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Molecular docking and dynamics studies of curcumin with COVID-19 proteins

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Abstract

Coronavirus disease 2019 (COVID-19) is caused by a Severe Acute Respiratory Syndrome-Coronavirus 2 (SARS-CoV-2), which is a positive-strand RNA virus. The SARS-CoV-2 genome and its association to SAR-CoV-1 vary from ca. 66 to 96% depending on the type of betacoronavirideae family members. With several drugs, viz. chloroquine, hydroxychloroquine, ivermectin, artemisinin, remdesivir, azithromycin considered for clinical trials, there has been an inherent need to find distinctive antiviral mechanisms of these drugs. Curcumin, a natural bioactive molecule has been shown to have therapeutic potential for various diseases, and its effect on COVID-19 is also currently being explored. In this study, we show the binding potential of curcumin targeted to a variety of SARS-CoV-2 proteins, viz. spike glycoproteins (PDB ID: 6VYB), nucleocapsid phosphoprotein (PDB ID: 6VYO), spike protein-ACE2 (PDB ID: 6M17) along with nsp10 (PDB ID: 6W4H) and RNA dependent RNA polymerase (PDB ID: 6M71) structures. Furthermore, representative docking complexes were validated using molecular dynamics simulations and mechanistic studies at 100 ns was carried on nucleocapsid and nsp10 proteins with curcumin complexes which resulted in stable and efficient binding energies and correlated with that of docked binding energies of the complexes. Both the docking and simulation studies indicate that curcumin has the potential as an antiviral against COVID-19.

Keywords Curcumin · COVID-19 · Nucleocapsid phosphoprotein · Membrane glycoprotein · Antiviral mechanism · nsp10

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1 Introduction

COVID-19 has caused unprecedented morbidity and mortality across the globe (www.who.int 2020). SARS-CoV-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. Whereas 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 et al. 2020).

A large number of potential therapeutic molecules that include antibiotics, antivirals, and antimalarials are being tested against COVID-19, which has caused global devastation (Oldekop et al. 2020). In a recent study, Sampangi-Ramaiah et al. (2020) have evaluated 27 natural compounds for binding affinities to both the proteases of COVID-19. A few 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. Turmeric as a spice is used extensively in India and is described in ancient literature for its wide medicinal use even as numerous studies describe that food supplements and nutraceuticals may turn out to be long-term options to prevent viral infection. For example, curcumin has been shown to have a strong anti-oxidative and anti-inflammatory properties and has potential therapeutic effects on chronic diseases (Padmanaban and Nagaraj 2018), Noroviruses (Yang et al. 2016), Hepatitis B infection (Hesari et al. 2018) and diabetes mediated cerebral infarction (Xia et al. 2018). In addition to its profound immunomodulatory effects, it may also bring about changes by directly binding to crucial viral proteins and preventing their function. Hence, we attempt to understand the molecular interactions and decipher the role of curcumin with SARS-CoV-2 structural and non-structural proteins. To provide molecular details of ligand recognition and a better understanding of the ligand-binding behaviour of different targets/receptors, we determined characteristic features on ligand binding, safe dosage, K_i values, and classify key factors that direct the docking complexes of drugs. The spike glycoproteins of SARS family to which SARS-CoV-2 and SARS-CoV belong to, have structure resemblance with 8 amino acids related to 14 binding residues, and are 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 SARS-CoV-2 (Hoffmann et al. 2020; Rossi et al. 2020). We deliberate on the role of curcumin, its antiviral drug binding efficiency and calculate the dosage concentration using molecular docking approaches targeted for therapeutics of SARS-CoV-2. This study, therefore, is designed to compare the binding efficacy of curcumin for potential antiviral and antibacterial drugs besides an affable regulatory molecule against COVID-19.

2 Materials and methods

2.1 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 experimentation in the treatment of COVID-19 patients, based on known potent antiviral/antimalarial properties 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 (https://www.mn-am.com/) using their SMILE nomenclature. Further, pdbqt files for the ligands were generated by OpenBabel (O'Boyle et al. 2011).

