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In-silico strategies for probing chloroquine based inhibitors against SARS-CoV-2

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ABSTRACT

The global health emergency of novel COVID-19 is due to severe acute respiratory syndrome corona-virus-2 (SARS-CoV-2). Currently there are no approved drugs for the treatment of coronaviral disease (COVID-19), although some of the drugs have been tried. Chloroquine is being widely used in treatment of SARS-CoV-2 infection. Hydroxychloroquine, the derivative of Chloroquine shows better inhibition than Chloroquine and has in vitro activity against SARS-CoV-2 also used to treat COVID-19. To study the interactions of Chloroquine and derivatives of Chloroquine with SARS-CoV-2, series of computational approaches like pharmacophore model, molecular docking, MM_GBSA study and ADME property analysis are explored. The pharmacophore model and molecular docking study are used to explore the structural properties of the compounds and the ligand-receptor (**PDB_ID: 6LU7**) interactions respectively. MM_GBSA study gives the binding free energy of the protein-ligand complex and ADME property analysis explains the pharmacological property of the compounds. The resultant best molecule (**CQD15**) further subjected to molecular dynamics (MD) simulation study which explains the protein stability (RMSD), ligand properties as well as protein-ligand contacts. Outcomes of the present study conclude with the molecule **CQD15** which shows better interactions for the inhibition of SARS-CoV-2 in comparison to Chloroquine and Hydroxychloroquine.

Introduction

Coronavirinae family is made up of four genera based on their genetic material, including Alpha, Beta, Gamma and Delta coronavirus genus. The coronavirus family is a positive-stranded RNA virus, which mainly causes respiratory and central nervous system disease (Perlman & Netland, 2009). Human coronaviruses namely HCoV-OC43, HCoV-229E, HCoV-NL63 and HCoV-HKU1 are responsible for mild respiratory diseases (Owusu et al., 2014). The SARS-CoV outbreak in 2002 demonstrated that coronaviruses would cross the barrier to species and emerge as highly pathogenic viruses (Perlman, 2020). The disease caused by SARS-CoV-2 is called COVID-19. The coronavirus has been named SARS-CoV-2 (Gorbalenya et al., 2020), because the RNA genome is about 82% identical to the SARS coronavirus (SARS-CoV); both viruses belong to the genus beta-coronavirus (Wu et al., 2020; Zhou et al., 2020). The novel COVID-2019 was first reported in Wuhan, China in December 2019. The outbreak has been rapidly spread all over the world. More than 200 countries were affected by this COVID-19. The world health Organization (**WHO**) announced on 11th march 2020 that the COVID-19 as pandemic disease. Currently, more than 4,310,786 number (as on 12th May 2020) of people were infected with COVID-19 which is increasing by approximately 100000+ cases per day and more than 290,455 people have died. Unfortunately, to date, there is no medication or vaccine approved by the World Health Organization (**WHO**), the

Centers for Disease Control and Prevention (**CDC**), and the **FDA** for the effective treatment of COVID-19 (CDC, 2020 WHO, 2020).

Many drugs have been tried recently in the treatment of COVID-19 due to the situation of "public-health emergency", that includes antimalarial drugs (Chloroquine and its derivative Hydroxychloroquine) (Colson et al., 2020; Gao et al., 2020; Wang et al., 2020; Yao et al., 2020) antiviral drugs (HIV Protease Inhibitors i.e. Lopinavir and Ritonavir) (Chu et al., 2004; Yao et al., 2020), investigational nucleoside analogue (Remdesivir (GS-5734)) (Agostini et al., 2018; Wang et al., 2020) and investigational RNA-dependent RNA polymerase inhibitor (Favipiravir) (Beijing Chao Yang Hospital, 2020; Peking University First Hospital, 2020; Wang et al., 2020). Some Adjunctive therapeutic drugs are also used for the treatment of COVID-19, which includes macrolide antibacterial drug (Azithromycin) (Amsden, 2005; Beigelman et al., 2010; Gautret et al., 2020), Interleukin-6 (IL-6) Receptor-Inhibiting Monoclonal Antibody (Tocilizumab, Sarilumab) (Assistance Publique – Hopitaux de Paris, 2020; Xu et al., 2020; Zhou et al., 2020) and Investigational Humanized Monoclonal Antibody to the Chemokine Receptor CCR5 (Leronlimab) (CytoDyn, 2020; Zhou et al., 2020). The detailed outcomes of the clinical trials are still awaited. However, among all these reported drugs Chloroquine and Hydroxychloroquine were used/being used widely for the treatment of COVID-19.
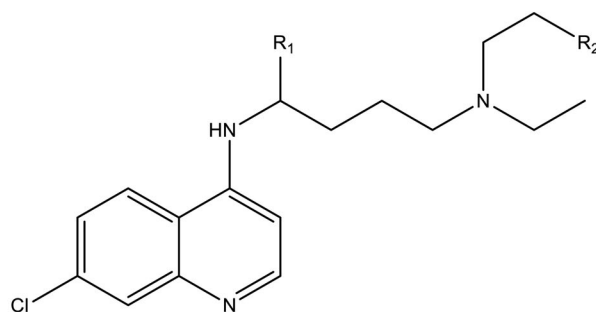
Sl. No.	Structure	R1	R2	Sl. No.	Structure	R1	R2
1	Chloroquine	CH_3	H	10	CQD8	Cl	CH_3
2	Hydroxychloroquine	CH_3	OH	11	CQD9	F	CH_3
3	CQD1	CH_3	Br	12	CQD10	NH_2	CH_3
4	CQD2	CH_3	Cl	13	CQD11	CH_3	
5	CQD3	CH_3	F	14	CQD12	OH	
6	CQD4	CH_3	NH_2	15	CQD13	NH_2	
7	CQD5	CH_3	NO_2	16	CQD14	CH_3	
8	CQD6	OH	CH_3	17	CQD15	OH	
9	CQD7	Br	CH_3	18	CQD16	NH_2	

