 doi:10.20944/preprints202005.0439.v1

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# Comparative Docking Studies on Curcumin with COVID-19 Proteins

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**Abstract:** Severe acute respiratory syndrome-coronavirus 2 (SARS-CoV-2) is a respiratory syndrome caused by positive RNA virus resulting in outbreak of corona virus disease 2019 (COVID-19). The SARS-CoV-2 genome and its association to SAR-CoV-1 vary from ca. 66% to 96% depending on the type of betacoronavirdeae family members. With several drugs, *viz.* chloroquine, hydroxychloroquine, ivermectin, quinidine, artemisinin, remdesivir, azithromycin considered for clinical trials, there has been an inherent need to find distinctive antiviral mechanisms of these drugs. On the other hand, curcumin, a natural bioactive molecule has been shown to have a therapeutic potential for various diseases, but no role of it in COVID-19 has been explored. In this work, we show the binding potential of curcumin targeted to a host of SARS-CoV-2 proteins, *viz.* spike glycoproteins (PDB ID: 6VYB), nucleocapsid phosphoprotein (PDB ID: 6VYO), membrane glycoprotein (PDB ID: 6M17) along with nsp10 (PDB ID: 6W4H) and RNA dependent RNA polymerase (PDB ID: 6M71) structures. Our results indicate that curcumin has potential antiviral protein binding affinity towards SARS-CoV-2 proteins which is comparable with other repurposed drugs that are considered for clinical trials.

**Keywords:** Curcumin, COVID-19, nucleocapsid phosphoprotein, membrane glycoprotein, antiviral mechanism

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## Introduction

COVID-19 has caused unprecedented morbidity and mortality in the globe. COV-SARS-2 contains 29 proteins that include 4 structural proteins, Spike, Envelope, Membrane and Nucleocapsid and the rest non-structural and adjunct proteins. A polyprotein is acted upon by an encoded protease, giving rise to 16 of these proteins. The viral RNA genome is close to 30 Kb in size. The structural proteins are mainly involved in entry, replication, assembly and propagation of the virus into lung and other cells carrying the ACE2 receptor (Chen etal., 2020). A large number of potential therapeutic molecules that include antibiotics, antiviral and anti-malarial properties are being tested against COVID-19, which has caused global devastation (R). In a recent study, Sampangi-Ramaiah et al., 2020, have evaluated 27 natural compounds for binding affinities to both the proteases of COVID-19. Fifteen compounds have been found to have good binding affinities ranging from –6.4 kcal/mol (for Coriandrin) to –8.0 kcal/ mol (for Glabridin) and –8.1 kcal/mol (for Glucobrassicin), comparable to the anti-HIV drug Saquinavir. In the present study, we have evaluated the binding affinities of 14 drug candidates with SARS-Cov-2 proteins: spike glycoproteins (PDB ID: 6VYB), nucleocapsid phosphoprotein (PDB ID: 6VYO), membrane glycoprotein (PDB ID: 6M17) nsp10 (PDB ID: 6W4H) and RNA dependent RNA polymerase (PDB ID: 6M71) structures. We find that the natural molecule, curcumin from turmeric, has good binding affinities to nucleocapsid and nsp-10, comparable to those of ivermectin, azithromycin, remedesivir and quinidine. The food supplements and nutraceuticals may turn out to be long-term option to prevent the viral infection. Turmeric is a spice used extensively in India and is described in ancient literature for its wide medicinal use ®. Curcumin, isolated from turmeric, has strong anti-oxidative and anti-inflammatory properties and has potential therapeutic effects on chronic diseases (Yan et al., 2015). Thus, curcumin, in addition to its profound immunomodulatory effects, may also bring about changes by directly binding to crucial viral proteins. The implications of these findings are discussed in this work.

