In silico analysis of modified quinine derivatives and their inhibitory implications on Covid variants

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Abstract

Corona viruses are a wide category of viruses classified as Orthoviridae. They were previously documented in the 1920s as an acute respiratory infection that causes disorders in chickens, bats and humans. Since 2019, it has posed a severe threat to the world. Many variants have emerged including Delta viruses, Omicron and others. The severity and side effects of these viral diseases are worsening by the day and no effective treatment is available. COVID-19 symptoms include fever, cough, weariness, loss of taste and smell, sour throat and other mild to severe symptoms such as difficulty in breathing or shortness of breath, loss of speech and chest pain. These viral diseases even have an impact on mortality rates. Vaccination is being utilised as a preventive measure. Because no single treatment is thought to be effective against the corona virus, antimalarial drugs such as hydroxychloroquine, chloroquine, amodiaquine, quinine, lumefantrine, mefloquine, primaquine and halofantrine were employed. There have been no studies on the modified samples of these chemicals and their efficiency against the covid virus.

As a result, the focus of this work was mostly on the utility of these anti-malarial drugs which were adjusted in silico using Gauusian view and optimised and afterward docked using Autodock vina to understand the binding affinity. The binding affinity is calculated using command prompt software. The inclusion of functional groups transformed these molecules. These modified and unmodified compounds were docked against the proteins Angiotensin-converting enzyme 2 (SARS-CoV 2) and Omicron spike protein in this study. A comparison study was also conducted between SARS-CoV 2 protein and Omicron spike protein and it was discovered that there is variation in affinity between different modified and unmodified antimalarial medicines. Docking research shows that modified Hydroxychloroquine, Chloroquine, Amodiaquine, Quinine, Lumefantrine, Mefloquine, Primaquine and Halofantrine medicines are more efficacious than unmodified ones. Additional clinical testing may be warranted.

Keywords: Covid, omicron, spike-protein, docking, *in silico*.

Introduction

Corona virus was already reported in late 1920s as acute respiratory infection of domesticated poultry in North America and was named as Infectious Bronchitis virus. Not only in poultry these viruses can cause disease in both humans and animals and can spread among camels, bats and through infected people[4,20]. Corona viruses cause diarrhea and other respiratory diseases in animals and severe problem or illness and even death in humans[7]. Novel corona virus (COVID-19) had become a serious threat around the world and was caused by a new pathogenic human severe acute respiratory syndrome corona virus 2 (SARS-COV-2) and was a member of Betacorona virus[14].

Its outburst was in Wuhan, China and the first case was reported in December, 2019[24]. In India, it was reported on January 27, 2020. From this time onwards, the severity of disease increased around the world and caused many people to lose their life. Three waves of COVID-19 worldwide included Covid-19 virus, Delta Corona virus and newly found variant named Omicron. This new variant is severely affecting the lives and is increasing the mortality rate. The major symptoms of COVID-19 include, fever, cough, tiredness, loss of taste and smell, sour throat etc. and some of the severe symptom include difficulty in beathing or shortness of breath, loss of speech and chest pain[10]. Different types of compounds have been clinically tested against corona virus but a completely effective drug was not established.

The drugs used for treatment against SARS-CoV-2 include remdesvir, molnupiravir[21], hydroxychloroquinine and chloroquine (anti-malarial drug) and various vaccines have been made by various laboratories and few were approved by WHO[15]. Many works have been carried out to find a drug that can be used to inhibit or to destroy the virus completely and now, not only *in vitro* techniques but also computational drug designing have also been widely carried out. Huang et al[9] suggested the structural and functional properties of SARS-CoV-2 spike protein.

It was found that the Covid 19 virus entry into the host is by several steps and for that, Covid virus makes use of its receptor binding domain (RBD) of the spike (S) glycoprotein which identifies the Angiotensin-converting enzyme 2 receptor which is present on the cell surface of human cells or tissues and by the fusion of host and virus membranes, viral entry is carried out[13]. To reduce the effect of pandemic, many agents are used as treatment which include the anti-inflammatory agents, anti-microbial therapies, various

vitamin supplements and drugs like remdesvir, steroids, tocilizumab have been used showing impact on patients. It has been also known that chloroquine and hydroxychloroquine were also used to prevent viral entry. Intake of vitamin C and D has also shown marked effect on the reduction of SARS CoV 2[16].

