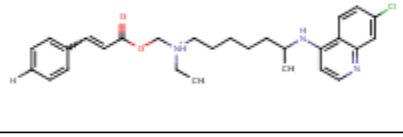
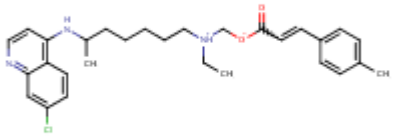
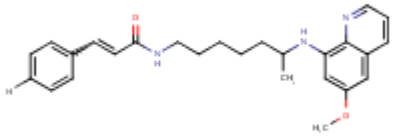
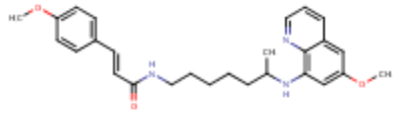
2.1. Hydroxychloroquine and Chloroquine derivatives as drug molecules

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, we have evaluated the binding potential of Chloroquine and its derivatives with different SARS-CoV-2 proteins involved in viral replication. 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.

Table1 Structures of Chloroquine derivatives and its synthesized compounds from Gomes et al., 2018.

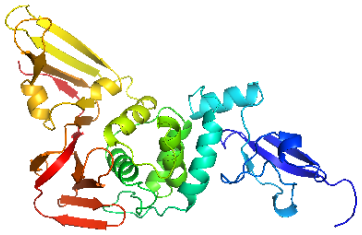
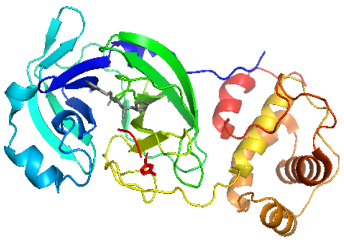
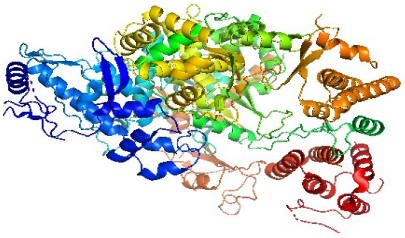
		
Chloroquine	Hydroxychloroquine	Chloroquine sulfate
		
Chloroquine mustard	Chloroquine pyrolidinyl	CQN2A
		
CQN2B	CQN2C	CQN2D
		
CQN2E	CQN2F	CQN2G
		
CQN2H	CQN2I	CQN2J

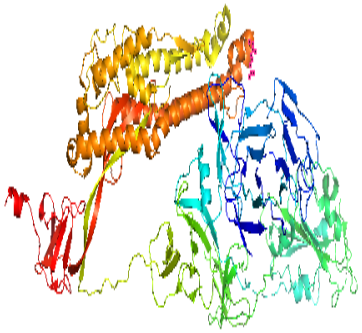
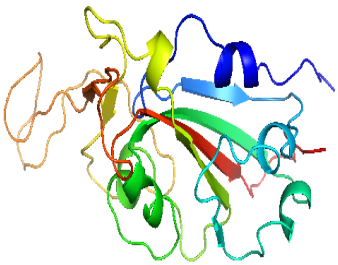
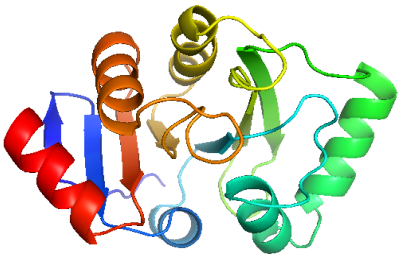
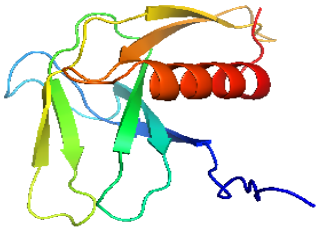
		
CQN21A	CQN21B	CQN1A
		
CQN1B		

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 Chloroquine and its analogue targets. 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: 3E9S; Ratia et al., 2020), Non-structural Protein 9 (Replicase protein; PDB ID: 6W4B; Littler et al., 2020), ADP-ribose-1 monophosphatase (PDB ID: 6VXS;) **(Table 2)**. Three-dimensional structures of the above drug targets were retrieved from the RCSB Protein Data Bank (www.rcsb.org).

Table 2 Structures of selected therapeutic targets for 2019-nCoV (RCSB Protein Data Bank).

		
3E9S_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	

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 proteins (3E9S, 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). Later, the protein structures were refined for hetero-atoms 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). Lastly, each drug target was saved in PDB (Protein Data Bank) format with their respective PDB IDs for docking studies.

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

In this study, molecular docking was performed at known active site of ligand form of proteins (3E9S, 6LU7), whereas, both blind docking and predicted active site docking (site-

specific docking) was performed to the remaining non-liganded form of proteins (6M71, 6VSB, 6VW1.E, 6VXS, and 6W4B) to examine the binding affinities of Chloroquine derivatives against each drug target used in this study. Docking was performed using PyRx 0.8 software consist of AutoDock 4.2 (Morris et al., 2009). Each prepared protein structure was uploaded and 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. After completion of docking, the dock results were saved for the observation of binding affinities and bonding interactions between ligand-target were analyzed by Ligplot software (Wallace et al., 1996).