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Over the last decade, there have been constant reports of curcumin showing healthy effects and specific therapeutic benefits with over 120 clinical trials under process (Gupta et al., 2013). It is believed to show immense potential even though it challenges the standard doctrine contemplated by medicinal chemists. While it has been shown to have served as an adjunct drug for malaria, as proven in animal experiments, there are reports on curcumin-arteether combination curing malaria, respiratory disorders with various formulations (Vathsala et al., 2012). Conversely, checking the bioavailability and rapid metabolism from a holistic approach, there is a need to resolve structural targets and the ligands associated with it. Hence, we attempt to understand the molecular interactions and decipher the role of curcumin with SARS-CoV-2 structural and nonstructural proteins. To provide molecular details of ligand recognition and better understanding of the ligandbinding behaviour of different targets/receptors, we further determined characteristic features on ligand binding, safe dosage, $K_i$ values, and classify key factors that direct the docking complexes of drugs. The SARS family to which SARS-CoV-2 and SARS-CoV were reported, have structure resemblance with 8 amino acids related to 14 binding residues, and were known to be conserved in SARS-CoV-2 (Walls et al., 2020). It was further shown that angiotensin converting enzyme-2 (ACE-2) interacts with the binding residues of SARSCoV-2 (Cynthia, et al., 2020, Abdul et al., 2020). We deliberate the role of curcumin, its antiviral drug binding efficiency and calculate the dosage concentration using molecular modelling approaches targeted for therapeutics of SARS-CoV-2.

Material and methods

**Preparation of ligands:** The molecular docking studies were performed using AutoDock 4.2 software (Morris et al. 2009). For the current study, 15 ligands were considered based on their current usage in treatment of COVID-19 patients based on known potent antiviral/antimalarial drugs and their plasma concentration (supplementary table 1). The ligands with 3D structures were retrieved from PubChem as sdf files, as others were subjected to a 3D structure generation on CORINA (MN-AM.com) using their SMILE nomenclature. Further, pdbqt files for the ligands were generated by OpenBabel (O'Boyle et al., 2011).

**Preparation of proteins and grid parameters:** The protein data bank (PDB) structures of different COVID19 proteins were retrieved from RCSB Membrane Protein (6M17), polymerase (6M71), spike

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(6VYB), nucleocapsid (6VY0) and nsp10 (6W4H). The protein structures were visualized on PyMOL 1.3 or 2.5 (DeLano, 2002) by demarcating the transmembrane regions if any, present in the protein. While the pdbqt files were generated for the proteins to initiate the grid parameters, we considered less than 1Å and x, y, z coordinates to establish the size of the protein for docking studies. MGL tool 1.5.6 (Morris et al., 2009) was used for generating necessary protein and ligand files along with the respective grids and dock files. The grid generation and docking were subsequently executed through AutoGrid 4.2 and AutoDock 4.2 respectively (Morris et al., 2009).To find the binding affinity of ligands with selected proteins, drug targets were screened from estimated free energy of binding and inhibition constant ($K_i$) at 298.15 K temperature. The docked ligand-protein complexes were visualized on Chimera 1.12 (Pettersen et al., 2004) for image construction. The binding energies and affinities for the ligands were obtained from the log files of the docks generated by AutoDock. A brief methodological overview is presented in Figure 1.

