Destabilizing the structural integrity of SARS-CoV2 receptor proteins by curcumin along with hydroxychloroquine: An *Insilco* approach for a combination therapy

Akhileshwar Kumar Srivastava[1*] and Divya Singh[2]

*[1]Corresponding author: CSIR-CFTRI, Mysuru, India, contact number: +91-8604664864, email: akhileshwar.kumar2@gmail.com

[2]CSRTI, Mysuru, India email: divya.ds012@gmail.com

Abstract:

Presently, an emerging disease (COVID-19) has been spreading across the world due to coronavirus (SARS-CoV2). For treatment of SARS-CoV2 infection, currently hydroxychloroquine has been suggested by researchers, but it has not been found enough effective against this virus. The present study based on in silico approaches was designed to enhance the therapeutic activities of hydroxychloroquine by using curcumin as an adjunct drug against SARS-CoV2 receptor proteins: main-protease and S1 receptor binding domain (RBD). The webserver (ANCHOR) showed the higher protein stability for both receptors with disordered score (<0.5). The molecular docking analysis revealed that the binding energy (-24.58 kcal/mol) of hydroxychloroquine was higher than curcumin (-20.47 kcal/mol) for receptor main-protease, whereas binding energy of curcumin (-38.84 kcal/mol) had greater than hydroxychloroquine (-35.87 kcal/mol) in case of S1 receptor binding domain. Therefore, this study suggested that the curcumin could be used as combination therapy along with hydroxychloroquine for disrupting the stability of SARS-CoV2 receptor proteins.

Keyword: SARS-CoV2, main-protease, S1 receptor binding domain, hydroxychloroquine, curcumin, ANCHOR, Molecular docking

Introduction

The outbreak of emerging COVID19 disease has been began in late December 2019 in Wuhan, China, by unique coronavirus (SARS-CoV-2) and now promptly disseminated in China and outside [1]. World Health Organization (WHO) acknowledged the epidemic of COVID-19 as a pandemic on March 12th March 2020 [2]. Presently Chinese studies explained on approximately 80% of patients were suffering with moderate disease, although the total case-fatality rate is approx. 2.3% including 8.0% in patients age group 70-79 years and 14.8% in age group >80 years [3]. Nonetheless, there are possibly several asymptomatic carriers present in the population, and hence the mortality rate from COVID19 is not possibly accounted.

Till now, three major pathogenic human coronaviruses (CoVs) have been recognized like Middle East respiratory syndrome coronavirus (MERS-CoV), severe acute respiratory syndrome (SARS) coronavirus (SARS-CoV), and a 2019 novel coronavirus (2019nCoV), as named by WHO earlier [4].

A coronavirus consists with four structural proteins: spike (S), envelope (E), membrane (M), and nucleocapsid (N) proteins [4, 5]. Of these proteins, S protein plays the essential role in viral adherence, integration and entry, and hence it acts as a target for synthesizing of antibodies, entry inhibitors and vaccines [6, 7]. The S protein acts as mediator for entry of viral into host cells, it first binds to a host receptor through the receptor-binding domain (RBD) of S1 subunit and then integration of viral protein with host membranes by S2 subunit [8, 9]. SARS-CoV and MERS-CoV RBDs have diverse group of receptors. SARS-CoV identifies angiotensin-converting enzyme 2 (ACE2) as its receptor and MERS-CoV has dipeptidyl peptidase 4 (DPP4) as its receptor [10, 11]. Like SARSCoV, SARS-CoV-2 also identified ACE2 as its host receptor binding to viral S protein. Hence, this is very difficult to explain the RBD in SARS-CoV-2 S protein as main target for the development of virus attachment inhibitors, neutralizing antibodies, and vaccines.

In a report, it has been explained that the recombinant RBD protein integrated firmly to human ACE2 and bat ACE2 receptors. Moreover, it inhibits the attachment of SARS-CoV-2 and SARS-CoV with their respective ACE2-expressing cells, elucidating that it can play role as inhibitor during viral attachment against SARS-CoV-2 and SARS-CoV infection. In addition, it has also been explained that SARS-CoV RBD polyclonal antibodies cross-reacted with SARS-CoV-2 RBD protein and blocked entry of SARS-CoV-2 into hACE2 (human ACE2)-expressing cells. The SARS-CoV RBD-specific polyclonal antibodies can also cross-

neutralize SARS-CoV-2 pseudovirus infection, explaining the potential to synthesis SARS-CoV RBD-based vaccine for stopping of infection by SARS-CoV-2 and SARS-CoV [12].

One of the well-known drug targets of coronaviruses is the main protease (Mpro) [13]. Like papain-like protease(s), the main protease requires for processing the polyproteins, which are translated from the viral RNA [14]. The Mpro needs at least 11 cleavage sites on the large polyprotein 1ab (replicase 1ab), the identified sequence of most sites is LeuGln↓(Ser,Ala,Gly) (↓ indicates the cleavage site). To stop the activity of this enzyme will inhibit the viral replication.