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.

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

3.2. 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. We used CASTp server to predicted active sites for the non-liganded form of proteins (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 is listed in **Table 4**. We 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.

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

Protein PDB ID	Active site predicted from CASTp
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

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 (3E9S, 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 our results, we have illustrated blind docked ligand-protein complexes 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.

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

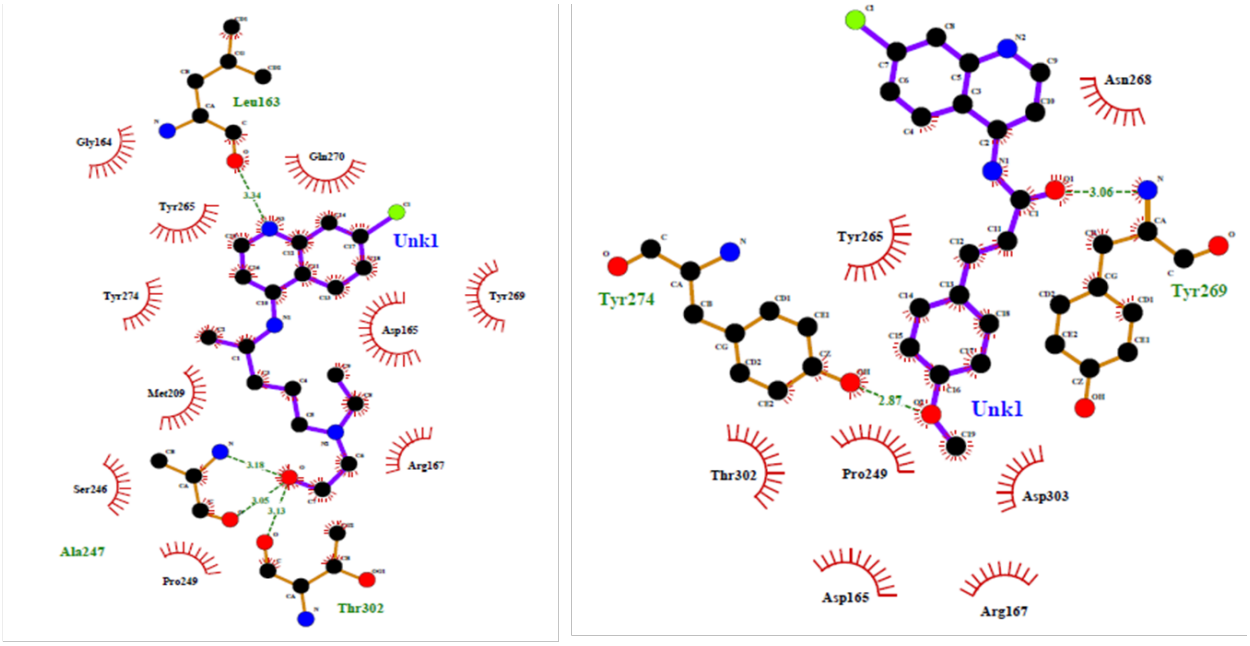
S. No.	Chloroquine derivatives and its synthesized compounds	3E9S	6LU7	6M71	6VSB	6VW1.E	6VXS	6W4B
1.	Chloroquine_2719	-7.1	-4.3	-5.6 (-5.0)	-4.5 (-5.4)	-5.1 (-5.4)	-5.9 (-5.9)	-4.5 (-3.9)
2.	Hydroxychloroquine_3652	-7.5	-4.8	-5.9 (-5.6)	-6.0 (-5.4)	-5.3 (-5.4)	-6.2 (-6.2)	-5.4 (-4.3)
3.	Chloroquine sulfate_ChEBI_50178	-6.9	-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	-7.7	-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	-7.2	-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 ($C_{25}H_{28}ClN_3O$)	-7.6	-4.8	-6.8 (-6.6)	-6.0 (-5.6)	-5.6 (-5.4)	-6.7 (-7.2)	-6.2 (-3.7)
7.	CQN2B ($C_{26}H_{30}ClN_3O$)	-8.2	-4.9	-6.9 (-6.9)	-5.3 (-6.7)	-5.2 (-6.6)	-7.0 (-7.3)	-6.1 (-4.2)
8.	CQN2C ($C_{28}H_{34}ClN_3O_2$)	-8.0	-4.7	-6.7 (-5.4)	-6.9 (-6.3)	-5.5 (-6.2)	-6.2 (-7.6)	-5.7 (-4.3)
9.	CQN2D ($C_{26}H_{30}ClN_3O_2$)	-8.1	-5.0	-6.5 (-6.2)	-6.6 (-5.3)	-6.3 (-6.6)	-7.1 (-7.2)	-5.6 (-4.1)
10.	CQN2E ($C_{25}H_{27}Cl_2N_3O$)	-7.8	-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	-0.9	-1.0 (-1.0)	-1.0 (-1.2)	-1.3 (-1.3)	-1.0 (-1.0)	-0.9 (-0.6)
12.	CQN2G ($C_{28}H_{34}ClN_3O_2$)	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 ($C_{19}H_{15}ClN_2O_2$)	-7.3	-6.0	-8.4 (-7.0)	-7.6 (-7.3)	-6.9 (-6.5)	-7.0 (-7.1)	-7.1 (-5.5)
14.	CQN2I ($C_{25}H_{34}ClN_3O$)	-8.1	-4.8	-5.3 (-	-6.2 (-5.3)	-6.6 (-	-7.4 (-7.5)	-6.1 (-3.9)