2.2 Preparation of proteins and grid parameters

The crystal structures of different COVID19 proteins, viz.s pike glycoproteins (6VYB), nucleocapsid phosphoprotein (6VYO), spike protein-ACE2 (6M17) along with nsp10 (6W4H) and RNA dependent RNA polymerase (6M71) structures were retrieved from protein data bank (PDB). PyMOL version 2.5 (DeLano 2002) was used for a quick view of the proteins and its complex with the docked ligand. While the pdbqt files were generated for the proteins to initiate the grid parameters, we considered less than 1 Å and x, y, z coordinates to establish the size of the protein for docking studies. MGL tool 1.5.6 was used for generating necessary protein and ligand files along with the respective grids and dock files and 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 the 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

Scheme 1 A pictorial methodology outlining molecular docking approach

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 Scheme 1.

2.3 Molecular dynamics simulations

To understand the potent complex dynamics, molecular dynamics simulations were considered for 100 ns. During the primary phase, the parameters set of libraries were developed for the protein as receptor complex and ligand molecules by Gromacs 5.1.4 suite (Van Der Spoel et al. 2005). Using, PRODRG server, the topology files were generated for ligands (Schüttelkopf and Van Aalten 2004). Furthermore, the complexes were solvated in a cubic box with an edge length 10 nm. The GROMOS96 43a1 force field was applied to know the intermolecular and intramolecular interactions. A cut-off radius of 9 Å was considered for Van der Waals and Coulombic interactions. In order to maintain a neutral system, sufficient numbers of ions were included and clashes were deselected. Optimization of energy was established at 1000 kJ/mol/nm. The long-range electrostatic interactions were accomplished via PME (Abraham and Gready 2011). The process of equilibrium was established in two phases. In which, the solvent and ion molecules were kept unrestrained and gradually reduced the NPT ensemble and equilibration attained for the complexes. Details of water, ions, NPT and NVT time for each docking complex are listed in Supplementary Table 1. After the coordinates were determined, all hydrogen bonds were constrained by the LINCS algorithm (Hess 2008; Hess et al. 1997). The simulation analysis was carried on 100 ns with 300 K temperature and 1 atm pressure was maintained in the system similar to that of Berendsen's temperature and Parrinello-Rahman pressure coupling was considered (Berendsen et al. 1984). The system stability was analysed from the simulated trajectories.

2.4 MMPBSA for binding free energies

The g_mmpbsa module (Kumari and Kumar 2014) of GROMACS was considered to evaluate receptor, ligand, receptor-ligand complex related to the solvation free energies and interaction energies by the MM-PBSA method of GROMACS-MM-PBSA to identify various transformations of bound and unbound molecules in a solvated complex. The polar solvation energy was calculated by the Poisson-Boltzmann equation to evaluate electrostatic energy distribution of the solvation state. The binding free energy between a receptor and a ligand during MM-PBSA analysis was calculated using the following equations:

$$\Delta G_{\text{MMPBSA}} = \left\langle G_{\text{complex}} - G_{\text{protein}} - G_{\text{ligand}} \right\rangle_{\text{complex}} \tag{1}$$

$$G_x = E_{\text{MM}} - T\langle S_{\text{MM}}\rangle + \Delta G_{\text{solv}} \tag{2}$$

$$E_{\text{MM}} = E_{\text{bonded}} + E_{\text{coul}} + E_{\text{LJ}} \tag{3}$$

where, G_{complex}, G_{protein} and G_{ligand} represent the free energies of the docking complex, receptor and ligand respectively.