Lv et al[11] suggested that Mpro, is also termed as 3CL protease which is about 33.8 K Da cysteine protease and which carries out the maturation of functional poypeptides involved in the replication transcription machinery. Vincent et al[22] followed bioinformatic way of designing drug to identify the inhibitor of Covid 19 Main Protease 3CLPro and for that, they selected about 145 phytocompounds from Kabasura Kudineer (KK) which is effective against some symptoms of Covid-19 and they obtained results from molecular docking and found some compounds from KK plants which helped to inhibit Covid-19, some of them include acetoside (-153.06), rutin (-133.06), chebulagic acid (-123.3), myricetin (-99.96) etc.

Ebenezer et al[6] carried out to find SARS CoV efficiency experiments, which were used to test reduction of virus load in mice using molecular docking and meta-analysis and found three compounds as best which include EIDD-2801, GS-5734 and Amadiaquine.

Cava et al[3] carried out the *in silico* discovery of candidate drugs against Covid-19 and found that the genes which are corelated with ACE2 the main cell receptor of SARS-CoV2, are enriched in the sterol biosynthetic process, acetate CoA, CoA ligase activity etc. and through experiment, they found 193 genes, 222 interactions and 36 drugs have best activity. Some drugs they identified include nimesulide, fluticasone propionate, photofrin and flutamide.

No specific Covid-19 drug has been discovered so far. In this computational analysis, some of the anti-malarial quinine derivatives are modified and used as ligand against the angiotensin-converting enzyme 2 (SARS-CoV2) spike protein and omicron spike protein to understand the efficacy.

Material and Methods

Determination of Ligand: The selection of quinine derivative compounds used as ligand in this study was based on the *in vitro* and *in silico* experiments that have been conducted by other researchers recently. The information was obtained from literature and digital library. The compounds include chloroquine, hydroxychloroquine, amodiaquine, quinine, mefloquine, primaquine, lumefantrine, halofantrine.

Determination of Receptors: One SARS-CoV-2 spike protein (PDB code: 7EFP) and one omicron spike protein (PDB code:7T9J) were chosen as drug discovery targets.

Ligand and Receptor Preparation: Ligand structures were downloaded from the PubChem (Pubchem.ncbi.nlm.nih.gov. http://pubchem.ncbi.nlm.nih.gov. Published 2022.) in sdf format. The sdf format file was then converted to pdb format using Avagadro software[8]. The pdb file is opened in Autodock tool 1.5.6 (Download AutoDock4. AutoDock. https://autodock.scripps.edu/download-autodock4/. Published 2022.) and is converted to pdbqt format.

Proteins were downloaded from RCSB PDB database (Bank R. RCSB PDB: Homepage. Rcsb.org. https://www.rcsb.org. Published 2022.) in pdb format. These proteins serve as the receptor for docking purpose. Pymol software deleted water molecules and other heteroatoms (https://pymol.org/). The same software added polar hydrogens and saved the file. Open Autodock tool, for converting pdb file to pdbqt file format.

Active-site Determination: Using BIOVIA Discovery studio visualizer 2020 client, the ligand and receptor binding location were determined (https://discover.3ds.com/discovery-studio-visualizer-download). Using this a 2-D interaction plot can be drawn with this software.

Receptor ligand Docking: The docking was carried out using VINA. The pdbqt files of ligand and receptor were copied to the vina folder. Edit the conf.txt file and save. Docking was performed using command prompt.

Ligand modification, Optimization and DFT calculation: Using Gaussian 09 program via Gauss view 05 software, the ligand is modified by the addition of functional groups in different positions. Then it is optimised for getting the lowest energy compound for further analysis using the same software. Frequency calculations were performed on ground state to make sure that there are no negative frequencies. The output files were visualized using Gauss view 05 software (https://gaussview.software.informer.com/5.0/).

Results and Discussion

The ligands and anti-malarial chloroquine derivatives identified by literature review include hydroxychloroquine, chloroquine[19], amodiaquine[17,5], quinine[1], lumefantrine[23], mefloquine[18], primaquine[2] and halofantrine[12] (Table 1).

