Hydroxychloroquine as Potent Inhibitor of COVID-19 Main Protease: Grid Based Docking Approach

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Abstract

Objectives: Coronavirus (COVID-19) is an enveloped RNA virus that occurs in various forms in humans and wildlife. A total of six disease-causing species have been identified in humans. Viral infections play a vital role in human diseases, and recent outbreaks have developed globally in the form of novel corona. The S5-RNA virus from the enveloped coronavirus family caused SARS (Severe Acute Respiratory Syndrome), a life-threatening viral infection. In many countries around the world, the spread of infection is rapid. As of March 26, 2020, there were 462,684 confirmed cases globally, and 20,834 deaths were recorded. The World Health Organization (WHO) described COVID-19 as a pandemic on March 11, 2020. There are numerous drug trials going on with some positive results. However, since no vaccine is available, the best way to combat the virus is to use preventive methods.

Methods: In this study, an attempt was made to find the new COVID-19 main protease inhibitor with a molecular docking approach. A grid-based docking approach was chosen to find the binding using VLife MDS software. The 2D structure of the compounds was created and then converted into the 3D, and then, it was energetically minimized up to the RMS gradient of 0.01, using the Merck Molecular Force Field (MMFF). By using cavity determination option, the enzyme’s cavities were determined. Cavity no.1 was selected for docking. The active site for docking was defined as all atoms within 5 Å radius.

Results: Hydroxychloroquine is a slow-acting antirheumatic drug. The value of hydroxychloroquine is analogous to that reported for other disease-modifying anti-rheumatic drugs. The docking score obtained was -4.308880 and the number of receptor atoms was 77, while the number of ligand atoms was 20, which shows that hydroxychloroquine binds effectively with Covid-19 protease.

Conclusion: Hydroxychloroquine was taken as drug following Lipinski’s rule of five, so it had a very good drug score and drug-likeness score as well. This study reveals that Hydroxychloroquine has good binding affinity with COVID-19 protease and thus can be used as prophylaxis and therapeutic treatment for corona patients.

Keywords: COVID-19, Hydroxychloroquine, Molecular Docking & Prevention measures

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cases and 13071 deaths reported from coronavirus.[1] The treatment of coronavirus-associated SARS has been still developing and consequently is no consent on an optimal regimen. The predictable therapeutic interventions for SARS involve broad-spectrum antibiotics and supportive care, as well as antiviral agents and immune-modulation therapy.[2] This time, nearly a decade after SARS, another highly pathogenic CoV, Middle East Respiratory Syndrome Coronavirus (MERS-CoV) appeared in the Middle Eastern countries.[3] Coronaviruses (CoVs) are the main group of viruses belonging to the order Nidovirales, which includes Coronaviridae, Arteriviridae, and Roniviridae families.[4] Coronavirus (Fig. 1) is an enveloped and single-stranded ribonucleic acid with 9-12 nm-long surface spikes. Various symptoms include fever, cough and shortness of breath.[5]

**Mode of transmission**[6]

The human-to-human transmission of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is thought to occur mainly through respiratory droplets. Infection (Fig. 2) can also occur when people touch their eyes, nose, or mouth after touching an infected surface.

**Co-morbidity associated with CO-V 19**

A logical analysis of CoV cases suggests that diabetes and hypertension are equally ubiquitous in approximately 50% of the patients. % of cases have CHD and 16% have obesity. These conditions downregulate the synthesis of proinflammatory cytokines and damage the innate and humoral immune systems of the host.[7]

- For prevention of COVID-19[8]
- Wash your hands; Use a hand sanitizer that contains at least 60% alcohol
- keep away from touching your eyes, nose, and mouth with unwashed hands
- Avoid close contact
- Cover your mouth and nose
- Wear a facemask
- Clean AND disinfect frequently touched surfaces daily

Symptoms are mainly like influenza and include fever, malaise, myalgia, headache, diarrhea, and shivering (rigors). Although fever is the most frequently reported symptom, it is sometimes not found on initial measurement, especially in elderly and immunocompromised patients.[9]

**Measures to prevent COVID-19**

Social distancing measures should be implemented by Govt. of India for spreading the epidemic. This can help disrupt the chains of human-to-human transmission.

