In silico docking studies of antimalarial drug Hydroxychloroquine to SARS-CoV proteins :An Emerging pandemic worldwide

Priyanka H. Jokhakar[1], Rishee K. Kalaria[2*], Hiren K. Patel[3]

[1]Department of Biosciences, Veer Narmad South Gujarat University, Surat (Gujarat)-395007

[2]ASPEE Shakilam Biotechnology Institute, Navsari Agricultural University, Surat (Gujarat)-395007

[3]School of Science, P. P. Savani University, Surat (Gujarat)-394125

* **Corresponding author:** risheekal@nau.in (ORCID-0000-0002-4481-3834)

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Abstract

This computational study comprises screening and prediction of interaction of selected antimalarial drug hydroxychloroquine with targeted two proteins of coronavirus. One is SARS enveloped E pantameric ion channel protein and another is SARS-CoV-2 main apoprotein protease. Both are vital for viral attachment and entry to the host cell for infection. After molecular protein docking with different confirmations, stable interacting complex of ligand and macromolecules were obtained. Interacting Lysine, Threonine and Tyrosine of E protein were found for participation of stable interaction with selected drug having docking affinity energy of -6.3kcal/mol. For apoprotein protease stable confirmation was screened out having bonding Threonine residue with same drug of energy -6.0 kcal/mol. Irreversible covalent bond formation and van der Waals interaction favours the selectivity and stability of both targeted proteins towards selected drug. Conventional as well as hydrophobic interactions are found in Ligplot and Discovery studio analysis also indicates stabilized confirmations between ligand and drug. Thus, this study delivers the putative mechanism of the drug interactions to target proteins hence comprising landmark

for future investigation for antimalarial hydroxychloroquine as anti COVID 19 drug in this experimental time.

Introduction

Numerous human viral infections are an outcome of a zoonotic event. A portion of the illnesses brought about by these zoonotic event have influenced a large number of individuals around the globe, some of which have brought about high paces of morbidity /mortality in people. The latest of these zoonotic occasions that the novel coronavirus (SARS-CoV-2) is a human pathogen as of late developed in China, causing a worldwide pandemic of severe respiratory illness (COVID19). In Wuhan, China was first reported to the WHO Country Office in China with respect to SARS-CoV-2. The quickly spreading, profoundly infectious and pathogenic SARS-coronavirus 2 (SARS-CoV-2) related Coronavirus Disease 2019 (COVID-19) has been pronounced as a pandemic by the World Health Organization (WHO). On May 02, 2020, All over the world all out 33,63,945 affirmed case and 2,37,458 passing was accounted for (https://mohfw.gov.in and WHO). Transmission of disease is due to either clustering or in sporadic (WHO report COVID 19). Finding the suitable candidate drug for the disease is an urgent need of this time. The one of the active part of pathogenesis of SARS is envelope E protein, which is more appropriate therapeutic target to developed drug and vaccine to combat COVID-19 (Surya et al., 2018). Additionally, main protease of SARS-CoV-2, in apo form is also likely to serve as a target receptor. Many countries are considering Hydroxychloroquine (HCQ) as a potential drug for the treatment of the disease. HCQ is approved as an anti-malarial drug, which also can be used for treatment of diabetic patients and is in clinical trial, for curing of SARS-CoV-2. Some in vitro studies indicate that it can inhibit entry and growth of coronavirus better than chloroquine (Singh et al., 2020). However, the exact process by which it hampers the virus' efficacy is not understood. In silico studies, the molecules offer insights into the chosen mechanism of action (Bhatt et al., 2017). With aim to identify potential drug against COVID-19, *In silico* study was done by interaction of the HCQ with SARS-CoV proteins such as envelop protein and main protease (in apo form) in order to understand the mechanism.

Material and Methods

Proteins Structures

Protein three dimensional structures of SARS envelop protein and SARS-CoV-2 main protease, in apo form (pdb id 5X29 and 6M03) were retrieved from PDB database in pdb format. Further water molecules were delete and hydrogen atoms added. Protein simulation was done by adding CHARMm force field and MMFF94 partial charges, bound ligand molecules were removed using Discovery Studio Visualizer 4.1.

Structure of Hydroxychloroquine

Three Dimensional SDF structure of HCQ was retrieved from PubChem database (https://pubchem.ncbi.nlm.nih. gov/). The conversion of these ligand file format was done using OpenBabel tool from sdf to pdb as needed for further procedures.

Docking

PyRx tool was use to dock the target proteins with ligand HCQ (Trott & Olson, 2010). PyRx is a virtual screening tool that uses Autodock Vina's enhanced features. Further, Autodock files are created in PyRx for target proteins and ligands (O'Boyle et al., 2011). Each macromolecule was docked separately with the ligand molecule. The docked conformations were obtained in PDB format and further visualized in PyMol. Discovery studio 4.0 and Ligplot were utilized to evaluate the docking sites identification.