Scheme 1. Chemical structure of Chloroquine scaffolds. []
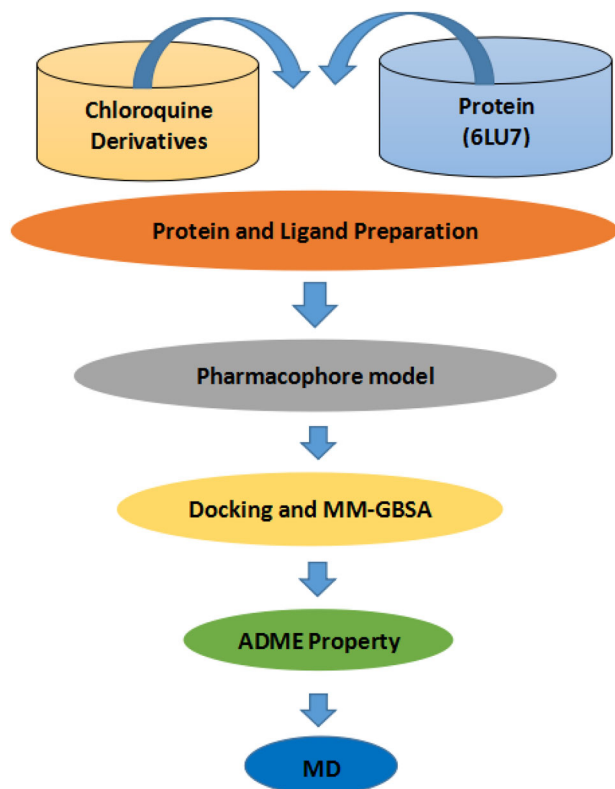
Scheme 2. Working methodology.

Many research institutes as well as pharmaceutical companies are working to identify potential candidate against SARS-CoV-2 worldwide. Some researcher reported that Chloroquine has the potential to treat COVID-19 with clinical study as well as in silico study (Adeoye et al., 2020) Boopathi et al., 2020; Gao et al., 2020). Department of Pathology, Vanderbilt University Medical Center, USA reported that antiviral Remdesivir (GS-5734) can treat SARS-CoV-2 (Agostini et al., 2018). The investigation of COVID-19 with Plasma therapy and its clinical trials are being conducted to evaluate the use of COVID-19 to treat patients with severe or immediately life-threatening COVID-19 infections (Duan et al., 2020). Hasan et al. reviewed the ongoing challenges and future prospects for the development of potential drugs to promote targeting specific activities of the CoV (Hasan et al., 2020). Some research groups are also working in the field of computational approach to find a solution for COVID-19. Khan et al. worked on a systematic drug repurposing approach to identify promising inhibitors against 3C-like proteinase and 2′-Oribose methyltransferase and proposed that Raltegravir, Paritaprevir, Bictegravir and Dolutegravir are excellent lead candidates for these crucial proteins and they could become potential therapeutic drugs against SARS-CoV-2 (Khan et al., 2020). 3,4 Dihydropyrimidone class molecule identified as a drug candidate against 2019-nCoV N-protein
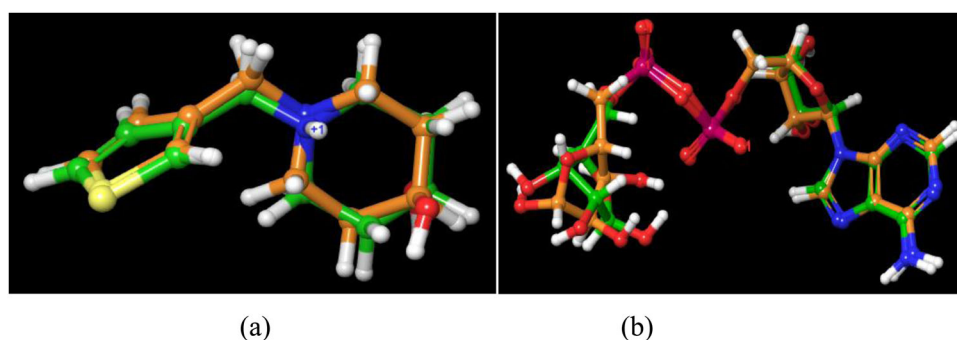
Figure 1. Superimpose of the self-docked ligand with native ligand in the protein grid (a) 5REB and (b) 6W02.