Table 1
Ligands with their compound ID

Ligands	Compound CID
Hydroxychloroquine	3652
Chloroquine	2719
Amodiaquine	2165
Quinine	3034034
Lumefantrine	6437380
Mefloquine	4046
Primaquine	4908
Halofantrine	37393

Molecular docking of these ligands with angiotensin-converting enzyme 2 SARS-CoV-2 spike protein (PDB code

7EFP) provided following data regarding affinity. Among the various unmodified ligands docked, mefloquine was found to be more effective in binding protein receptors 7EFP and 7T9J with an affinity value -9.0 and -8.6 respectively. Halofantrine, quinine, amodiaquine and lumefantrine are the other active compounds in the list which exhibited good binding affinity towards 7EFP receptor but less compared to mefloquine (table 2). Considering, 7T9J spike protein binding context, amodiaquine has an affinity value of -8.2 which is considered respectable in docking. For other ligands such as quinine, chloroquine, lumefantrine, primaquine, hydroxychloroquine, halofantrine affinity values for 7T9J spike protein are less preferable according to the docking result (Table 3).

Subsequently, the ligands were modified by adding CF_3, $COCH_3$ and $CONH_2$ functional groups in respective positions. On modification of the ligands there are quite changes in the binding affinity between the modified and unmodified ligands. In 7EFP protein receptor, there is a significant increase in binding affinity of amodiaquine CF_3, lumefantrine CF_3, lumefantrine $COCH_3$ and lumefantrine $CONH_2$ when compared to other ligands (Table 4, figure 1 and figure 3). Comparing these lumefantrine and amodiaquine modified ligands, modified lumefantrine $CONH_2$ is more preferable with a highest affinity of -9.8 and following this lumefantrine $COCH_3$-9.6, lumefantrine CF_3 -8.8 and amodiaquine CF_3 -8.9 affinity values.

Also, in 7T9J receptor protein, quinine CF_3 is found to be more effective with an affinity value of -8.8 followed by amodiaquine $COCH_3$ (-8.7), lumefantrine $COCH_3$ and mefloquine $COCH_3$ (-8.3) (Figure 2). The above values inferred that the efficiency of modified compounds is more than that of unmodified compounds (table 5).

The interaction of LumefantrineCONH2 shows ASN 277, LEU 370, THR 445, THR 371, PRO 346, PHE 274, GLU 375, GLN 442, HIS 346 residues of protein at the pocket site. The interaction of Qunine CF3 shows HIS 954, GLU 1017 ALA 766, ARG 765, ARG 1014, GLN 1010, LEU 1012, VAL 1008, ILE 1013 residues of protein at the pocket site (table 6).

Conclusion

The present study investigated the *in vitro* analysis of modified quinine derivatives and its inhibitory implications on Covid variants. Here eight quinine derivatives and twenty modified quinine derivatives were docked against the two Covid variants: 7EFP and 7T9J spike proteins for analyzing the best drug that can be substituted in place of quinine compounds like hydroxychloroquine which was mostly used for the treatment. From the analysis of the result, unmodified mefloquine was found to be the best drug that can be used against the two spike protein receptors 7EFP and 7T9J with -9.0 and -8.6 binding energy.