Earlier, hydroxychloroquine (HCQ) has been elucidated as an anti SARS-CoV activity *in vitro* [15]. It has been explained that hydroxychloroquine has potential to eliminate the viral nasopharyngeal carriage of SARS-CoV-2 in COVID-19 infected persons within three to six days. A significant difference of this medication had been addressed between HCQ-treated patients and normal one even on day 3 post-inclusion. Such results revealed a great value by explaining the mean duration of viral shedding in patients infecting from COVID-19 in China was 20 days [2].

Hydroxychloroquine stops receptor binding and membrane fusion; two main events steps involve for cell entry by coronaviruses. Hydroxychloroquine has revealed an antiviral effect while pre- and post-infection time through altering with the glycosylation of angiotensin-converting enzyme 2 (ACE2) and stopping the virus fusion with the host cell. The altered terminal glycosylation of ACE2 might decline the binding capacity between ACE2 on host cells and the SARS-CoV spike protein. The attachment of the virus to the receptors of cells is curbed and so the infection could be prevented at early stage. Once hydroxychloroquine enters a cell, it is countered in organelles at low pH like endosomes, Golgi vesicles and lysosomes. The virus employs endosomes for entry mechanism in cells, rising the pH of endosomes through hydroxychloroquine treatment creates a negative effects on the fusion step of virus and endosome [16]. Lysosomal proteases trigger the fusion method between host and viral membranes through cleaving surface spike proteins of coronavirus [17]. The rising the pH level in lysosome stops the protease activity and consequently this fusion process is inhibited [18]. It has been reported that the spread of SARS-CoV was inhibited in cells treated with hydroxychloroquine before or after infection, explaining both prophylactic and therapeutic significance of hydroxychloroquine in fighting against SARS-CoV [19].

Curcumin (CUR) is the main bioactive molecule of turmeric (*Curcuma longa*), one of the highly potential nutritional supplements and traditional medicine [20]. Several studies explained that curcumin contains anti-inflammatory, anti-oxidant and anti-neoplastic properties. Many literatures on curcumin have stated potential actions of this component in the treatment and prevention of certain ailments like oxidative and inflammatory conditions, metabolic syndrome, arthritis, anxiety, and cancers [21]. It has been recommended as generally recognized as safe (GRAS) by Food and Drug Administration (FDA) the maximum dose of curcumin up to 12g/day is to be safe for human consumption while the clinical trials without producing any side effects [21].

The antiviral properties of curcumin had been detected against several viruses e.g. Parainfluenza virus type 3, Feline infectious peritonitis virus , vesicular stomatitis virus, herpes simplex virus, flock house virus and respiratory syncytial virus [22]. It is very challenging to combating against viral diseases, particularly those produced by the emerging viruses or variants. Even lack of the proofreading capability, the high mutation rate of viral RNA polymerase [23] comforts viral pathogens with RNA genome to develop resistance against pre-existing antiviral drugs. If a new mutated variant of a RNA viral pathogens evolves then cost, time and effort to generate an antiviral drug takes its toll [24]. Further, long-term administration of antiviral drugs produces side effect such as nausea, vomiting, mitochondriaxicity and insomnia depending upon the antiviral drug.

The attachment is the initial step which takes viruses to the surface of cell membrane. Earlier, it has been explained that if curcumin is added to cells before to or upon infection, it inhibits the infection processes of enveloped viruses e.g. poxvirus, flavivirus, herpesvirus, and orthomyxovirus, however plaque formation capacity of the nonenveloped enterovirus like 71 (EV71) is unaltered [25]. Moreover, when curcumin is applied directly with viruses, it damages the functional activity of viral envelope proteins such as haemagglutinin-neuraminidase protein of New Castle disease virus and haemagglutinin protein of IAV [25]. Likewise, a present study suggested curcumin prevents two arthropod-borne viruses zika and chikungunya virus infections by inhibiting the binding process of viruses on the cell surface [26]. Due to the rapid spread of SARS-CoV-2, affecting more than 199 countries, therapeutic alternatives are urgently required. Hence this study emphasized to develop a novel idea for combination therapy by examining the inhibitory potential of curcumin along with hydroxychloroquine targeting the main-protease (Mpro) and S1 receptor binding domain (RBD) of SARS-CoV2.

Material and methods

Selection of ligands and proteins

The 3D structure of curcumin (PubChem ID: 969516) and hydroxychloroquine (PubChem ID: 3652) were retrieved from the database PubChem [27] for interactive study. The crystal structure of COVID-19 main protease (PDB ID: 6Y84) with resolution 1.39 Å and S-receptor binding domain (PDB ID: 2GHV) [12] with 2.2 Å of SARS-CoV2 were downloaded from database Protein Data Bank (PDB) [28].

Determination of proteins stability

The stability of protein receptors was determined through the web server (http://iupred.enzim.hu and http://iupred.elte.hu) by using algorithm IUPred2 and ANCHOR. The FASTA file of protein receptors COVID-19 main protease (PDB ID: 6Y84) containing 306 residues and S-receptor binding domain (PDB ID: 2GHV) with 203 residues were uploaded on webserver (https://iupred2a.elte.hu/) for prediction of protein disorders [29, 30].