3 Results and discussion

Comprehensive docking studies were carried out on 14 drug molecules with antiviral properties that are considered for clinical trials along with 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 ($\Delta G = -$ 8.75 kcal/mol, $K_i = 0.39$ μM) and nsp10 ($\Delta G = -$ 7.85 kcal/mol, $K_i = 1.77$ μM) which is comparable to azithromycin with nucleocapsid ($\Delta G = -$ 8.71 kcal/mol, $K_i = 0.41$ μM) and nsp10 ($\Delta G = -$ 8.03 kcal/mol, $K_i = 1.29$ μM), ivermectin with nucleocapsid ($\Delta G = -$ 7.11 kcal/mol, $K_i = 6.17$ μM) and nsp10 ($\Delta G = -$ 9.82 kcal/mol, $K_i = 63.20$ nm), quinidine with nucleocapsid ($\Delta G = 11.74$ kcal/mol, $K_i = 2.50$ nM) and nsp10 ($\Delta G = -$ 8.45 kcal/mol, $K_i = 639$ nm), and remdesivir with nucleocapsid ($\Delta G = -$ 6.30 kcal/mol, $K_i = 23.94$ μM) and nsp10 ($\Delta G = -$ 6.54 kcal/mol, $K_i = 16.02$ μM) (Fig. 1;
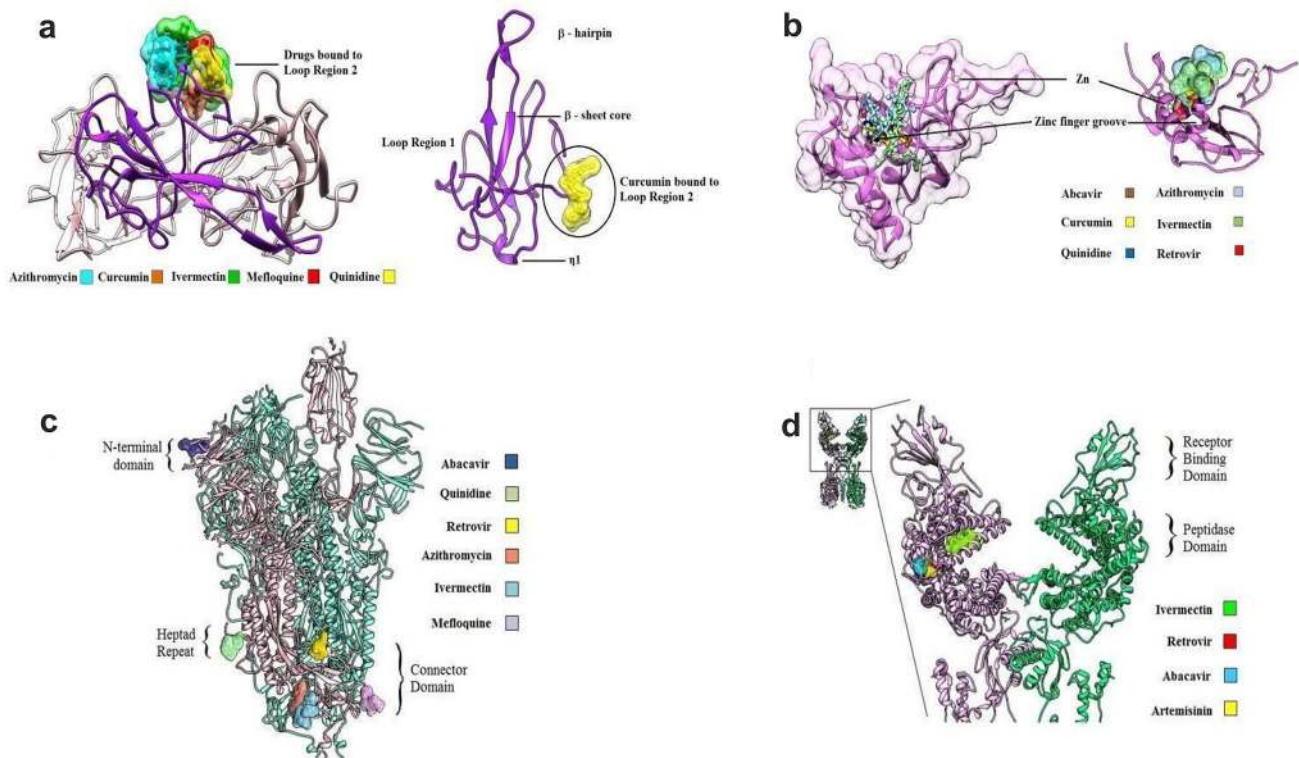
Fig. 1 Protein–ligand interactions of (**a**) nucleocapsid phosphoprotein (PDB ID: 6VYO) (**b**) nsp10 (**c**) spike glycoproteins (PDB ID: 6VYB) and (**d**) membrane glycoprotein (PDB ID: 6M17)

Supplementary Information, Tables 1 and 2). Ivermectin showed the best affinity towards all the targeted proteins studied and significant efficient binding to the non-structural proteins. The efficient binding to non-structural proteins is not much in the case of chloroquine, efavirenz, favipiravir, tenofovir and disoproxil.