Table 1. Phase generated pharmacophore hypothesis models for chloroquine derivatives (**CQD**).

S. No.	Title	BEDROC Score	Survival Score	PhaseHypoScore
1	DHHHRR_1	0.999	6.392	1.383
2	DHHHRR_2	1	6.374	1.382
3	DHHHRR_3	1	6.372	1.382
4	DHHHRR_4	0.998	6.364	1.380
5	DHHHRR_5	1	6.362	1.382
6	DHHHRR_6	0.995	6.341	1.376
7	DHHHRR_7	1	6.334	1.380
8	DHHHRR_8	1	6.331	1.380
9	DHHHRR_9	1	6.299	1.378
10	DHHHRR_10	0.999	6.288	1.376
11	DHHRR_1	1	5.869	1.352
12	DHHRR_2	0.995	5.853	1.346
13	DHHRR_3	1	5.814	1.349
14	DHHRR_4	1	5.804	1.348
15	DHHRR_5	1	5.792	1.348
16	DHHRR_6	0.994	5.768	1.341
17	DHHRR_7	0.996	5.765	1.342
18	DHHRR_8	0.987	5.765	1.333
19	DHHRR_9	1	5.759	1.346
20	DHHRR_10	1	5.744	1.345

(N terminal domain) (Sarma et al., 2020). Enayatkhani et al. worked on reverse vaccinology approach to design a novel multi-epitope vaccine candidate against COVID-19 and conclude with NOM-TLR4 and NOM-HLA-A11:01 docked model (Enayatkhani et al., 2020). Elfiky et al. suggested guanosine derivatives (Guanosine triphosphate (GTP), Uridine triphosphate (UTP), IDX-184 (GTP derivative), sofosbuvir (UTP derivative), ribavirin (wide acting antiviral drug) can be the potential candidate against COVID-19 (Elfiky & Azzam, 2020). Zhou et al. and Joshi et al. reported the network-based methodologies for rapid identification of candidate repurposable drugs and potential drug combinations targeting 2019-nCoV/SARS-CoV-2 (Joshi et al., 2020; Zhou et al., 2020). Zhang et al. from Institute of Biochemistry, Center for Structural and Cell Biology in Medicine, University of Lübeck, Germany designed the improved α-ketoamide inhibitors for the inhibition of SARS-CoV-2 (Zhang et al., 2020). Yogi Vemana University, India, Government Medical College, Anantapur, India and ICAR-National Rice Research Institute, India collaboratively worked on In-silico approaches to detect inhibitors of the human severe acute respiratory syndrome coronavirus envelope protein ion channel (Gupta et al., 2020). A few in silico research has also been done by considering FDA approved drugs and concludes with Lopinavir
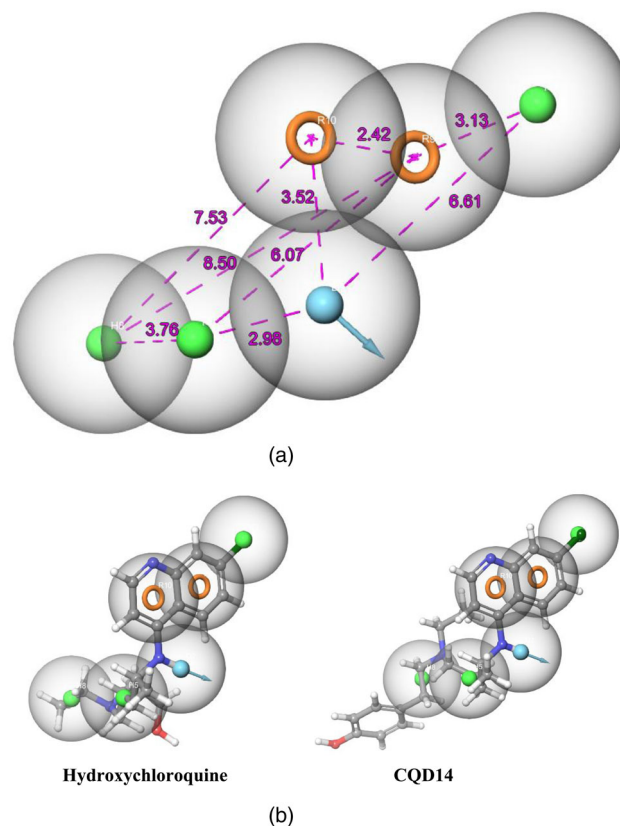

Figure 2. (a) Arrangement of individual features in a fixed distance (in Å) of pharmacophore hypothesis model DHHHRR_1 for Chloroquine derivatives. (b) Mapping of molecules on pharmacophore hypothesis model DHHHRR_1.