				5.6)		6.2)		
15.	CQN2J ($C_{21}H_{30}ClN_3O$)	-7.3	-5.3	-6.5 (-5.7)	-6.2 (-5.8)	-4.7 (-5.4)	-6.4 (-5.8)	-5.8 (-4.2)
16.	CQN21D ($C_{28}H_{35}ClN_3O_2$)	-8.2	-4.7	-6.1 (-6.0)	-5.9 (-5.6)	-5.7 (-6.3)	-6.2 (-7.3)	-5.8 (-3.7)
17.	CQN21A ($C_{29}H_{37}ClN_3O_2$)	-7.9	-4.7	-6.0 (-6.2)	-5.9 (-5.7)	-4.7 (-6.5)	-6.0 (-7.4)	-5.7 (-3.9)
18.	CQN1A ($C_{26}H_{31}N_3O_2$)	-8.2	-5.1	-6.5 (-6.0)	-6.8 (-7.0)	-7.6 (-6.9)	-6.6 (-6.9)	-6.8 (-3.8)
19.	CQN1B ($C_{27}H_{33}N_3O_3$)	-7.3	-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.

3.3.1. Binding to Non-structural protein3 (NSP3)

Docking results demonstrate that Hydroxychloroquine can bind to NSP3 with a binding affinity (-7.5 kcal/mol) by forming hydrogen bonding with amino acid residues LEU163, ALA247, and THR302 **(Table 5) (Figure 1)**. In parallel an even stronger binding to NSP3 when compared to Hydroxychloroquine was demonstrated by chemically synthesized Chloroquine derivatives viz. CQN2B, CQN21D, and CQN1A with CQ21D showing the maximum binding affinity (-8.2 kcal/mol) (Table 5). CQN2B shows hydrogen bonding with the residues TYR269 and TYR274 **(Figure 1)**. Overall, Chloroquine and its derivatives demonstrated 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.



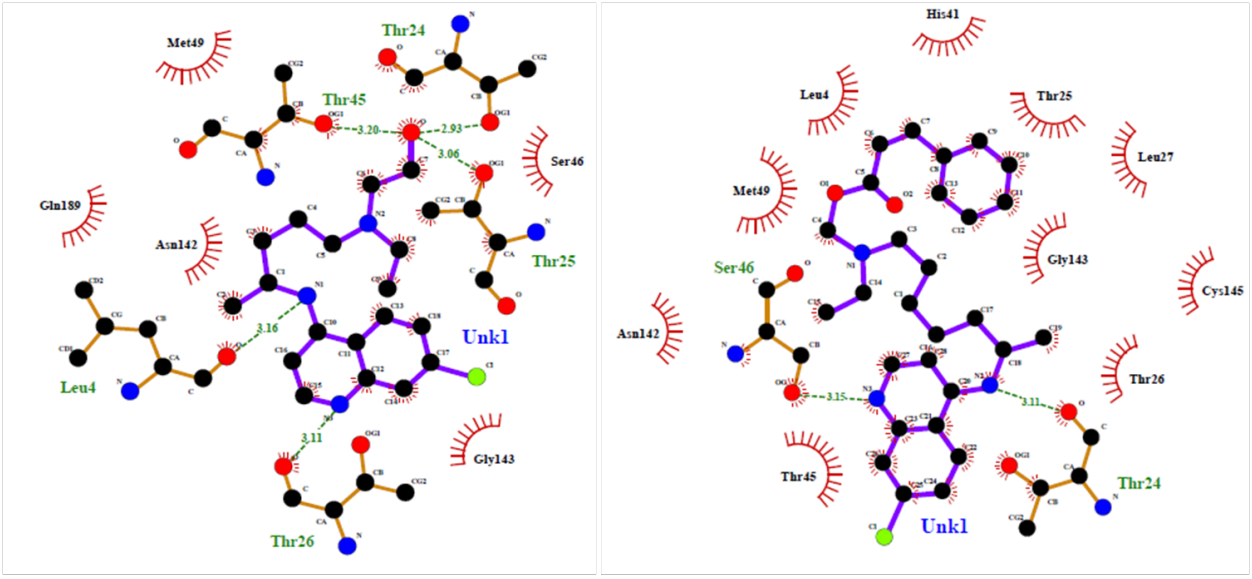
3E9S_Hydroxychloroquine **3E9S- CQN2B**

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

3.3.2. Binding to main protease

The main protease is moderately inhibited by Hydroxychloroquine with the binding affinity (-4.8 kcal/mol) **(Table 5)**. 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 **(Table 5)**. 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.