IUPred employs an energy estimation method at its core and this methods uses a low-resolution statistical potential to identify the capability of amino acid pairs to form contacts and examined as globular protein structures [31]. If the structure is known, the statistical potential calculates the energy for all residues associated with its interactions with other contacting residues of structure. The sum of such residue-level energy terms could be employed to account the total stabilizing energy addition of intrachain interactions in protein structure. Therefore this novel method has been developed to determine these energies precisely from the amino acid sequence of unknown structure [32]. By using this model, the energy of every single residue in the amino acid sequence is calculated by following formula:

$$e_i^k = \sum_j =1^{20} P_{ij} C_j^k$$

where e_i^k is the energy of the residue in position k of type i, P_{ij} is the ijth element of the energy predictor matrix, and cj is the jth element of amino acid composition vector, indicating the ratio of amino acid type j in the adjacent sequence of position k. P is a 20 × 20 energy predictor matrix which links with the amino acid composition vector to the energy of the residue.

Like IUPred, ANCHOR also uses the energy calculation method for knowing the disordered binding sites. Inspite the general disorder tendency, two additional terms have also been

added into the method, which calculate the energy based on the interaction with a globular protein and with the neighbored disordered sequence [33]. These tendencies had been combined by a linear combination and eventually were transformed to produce a normalized score between 0 and 1 expressing the probability of a residue being part of a disordered binding region. In this work, residues of disordered binding sites fulfill two different criteria: (i) they are capable to form favorable interactions with binding surface of an ordered protein and (ii) they should allow to fix in a disordered sequence environment. Following formula has been given by these two criteria:

$$S_k = (E_{gain,\,k}(w_1) - E_{gain,\,0})\,(I_k(w_2) - I_0)$$

where S_k is the score of residue, w_1 half-window sequential of adjacent residue k, $I_k(w_2)$ is the averaged IUPred score, $E_{gain,\,0}$ and I_0 are parameters which calculate the minimum energy gain and minimum average disorder tendency a residue.

Molecular docking

The molecular docking has been performed to understand the interactive mode of hydroxychloroquine and curcumin with COVID-19 main protease and S-receptor binding domain of SARS-CoV2. The interactive study of each leading molecules was accomplished through PatchDock online server. The complex type had been fixed to default with the clustering RMSD: 4.0Å. PatchDock created the results and their rank were based on geometric shape complementarily score. Next, the obtained results from PatchDock had been subjected for refinement and rescoring of ten best solutions among 1000 top scoring complexes using FireDock and expressed the energy as global binding energy involving in complex formation. Each solution of FireDock had been provided rank on the basis of minimum global binding energy. For the visualization of interactive mode of the SARS-CoV2 receptors proteins and ligands (hydroxychloroquine and curcumin), a visualizer software Discovery Studio 4.5 Client has been used.

Result and discussion

Due to the rapid spread of SARS-CoV-2, affecting more than 199 countries, therapeutic alternatives are urgently required. Viral main protease plays a pivotal role in coronavirus replication. This enzyme is responsible for the cleavage of the polyprotein, producing functional proteins that will be packed into the virion. The molecular study of SARSCoV-2 protease showed that this virus has high homology with SARS-CoV protease (Joseph Thomas

Ortega, Maria Luisa Serrano, Flor Helene Pujol, 2020). The coronaviral surface spike protein S is a type I transmembrane glycoprotein which leads for initial host binding through the cell surface receptor angiotensin-converting enzyme 2 (ACE2), and consequently membrane fusion step is occurred for cell entry [35, 36]. The S protein works as the mediator of host-specific SARS infection as well as viral surface antigen, hence the S protein has become a novel targeting candidate for both vaccine development and immunotherapy [35].

Prediction of protein stability

Most of the proteins have intrinsically disordered regions (IDRs), functional polypeptide segments which gain a highly flexible conformational orchestra despite of a single, well-known structure. Disorder prediction process could differentiate ordered and disordered regions from the amino acid sequence, have attributed valuable data for understanding the different characteristics of intrinsically disordered proteins through enabling the characterization of individual examples and large-scale assessment of such protein regions. One well-known method, IUPred gives a vigorous method for prediction of protein disorder by following an energy estimation method which brings the basic differences between the biophysical properties of ordered and disordered regions [30]. Like IUPred, ANCHOR method based on the energy estimation approach is also applied to identify the disordered tendency and binding capacity of protein segments.

The graph obtained from webserver (https://iupred2a.elte.hu) (Fig. 1) after submission of FASTA file of each protein receptors achieved score less than 0.5 for all residues of COVID-19 main protease (PDB ID: 6Y84) (Fig. 1a) and S-receptor binding domain (PDB ID: 2GHV) (Fig. 1b) that indicated the integrity of residues in selected protein receptors of SARS-CoV2 is very high [29], hence individual drug like hydroxychloroquine may not capable to disrupt the stability of COVID-19 main protease (PDB ID: 6Y84) and S-receptor binding domain.

Molecular docking

Molecular docking (MD) plays a major role to understand the binding mode of small molecules with active region of the target receptor protein [37]. Fig. 2 represented the interactive potential of hydroxychloroquine and curcumin with COVID-19 main protease and S-receptor binding domain of SARS-CoV2 in form of 3D-Structures of each complex. The interactive residues of SARS-CoV2 protein receptors with selected ligands were explained in Table 1.