Curcumin is a molecule with symmetrical halves containing a ring at both the edges (Supplementary Information, Fig. 2). 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 modulator of stability. Curcumin does not show a remarkably high C_{max} value (Supplementary Information, Table 3) but the K_i values correlate with binding to nucleocapsid and nsp10 proteins. We have visualised it using PyMOL and identified the best pose of curcumin with binding residues at LEU161, GLN163, ALA173 of Loop 2 in nucleocapsid protein (Fig. 2a). We argue that the binding residues of loop 2, viz. GLN163, LEU161, ALA173 in nucleocapsid protein may involve a distinct signalling system associated with vertebrate evolution, as evident from the complex interaction pathways (Fig. 1). We have also identified LYS4281, LEU4365 of

zinc finger groove in nsp10 protein as the binding residues (Fig. 2b). The zinc-binding sites in nsp10 protein are HIT-type zinc finger families that are reported to bind to transcription regulatory proteins which help in recognizing RNA and also in protein–protein recognition (Su et al. 2006).

Consequently, nsp10 plays a major role in viral transcription wherein 3'-5' exoribonuclease activity of nsp14 and 2'-O-methyltransferase activity of nsp16 are stimulated by playing a lead role in viral mRNAs cap methylation (Bouvet et al. 2014). While nucleocapsid protein regulates replication and transcription of viral RNA, it also inhibits the EF1α-mediated protein translation, altering the cell cycle which further leads 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 GLN163, ALA173, GLN70, GLN163, GLY164, LEU161, THR165, LEU167 in loop 2 which serves as latent targets, activating T-cell cytotoxicity for candidate drug therapeutics. 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 (β-sheet core dominantly) at the RNA binding. As loops are known to play a major role in the stability of a
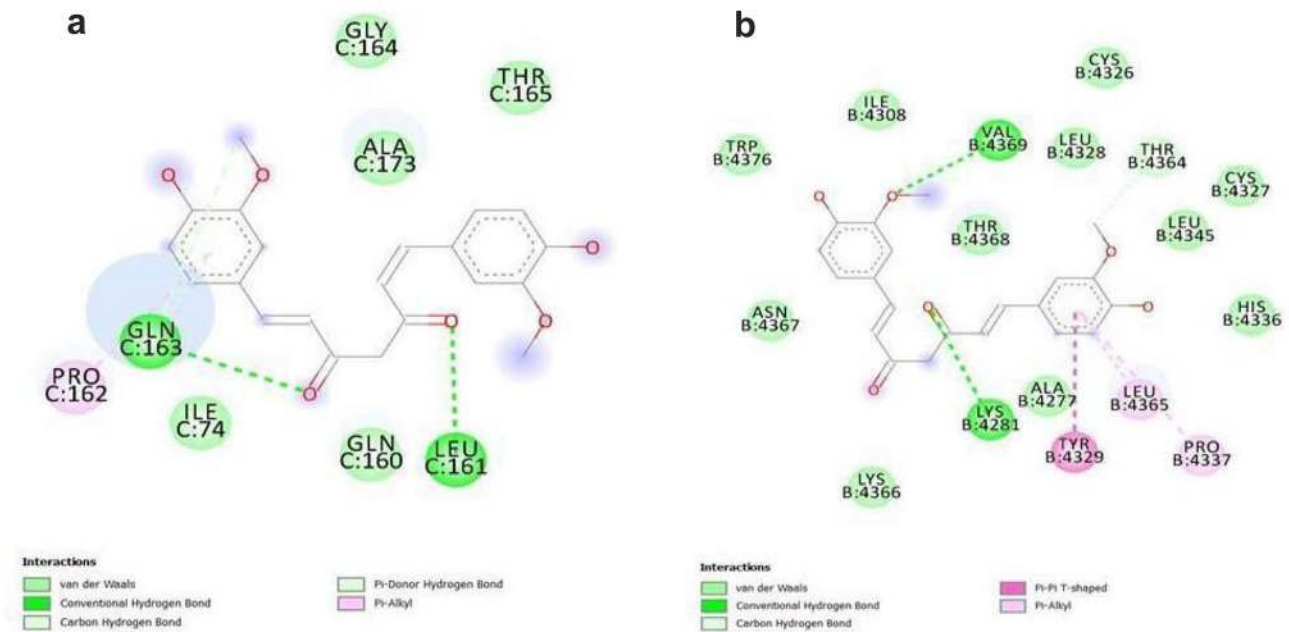
Fig. 2 Curcumin protein-binding residues with (**a**) nucleocapsid and (**b**) nsp10

protein, they are flexible, and can relatively move with ease (Balasco et al. 2013). We could, hence, predict the role of this loop in affecting the binding of RNA to the palm groove of the nucleocapsid (Fig. 1). 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 methyltransferase 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 SARS-CoV-2. At the same time, inhibiting nsp10 would not allow the viral RNA to camouflage with the eukaryotic RNA of humans and eventually inhibit the replication of the 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 K_i constant. The binding of all these drugs is observed on the loop in the groove between the zinc fingers. Such strong affinity indicates that it is a probable target site for COVID-19.