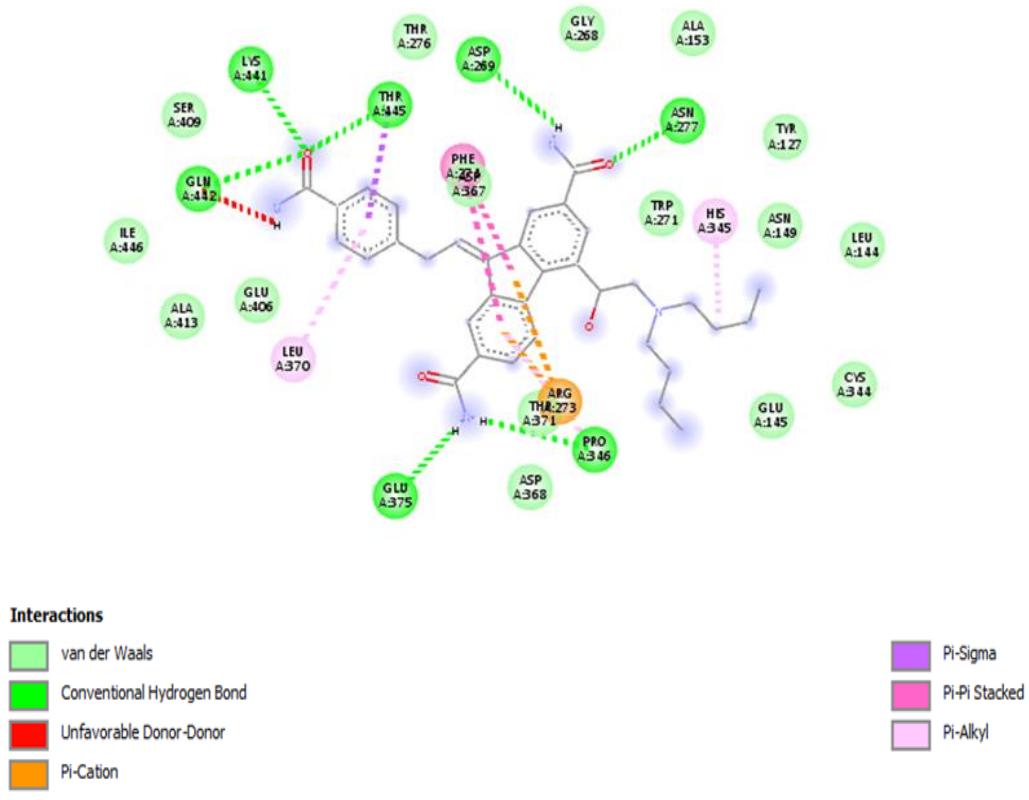
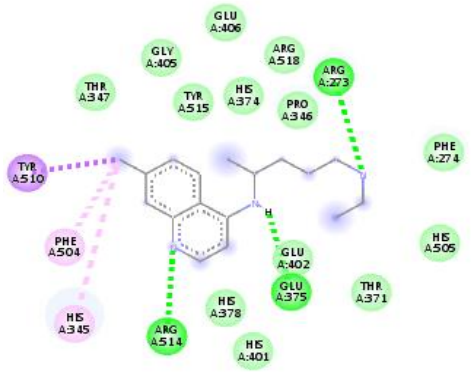
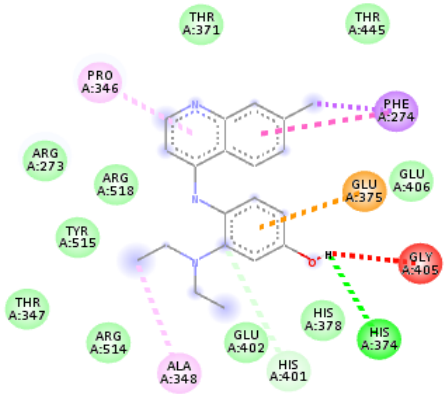
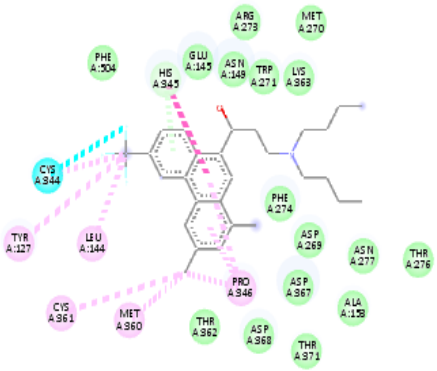
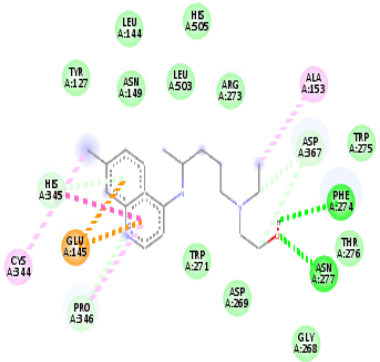


Figure 1: Showing 2-D inetraction plot of Lumefantrine CONH2 towards 7EFP protein receptor.

Table 2
Showing affinity and interaction of compounds on Angiotensin-converting enzyme 2 SARS-CoV 2 [7EFP] spike protein

Ligands	Affinity of ligands towards protein receptor [7EFP]	2D- Interaction plot
Chloroquine	-6.7	
Amodiaquine	-8.0	
Halofantrine	-8.3	
Hydroxychloroquine	-7.0	