Hydroxychloroquine made hydrophobic interactions with COVID-19 main protease (Fig. 2a) through residues PRO9 and MET6 (Table 1) and indicated the weak interactions of hydroxychloroquine due to absence of H-bond. In contrast to hydroxychloroquine, a single atom (C26) of curcumin formed two H-bond by interacting with two atoms OG1 (THR25) and O (THR24) of COVID-19 main protease (Fig. 2a) and other interaction was hydrophilic with residue GLN189 (NE2) (Table 1), which suggested the strong interaction of curcumin toward the COVID-19 main protease. Mainly, the COVID-19 main protease involves in replication process of SARS-CoV2 [14] that could be blocked by curcumin as explained in a report that curcumin inhibits the activity of protease by direct intermolecular interactions [38].

In S-receptor binding domain of SARS-CoV2, the atoms C (ASN330), O (ALA331), NH1 (complex of ARG495 and PHE334) were strongly binding with single atom CL1 of hydroxychloroquine by forming 3-H bonds, whereas atom O (ASN357) and OG1 (THR259) bind with C20 atom of hydroxychloroquine through formation of Pi-bond (Fig. 2b). Also, C8 atom of hydroxychloroquine made interaction with SER358 by Pi-bond formation. The involvement of H-bond and Pi-bond in making complex showed that the hydroxychloroquine has stronger capability to destabilize the 3D-structure of S-receptor binding domain of SARS-CoV2. Curcumin also has efficient interactive potential with S-receptor binding domain, where ILE428 (O) of S-receptor binding domain made H-bond with atom C26 of curcumin. The atom ND1 (ASN437) involved to make complex with S-receptor binding domain through carbon-hydrogen bond, whereas TYR438 and LYS333 made hydrophobic interaction with curcumin (Fig. 2b). The interactive potential of curcumin with S-receptor binding domain revealed that the curcumin could prevent the attachment of SARS-CoV2 with host-surface. The attachment of virus on host cell membrane is first step of viral infection. Previously, it has been explained that when curcumin is employed to cells before or to upon infection, it stops the series of infections form enveloped viruses like poxvirus, flavivirus and orthomyxovirus [25]. Recently, it has been reported that curcumin stops two arthropod-borne viruses zika and chikungunya virus infections by inhibiting the binding of viruses on the host-cell surface [26]. The treatment of curcumin at different doses and periods exhibited the antiviral events, for instance viral entry, rather than RNA replication of HuNoV [39].

The global energy of interacted molecules was associated with free binding energy and their higher negative value explains higher binding probability [28]. Based on molecular docking study, it was worth noticed that the predicted antiviral activity of curcumin against SARS-

CoV2 infection targeting remarkable COVID-19 main protease and S-receptor binding domain is encouragingly similar to hydroxychloroquine. The obtained binding energy (-24.58 kcal/mol) of hydroxychloroquine was higher than curcumin (-20.47 kcal/mol) for COVID-19 main protease (Table 2). In case of S-receptor binding domain, the curcumin had higher binding energy (-38.84 kcal/mol) than hydroxychloroquine (-35.87 kcal/mol) (Table 2). These results of molecular docking indicated that the curcumin as an adjunct drug could be a potent antiviral molecule along with hydroxychloroquine or other antiviral conventional drugs for disruption the integrity of SARS-CoV2 protein receptors (Fig. 3.).

Conclusion:

Several antiviral or other conventional drugs are being investigated against COVID-19, but not yet positive results have come. This study has emphasized to know exact reason by considering the two receptor proteins of SARS-CoV2: COVID-19 main protease and S-receptor binding domain, why FDA (Food and Drug Administration) approved drugs or other conventional drugs are not working against SARS-CoV2? The protein disordered results from ANCHOR showed that the COVID-19 main protease and S-receptor binding domain are highly stable proteins, so it is a quite difficult to unstable the integrity of these proteins by using individual drugs. The molecular docking analysis revealed that the hydroxychloroquine may not have adequate potential to destabilize the COVID-19 main protease. However, curcumin showed the remarkable interactive activities with both receptors COVID-19 main protease and S-receptor binding domain, so the antiviral activity of hydroxychloroquine could be increased against SARS-CoV2 by using curcumin as combination therapy.

Conflicts of interest

No any conflicts in authors

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References:

1. Lai CC, Shih TP, Ko WC, et al (2020) Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and coronavirus disease-2019 (COVID-19): The epidemic and the challenges. Int. J. Antimicrob. Agents

2. Gautret P, Lagier J-C, Parola P, et al (2020) Hydroxychloroquine and azithromycin as a

treatment of COVID-19: results of an open-label non-randomized clinical trial. Int J Antimicrob Agents. https://doi.org/10.1016/j.ijantimicag.2020.105949

3. Wu Z, McGoogan JM (2020) Characteristics of and Important Lessons from the Coronavirus Disease 2019 (COVID-19) Outbreak in China: Summary of a Report of 72314 Cases from the Chinese Center for Disease Control and Prevention. JAMA - J Am Med Assoc. https://doi.org/10.1001/jama.2020.2648