Thus, curcumin acts as a potential inhibitor of the nucleocapsid (6VYO), which is a multifunctional pro-inflammatory protein (McBride et al. 2014). The regions are also exposed further considering them as epitope targets and this 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 (Dolati et al. 2018). Furthermore, curcumin is reported to suppress components of cellular signalling transduction pathways that play a key role in infected cell growth, and transformation by inhibiting protein kinases, activating enzyme cyclooxygenase (COX-2) (Prasad et al. 2014). Studies reveal that spike proteins interact with ACE2 through their SB domain (at the N-terminus) to make an entry into the host cell. This makes spike proteins, and more precisely the SB 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 and it helps in stabilizing the post-fusion structure (Walls et al. 2017). We screened six ligands keeping less than 50 μM K_i value as the cut-off criteria. Curcumin although shares site with mefloquine, is not a good inhibitor of the spike protein, but binds to the loop of the connector domain of spike protein with fair binding energy of − 4.9 kcal/mol corresponding to ∼ 250 μM Ki value. Curcumin falls in the top 3 hydrophobic molecules with the logS value making it one of the best drug candidate molecules (Supplementary Information, 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 μg/mL, even the smallest compound with a molecular

weight of ~ 100 g/mol, e.g. favipiravir (~ 150 g/mol), would be present at a concentration of 50 μM (the actual C_{max} of favipiravir is ~ 40 mg/mL). If 5% of the drug manages to reach inside the viral coat, it would amount to 2.5 μM of the drugs available to act on nucleocapsid, nsp10, and/or RDRP, thus providing the cut-off value for core protein inhibition.

We further argue that ivermectin is relatively a complex molecule with a reasonably high number of rings, with least C_{max} and relatively most insoluble compound among the ones used here. The low Ki values observed for ivermectin correlates with its low physiological C_{max}, which therefore could make a viable target drug. Curcumin, although has a binding site on both the human domain and the viral domain of the complex, would be needed at 10 times more concentration to act on them, given its C_{max} value. A binding at the linker region would have been of great value for the therapeutics purpose, which was not observed for the molecules under consideration. 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 (Nelson et al. 2017). Receptor binding domain (RBD), above the peptidase domain of ACE2, is identified only in the closed state, probably indicating the role of the closed state in anchoring, hence the necessity of transition between the conformations for the invasion.

3.1 MD simulations

The stability of curcumin-based nucleocapsid, nsp10 viral proteins docked complexes was evaluated by optimisation process via MD simulations. The simulation was carried to analyse the root mean square deviation (RMSD), root mean square fluctuation (RMSF), radius of gyration (Rg), solvent accessible surface area (SASA) and MM-PBSA binding free energies. While the calculated energies generated as trajectories, the system-maintained equilibrium after 10 ns and remained constant till 100 ns simulation time.