- Immediate isolation of suspected or confirmed symptomatic persons.
- Suspension of the mass gathering.
- Social distancing events at workplaces

Hydroxychloroquine (Fig. 3) is known as disease modifying drugs[10]. Hydroxychloroquine sulfate chemically known as 2-[[4-[(7-Chloro-4-quinolyl)amino]pentyl] ethylamino]
ethanol sulfate (1:1) having molecular weight 433.95 and molecular formula is $C_{18}H_{26}ClN_{3}H_{2}SO_{4}$. It is used in Malaria, Lupus Erythematosus & Rheumatoid Arthritis.\textsuperscript{[11]}

**Experimental Works:**

In this research study, hydroxychloroquine binding affinity with Covid-19 main protease was accessed through Grid-Based Docking studies by using VLife MDS software.\textsuperscript{[12]} Covid-19 main protease structure was downloaded from RCSB Protein Data Bank (PDB ID: 6LU7)\textsuperscript{[13]} and saved in PDB file format. The structure was present in the complex with an inhibitor N3, therefore the ligand was first removed and then the protease structure was used for further docking studies. Hydroxychloroquine structure was drawn using Marvin JS (Chem Axon) at RCSB PDB and it was also saved in PDB file format. The structure of Hydroxychloroquine and Covid-19 main protease is shown in figure 4 and figure 5.

Docking studies were performed by using the Biopredicta software tool, where grid-based docking was made by selecting Hydroxychloroquine as the ligand molecule and Covid-19 main protease as the receptor molecule. The cavity number was set as 1, the angle of rotation was set as 25.0, and then the docking score was calculated. After successful completion of the docking process, a docked complex was obtained, as shown in figure 6.

![Figure 4. Hydroxychloroquine.](image1)

![Figure 5. Crystal Structure of Covid 19 main protease.](image2)

![Figure 6. Structures of Covid 19 Protease and Hydroxychloroquine before docking.](image3)

![Figure 7. Docked Complex (Hydroxychloroquine is shown in golden colour ball and stick model).](image4)

![Figure 8. Hydrophobic Interaction.](image5)
complex was formed and then the interaction between the ligand and receptor molecule was detected.

**Result and Discussion**

The current docking studies revealed that Hydroxychloroquine has a good binding affinity for Covid-19 main protease (Figs. 6, 7). The resulting docking score was -4.308880 and the number of receptor atoms was 77, while the number of ligand atoms was 20, indicating that Hydroxychloroquine binds effectively with Covid-19 protease. The binding affinity was confirmed by hydrophobic, charged and Van der Waals forces.

**Figure 9.** Van der Waals Interaction.

**Figure 11.** Cavity Surface with Ligand and Receptor Atoms.

**Figure 10.** Charge Interaction.

**Figure 12.** Cavity points Mapped properties and Grid Box.

**Figure 13.** Ramachandran Plot for Ligand-Receptor Complex.
type of interaction between ligand and receptor molecules with a total of 123 interactions. Amino acid residues actively involved in binding with ligand atoms were THR (Threonine), HIS (Histidine), MET (Methionine), ASN (Asparagine), ASP (Aspartic acid), ARG (Arginine), GLN (Glutamine), CYS (Cysteine), PRO (Proline), TYR (Tyrosine) and all of these showed different types of interactions. Detailed data on main interactions with residue atom, ligand atom and distance are shown in Table: 1 and Figure 8, Figure 9, Figure 10, Figure 11, Figure 12 and Figure 13 for various types of interactions.

Conclusion
The 2019-Novel coronavirus (nCoV) is the main source of disaster in the 21st century. However, the lack of specific drugs to prevent/treat an attack is a major need at this time. Drug discovery against the CoV is a challenging task owing to recurrent recombination events. Developing a vaccine is another important issue. However, preventive measures need to be taken to spreading the SARs. From current molecular docking studies, it is concluded that hydroxychloroquine may act as a preventive drug for the treatment of SARS, as it acts as a potent inhibitor of the Covid-19 main protease and shows good binding affinity with the macromolecule with a very good docking score and various binding interactions. Prophylactic and therapeutic treatment can be done using Hydroxychloroquine to combat Covid-19 infections.

Disclosures
Ethics Committee Approval: The study was approved by the Local Ethics Committee.
Peer-review: Externally peer-reviewed.
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Authorship Contributions: Concept – J.K.M.; Design – H.S.; Supervision – J.K.M.; Materials – S.S.; Data collection &/or processing – J.K.M.; Analysis and/or interpretation – S.S.; Literature search – H.S.; Writing – S.S.; Critical review – J.K.M.