4. Wang N, Shang J, Jiang S, Du L (2020) Subunit Vaccines Against Emerging Pathogenic Human Coronaviruses. Front Microbiol. https://doi.org/10.3389/fmicb.2020.00298

5. Zhou Y, Jiang S, Du L (2018) Prospects for a MERS-CoV spike vaccine. Expert Rev. Vaccines

6. Du L, Yang Y, Zhou Y, et al (2017) MERS-CoV spike protein: a key target for antivirals. Expert Opin. Ther. Targets

7. Wang Q, Wong G, Lu G, et al (2016) MERS-CoV spike protein: Targets for vaccines and therapeutics. Antiviral Res.

8. Liu S, Xiao G, Chen Y, et al (2004) Interaction between heptad repeat 1 and 2 regions in spike protein of SARS-associated coronavirus: Implications for virus fusogenic mechanism and identification of fusion inhibitors. Lancet. https://doi.org/10.1016/S0140-6736(04)15788-7

9. Li F, Li W, Farzan M, Harrison SC (2005) Structural biology: Structure of SARS coronavirus spike receptor-binding domain complexed with receptor. Science (80-). https://doi.org/10.1126/science.1116480

10. Li W, Moore MJ, Vasllieva N, et al (2003) Angiotensin-converting enzyme 2 is a functional receptor for the SARS coronavirus. Nature. https://doi.org/10.1038/nature02145

11. Raj VS, Mou H, Smits SL, et al (2013) Dipeptidyl peptidase 4 is a functional receptor for the emerging human coronavirus-EMC. Nature. https://doi.org/10.1038/nature12005

12. Tai W, He L, Zhang X, et al (2020) Characterization of the receptor-binding domain (RBD) of 2019 novel coronavirus: implication for development of RBD protein as a

viral attachment inhibitor and vaccine. Cell Mol Immunol. https://doi.org/10.1038/s41423-020-0400-4

13. Anand K, Ziebuhr J, Wadhwani P, et al (2003) Coronavirus main proteinase (3CLpro) Structure: Basis for design of anti-SARS drugs. Science (80-). https://doi.org/10.1126/science.1085658

14. Hilgenfeld R (2014) From SARS to MERS: crystallographic studies on coronaviral proteases enable antiviral drug design. FEBS J.

15. Biot C, Daher W, Chavain N, et al (2006) Design and synthesis of hydroxyferroquine derivatives with antimalarial and antiviral activities. J Med Chem. https://doi.org/10.1021/jm0601856

16. Vincent MJ, Bergeron E, Benjannet S, et al (2005) Chloroquine is a potent inhibitor of SARS coronavirus infection and spread. Virol J. https://doi.org/10.1186/1743-422X-2-69

17. Millet JK, Whittaker GR (2015) Host cell proteases: Critical determinants of coronavirus tropism and pathogenesis. Virus Res. https://doi.org/10.1016/j.virusres.2014.11.021

18. Schrezenmeier E, Dörner T (2020) Mechanisms of action of hydroxychloroquine and chloroquine: implications for rheumatology. Nat. Rev. Rheumatol.

19. Dan Zhou S-MD and QT (2020) COVID-19: a recommendation to examine the effect of hydroxychloroquine in preventing infection and progression. J Antimicrob Chemother doi:10.109:1–4

20. Li H, Zhong C, Wang Q, et al (2019) Curcumin is an APE1 redox inhibitor and exhibits an antiviral activity against KSHV replication and pathogenesis. Antiviral Res. https://doi.org/10.1016/j.antiviral.2019.04.011

21. Gupta SC, Patchva S, Aggarwal BB (2013) Therapeutic roles of curcumin: Lessons learned from clinical trials. AAPS J.

22. Zorofchian Moghadamtousi S, Abdul Kadir H, Hassandarvish P, et al (2014) A review on antibacterial, antiviral, and antifungal activity of curcumin. Biomed Res. Int.

23. Elena SF, Sanjuan R (2005) Adaptive Value of High Mutation Rates of RNA Viruses:

Separating Causes from Consequences. J Virol. https://doi.org/10.1128/jvi.79.18.11555-11558.2005

24. Bolken TC, Hruby DE (2008) Discovery and development of antiviral drugs for biodefense: Experience of a small biotechnology company. Antiviral Res.

25. Chen TY, Chen DY, Wen HW, et al (2013) Inhibition of Enveloped Viruses Infectivity by Curcumin. PLoS One. https://doi.org/10.1371/journal.pone.0062482

26. Mounce BC, Cesaro T, Carrau L, et al (2017) Curcumin inhibits Zika and chikungunya virus infection by inhibiting cell binding. Antiviral Res. https://doi.org/10.1016/j.antiviral.2017.03.014

27. Srivastava AK, Tewari M, Shukla HS, Roy BK (2015) In Silico Profiling of the Potentiality of Curcumin and Conventional Drugs for CagA Oncoprotein Inactivation. Arch Pharm (Weinheim). https://doi.org/10.1002/ardp.201400438