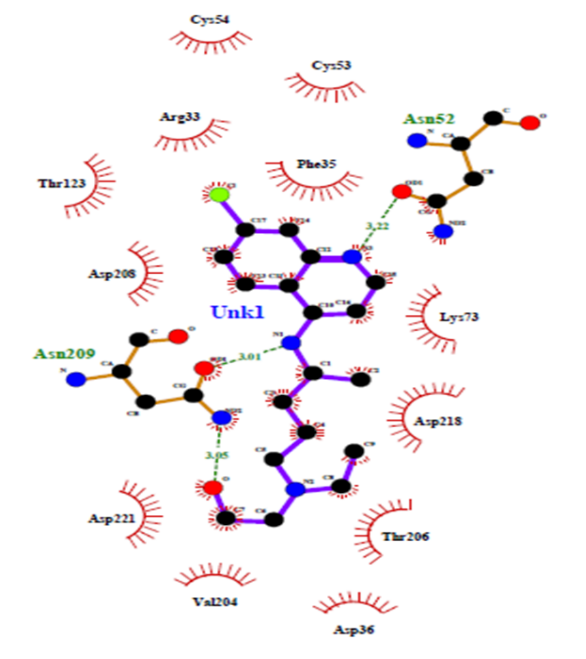


6LU7- Hydroxychloroquine **6LU7- CQN2H**

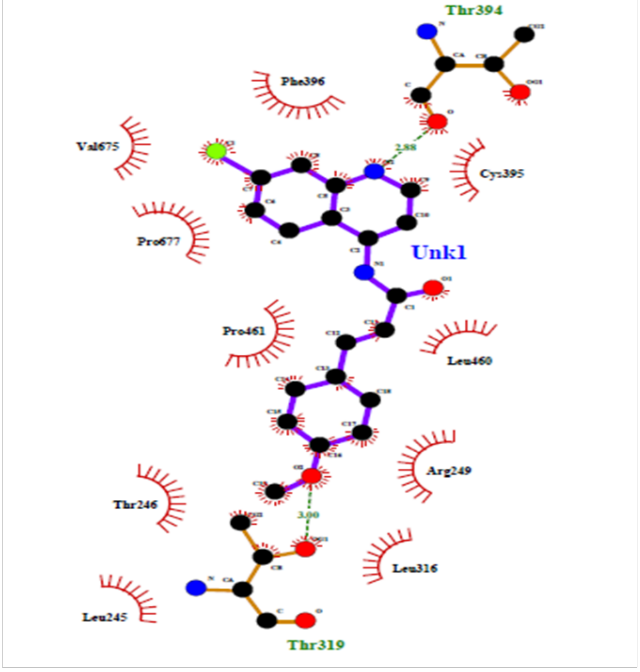
Figure 2. Hydroxychloroquine and CQN21D showing various interactions with Main protease.

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) **(Table 5)**. 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.