3.2 RMSD and RMSF analysis

The RMSD was calculated for backbone Cα atoms was obtained in between 0.3 and 0.51 nm for nucleocapsid-curcumin complex (blue); 0.3 and 0.48 nm for nsp10-curcumin complex (red) and nucleocapsid protein (green) respectively (Figs. 3a, b). Minor fluctuations observed at various intervals were due to conformational changes of ligand at the active sites. Furthermore, the average RMSF of the
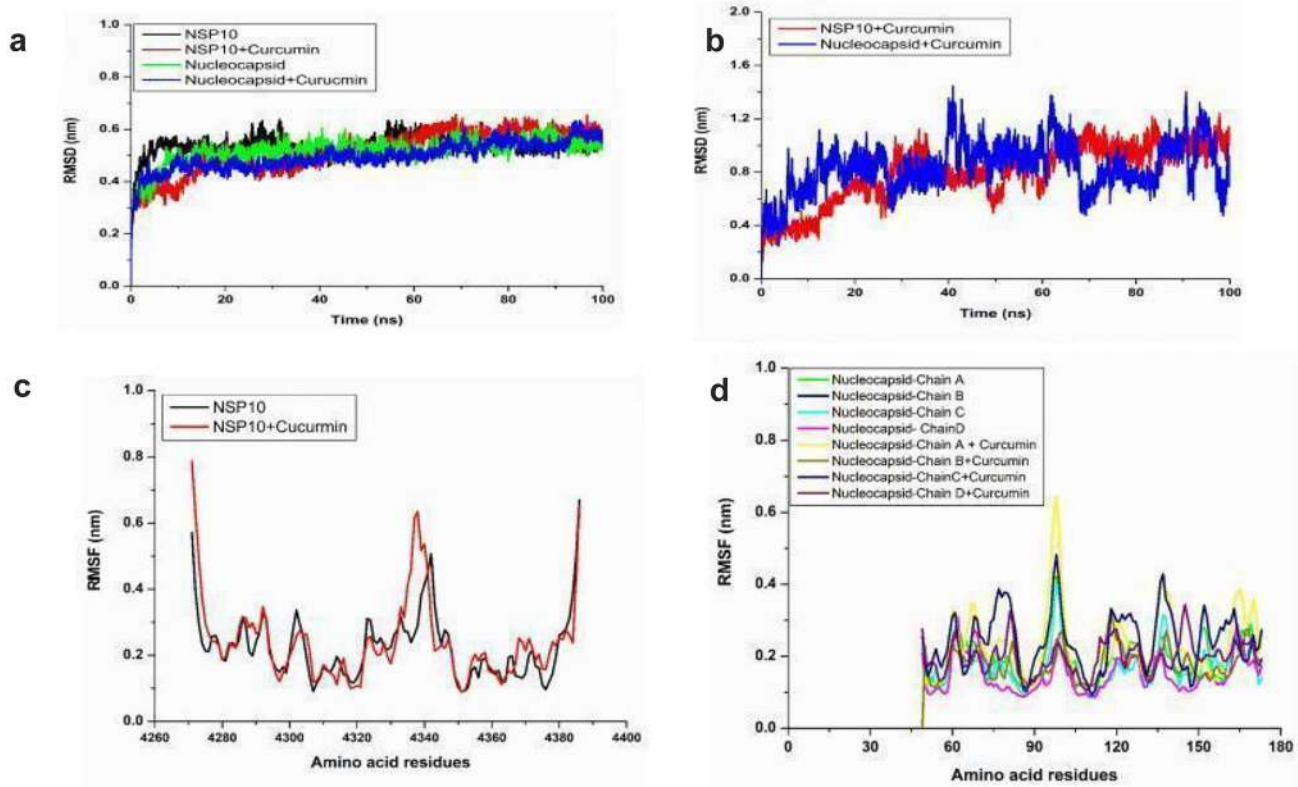

Fig. 3 RMSD and RMSF analysis: The nsp10 protein (black), nucleocapsid (green), nsp10-curcumin (red), nucleocapsid-curcumin (blue) RMSD data (**a**, **b**) and RMSF data (**c**, **d**) was evaluated, respectively

backbone Cα atoms for each complex were proved to form stable complexes.

3.3 Rg and SASA analysis

The intrinsic dynamics was calculated by Rg was carried on docked complexes with an average Rg ~ 1.3 nm for curcumin-nsp10 protein complex and an average Rg of ~ 2.5 nm for curcumin-nucleocapsid complex respectively (Fig. 4a, b). In addition, stability-based SASA analysis was evaluated as 55–75 nm^2 for curcumin-nsp10 protein and 230–260 nm^2 for

curcumin-nucleocapsid complexes had no folding effect on the complex stability. These studies conclude that curcumin-nsp10 protein and curcumin-nucleocapsid complexes exhibited potential binding affinity and simulation studies proved the most stable and suitable complexes formed.