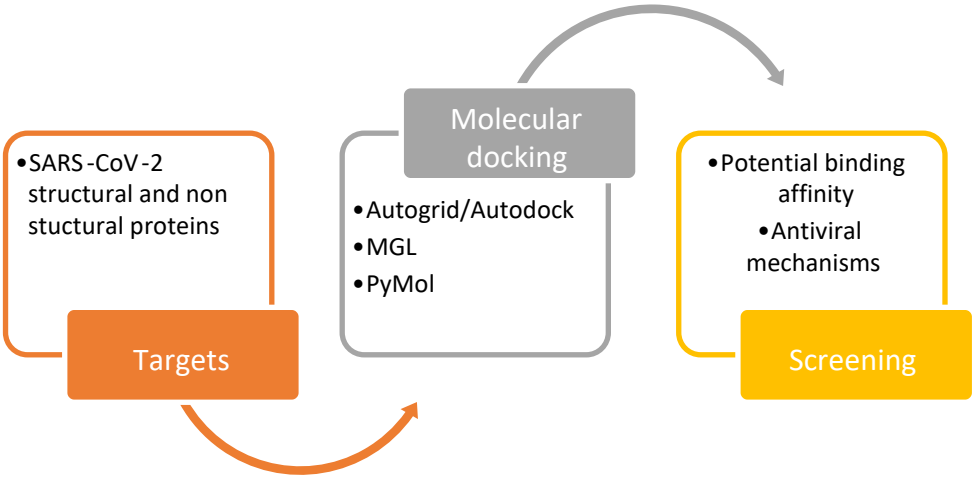


Figure 1: A pictorial methodology outlining molecular docking approaches

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**Results and Discussion**

A comprehensive docking studies were carried on14 drug molecules with antiviral properties that are considered for clinical trials along with a natural bioactive molecule (curcumin) using AutoDock 4.2. This resulted in free energy (ΔG) and inhibition constant ($K_{i)}$ values showing the binding interaction of the ligand molecules with different structural and non-structural proteins of SARS-CoV-2. From the docking studies, we observe that curcumin shows good binding affinity with nucleocapsid (ΔG =-8.75 kcal/mol, $K_i$=0.39μM ), nsp10 (ΔG =-7.85kcal/mol, $K_i$ = 1.77μM) which is comparable to quinidine with nucleocapsid (ΔG = 11.74kcal/mol, $K_i$ = 2.50 nM ) and nsp10 (ΔG = -8.45 kcal/mol, $K_i$ = 639nm), ivermectin with nucleocapsid(ΔG =-7.11 kcal/mol,$K_i$ = 6.17 μM) and nsp10 (ΔG =-9.82 kcal/mol, $K_i$ = 63.20nm ), azithromycin with nucleocapsid(ΔG = -8.71 kcal/mol, $K_i$ = 0.41 μM) and nsp10(ΔG = -8.03 kcal/mol, $K_i$ = 1.29 μM) in nsp10), and remdesivir with nucleocapsid(ΔG = -6.30 kcal/mol, $K_i$ = 23.94) and nsp10(ΔG = -6.54 kcal/mol, $K_i$ = 16.02 μM)(Figure 2; Supplementary table-2). While we validate that ivermectin showed the best affinity towards all the targeted proteins we studied, we also find significant efficient binding to the non-structural proteins (nuclocapsid and nsp10).The efficient binding to non-structural proteins is not much in the case of chloroquine, efavirenz, favipiravir and tenofovir, disoproxil. We argue that curcumin serves as the molecule of interest to us because (i) it is a natural product, and (ii) it is non-toxic to humans at dosage as high as 5 g/day (Soleimani et. al., 2018).