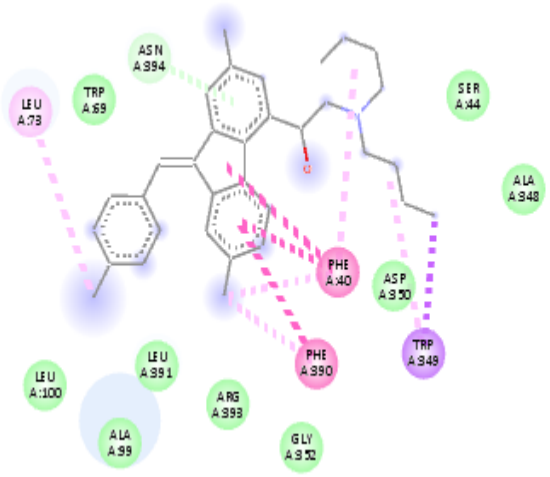
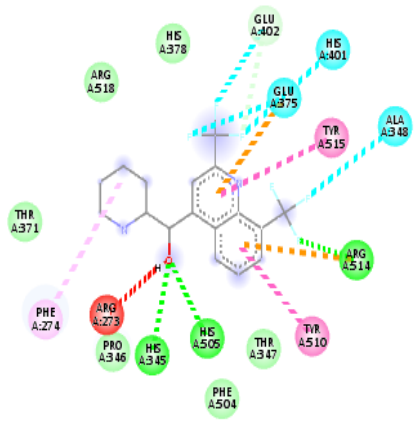
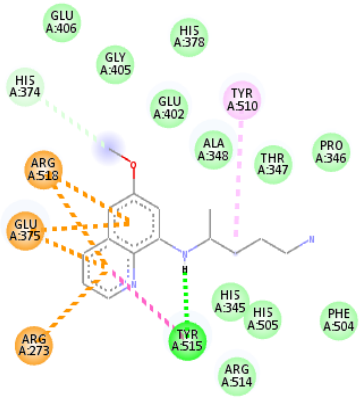
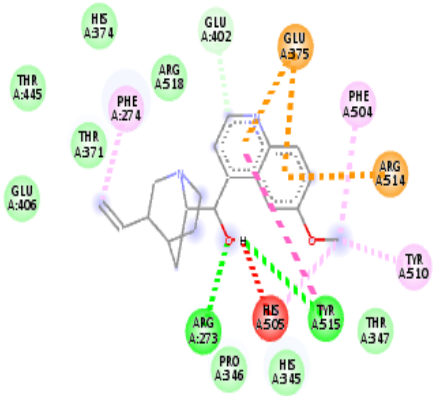
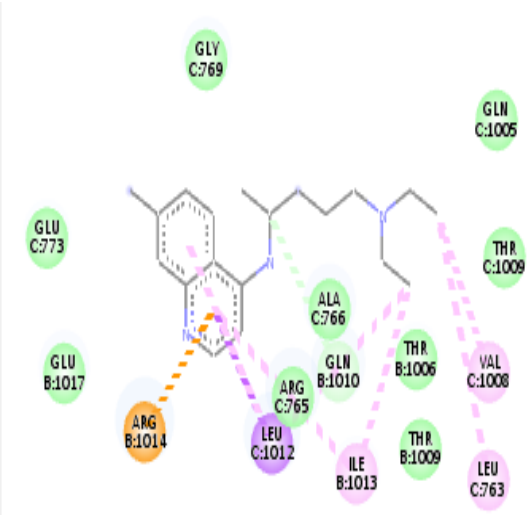
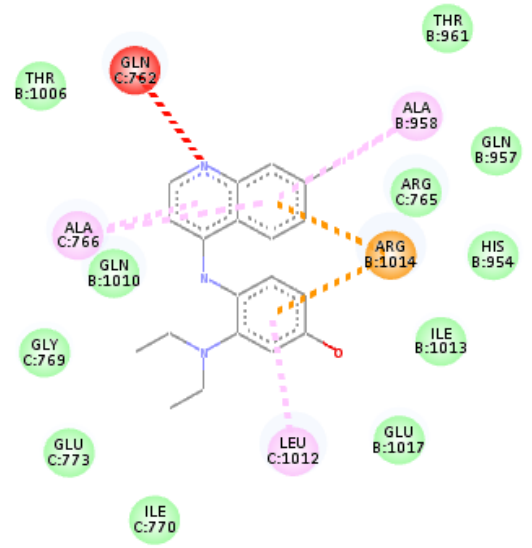