3.4 Free binding energy analysis/MM-PBSA

The intermolecular interactions impact on the potent complex's stability was evaluated by MM-PBSA studies and the trajectories were generated. The distribution
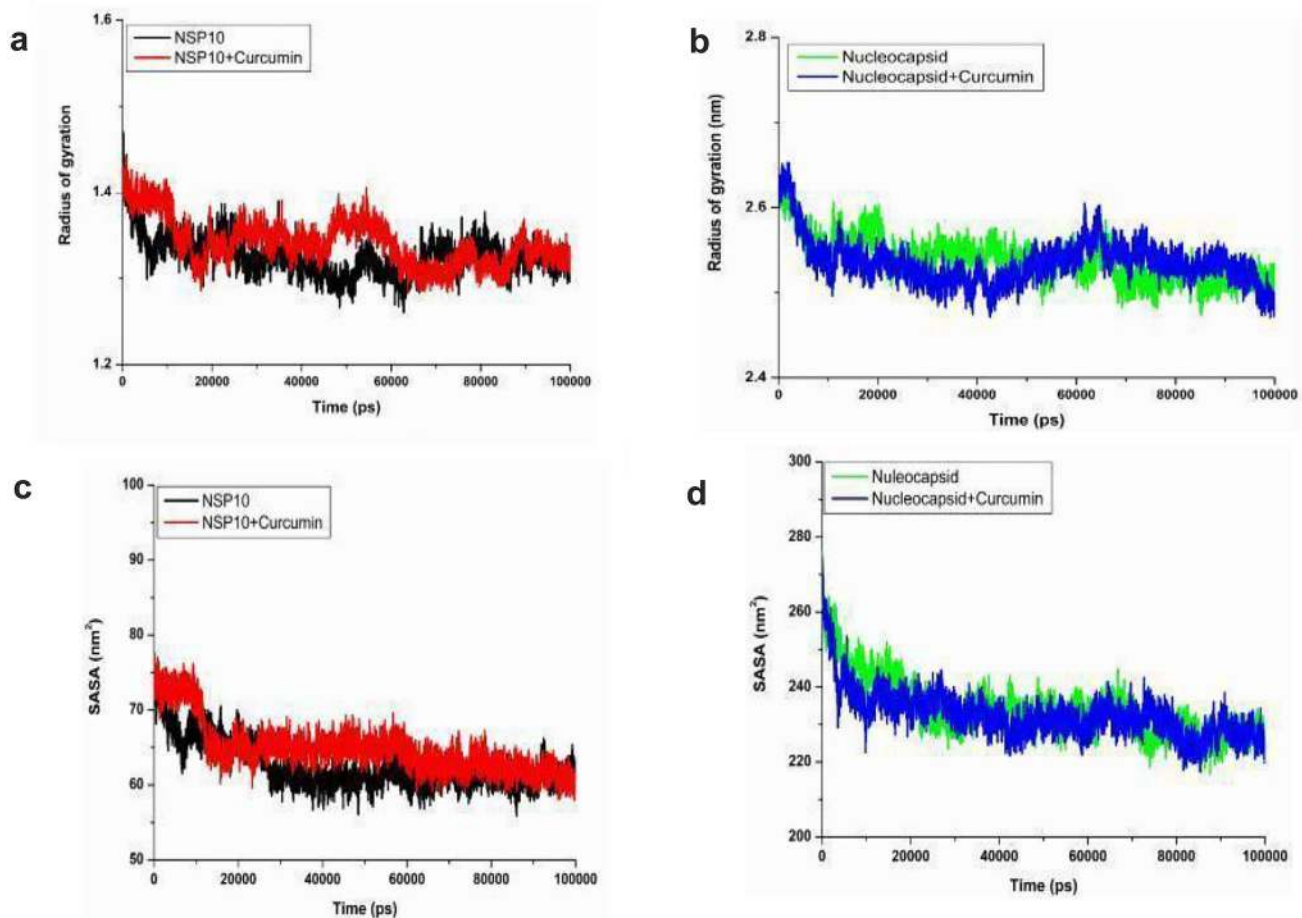

Fig. 4 Rg and SASA analysis: The nsp10 protein (black), nucleocapsid (green), nsp10-curcumin (red), nucleocapsid-curcumin (blue) Radius of gyration (Rg) studies (**a**, **b**) and SASA studies (**c**, **d**) were evaluated, respectively

Table 1 Free binding energy calculations of curcumin-nsp10 and nucleocapsid viral proteins complexes respectively