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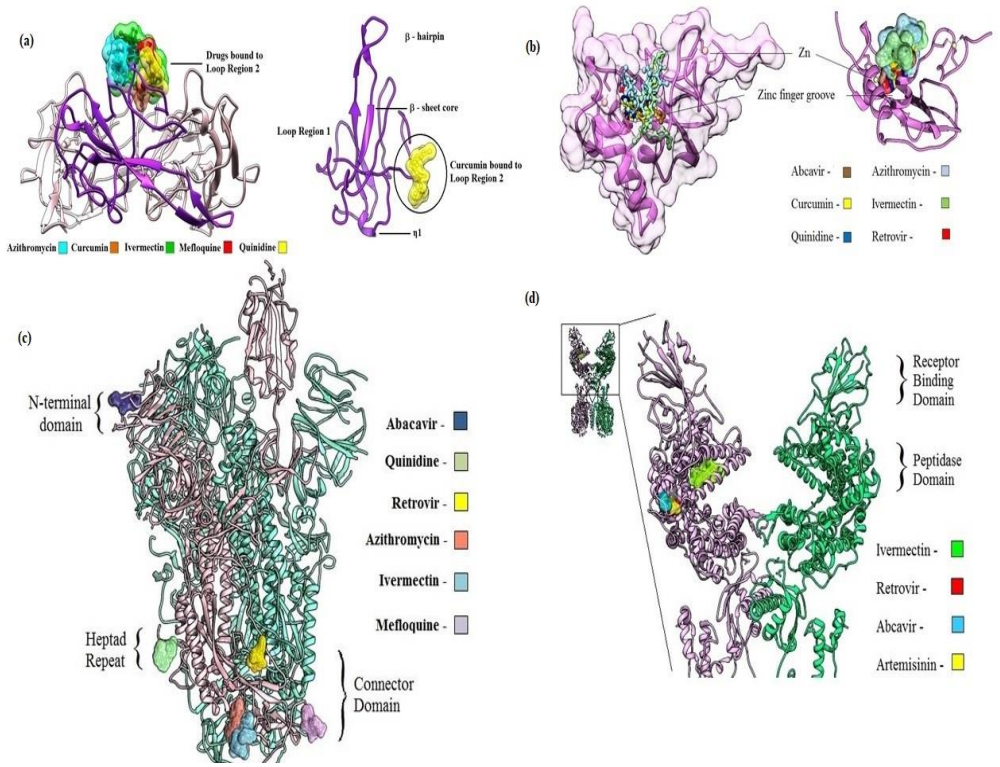


**(e)**

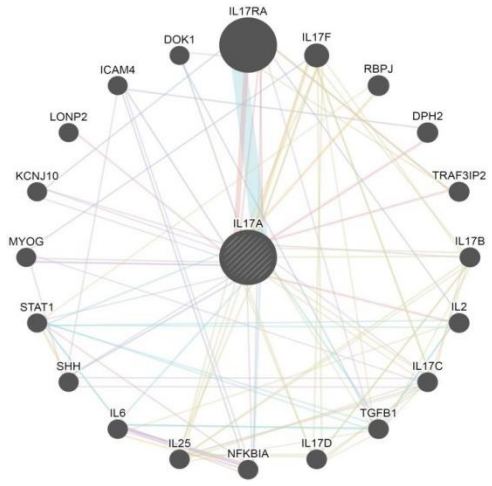


Figure 2: Protein-ligand interactions of spike (a) nucleocapsid phosphoprotein (PDB ID: 6VYO) (b) nsp10 (c) spike glycoproteins (PDB ID: 6VYB) and (d) membrane glycoprotein (PDB ID: 6M17). (e) Protein interaction map of interleukin-17 as a potent proinflammatory cytokine in which potential candidates are known to be associated with inflammation.