6M71- Hydroxychloroquine

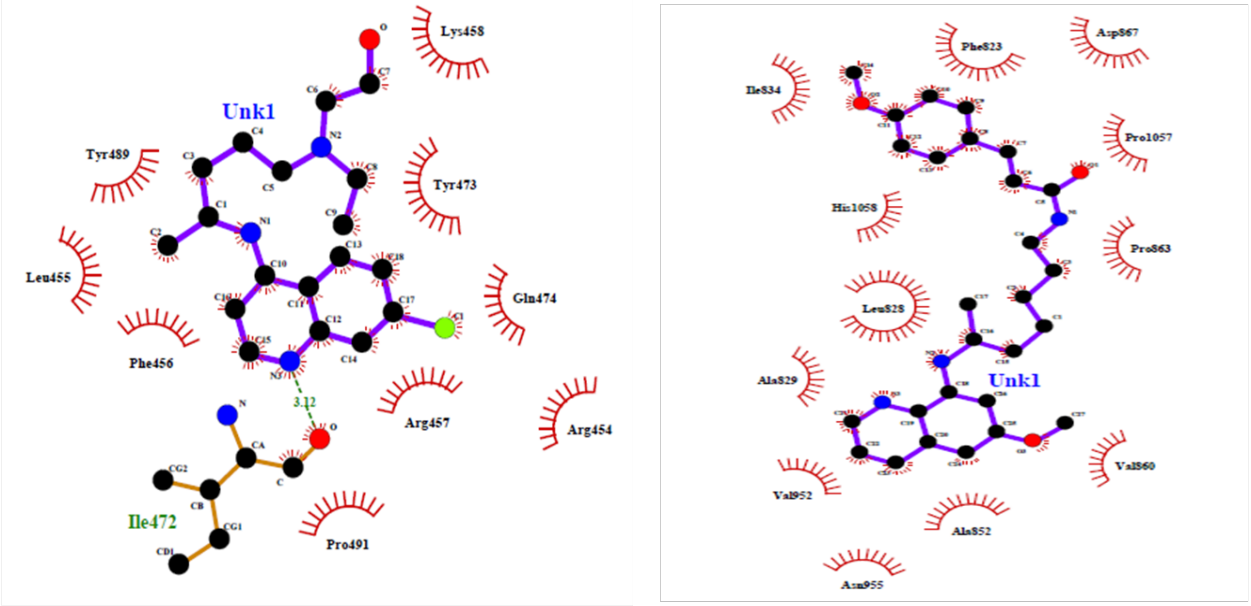


6M71- CQN2H

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

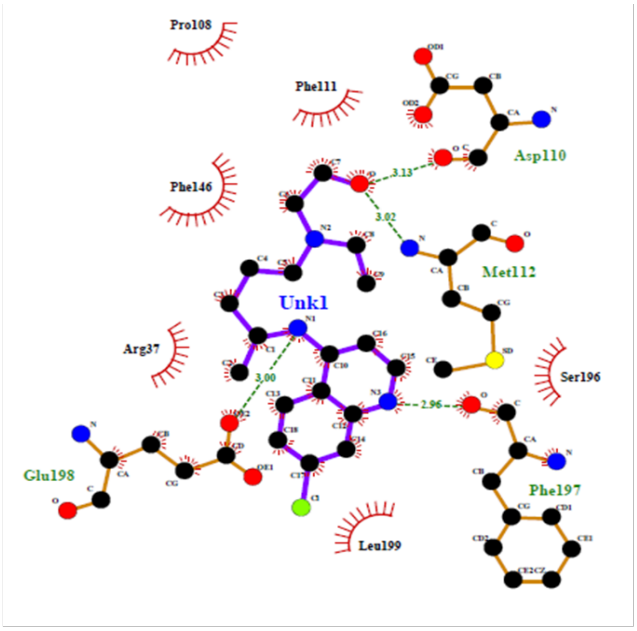


6VSB- Hydroxychloroquine **6VSB- CQN1B**

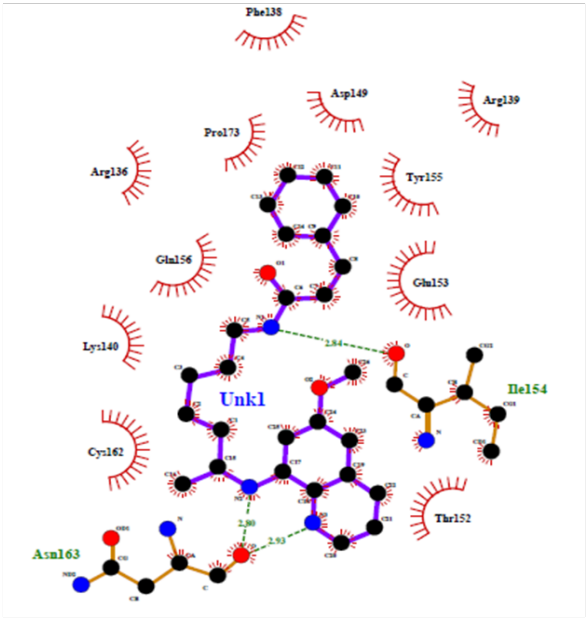
Figure 4. Hydroxychloroquine and CQN1B showing various interactions with SARS-CoV-2 spike glycoprotein.

3.3.5. Binding to Spike protein-receptor binding domain

Our studies demonstrate that Hydroxychloroquine can also bind to the receptor binding domain with 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 receptor binding domain involved in the hydrogen bonding are ASP110, MET112, PHE197, and GLU198 **(Figure 5)**. 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)**.