Results and Discussion

Knowledge based method was used for scoring of drug and target protein interaction screening. For inhibition of targeted E protein (5X29) several sites has been studied. From all nine confirmations obtained after docking binding of amino acid residue Tyrosine 57 and hydroxychlorquine shows stable confirmation with affinity -6.3 kcal/mol through van der Waals bond. In another binding was found between amino acid residue of Threonine 35 and Lysine 60 with hydroxychloroquine through covalent bond. Both residues bind with selected drug through pi interaction (Figure 1a). Other obtained poses shows interaction of drug with selected target E protein through Phenylalanine 26, Tyrosine

57, Phenylalanine 23, Alanine 40, Arginine 61, Serine 60 and Alanine 22 (Supplementary data: Figure 3 and Figure 4). The ligplot is a tool to visualize atomic interactions between ligand and protein residues. The interactions shown are those mediated by hydrogen bonds and hydrophobic contacts. Pro 45, Thr 35, Leu 31, Tyr 57, Arg 61, Ala 40 and Ser 60 residues of 5X29 were involved in hydrophobic bonding with the ligand HCQ (Figure 1b).

Figure 1.(a): Modeled 5X29 SARS corona virus enveloped (E) protein docked with drug hydroxychloroquine (green colored residues of amino acids indicate van der Waals interaction and purple color indicate covalent interaction of target protein with the drug)

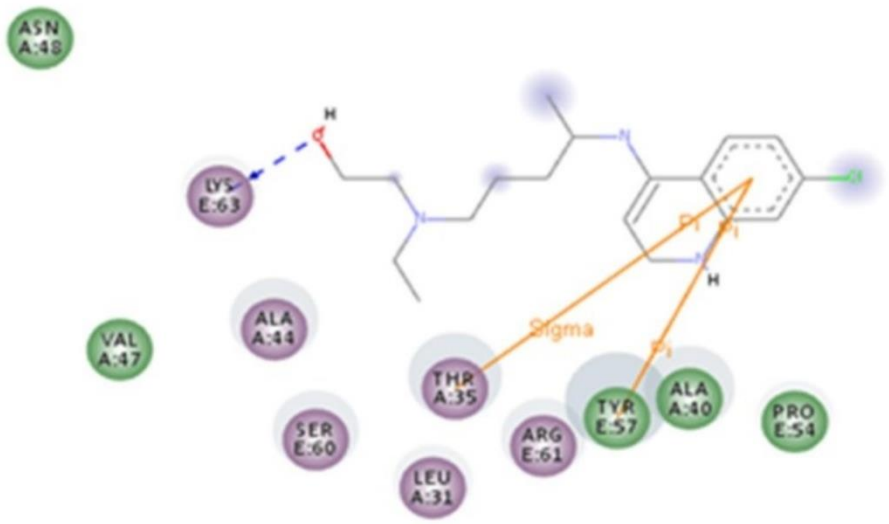
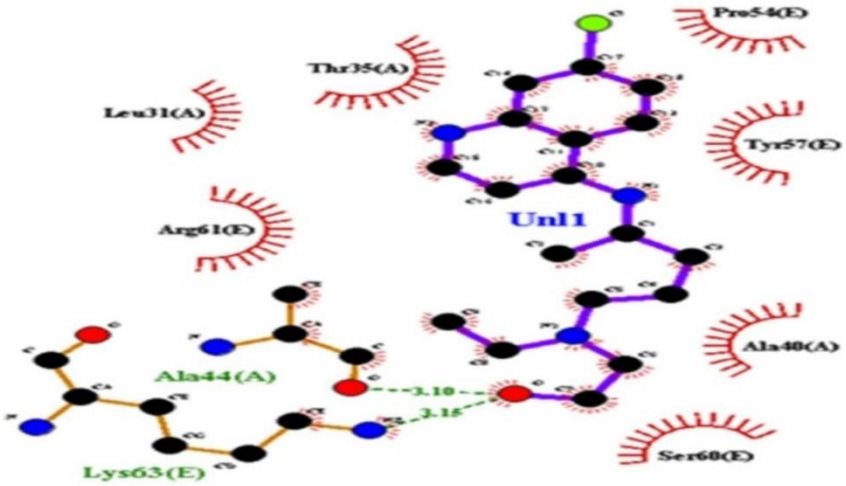


Figure 1.(b): Ligplot of molecular docking of targeted E protein with hydroxychloroquine (dashed lines indicate hydrogen bond between the atoms involved, while hydrophobic contacts are represented by an arc with spoke radiating towards the ligand atoms they contact. The contacted atoms are shown with spoke radiating back**)**