28. Srivastava AK, Singh D, Roy BK (2017) Structural Interactions of Curcumin Biotransformed Molecules with the N-Terminal Residues of Cytotoxic-Associated Gene A Protein Provide Insights into Suppression of Oncogenic Activities. Interdiscip Sci Comput Life Sci. https://doi.org/10.1007/s12539-016-0142-2

29. Mészáros B, Erdös G, Dosztányi Z (2018) IUPred2A: Context-dependent prediction of protein disorder as a function of redox state and protein binding. Nucleic Acids Res. https://doi.org/10.1093/nar/gky384

30. Dosztányi Z (2018) Prediction of protein disorder based on IUPred. Protein Sci. https://doi.org/10.1002/pro.3334

31. Thomas PD, Dill KA (1996) An iterative method for extracting energy-like quantities from protein structures. Proc Natl Acad Sci U S A. https://doi.org/10.1073/pnas.93.21.11628

32. Dosztányi Z, Csizmók V, Tompa P, Simon I (2005) The pairwise energy content estimated from amino acid composition discriminates between folded and intrinsically unstructured proteins. J Mol Biol. https://doi.org/10.1016/j.jmb.2005.01.071

33. Mészáros B, Simon I, Dosztányi Z (2009) Prediction of protein binding regions in disordered proteins. PLoS Comput Biol. https://doi.org/10.1371/journal.pcbi.1000376

34. Joseph Thomas Ortega1, Maria Luisa Serrano2, Flor Helene Pujol3 HRR (2020) Unrevealing sequence and structural features of novel coronavirus using in silico approaches: The main protease as molecular target. EXCLI J 19:400–409

35. Hwang WC, Lin Y, Santelli E, et al (2006) Structural basis of neutralization by a human anti-severe acute respiratory syndrome spike protein antibody, 80R. J Biol Chem. https://doi.org/10.1074/jbc.M603275200

36. Letko M, Marzi A, Munster V (2020) Functional assessment of cell entry and receptor usage for SARS-CoV-2 and other lineage B betacoronaviruses. Nat Microbiol. https://doi.org/10.1038/s41564-020-0688-y

37. Thangavel M, Chandramohan V, Shankaraiah LH, et al (2020) Design and Molecular dynamic Investigations of 7,8-Dihydroxyflavone Derivatives as Potential Neuroprotective Agents Against Alpha-synuclein. Sci Rep. https://doi.org/10.1038/s41598-020-57417-9

38. Vajragupta O, Boonchoong P, Morris GM, Olson AJ (2005) Active site binding modes of curcumin in HIV-1 protease and integrase. Bioorganic Med Chem Lett. https://doi.org/10.1016/j.bmcl.2005.05.032

39. Yang X, Jiang H, Shi Y (2017) Upregulation of heme oxygenase-1 expression by curcumin conferring protection from hydrogen peroxide-induced apoptosis in H9c2 cardiomyoblasts. Cell Biosci. https://doi.org/10.1186/s13578-017-0146-6

Tables:

Table 1. The interacted residues of SARS-CoV2 receptor proteins with selected ligands

Compounds	Protease	S1 receptor binding domain (RBD)
Hydroxychloroquine	PRO9-UNK0	ASN330:C-UNK:CL1
	MET6-UNK0	ALA331:O-UNK:CL1
		ARG495:NH1-UNK:CL1
		PHE334-UNK:CL1
		THR395:OG1-UNK
		SER358-UNK:C8
		ASN357:O-UNK:C20
Curcumin	THR25:OG1-UNK0:C26	ASN437:ND2-UNK
	THR24:O-UNK:C26	TYR438-UNK:O5
	GLN189:NE2-UNK0	ILE428:O-UNK:C26
		LYS333-UNK

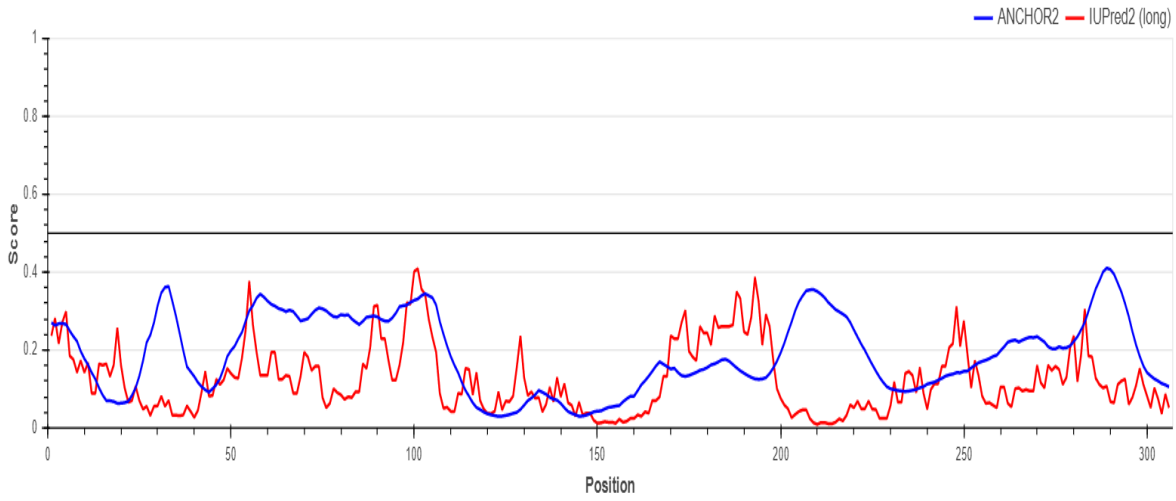
Table 2. The obtained binding energy of molecules after interacting with active sites of receptor proteins of SARS-CoV2