6VW1- Hydroxychloroquine

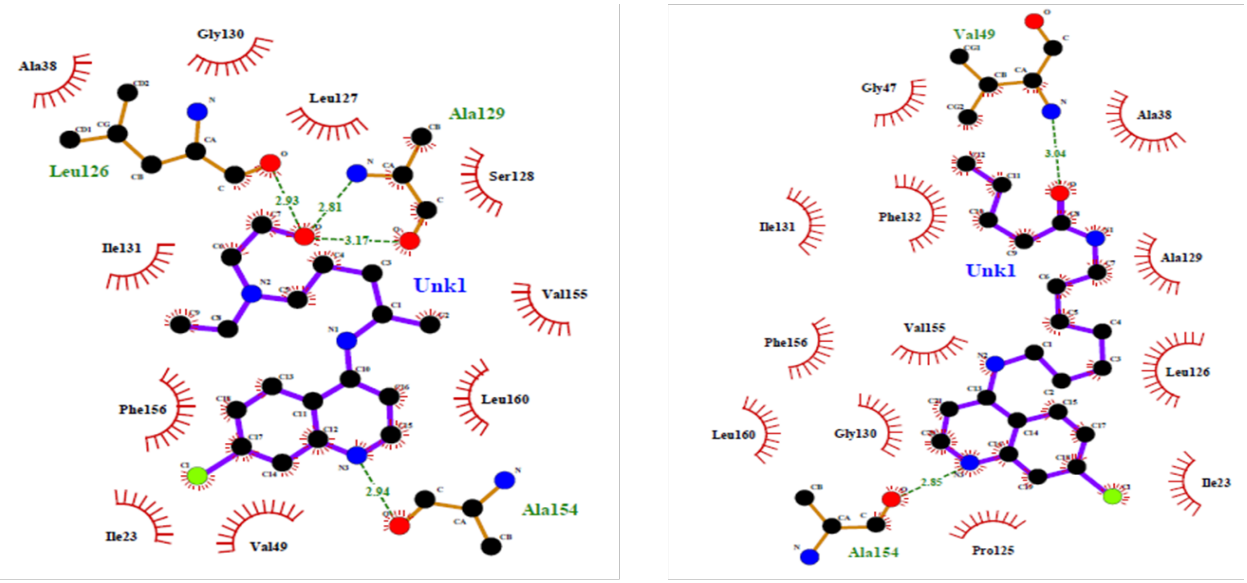


6VW1- CQN1A

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

3.3.6. Binding to ADP-ribose-1 monophosphatase

Molecular docking studies with Chloroquine and its derivatives withADP-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.



6VXS- Hydroxychloroquine **6VXS- CQN2I**

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

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 -4.3kcal/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 -5.5kcal/mol, respectively **(Table 5)** and forms hydrogen bonds with ARG40, PRO58, and THR68 residues of the replicase protein **(Figure 7)**.

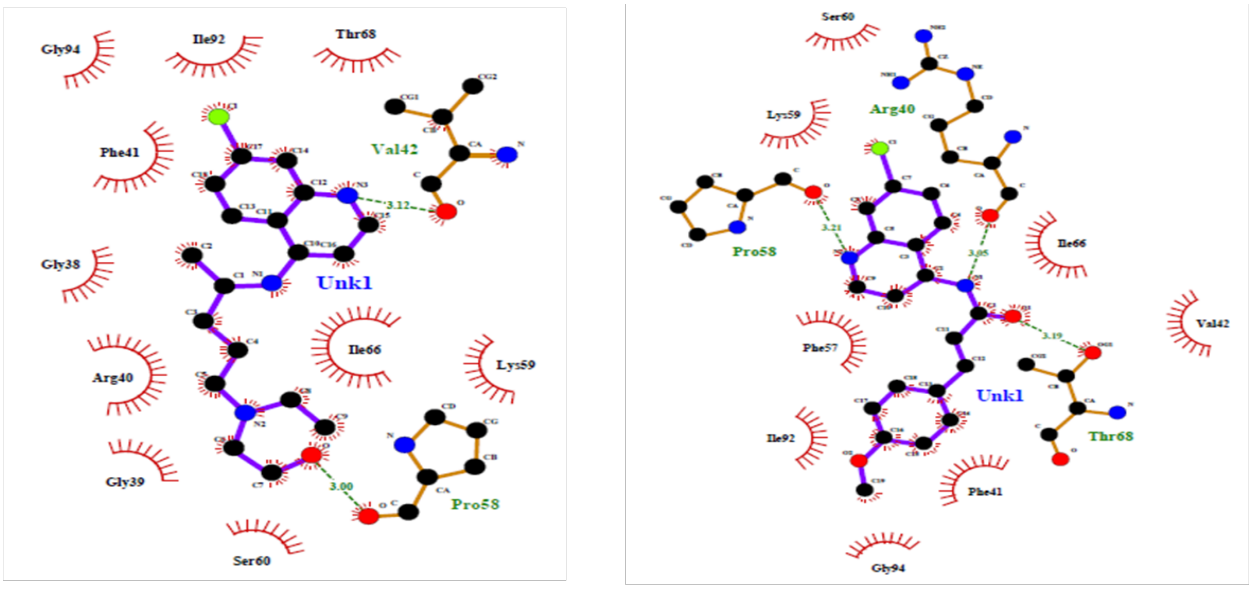


Figure 7. Hydroxychloroquine and CQN2H showing various interactions with Non-structural protein-9 (Replicase protein).

4. Discussion:

In the present work, with the use of molecular docking techniques we have studied molecular interactions between Chloroquine derivatives and selective SARS-CoV-2 viral proteins. Since most of these viral proteins are 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. This 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 viral drug targets more efficiently than Chloroquine and this opens a case for Chloroquine remodeling for better inhibition of SARS-C0V-2 multiplication.

Based on the recent reports, we have selected a few essential regulatory proteins and enzymes associated with the pathogenesis of SARS-CoV-2 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 Protein10, Non-structural Protein 9 (Replicase protein) and ADP-ribose-1 monophosphatase. In order to have a detailed understanding of interaction of Chloroquine and allied compounds with viral proteins we first identified the potential active site of these proteins and the amino acid residues involved in these active sites. Our docking studies were able to specifically identify the hydrogen bonds and van der wall interactions formed as a result of binding of drugs to the viral proteins. Since Chloroquine and its derivatives can effectively bind to active site of most of the viral proteins it might not be an overstatement to postulate that these drugs can also inhibit the natural function of these proteins.