(Pant et al., 2020), Setrobuvir (Elfiky, 2020a), Saquinavir (Al-Khafaji et al., 2020) Remdesivir, Saquinavir, Darunavir, (Khan et al., 2020) and Disulfiram (Lobo-Galo et al., 2020) which have better interactions with SARS CoV-2. Some compounds like Crocin, Digitoxigenin, b-Eudesmol, (Aanouz et al., 2020) Andrographolide, (Enmozhi et al., 2020) phytoestrogens (Diadiazin, Genistein, Formontein and Biochanin A), (Elfiky, 2020b) phytochemicals (hypericin, cyanidin 3-glucoside, baicalin, glabridin, and α-ketoamide-11r), (Islam et al., 2020) Resveratrol, (Wahedi et al., 2020) Rutin, (Das et al., 2020) Ammoides verticillata, (Abdelli et al., 2020) alkaloids (10–Hydroxyusambarensine, and Cryptoquindoline) and terpenoids (6-Oxoisoiguesterin and 22-Hydroxyhopan-3-one) (Gyebi et al., 2020) also reported as a better candidate
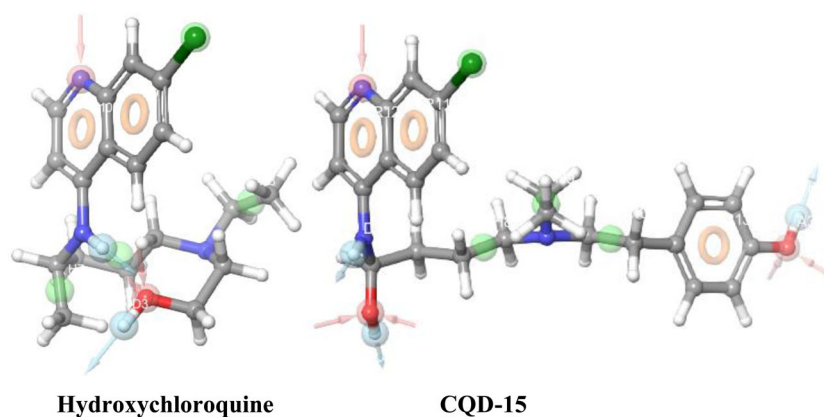
Hydroxychloroquine   **CQD-15**

Figure 3. Pharmacophre model generated structural features present in the ligands (**Hydroxychloroquine** and **CQD15**).

Table 2. Docking score and functional parameters of all 18 molecules including Chloroquine and Hydroxychloroquine with SARS-CoV-2 main protease (PDB ID: 6LU7).

S. No.	Ligand	DockScore	LipophilicEvdW	PhobEnHB	HBond	Electro	LowMW
1	CQD15	−6.17	−2.24	−1.5	−1.87	−1.1	−0.12
2	CQD14	−5.14	−1.93	−1.5	−1.35	−0.77	−0.13
3	CQD16	−4.19	−2.78	0	−1.11	−0.63	−0.12
4	CQD6	−4.15	−2.57	0	−1.15	−0.63	−0.38
5	CQD5	−3.92	−1.45	−1.5	−0.7	−0.42	−0.28
6	Hydroxychloroquine	−3.48	−2.38	0	−1.28	−0.48	−0.38
7	CQD13	−3.44	−2.68	0	−1.15	−0.6	−0.18
8	CQD12	−3.41	−2.49	0	−1.25	−0.47	−0.17
9	CQD9	−3.39	−3.26	0	−0.04	−0.14	−0.37
10	CQD10	−3.3	−2.64	0	−0.52	−0.21	−0.38
11	Chloroquine	−3.27	−2.86	0	−0.34	−0.15	−0.43
12	CQD7	−3.08	−3.36	0	0	−0.14	−0.17
13	CQD4	−3.04	−2.46	0	−1.68	−0.62	−0.38
14	CQD1	−3.01	−2.78	0	−0.45	−0.14	−0.17
15	CQD8	−2.93	−3.24	0	−0.02	−0.12	−0.32
16	CQD2	−2.9	−2.84	0	0	−0.14	−0.32
17	CQD11	−2.78	−2.83	0	0	−0.26	−0.18
18	CQD3	−2.73	−2.75	0	0	−0.24	−0.37

LipophilicEvdW: Lipophilic term derived from hydrophobic grid potential and fraction of the total protein-ligand vdW energy
PhobEnHB: Reward for hydrophobicallypacked H-bond
HBond: ChemScore H-bond pair term
Electro: Electrostatic rewards
LowMW: Reward for ligands with low

against COVID-19. Umesh et al. Worked on Indian spices to study structure based interaction with COVID-19 and conclude with three compounds Carnosol, Arjunglucoside-I and Rosmanol (Umesh et al., 2020). Sinha et al. evaluated different Saikosaponins for their potency against SARS-CoV-2 using NSP15 and fusion spike glycoprotein as targets (Sinha et al., 2020).