Compounds	Protease (kcal/mol)	S1 receptor binding domain (RBD) (kcal/mol)
Hydroxychloroquine	-24.58	-35.87
Curcumin	-20.47	-38.84

Figures:

Fig. 1. Prediction of protein disorder using the IUPred web server for receptors of SARS-CoV2

(a) Main-protease



(b) Receptor binding protein (Spike protein)

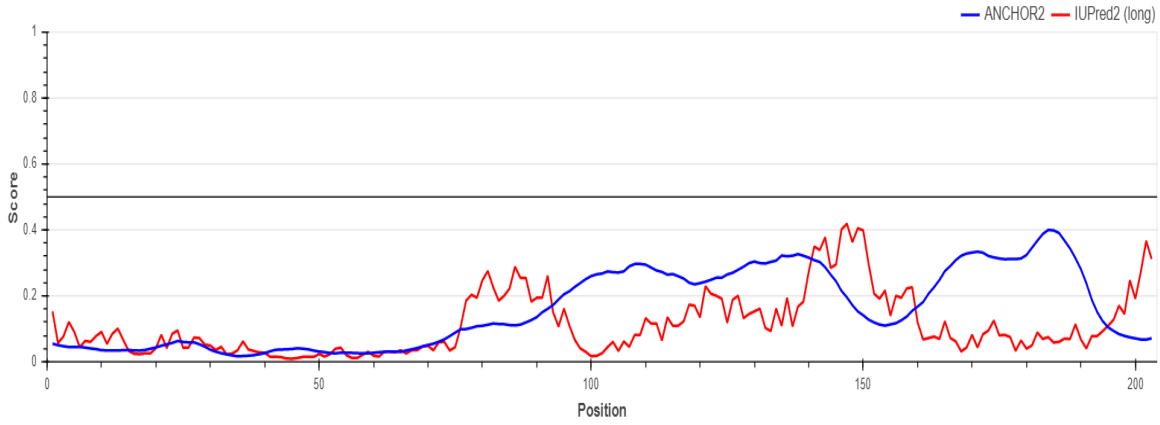
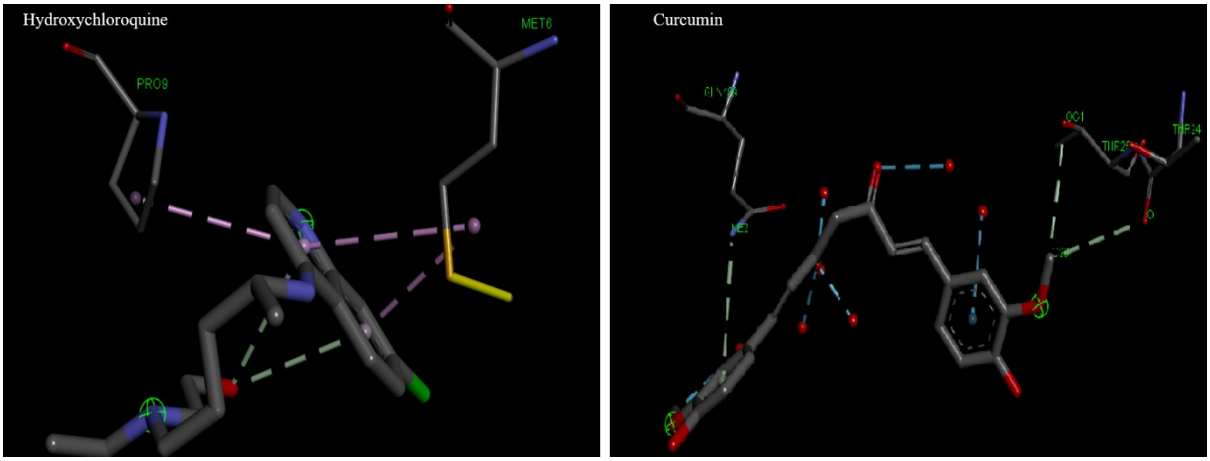


Fig. 2. The 3D representations showing the main interaction between the inhibitor and the receptor are displayed after molecular docking analysis.