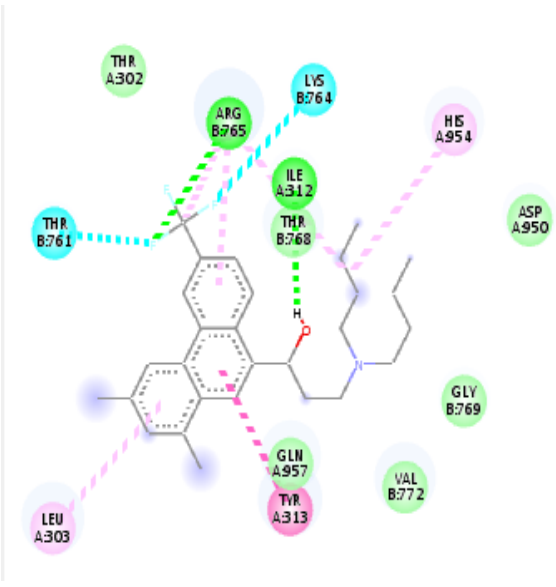
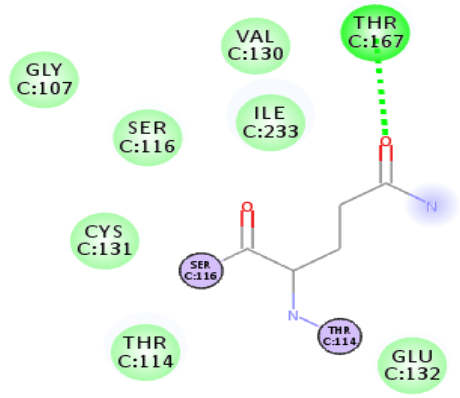
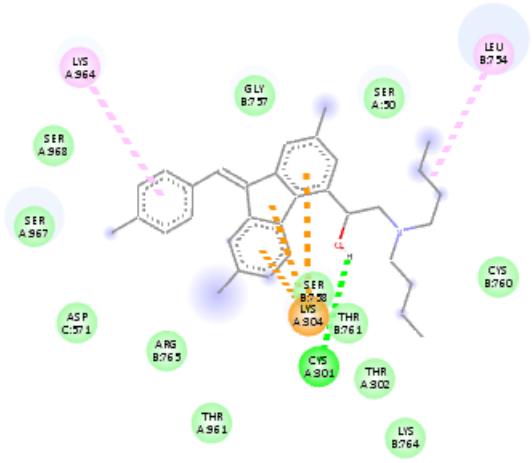
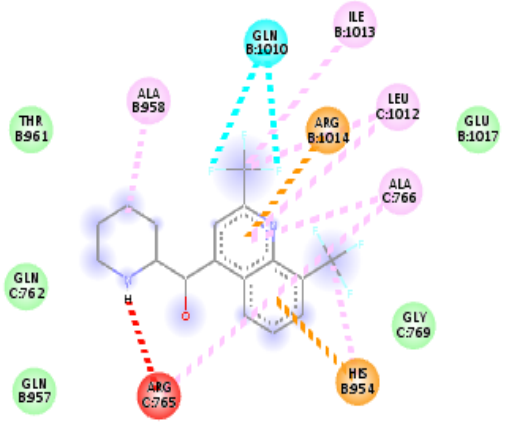
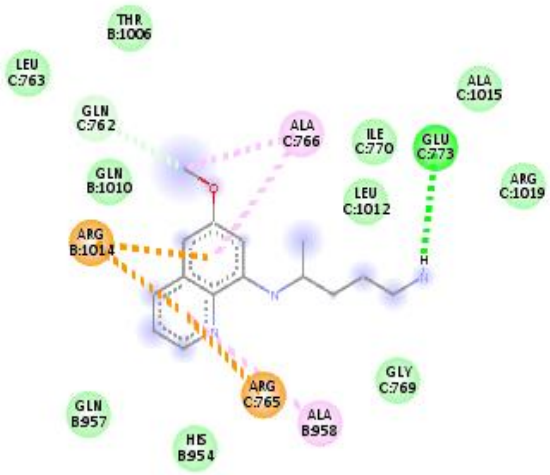
Lumefantrine	-7.9	
Mefloquine	-9.0	
Primaquine	-6.6	
Quinine	-8.1	