As Hydroxychloroquine is the derivative of Chloroquine and shows better inhibition in comparison to Chloroquine against SARS CoV-2 (Pastick et al., 2020; Yao et al., 2020), the current study is emphasized on the derivatives of Chloroquine against COVID-19. A set of 18 molecules based on Chloroquine scaffold (Scheme 1) are designed and optimized computationally by using Schrodinger software. These molecules were designed by substituting different groups (like, methyl (CH_3), hydroxyl group (OH), NO2, Amino group (NH2), halogens (F, Cl and Br) and ring aromatic (benzene, phenol)) at the position of R1 and R2 (Scheme 1). These groups were considered due to its potential to interact with the amino acids (like 'hydroxyl group, methyl and amine can

interact with the amino acid through H-bond', 'halogens; Cl, Br, F can interact through halogen bonding', 'NO2 can show H-bond as well as salt bridges' and 'ring aromatic compounds can show pi-pi and pi-cation interactions. In addition to pi-pi and pi-cation interactions the phenol can also show H-bonding due to OH group') (Rawel et al., 2005; Zavodszky & Kuhn, 2005). The generated pharmacophore model can efficiently explain the essential structural features present in the molecules having the potency to interact with the target protein. Molecular docking, MM/GBSA study, ADME property and MD simulation analysis have been employed to study and identify the candidate having better interactions and binding affinity with the SARS-CoV-2.

Methodology

Molecular simulation studies were performed by using Maestro 12.3 module (Schrodinger Release 2020-1, 2020). All the 18 designed molecules (including chloroquine and hydroxychloroquine) and protein (**6LU7**) (Jin et al., 2020;
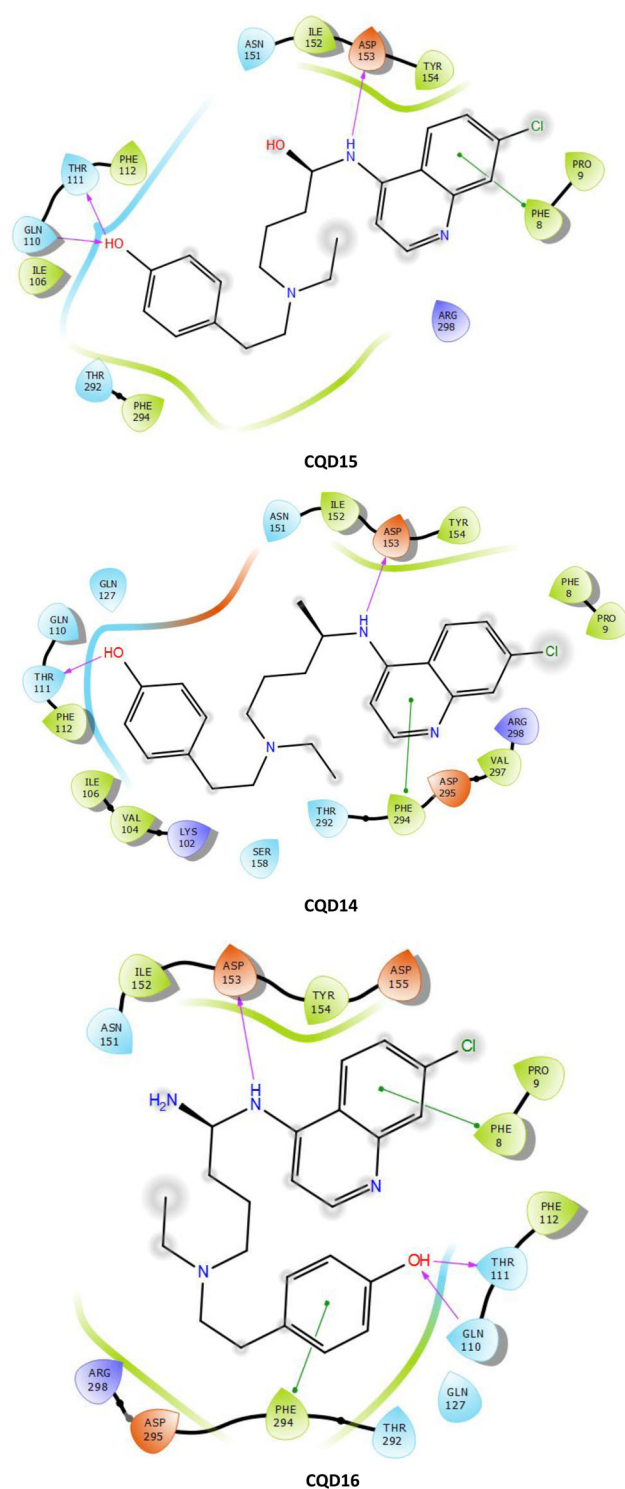
Figure 4. Ligand-receptor interaction diagram of chloroquine derivative molecules (**CQD14**, **CQD15** and **CQD16**) with the SARS-CoV-2 (**PDB ID: 6LU7**).

For the validation of theoretical results, a set of different PDB_IDs i.e. 5REB (Fearon et al., 2020), 6W02 (Michalska et al., 2020) were downloaded from protein data bank and re-docking of the self ligand with the protein was performed by using 'Ligand Docking' module with extra precision (XP) mode. RMSE values were calculated between crystal and re-dock pose by using the 'superimpose' module. RMSD value generated by re-docking the native ligands in the active site of the respective protein was 1.33 Å, and 1.29 Å. As the RMSD values are less than X-ray resolution of the corresponding protein which is 1.68 Å, and 1.5 Å. It indicates that the generated model has a good correlation with the experimental X-ray crystal structures (Figure 1).