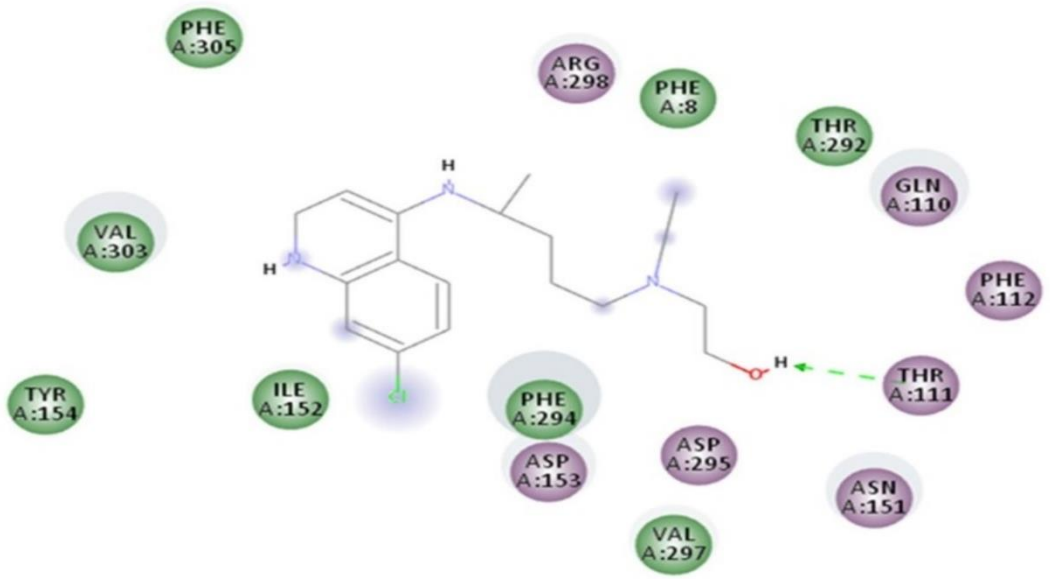
Another targated protease protein in apo form (6M03) of COVID 19 shows most stabilized form after binding with the selected drug through Threonine 111 residue by covalent bond interaction with -6 kcal/mol docking affinity (Figure 2a). Other docked poses indicate interaction of hydroxychloroquine with apo protease by binding through Isoleucine 152, Threonine 111, Phenylalanine 294, Aspartate 295, Serine 158, Isoleucine 152, Tyrosine 237, Arginine 131, Serine 144 and Threonine 25 amino acid residues (supplementary data). Ligplot analysis suggest Asp 153, Ile 152, Val 303, Phe 305, Arg 298, Phe 8, Phe 294, Glu 110 and Asp 295 of 6M03 interact with HCQ through conventional and hydrophobic interaction (Figure 2b). Hydrophobic interactions between drug and targeted protein suggest increasing biological activity. Drug with low affinity can also efficiently work due to presence of hydrophobic interactions in some diseased conditions. Hydrogen bond can optimize hydrophobic interaction. Weak interactions stabilize ligand in terms of energy and improve drug efficiency (Patil et al., 2010). Presence of hydrogen bonds in various positions indicate the efficacy of the ligand enhance the binding. The following Table: 1 shows the comparative energy value and the interaction between amino acid residues obtained for selected drug hydroxychloroquine and the two proteins targeted (1) enveloped E protein of SARS Corona virus (5X29) and (2) COVID 19 main apo protease (6M03). Covalent bond formation between selected drug and both targeted protein's amino acids residues indicate irreversible binding advantage in

pharmacological studies. Covalent bond interactions also signify target specificity and prolonged time of interaction (Singh et al., 2011).

Table 1. Comparative parameters for selected two different targets and a common ligand

Sr. No.	Targeted protein	Binding/ Docking affinity	Amino acid residues	Interaction type
1.	SARS enveloped E protein (pentameric ion channel)	-6.3 kcal/mol	Lysine 63 Threonine 35 Tyrosine 57	Covalent bond Van der Waals bond
2.	COVID 19/ SARS-CoV-2 main apo protein protease	-6.0 kcal/mol	Threonine 111	Covalent bond

Figure 2. (a): Modeled 6M03 COVID 19 main protease protein docked with drug hydroxychloroquine