In order to understand and evaluate the possibility of Chloroquine and its derivative Hydroxychloroquine bind to the above mentioned viral proteins we have used molecular docking techniques and study the molecular interaction of the drugs with the viral proteins. Our results clearly demonstrate that Chloroquine and its derivatives can bind to SARS-C0V-2 virus proteins and thereby can disrupt the normal functioning of these proteins. Also we 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 identification of an effective inhibitor of SARS-CoV-2 virus.

As we write this report extensive research is ongoing around the globe to discover a specific and effective drug that can halt the spread of COVID-19 pandemic. Computationally, several natural compounds, anti-viral compounds, anti-malarial drugs, antibiotics, and pharmacologically active compounds have been screened and are being investigated for their potential towards inhibiting SARS-CoV-2 infection (Elfiky, 2020; Wu et al., 2020; Fantini et al., 2020). However, to the best of our knowledge not much research has gone in to probe the effect of Chloroquine derivatives on various drug targets of coronavirus.

Both non-structural protein 3 & 5 are involved in the formation of multi-domain M protease, which has a key role in the replication process (Stobart et al., 2013). In this context, we have targeted non-structural protein3 in an attempt to terminate the life cycle of the coronavirus in the host cell. Our results indicate that Chloroquine and its derivatives can effectively bind to the NSP 3. Also, we found that Chloroquine derivatives CQN2B, CQN21D, and CQN1Acan

strongly bind to NSP3 with the binding affinity of-8.2 kcal/mol, which is even higher than the binding affinity of Hydroxychloroquine to NSP3.

The main protease is also a critical enzyme involved in the cleavage of polyprotein (PP) at the C-terminal end and leads to the formation of non-structural proteins (Linder 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 potentially inhibit its activity.

Non-structural protein12 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 including the hepatitis C virus and the Zika virus (Ganesan and Barakat, 2017; Elfiky, 2016). 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 strongly inhibits RdRp with high binding affinity (-8.2 kcal/mol) than the Hydroxychloroquine. From the results, it is evident that CQN2H is capable of acting as a potent drug molecule to inhibit RdRp function.

Spike glycoprotein plays an essential role in the attachment of coronavirus with the ACE2 receptor of the host cell. 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 derivatives act are capable of binding to Spike glycoprotein and can also potentially inhibit Spike protein interaction with the ACE-2 receptor. A recent study found that new mechanism of action for both Chloroquine and Hydroxychloroquine on spike protein, which reveals that these two drugs prevent the binding of spike protein with ACE2 receptor by interacting with sialic acids and gangliosides of the host cell surface. (Fantini et al., 2020).

Non-structural protein 9 (nsp9) acts as a replicase protein (RNA-binding protein) and the dimerization of these proteins is essential for viral propagation. 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 a 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.

Among the various drug targets of SARS-CoV-2, Chloroquine derivatives have been shown to be a promising drug in containing the SARS-CoV-2 spread. However, still these results need to be substantiated with binding experiments to ascertain other drug-protein interaction parameters. Still the specificity of these studies can be ascertained from the fact that one of the derivative of chloroquine, CQN2Fdid not show binding to any viral protein included in the study. Also, as mentioned earlier that some of the Chloroquine derivatives can be toxic in present form but from our study informed approach can be taken to understand the drug protein interaction and then further modify the drug structure which will have minimal toxic burden and maximal inhibitory potential against all of some of the viral proteins. Still, our results provide strong basic knowledge on the interaction of Chloroquine derivatives with the various drug targets of SARS-CoV-2. Further, these findings are useful for constructive research to identify a potent drug to treat COVID-19.

5. Conclusion:

From our results, we can infer that Hydroxychloroquine considerably inhibits all the drug targets of SARS-COV-2. Further, we 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.

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References:

1. 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. doi:10.1038/srep42717.
2. DeLano, W. L. (2002). The PyMOL molecular graphics system. Retrieved from http://www.pymol.org.
3. 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*, doi: 10.1016/j.ijantimicag.2020.105938.
4. Egloff, M. P., Ferron, F., Campanacci, V., Longhi, S., Rancurel, C., Dutartre H, Eric, J. S., & Alexander, E. (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 National Academy of Sciences U S A*, 2004;101:3792–6.
5. 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*, 117592.
6. Elfiky., A. A. (2016). Zika viral polymerase inhibition using anti-HCV drugs both in market and under clinical trials. *Journal of Medical Virology*, 88, 2044-2051.
7. 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*, 105960.
8. Ganesan, A., Barakat., K. (2017). Applications of computer-aided approaches in the development of hepatitis C antiviral agents. *Expert Opinion in Drug Discovery*, 12, 407-425.
9. Gao, Y., Yan, L., Huang, Y., Liu, F., Zhao, Y., Cao, L., Wang, T., Sun, Q., Ming, Z., Zhang, L. & Ge, J. (2020). Structure of the RNA-dependent RNA polymerase from COVID-19 virus. *Science*.