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Studies related to spike proteins through their $S^B$ domain (at the N-terminus) interact with ACE2 to make an entry in the host cell (Walls et al., 2020). This makes spike proteins, and more precisely the $S^B$ domain, a hot spot for therapy. Other two important regions of spike proteins are heptad repeats (HR1 and HR2), involved in viral fusion (Bosch et al., 2003), and connector domain, which connects the two heptad repeats (Wrapp et al., 2020) and it helps in stabilizing the post fusion structure (Walls et al., 2017). The basis for screening these ligands was less than 50 µM $K_i$ value, based on the reason discussed above. We obtained six ligands fulfilling the criteria and curcumin although shares site with mefloquine, even as it does not qualify with the constraint we set-up, but is shown to bind to the loop of the connector domain of spike protein with a fair binding energy of -4.9 kcal/mol corresponding to ~250 □M $K_i$ value. Ivermectin and azithromycin both have a complex structure with multiple rings and they bind at the same locus with similar affinities at the C-terminal of the spike protein. These drugs show interaction with the connector domain, indicating their stability and transition from prefusion to post fusion state rather than a preferable ACE2 receptor blocker. On the other hand, it seems plausible that the nucleocapsid (6VYO) has a potent homologue of interleukin-17a (IL17a) in humans with proinflammatory cytokines produced by activated memory T cells. We argue that the binding residues of loop 2, *viz.* Q163, L161, Q163, A173 in nucleocapsid protein may involve in a distinct signaling system associated with vertebrate evolution, as evident from the protein association pathway map (Figure 2, panel e). The regions are also exposed further considering them as epitope targets and is in agreement with the reports that curcumin,-nanocurcumin inhibits transcription factor NFκB thereby leading to instability of pro-inflammatory cytokines such as interleukins and TNF-R (Bisht et al 2007). Furthermore, curcumin is reported to suppress components of cellular signaling transduction pathways that play a key role in infected cells growth, and transformation by inhibiting protein kinases, activating enzyme cyclooxygenase (COX)-2 (Sukandar et al., 2016).

The *in silico* docking studies revealed considerable affinity towards a few drugs, *viz.* abcavir, artemisinin, ivermectin and retrovir at the peptidase domain of ACE2 segment of the complex. Whereas the viral domain ($B^0AT1$) showed considerable affinity only towards ivermectin ($\Delta G$ = -7.66, $K_i$ = 2.44 □M), yet that was not at the contact site of ACE2 and $B^0AT1$. Curcumin, although has a binding site on both the human domain and the viral domain of the complex, it would need 10 times more concentration to act on them, given its $C_{max}$ value. A binding at the linker region would

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have been of great value for the therapeutics purpose, which was not observed for the given molecules. In addition, binding of these drugs even at the peptidase domain of ACE2 would inhibit the rotation of the same and hence the change between open and closed conformation, which facilitates the viral attachment (Yan et al., 2020). Receptor binding domain (RBD), above the peptidase domain of ACE2, is identified only in the closed state, probably indicating the role of closed state in anchoring, hence the necessity of transition between the conformations for the invasion.

We further argue that ivermectin is relatively a complex molecule with reasonably high number of rings, with least $C_{max}$and relatively most insoluble compound among the ones used here. The low $K_i$ values observed for ivermectin correlates with its low physiological $C_{max}$ which therefore could make a viable target drug. Curcumin, on the other hand is a molecule with symmetrical halves containing a ring at both the edges (Supplementary figure 1). The bendable chain and the rigid rings of curcumin would serve a good clamp or a hook formation on the loop regions of targeting SARS-CoV-2 proteins. On the other hand, loops are known to play a major role in the stability of the protein structure (Balasco et al., 2013) and hence a molecule binding to loop is a potential affecter of stability. Curcumin does not show a remarkably high $C_{-max}$ value (Cas and Ghidoni, 2019) but the $K_i$ values correlates with nucleocapsid and nsp10 proteins, we have visualised it using PYMOL and identified the best pose of curcumin with binding residues at L161, Q163, A173 of Loop 2 in nucleocapsid protein and K4281, L4365 of Zinc finger groove in nsp10protein.The zinc binding sites in nsp10 protein are HIT-type zinc finger family that are reported to bind to transcription regulatory proteins which helps in recognizing RNA and also in protein-protein recognition (Su et al., 2006). Consequently, nsp10 plays a major role in viral transcription wherein nsp14 3'-5' exoribonuclease and nsp16 2'-O-methyltransferase are stimulated by playing a lead role in viral mRNAs cap methylation (Bouvet et al., 2012). Whereas, nucleocapsid protein regulates replication and transcription of viral RNA, it also inhibits the EF1α-mediated protein translation, altering the cell cycle and further lead to apoptosis in host cells (McBride et al., 2014, Hilgenfeld 2014, Zhou et al., 2008). From our studies, we demonstrate that the drugs such as azithromycin, mefloquine, quinidine, ivermectin and curcumin show hydrogen binding interactions with Q163, A173, Q70, Q163, G164, L161, T165, L167 in loop 2