(a) Main-protease



(b) Receptor binding protein (Spike protein)

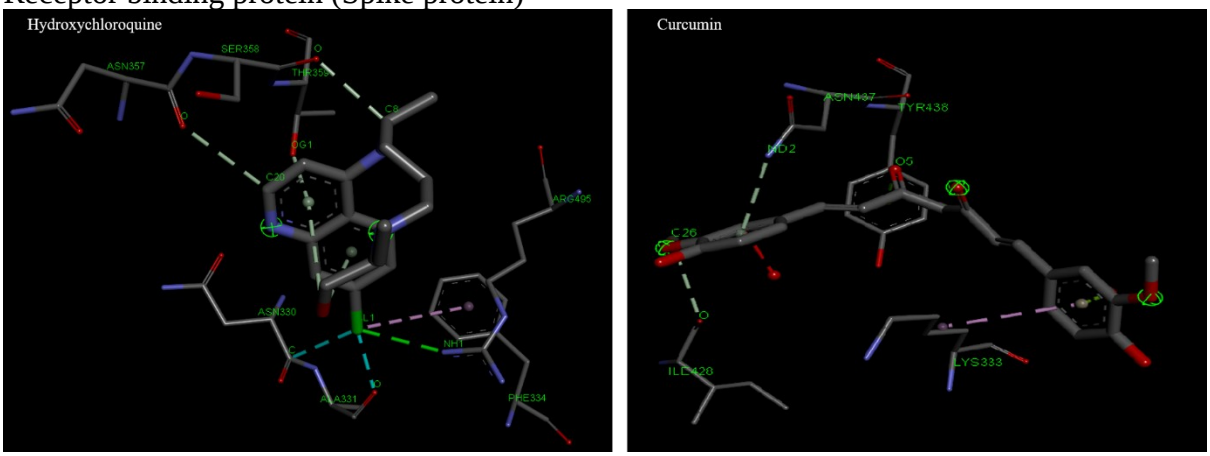
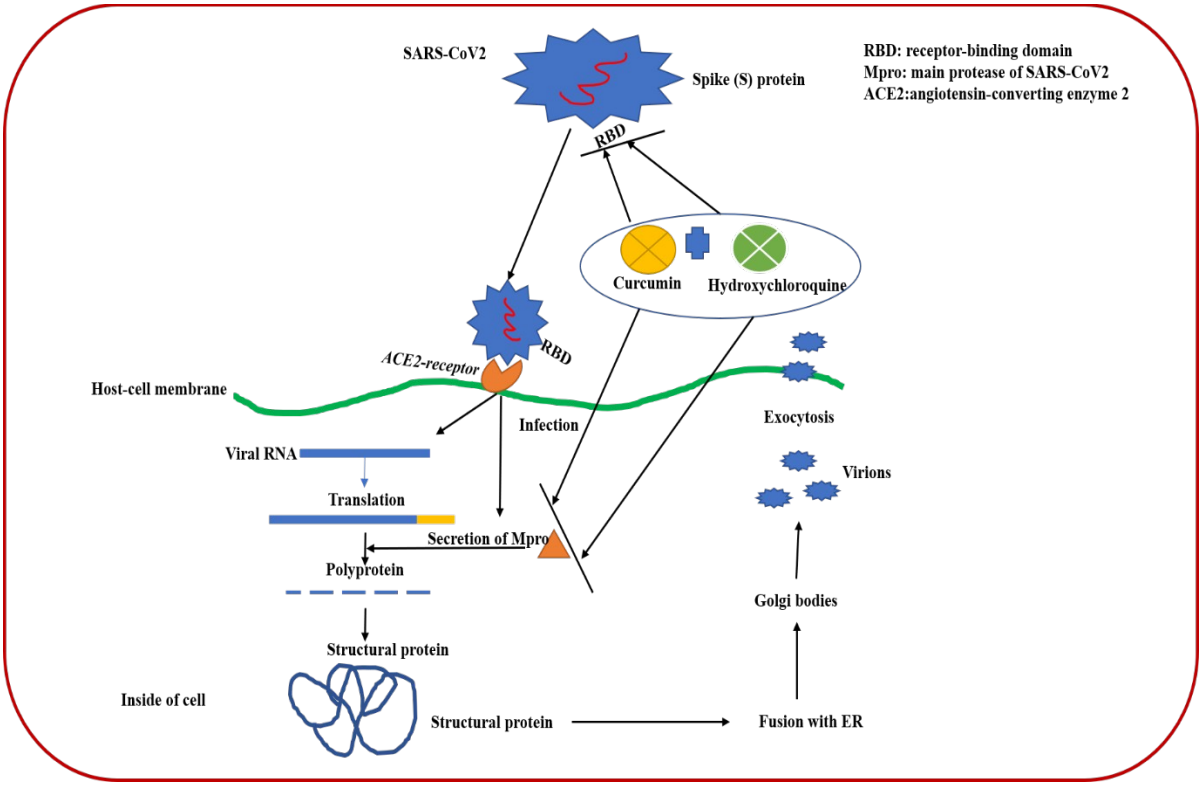


Fig. 3. The schematic presentation for a combination therapy by hydroxychloroquine and curcumin suppressing activity of main-protease and receptor binding protein playing essential role in completion of SARS-CoV2 life cycle.


SARS-CoV2
Spike (S) protein
RBD: receptor-binding domain
Mpro: main protease of SARS-CoV2
ACE2:angiotensin-converting enzyme 2
RBD
Curcumin
Hydroxychloroquine
RBD
ACE2-receptor
Host-cell membrane
Infection
Exocytosis
Viral RNA
Virions
Translation
Secretion of Mpro
Polyprotein
Golgi bodies
Structural protein
Inside of cell
Structural protein
Fusion with ER