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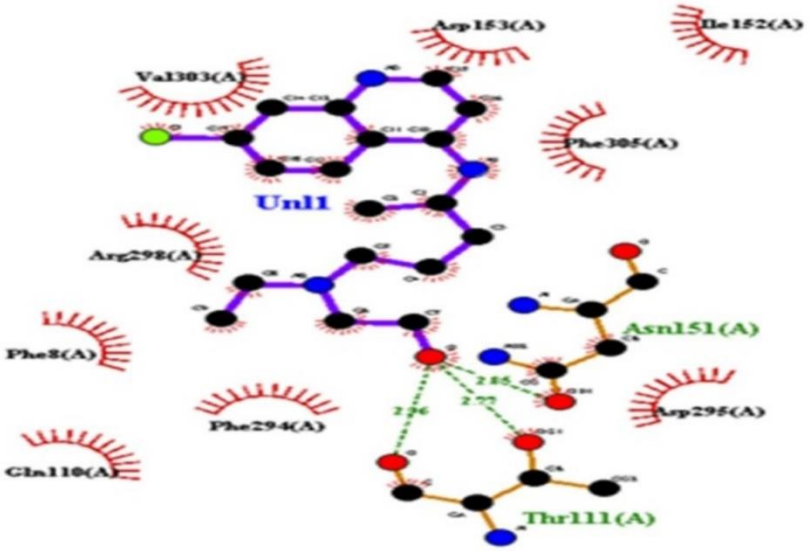
124 **Figure 2. (b):** Ligplot of molecular docking of targeted protease protein with hydroxychloroquine



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126 **Conclusion**

127 COVID 19 which is officially named as SARS-CoV-2 is recently becoming
128 pandemic with destructions in various areas of living and nonliving aspects. To order to
129 maintain control over it, various drugs were studied at the clinical level using both methods

of cell culture and the silico approach. According to the docking interpretation selected HCQ can interact successfully with E protein and protease protein by forming a covalent bond with residues of Lysine 63, Threonine 35 and Threonine 111. It also binds successfully to E protein through the interaction of Threonine residue with van der Waals. These interactions are with minimal energy, suggested strong confirmatory presence over others. First hydroxychloroquine was synthesised in the year 1950. From 1955 FDA has been approved use of hydroxychloroquine for medicinal purpose (Schrezenmeier & Dörner, 2020). This *in silico* computational approach indicates that HCQ can successfully modifies crucial residues which possibly lead to deteriorated virulence and inhibition from penetration in the cell. Some studies indicate that HCQ can inhibit viral growth through modification of host cells also. Exact mechanism of selected drug hydroxychloroquine over our target proteins is not known, but studies indicate that it prevent glycosylation of angiotensin converting enzyme 2, which is receptor molecule of SARS-CoV-2 on host cells. Due to inhibition of such modification spike proteins of the virus cannot attach to the host cells (Wang et al., 2020). For entry of virus spike attachment and adherence to host cell should be done successfully, in turn spike proteins require activated protease (Mousavizadeh & Ghasemi, 2020). Stable binding of selected drug with protease in apo form results in inactivation of proteases. As hydroxychloroquine can affect both host cells modifications and targeted proteins of virus by binding with amino acid residues it can be proven effective and potential drug to treat emerging SARS-CoV-2 disease.

References

Bhatt, M.H., Prajapati, C.K., Reddy, M. (2017). In silico docking studies of Lupeol with MAPK pathway proteins-Raf-1, MEK & ERK. Journal of experimental therapeutics & oncology, Vol.12,No.2.,pp.137-140.

Mousavizadeh, L., Ghasemi, S. (2020). Genotype and phenotype of COVID-19: Their roles in pathogenesis. Journal of Microbiology, Immunology and Infection. (In press)

O'Boyle, N.M., Banck, M., James, C.A., Morley, C., Vandermeersch, T., Hutchison, G.R. (2011). Open Babel: An open chemical toolbox. Journal of cheminformatics, Vol.3, No.1, pp.33.

Patil, R., Das, S., Stanley, A., Yadav, L., Sudhakar, A., Varma, A.K. 2010. Optimized hydrophobic interactions and hydrogen bonding at the target-ligand interface leads the pathways of drug-designing. PloS one, Vol.5, No.8.pp.1-10.

Schrezenmeier, E., Dörner, T. 2020. Mechanisms of action of hydroxychloroquine and chloroquine: implications for rheumatology. Nature Reviews Rheumatology, 1-12.

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. (In press)

Singh, J., Petter, R.C., Baillie, T.A., Whitty, A. 2011. The resurgence of covalent drugs. Nature reviews Drug discovery, Vol.10, No.4,pp. 307-317.

Surya, W., Li, Y., Torres, J. 2018. Structural model of the SARS coronavirus E channel in LMPG micelles. Biochimica et Biophysica Acta (BBA)-Biomembranes, Vol.1860, No.6, pp. 1309-1317.

Trott, O., Olson, A.J. 2010. AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. Journal of computational chemistry, Vol. 31, No.2,pp. 455-461.

Wang, M., Cao, R., Zhang, L., Yang, X., Liu, J., Xu, M., Shi, Z., Hu, Z., Zhong, W., Xiao, G. 2020. Remdesivir and chloroquine effectively inhibit the recently emerged novel coronavirus (2019-nCoV) in vitro. Cell research, Vol.30, No.3, pp.269-271.