Result and discussion

Pharmacophore model

A total of 20 pharmacophore hypothesis models (Table 1) were generated by using the 'phase module.' The hypotheses have consisted of all active match molecules. There were 2 types of different models generated like DHHHRR (model 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10) and DHHRR (model 11, 12, 13, 14, 15, 16, 17, 18, 19 and 20). Out of 20 models, DHHHRR_1 was considered as the best model as this six featured models shows the best PhaseHypoScore (1.383), Survival Score (6.392) and good BEDROC score (0.999). Pharmacophore hypothesis model DHHHRR_1 consists of one H-bond donor (blue color), three hydrophobic sites (green color) and two aromatic rings (orange color), shown in Figure 2(a) (also shown the position of features concerning each other in Figure 2(a)). DHHHRR_1 model shows proper fit with all designed ligands. For example; Hydroxychloroquine and **CQD14** shows a proper fit with the best pharmacophore model DHHHRR_1 (Figure 2(b)) which indicates all the optimized Chloroquine derivatives are having nearly similar orientations.

The Pharmacophore models were generated to study the maximum number of structural features present in the designed molecules. The ligand-receptor interactions depends upon these structural features present in the ligand like, H-bond acceptor (A, red color ball), H-bond donor (D, blue color ball), Aromatic rings (R, orange color ring) and Hydrophobic region (H, green color ball) (Figure 3). The selected molecules **CQD-15**, **CQD-14** and **CQD-16** shows 13, 12 and 13 number of structural features whereas Chloroquine and Hydroxychloroquine shows 9 and 10 number of structural features. It indicates that the selected molecules **CQD-15**, **CQD-14** and **CQD-16**, have the potential to interact with the enzyme (Figure 3 and Figure S1).

Molecular docking

The generated active site of SARS-CoV-2 main protease (**PDB ID: 6LU7**) is occupied by charged amino acids (ARG-298, LYS-102, ASP-153 and ASP-295), polar amino acids (THR-111, GLN-110, GLN-127, THR-292, ASN-151 and SER-158) and hydrophobic amino acid (ILE-152, TYR-154, PRO-9, PHE-8,
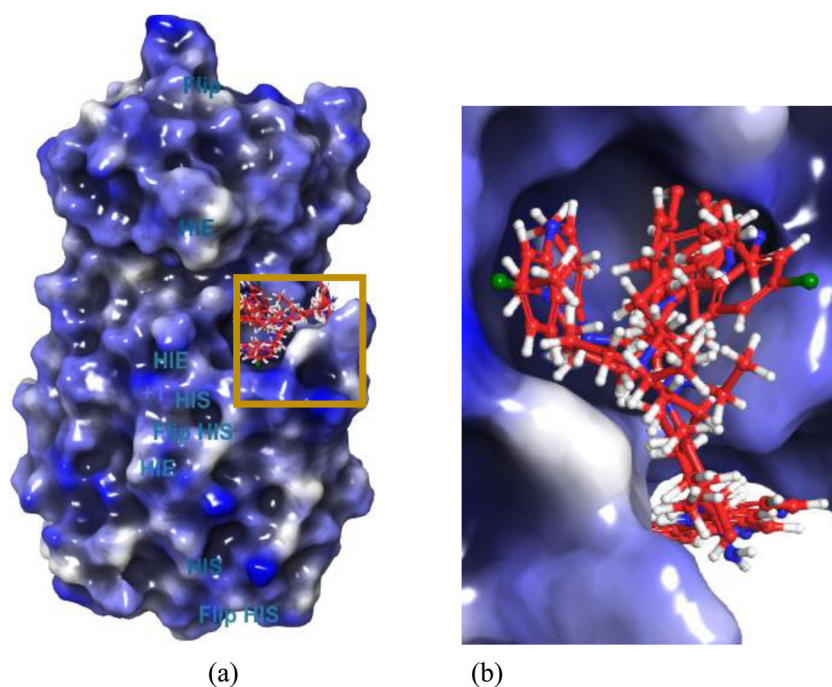
Muralidharan et al., 2020; Elmezayen et al., 2020; Kumar et al., 2020) were optimized and followed the methodology of pharmacophore hypothesis, molecular docking, MM-GBSA study, ADME property analysis and molecular dynamics (MD) simulations as per the previous reports (Beura & Chetti, 2020; Ganjoo et al., 2019) and detailed methodologies are given in supporting information. The detailed working methodology flowchart is shown in Scheme 2.

Figure 5. Molecular docking pose of Chloroquine, Hydroxychloroquine, **CQD14**, **CQD15** and **CQD16** with the SARS-CoV-2 (**PDB ID: 6LU7**). (a) Complete image of ligands binding with the protein (b) Magnified image of ligands binding at the active site.