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| Residue Atom | Ligand Atom | Distance | Interaction Type |
|--------------|-------------|----------|------------------|
| THR25A 197C  | 19C         | 4.934    | HYDROPHOBIC_INTERACTION |
| THR25A 197C  | 20C         | 3.754    | HYDROPHOBIC_INTERACTION |
| THR25A 200C  | 19C         | 4.927    | HYDROPHOBIC_INTERACTION |
| THR25A 200C  | 20C         | 4.065    | HYDROPHOBIC_INTERACTION |
| THR25A 202C  | 19C         | 4.187    | HYDROPHOBIC_INTERACTION |
| THR25A 202C  | 20C         | 3.766    | HYDROPHOBIC_INTERACTION |
| THR26A 209C  | 22C         | 4.708    | HYDROPHOBIC_INTERACTION |
| HIS41A 330C  | 10C         | 4.949    | HYDROPHOBIC_INTERACTION |
| HIS41A 330C  | 11C         | 4.100    | HYDROPHOBIC_INTERACTION |
| MET49A 388C  | 3C          | 4.442    | HYDROPHOBIC_INTERACTION |
| MET49A 388C  | 11C         | 4.054    | HYDROPHOBIC_INTERACTION |
| MET49A 391C  | 3C          | 4.215    | HYDROPHOBIC_INTERACTION |
| MET49A 391C  | 4C          | 4.847    | HYDROPHOBIC_INTERACTION |
| MET49A 391C  | 9C          | 4.766    | HYDROPHOBIC_INTERACTION |
| MET49A 391C  | 10C         | 4.025    | HYDROPHOBIC_INTERACTION |
| MET49A 391C  | 11C         | 3.235    | HYDROPHOBIC_INTERACTION |
| MET49A 391C  | 14C         | 4.746    | HYDROPHOBIC_INTERACTION |
| MET49A 392C  | 3C          | 4.500    | HYDROPHOBIC_INTERACTION |
| MET49A 392C  | 9C          | 4.729    | HYDROPHOBIC_INTERACTION |
| MET49A 392C  | 10C         | 3.567    | HYDROPHOBIC_INTERACTION |
| MET49A 392C  | 11C         | 2.618    | HYDROPHOBIC_INTERACTION |
| MET49A 392C  | 14C         | 4.009    | HYDROPHOBIC_INTERACTION |
| MET49A 394C  | 10C         | 3.483    | HYDROPHOBIC_INTERACTION |
| MET49A 394C  | 11C         | 3.178    | HYDROPHOBIC_INTERACTION |
| MET49A 394C  | 13C         | 4.182    | HYDROPHOBIC_INTERACTION |
| MET49A 394C  | 14C         | 2.889    | HYDROPHOBIC_INTERACTION |
| MET49A 394C  | 15C         | 4.563    | HYDROPHOBIC_INTERACTION |
| MET49A 394C  | 16C         | 3.875    | HYDROPHOBIC_INTERACTION |
| MET49A 394C  | 19C         | 4.197    | HYDROPHOBIC_INTERACTION |
| ASN142A 1124C| 17C         | 4.743    | HYDROPHOBIC_INTERACTION |
| ASN142A 1124C| 21C         | 4.817    | HYDROPHOBIC_INTERACTION |
| MET165A 1297C| 6C          | 4.625    | HYDROPHOBIC_INTERACTION |
| MET165A 1297C| 7C          | 4.117    | HYDROPHOBIC_INTERACTION |
| MET165A 1297C| 8C          | 4.784    | HYDROPHOBIC_INTERACTION |
| MET165A 1300C| 4C          | 4.959    | HYDROPHOBIC_INTERACTION |
| MET165A 1300C| 6C          | 3.304    | HYDROPHOBIC_INTERACTION |
| MET165A 1300C| 7C          | 3.200    | HYDROPHOBIC_INTERACTION |
| MET165A 1300C| 8C          | 4.091    | HYDROPHOBIC_INTERACTION |
| MET165A 1300C| 9C          | 4.890    | HYDROPHOBIC_INTERACTION |
| MET165A 1301C| 6C          | 3.885    | HYDROPHOBIC_INTERACTION |
| MET165A 1301C| 7C          | 4.296    | HYDROPHOBIC_INTERACTION |