Table 3
Showing affinity and interaction of Omicron spike protein[7T9J]

Ligands	Affinity of ligands towards protein receptr [7T9J]	2D interaction plot
Chloroquine	-7.1	
Amodiaquine	-8.2	
Halofantrine	-6.3	

Hydroxychloroquine	-6.4	
Lumefantrine	-6.9	
Mefloquine	-8.6	
Primaquine	-6.6	

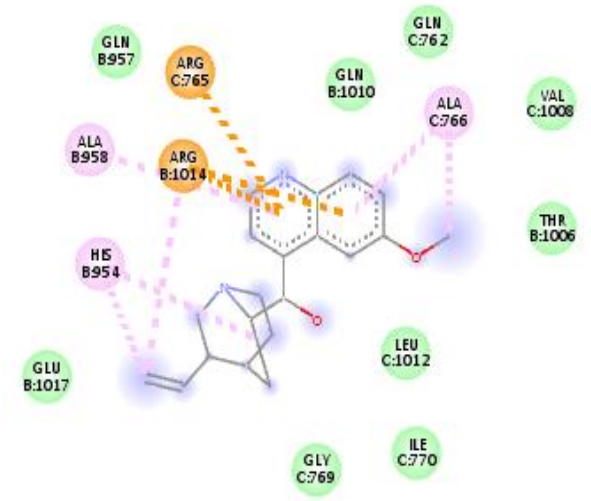
Quinine	-7.5	

Table 4
Showing affinity of unmodified and modified ligands towards Angiotensin-converting enzyme 2 (SARS-CoV 2) spike receptor protein.

Unmodified ligands	**Affinity of protein receptor [7EFP]**	**Modified ligands**	**Affinity of protein receptor [7EFP]**
Hydroxychloroquine	-7.0	Hydroxychloroquine CF3 Hydroxychloroquine COCH3 Hydroxychloroquine CONH2	-7.7 -6.9 -7.1
Chloroquine	-6.7	Chloroquine CF3 Chloroquine COCH3 Chloroquine CONH2	-6.5 -7.1 -6.9
Amodiaquine	-8.0	Amodiaquine CF3 Amodiaquine COCH3 Amodiaquine CONH2	-8.9 -7.8 -7.8
Quinine	-8.1	Quinine CF3 Quinine COCH3 Quinine CONH2	-8.5 -8.5 -7.9
Lumefantrine	-7.9	Lumefantrine CF3 Lumefantrine COCH3 Lumefantrine CONH2	-8.8 -9.6 -9.8
Mefloquine	-9.0	Mefloquine COCH3 Mefloquine CONH2	-8.8 -8.8
Primaquine	-6.6	Primaquine CF3 Primaquine COCH3 Primaquine CONH2	-6.9 -6.7 -7.1
Halofantrine	-8.3	----------	--------

Table 5
Showing affinity of unmodified and modified ligands towards Omicron spike protein.

Unmodified ligands	Affinity of protein receptor [7T9J]	Modified ligands	Affinity of protein receptor [7T9J]
Hydroxychloroquine	-6.4	Hydroxychloroquine CF3 Hydroxychloroquine COCH3 Hydroxychloroquine CONH2	-6.8 -6.9 -6.9
Chloroquine	-7.1	Chloroquine CF3 Chloroquine COCH3 Chloroquine CONH2	-6.8 -6.6 -7.2
Amodiaquine	-8.2	Amodiaquine CF3 Amodiaquine COCH3 Amodiaquine CONH2	-7.2 -8.7 -7.4
Quinine	-7.5	Quinine CF3 Quinine COCH3 Quinine CONH2	-8.8 -6.8 -7.5
Lumefantrine	-6.9	Lumefantrine CF3 Lumefantrine COCH3 Lumefantrine CONH2	-7.7 -8.3 -7.4
Mefloquine	-8.6	Mefloquine COCH3 Mefloquine CONH2	-8.3 -7.6
Primaquine	-6.6	Primaquine CF3 Primaquine COCH3 Primaquine CONH2	-6.9 -6.9 -7.0
Halofantrine	-6.3	----------	--------