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which serves as latent targets activating T-cell cytotoxicity for candidate drug therapeutics. Further, curcumin falls in the top 3 hydrophobic molecules with a logS value making it one of the best drug candidate molecules (Supplementary table 4). The spike proteins and membrane proteins readily come in contact with the cytoplasm of the host cell and therefore would have to come across a higher concentration of the drugs administered to the patient as compared to the proteins inside the coat (nucleocapsid, nsp10 and RDRP) given that the drug has to cross the surface protein and lipid layer of the virus. Taking into account this fact, we observed that most of the drugs do not have a $C_{max}$ value of more than 5 mg/mL, even the smallest compound with a molecular weight of ~100 g/mol, e.g. favipiravir (~150 g/mol), would be found at a concentration of 50 mM (disregarding the actual $C_{mzx}$ of favipiravir, which is ~40 mg/mL). Of that, if 5% of the drug manages to reach inside the viral coat, it would account to 2.5 µM of the drugs available to act on nucleocapsid, nsp10 and/or RDRP, hence making the cut-off value for core proteins. The crystal structure of the RNA binding domain of nucleocapsid protein of SARS-CoV-2 was recently published (Kang et. al., 2020) which details the groove between the palm (loop region 2) and finger region (bsheet core dominantly) at the RNA binding. As loops are known to play a major role in the stability of a protein (Balasco et. al., 2013) they are flexible, and can relatively move with ease. We could hence, predict the role of this loop in affecting the binding of RNA to the palm groove of nucleocapsid (Figure 2). Curcumin has shown exceptional binding affinity to this region, which therefore makes it a potent target drug for RNA binding domain or nucleocapsid of SARS-CoV-2. On the other hand, nsp16, which methylates the RNA cap with its S-adenosyl-L-methionine dependent methyl transferase activity, requires nsp10 as its stimulatory factor (Chen et al., 2011) as demonstrated in SARS-CoV, and the same is expected to be established in SARSCoV-2. On the contrary, inhibiting nsp10 would not allow the viral RNA to camouflage with the eukaryotic RNA of humans and eventually inhibit the replication of virus in the host cell. Among six other drug molecules, we observed that curcumin also shows a very strong affinity to nsp10 and less than 2 mM of the same is adequate concentration correlated with its its $K_i$constant. The binding of all these drugs is observed on the loop in the groove between the Zn fingers. Such strong affinity indicates that it is a good probable target site for COVID-19.

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## Conclusions

We propose curcumin as a therapeutic target for anti-corona virus drug development. In this work, we screened 14 ligands against SARS-CoV-2 structural and non-structural proteins and evaluate the binding affinity of curcumin to that of other known drugs that are commercially available. From the current study, it is evident that curcumin could play a major role in regulating the activity of nucleocapsid and nsp10, both of which are indirectly related to the detection and processing of viral RNA. At very high concentrations, curcumin acts on the spike and membrane proteins and hence invasion itself is difficult to achieve with the given solubility and plasma concentrations of curcumin. Further study on a combinatorial administration of drugs with curcumin would demonstrate if lesser concentrations of curcumin could be effectual at the surface proteins. We hope that our studies provide an imperative role of curcumin as a potential therapeutic agent for COVID-19 treatment.

**Author contributions:** GP, PBK and RP ideated the project. RS and AP jointly analysed the structures and modelled the docking complexes. PS did the protein interaction analyses. AP and RS wrote the first draft with PS, PBK and RP. PS, GP, PBK and RP proofread the manuscript.

**Acknowledgements:** Renuka Suravajhala gratefully acknowledges Manipal University Jaipur towards Ramdas Pai fellowship rendered to her.

**Funding:** None.

**Competing interests:** None

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