Hydroxychloroquine

Chloroquine

Charged (negative)	Polar	Distance · · · ·
Charged (positive)	Unspecified residue	H-bond
Glycine	Water	Halogen bond
Hydrophobic	Hydration site	Metal coordination
Metal	Hydration site (displaced) ✕	Pi-Pi stacking

Pi-cation
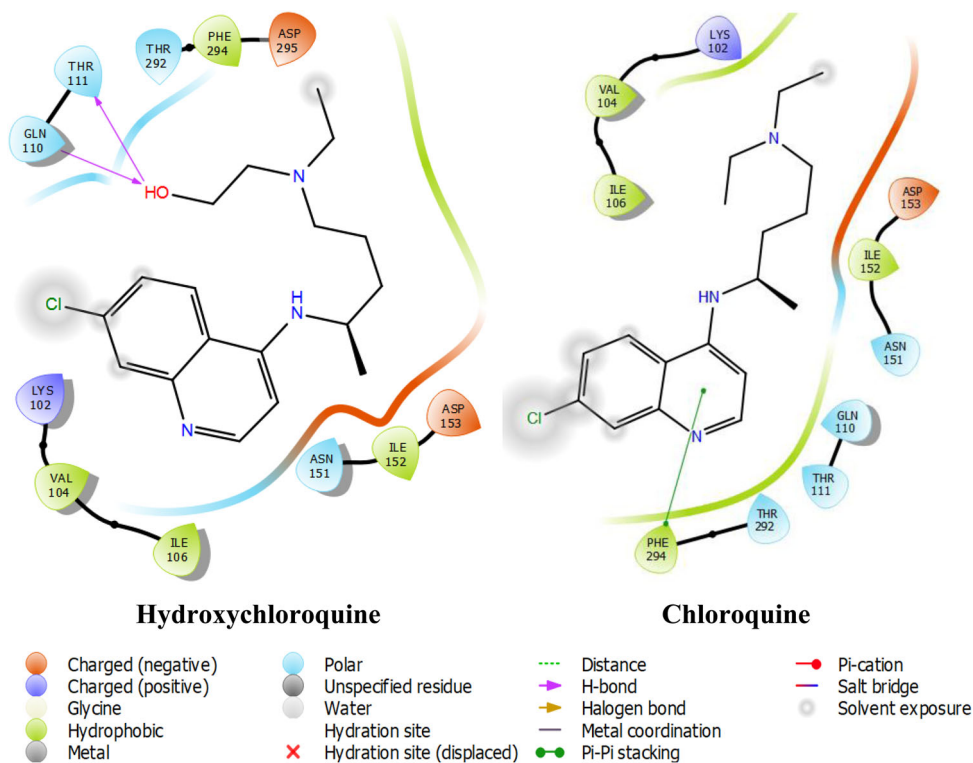Salt bridge
Solvent exposure

Figure 6. Ligand-receptor interaction diagram of chloroquine and hydroxychloroquine with the SARS-CoV-2(PDB ID: 6LU7).

PHE-112, PHE-294, VAL-104, VAL-297 and ILE-106) residue. To ensure the excavation of the best candidates, molecular interaction study was performed through ligand docking into the active site of the SARS-CoV-2. The designed 18 molecules were visually inspected based on the docking score, ligand-receptor interactions, H-bonding, hydrophobic potential, electrostatic rewards and rewards for the ligand of low molecular weight. This deep study concludes with 3 best

molecules i.e. **CQD15**, **CQD14** and **CQD16** and are showing docking scores of -6.17, −5.14 and -4.19 respectively (Table 2). **CQD15** shows three H-bonds with two polar amino acids (THR-111 and GLN-110) and one charged amino acid (ASP-153), two H-bonds due to the H-bond donor group and one due to H-bond acceptor site present in the ligand. Additionally, **CQD15** shows one pi-pi interaction with hydrophobic amino acid (PHE-8) (Figure 4). **CQD14** shows 2 H-

bond interactions due to two H-bond donor groups present in the ligand along with one pi-pi interaction. The H-bond interactions take place with one polar amino acid (THR-111) and one charged amino acid (GLN-110) present in the active site (Figure 4). **CQD16** shows similar interactions like **CQD15** and additionally, it shows one pi-pi interaction with hydrophobic PHE-294 amino acid (Figure 4). All 3 new ligands showing a good range of solvent exposures (Figure 4) and the docked pose shows the best fit of the ligand in the active site cavity of the enzyme (Figure 5).

The comparative analysis of selected molecules (**CQD15**, **CQD14** and **CQD16**) with Chloroquine and Hydroxychloroquine were studied. Chloroquine and Hydroxychloroquine are showing a docking score of -3.27 and -3.48 respectively (Table 2). Chloroquine shows one pi-pi stacking with hydrophobic region (PHE-294) along with a good range of solvent exposure where as Hydroxychloroquine shows two H-bond interactions, one is due to H-bond acceptor and another is due to H-bond donor group present in the active site of SARS-CoV-2. The selected molecules (**CQD15**, **CQD14** and **CQD16**) were showing better docking scores (-6.17, −5.14 and -4.19 respectively) as well as better ligand-receptor interactions in comparison to chloroquine and hydroxychloroquine (Figure 6).