| MET165A 1303C| 6C          | 4.981    | HYDROPHOBIC_INTERACTION |
| ASP187A 1462C| 3C          | 4.371    | HYDROPHOBIC_INTERACTION |
| ASP187A 1462C| 4C          | 4.758    | HYDROPHOBIC_INTERACTION |
| ASP187A 1465C| 3C          | 4.895    | HYDROPHOBIC_INTERACTION |
| ASP187A 1470C| 3C          | 2.830    | HYDROPHOBIC_INTERACTION |
| ARG188A 1470C| 4C          | 3.584    | HYDROPHOBIC_INTERACTION |
| ARG188A 1470C| 6C          | 4.863    | HYDROPHOBIC_INTERACTION |
| ARG188A 1473C| 3C          | 4.142    | HYDROPHOBIC_INTERACTION |
| GLN189A 1481C| 3C          | 4.262    | HYDROPHOBIC_INTERACTION |
| Residue Atom | Ligand Atom | Distance | Interaction Type            |
|-------------|-------------|----------|----------------------------|
| 51 GLN189A 1481C | 4C         | 3.938    | HYDROPHOBIC_INTERACTION    |
| 52 GLN189A 1481C | 6C         | 4.085    | HYDROPHOBIC_INTERACTION    |
| 53 GLN189A 1481C | 7C         | 4.942    | HYDROPHOBIC_INTERACTION    |
| 54 GLN189A 1481C | 9C         | 4.821    | HYDROPHOBIC_INTERACTION    |
| 55 GLN189A 1484C | 3C         | 4.416    | HYDROPHOBIC_INTERACTION    |
| 56 GLN189A 1484C | 4C         | 4.036    | HYDROPHOBIC_INTERACTION    |
| 57 GLN189A 1484C | 6C         | 4.508    | HYDROPHOBIC_INTERACTION    |
| 58 GLN189A 1484C | 7C         | 4.909    | HYDROPHOBIC_INTERACTION    |
| 59 GLN189A 1484C | 8C         | 4.896    | HYDROPHOBIC_INTERACTION    |
| 60 GLN189A 1484C | 9C         | 4.480    | HYDROPHOBIC_INTERACTION    |
| 61 GLN189A 1485C | 3C         | 3.632    | HYDROPHOBIC_INTERACTION    |
| 62 GLN189A 1485C | 4C         | 3.023    | HYDROPHOBIC_INTERACTION    |
| 63 GLN189A 1485C | 6C         | 3.849    | HYDROPHOBIC_INTERACTION    |
| 64 GLN189A 1485C | 7C         | 3.907    | HYDROPHOBIC_INTERACTION    |
| 65 GLN189A 1485C | 8C         | 3.556    | HYDROPHOBIC_INTERACTION    |
| 66 GLN189A 1485C | 9C         | 3.096    | HYDROPHOBIC_INTERACTION    |
| 67 GLN189A 1485C | 10C        | 3.754    | HYDROPHOBIC_INTERACTION    |
| 68 GLN189A 1485C | 11C        | 4.259    | HYDROPHOBIC_INTERACTION    |
| 69 GLN189A 1485C | 13C        | 4.972    | HYDROPHOBIC_INTERACTION    |
| 70 GLN189A 1485C | 14C        | 4.653    | HYDROPHOBIC_INTERACTION    |
| 71 HIS41A 330C  | 1Cl        | 4.166    | CHARGE_INTERACTION         |
| 72 CYS44A 355C  | 1Cl        | 4.384    | CHARGE_INTERACTION         |
| 73 CYS44A 356S  | 1Cl        | 3.951    | CHARGE_INTERACTION         |
| 74 MET49A 387N  | 1Cl        | 4.273    | CHARGE_INTERACTION         |
| 75 MET49A 388C  | 1Cl        | 3.236    | CHARGE_INTERACTION         |
| 76 MET49A 391C  | 1Cl        | 3.553    | CHARGE_INTERACTION         |
| 77 MET49A 392C  | 1Cl        | 3.445    | CHARGE_INTERACTION         |
| 78 PRO52A 415C  | 1Cl        | 4.489    | CHARGE_INTERACTION         |
| 79 PRO52A 416C  | 1Cl        | 3.391    | CHARGE_INTERACTION         |
| 80 PRO52A 417C  | 1Cl        | 4.011    | CHARGE_INTERACTION         |