S. no	Molecules	Van der waals (kJ/mol)	Electrostatic energy (kJ/mol)	Polar solvation energy (kJ/mol)	SASA energy (kJ/mol)	Binding energy (kJ/mol)
1	nsp10-Curcumin	− 165.083 ± 11.041	− 36.534 ± 13.271	102.404 ± 23.312	− 14.715 ± 1.033	− 113.928 ± 19.000
2	Nucleocapsid-Curcumin	− 168.101 ± 17.564	− 26.744 ± 14.913	86.759 ± 24.612	− 14.985 ± 1.320	− 123.071 ± 17.861

of MM-PBSA binding free energies were tabulated as (Table 1). The net lowest binding energy illustrated during the studies had more significance on the complex stability. The binding energy of curcumin-nucleocapsid complex (-123.071 ± 17.861) is lower than curcumin-nsp10 complex, i.e., (-113.928 ± 19.000) signifying strong binding affinity. The gas-phase energy showed a significant impact on the total solvation energy of the system. The best conformation of the receptor with respect to the ligand have stabilised complexes. The net gas phase energy for MM-PBSA method was the same curcumin-nsp10, curcumin-nucleocapsid i.e. 102.404 and 86.759 kcal/mol and the Van der Waal energy of nsp10-curcumin complex with -165.083 ± 11.041 kJ/mol was observed with less effect on binding affinity while nucleocapsid-curcumin (-168.101 ± 17.564) has a strong binding affinity (Table 1). The electrostatic energy and SASA energy are shown a moderate effect on complex binding energies (shown in Table 1). The MM-PBSA analysis revealed the contribution of different energies that has a significant impact on the stability of curcumin-nucleocapsid and curcumin-nsp10 complexes.

Despite extensive studies and clinical trials for a variety of diseases, mostly cancers, there has been a view that curcumin is not an ideal drug candidate in view of its low bioavailability and stability in plasma. Nevertheless, even with low bioavailability, it is a very effective immunomodulator to cure experimental malaria, cerebral malaria, and infectious diseases (Padmanaban and Nagaraj 2017) even as many derivatives with improved bioavailability are also available (Prasad et al. 2014). The present study shows that in addition to being a potent immunomodulator, it can effectively bind and inhibit the nucleocapsid and nsp10 proteins of SARS-CoV-2. Some of the recent reviews also have emphasized on the ability of curcumin against SARS-CoV-2 by mostly targeting the protease activity and/or being an inhibitor of the receptor-binding domain (RBD) on spike protein (Manoharan et al. 2020; Zahedipour et al. 2020; Babaei et al. 2020; Liu and Ying 2020). Although, in the current study, we found that the affinity of curcumin towards protease or spike protein is not par with the affinity towards nucleocapsid and nsp10, it is still observed that the K_i values are sufficient to compete as a potent drug candidate against SARS-CoV-2. Curcumin can be an ideal adjunct drug, being a food additive and non-toxic to humans at a dosage as high as 8 g/day (Soleimani et al. 2018). We find that the natural molecule, curcumin from turmeric, has good binding affinities to nucleocapsid and nsp10, comparable to those of ivermectin, azithromycin and remdesivir.

4 Conclusion

In summary, we propose curcumin as a therapeutic option for anti-corona virus drug development. In this work, we screened 14 ligands against SARS-CoV-2 structural and non-structural proteins binding affinity related molecular docking studies. It was observed that curcumin has a significant impact on nucleocapsid and nsp10 of SARS-CoV-2 proteins. Furthermore, the conformational complex structure stability was evaluated by considering molecular dynamics simulations and MM-PBSA. Studies revealed that the strong binding with residues could enhance the possibility of in vitro and in vivo studies. In conclusion, our studies resulted in considering curcumin as a combinatorial administration of drugs against SARS-CoV-2. This can be interesting to know the major role played by curcumin in regulating the activity of nucleocapsid and nsp10 proteins, which are known to have a significant impact on the detection and processing of viral RNA. The present findings are promising and supplemented with the fact that curcumin can be a natural bioactive and safe compound.

Author contributions GP, PBK, and RP ideated the project. RS and AP jointly analysed the structures and modelled the docking complexes. PS performed the protein interaction analyses. AK and GC performed the MD simulation analysis. AP and RS wrote the first draft with AK, PS, PBK, BM and RP. GP, PBK, PS, VAN and RP proofread the manuscript.

Declarations

Conflict of interests The authors declare that the research was conducted in the absence of any commercial or financial relationship that could be constructed as a potential conflict of interest.

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