10. Gautret, P., Lagier, J.C., Parola, P., Meddeb, L., Mailhe, M., Doudier, B., Courjon, J., Giordanengo, V., Vieira, V.E., Dupont, H.T. & Honoré, S. (2020). Hydroxychloroquine and azithromycin as a treatment of COVID-19: results of an open-label non-randomized clinical trial. *International Journal of Antimicrobial Agents*, doi:10.1016/j.ijantimicag.2020.105949.
11. 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), e00983-18.
12. 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. doi:10.1038/s41564-020-0695-z.
13. Hu, T., Chen, C., Li, H., Dou, Y., Zhou, M., Lu, D. (2017). Structural basis for dimerization and RNA binding of avian infectious bronchitis virus nsp9. *Protein Science*, 26, 1037-48.
14. 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(W1), W478–W484. doi:10.1093/nar/gku377.
15. 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*.
16. Kim, Y., Jedrzejczak, R., Maltseva, N., Endres, M., Mececar, A., Michalska, K., Joachimiak, A. (2020) Center for Structural Genomics of Infectious Diseases (CSGID). Crystal Structure of ADP ribose phosphatase of NSP3 from SARS CoV-2. doi: 10.2210/pdb6vxs/pdb.
17. 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. doi:10.1089/vbz.2008.0049.
18. 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, 15199–208.
19. Littler, D., Gully, B., Colson, R. N., & Rossjohn, J. (2020). Crystal structure of the SARS-CoV-2 non-structural protein 9, Nsp9. *bioRxiv*.
20. Mallikarjuna Nimgampalle, Hemanth Naick Banavath, Harshini Chakravarthy, Ambrish Saxena & Vasudharani Devanathan (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*, DOI: 10.1080/07391102.2019.1686066.

21. 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*, 16, 2785–2791. doi:10.1002/jcc.21256.
22. Prajapat, M., Sarma, P., Shekhar, N., Avti, P., Sinha, S., Kaur, H., & Medhi, B. (2020). Drug targets for corona virus: A systematic review. *Indian journal of pharmacology*, 52(1), 56.
23. 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.
24. Ratia, K., Pegan, S., Takayama, J., Sleeman, K., Coughlin, M., Baliji, S., & Baker, S. C. (2008). A noncovalent class of papain-like protease/deubiquitinase inhibitors blocks SARS virus replication. *Proceedings of the National Academy of Sciences*, *105*(42), 16119-16124.
25. Savarino A, Shytaj IL. (2015). Chloroquine and beyond: exploring anti-rheumatic drugs to reduce immune hyperactivation in HIV/AIDS. *Retrovirology*, 12:51. doi:10.1186/s12977-015-0178-0.
26. Scholz, M., & Derwand, R. (2020). Does Zinc Supplementation Enhance the Clinical Efficacy of Chloroquine/Hydroxychloroquine to Win Todays Battle Against COVID-19?.
27. 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.
28. 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*.
29. Stobart, C. C., Sexton, N. R., Munjal, H., Lu, X., Molland, K. L., Tomar S. (2013). Chimeric exchange of coronavirus nsp5 proteases (3CLpro) identifies common and divergent regulatory determinants of protease activity. *Journal of Virology*, 87, 12611-8.
30. 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..

31. Touret, F., & de Lamballerie, X. (2020). Of chloroquine and COVID-19. *Antiviral research*, 104762.
32. Tricou, V., Minh, N. N., Van, T. P., Lee, S. J., Farrar, J., Wills, B., Hien, T. Tran., & Cameron, P. S. (2010). A randomized controlled trial of chloroquine for the treatment of dengue in Vietnamese adults. *PLoS neglected tropical diseases*, 4(8).
33. 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*, doi:10.1186/1743-422X-2-69.
34. Wallace A C, Laskowski R A, Thornton J M. (1996). LIGPLOT: a program to generate schematic diagrams of protein-ligand interactions. *Protein Engineering*, 8, 127-134.
35. Wee, S. L., McNeil, D. G., & Hernandez, J. C. (2020). WHO declares global emergency as Wuhan coronavirus spreads. New York Times.
36. Wrapp, D., Wang, N., Corbett, K. S., Goldsmith, J. A., Hsieh, C. L., Abiona, O., & McLellan, J. S. (2020). Cryo-EM structure of the 2019-nCoV spike in the prefusion conformation. *Science*, 367, (6483), 1260-1263.
37. Wu, C., Liu, Y., Yang, Y., Zhang, P., Zhong, W., Wang, Y., & Zheng, M. (2020). Analysis of therapeutic targets for SARS-CoV-2 and discovery of potential drugs by computational methods. *Acta Pharmaceutica Sinica B*.
38. 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.
39. Yuki, K., Fujiogi, M., & Koutsogiannaki, S. (2020). COVID-19 pathophysiology: A review. *Clinical immunology (Orlando, Fla.)*, *215*, 108427. Advance online publication. https://doi.org/10.1016/j.clim.2020.108427.