| 81 TYR54A 434C  | 1Cl        | 4.357    | CHARGE_INTERACTION         |
| 82 TYR54A 435C  | 1Cl        | 4.783    | CHARGE_INTERACTION         |
| 83 TYR54A 437O  | 1Cl        | 2.861    | CHARGE_INTERACTION         |
| 84 ARG188A 1469N| 1Cl        | 4.826    | CHARGE_INTERACTION         |
| 85 ARG188A 1470C| 1Cl        | 4.307    | CHARGE_INTERACTION         |
| 86 ARG188A 1473C| 1Cl        | 4.932    | CHARGE_INTERACTION         |
| 87 THR25A 197C  | 20C        | 3.754    | VDW_INTERACTION            |
| 88 THR25A 202C  | 20C        | 3.766    | VDW_INTERACTION            |
| 89 CYS44A 356S  | 1Cl        | 3.951    | VDW_INTERACTION            |
| 90 MET49A 388C  | 2C         | 3.630    | VDW_INTERACTION            |
| 91 MET49A 389C  | 1Cl        | 3.908    | VDW_INTERACTION            |
| 92 MET49A 390O  | 1Cl        | 3.698    | VDW_INTERACTION            |
| 93 MET49A 390O  | 2C         | 3.674    | VDW_INTERACTION            |
| 94 MET49A 390O  | 3C         | 3.576    | VDW_INTERACTION            |
| 95 MET49A 391C  | 1Cl        | 3.553    | VDW_INTERACTION            |
| 96 MET49A 392C  | 10C        | 3.567    | VDW_INTERACTION            |
| 97 MET49A 394C  | 10C        | 3.483    | VDW_INTERACTION            |
| 98 MET49A 394C  | 16C        | 3.875    | VDW_INTERACTION            |
| 99 TYR54A 436C  | 1Cl        | 3.915    | VDW_INTERACTION            |
| 100 TYR54A 437O | 2C         | 3.682    | VDW_INTERACTION            |
| Residue Atom | Ligand Atom | Distance | Interaction Type |
|--------------|-------------|----------|------------------|
| MET165A 1301C | 6C          | 3.885    | VDW_INTERACTION  |
| MET165A 1302S | 5N          | 3.713    | VDW_INTERACTION  |
| ASP187A 1464O | 1Cl         | 3.629    | VDW_INTERACTION  |
| ASP187A 1464O | 3C          | 3.261    | VDW_INTERACTION  |
| ARG188A 1469N | 4C          | 3.702    | VDW_INTERACTION  |
| ARG188A 1469N | 5N          | 3.322    | VDW_INTERACTION  |
| ARG188A 1470C | 4C          | 3.584    | VDW_INTERACTION  |
| ARG188A 1470C | 5N          | 3.420    | VDW_INTERACTION  |
| ARG188A 1472O | 6C          | 3.703    | VDW_INTERACTION  |
| GLN189A 1480N | 3C          | 3.262    | VDW_INTERACTION  |
| GLN189A 1480N | 4C          | 3.403    | VDW_INTERACTION  |
| GLN189A 1480N | 5N          | 3.171    | VDW_INTERACTION  |
| GLN189A 1481C | 5N          | 3.497    | VDW_INTERACTION  |
| GLN189A 1485C | 3C          | 3.632    | VDW_INTERACTION  |
| GLN189A 1485C | 5N          | 3.428    | VDW_INTERACTION  |
| GLN189A 1485C | 6C          | 3.849    | VDW_INTERACTION  |
| GLN189A 1485C | 8C          | 3.556    | VDW_INTERACTION  |
| GLN189A 1485C | 10C         | 3.754    | VDW_INTERACTION  |
| GLN189A 1486C | 8C          | 3.676    | VDW_INTERACTION  |
| GLN189A 1486C | 9C          | 3.578    | VDW_INTERACTION  |
| GLN189A 1487O | 10C         | 3.418    | VDW_INTERACTION  |
| GLN189A 1487O | 13C         | 3.273    | VDW_INTERACTION  |
| GLN189A 1487O | 14C         | 3.454    | VDW_INTERACTION  |