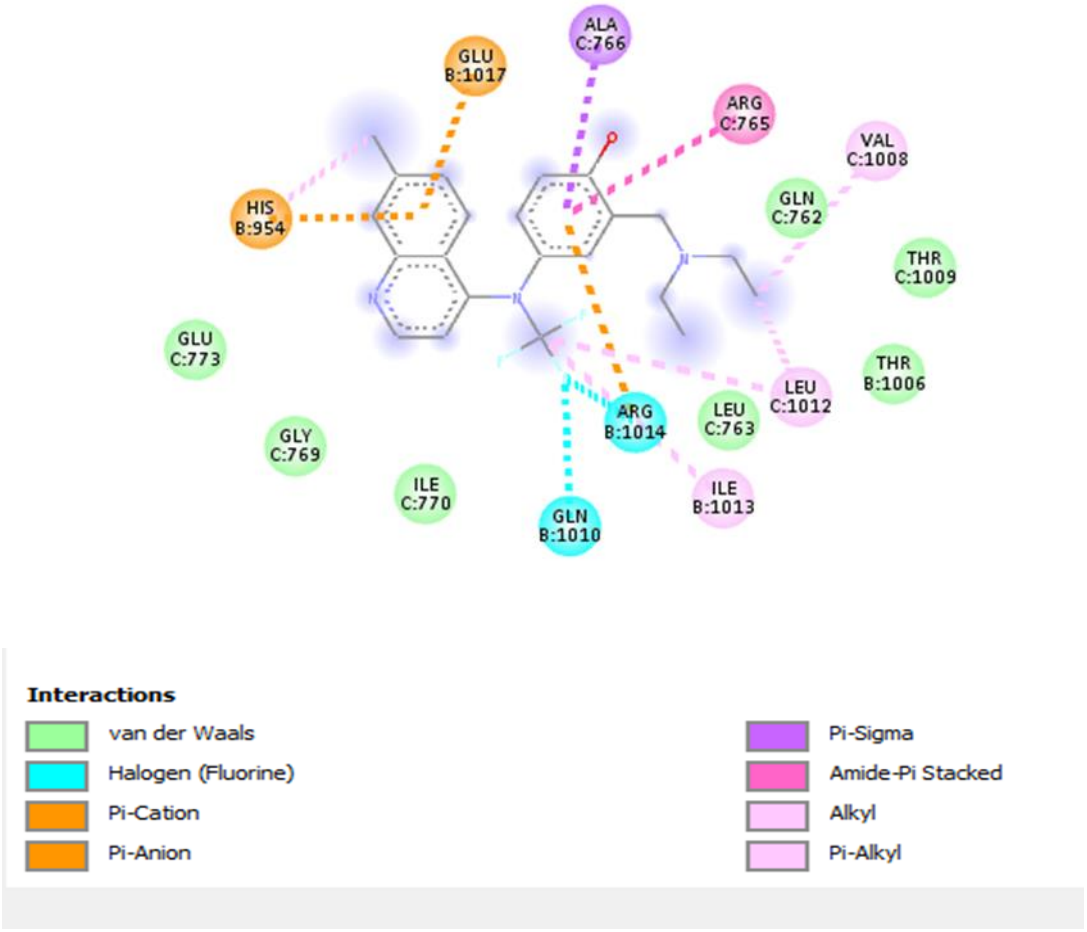


Figure 2: Showing 2-D interaction plot of Qunine CF3 towards 7T9J receptor protein.

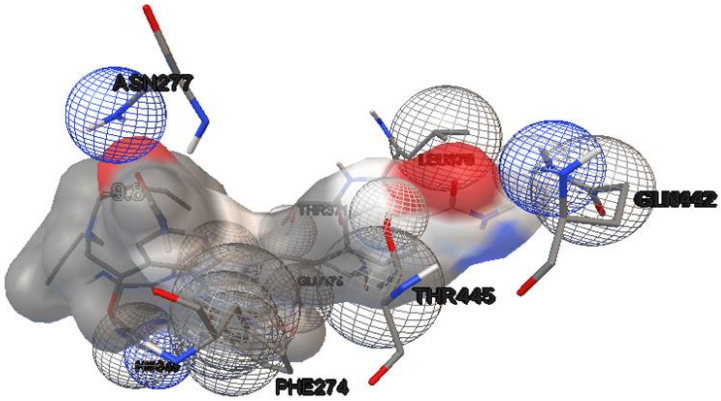


Figure 3: Interation of LumefantrineCONH2 towards 7EFP protein receptor

Table 6
Showing amino acid residues on interaction of highly effective ligands towards respective protein receptor.

Result analysis software	Protein	Ligand	Docking score	Amino acid residue
Autodock Vina	7EFP	LumefantrineCONH2	-9.8	ASN 277, LEU 370, THR 445, THR 371, PRO 346, PHE 274, GLU 375, GLN 442, HIS 346
Autodock Vina	7T9J	QunineCF3	-8.8	HIS 954, GLU 1017 ALA 766, ARG 765, ARG 1014, GLN 1010, LEU 1012, VAL 1008, ILE 1013

Modified Lumefantrine CONH2 and modified QunineCF3 show best binding energy among the modified compounds against 7EFP and 7T9J with -9.8 and -8.8 values. So, these compounds can be substituted for the treatment against Covid-19. Additional clinical testing may be warranted.

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