Further, the designed ligands were also docked with coronavirus SARS Spike Glycoprotein - human ACE2 complex (PDB ID: 6CS2) (Kirchdoerfer et al., 2018) to study the impact of ligands on SARS Spike Glycoprotein - human ACE2 complex. The docking study of "ligand and SARS Spike Glycoprotein - human ACE2 complex" shows similar result like "ligand and COVID-19 main protease". The ligands **CQD-15**, **CQD-14** and **CQD-16** showing docking score of -6.47, −5.48, and 5.35 respectably where as Chloroquine and Hydroxychloroquine shows -3.56 and -3.6 respectively (Table 3). The detailed interaction diagrams are shown in Figure S2a–S2e. Docking study is also performed with SARS-CoV-2 main protease (PDB ID: 7BUY) (Zhao et al., 2020) to validate the ligand-main protease interactions. The study also concludes with same ligands **CQD-16**, **CQD-15** and **CQD-14** which shows better docking score and ligand-receptor interactions in comparison to Chloroquine and Hydroxychloroquine which follows the trend of ligand-6LU7 docking study. The detailed interactions and docking score are shown in Figure S3a–S3e and Table S1 respectively.
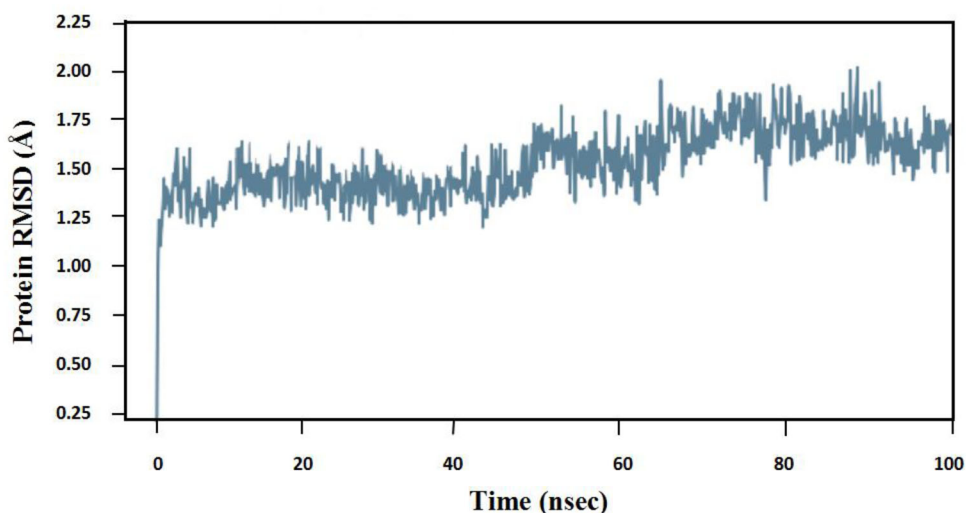
Table 3. Docking score and functional parameters of all 18 molecules including Chloroquine and Hydroxychloroquine with coronavirus SARS Spike Glycoprotein - human ACE2 complex (PDB ID: 6CS2).

Ligand	DockScore	LipophilicEvdW	PhobEnHB	HBond	Electro	LowMW
CQD-15	−6.47	−3.77	−1.37	−1.25	−0.55	−0.12
CQD-14	−5.48	−3.74	−1.5	−1.28	−0.38	−0.13
CQD-16	−5.35	−3.32	−1.5	−2.2	−0.81	−0.12
CQD-11	−5.33	−4.56	−0.75	−0.7	−0.38	−0.18
CQD-12	−4.91	−3.21	−1.5	−0.94	−0.44	−0.17
CQD-13	−4.88	−3.93	0	−1.2	−0.39	−0.18
CQD-6	−4.78	−2.79	−1.5	−0.63	−0.34	−0.38
CQD-7	−4.68	−2.69	−1.5	−0.7	−0.22	−0.17
CQD-5	−4.44	−3.51	−0.75	−0.7	−0.49	−0.28
CQD-4	−4.06	−3.38	0	−0.7	−0.41	−0.38
CQD-9	−3.9	−3.38	0	−0.65	−0.32	−0.37
CQD-10	−3.7	−3.44	0	−0.11	−0.25	−0.38
CQD-2	−3.6	−3.53	0	0	−0.14	−0.32
Hydroxychloroquine	−3.6	−3.28	0	0	−0.19	−0.38
Chloroquine	−3.56	−3.48	0	0	−0.16	−0.43
CQD-1	−3.54	−3.62	0	0	−0.07	−0.17
CQD-3	−3.52	−3.62	0	0	−0.14	−0.37
CQD-8	−3.51	−3.81	0	0	−0.05	−0.32

Table 4. MM-GBSA_dG_Bindvalues of 18 molecules in (kcal/mol) obtained through MM-GBSA analysis.

Ligand	r_psp_MMGBSA_dG_Bind
CQD15	−47.74711519
CQD14	−58.40628355
CQD16	−41.70415476
CQD6	−51.56851776
CQD5	−39.24253856
Hydroxychloroquine	−40.34126469
CQD13	−52.76079182
CQD12	−49.54062105
CQD9	−53.07817314
CQD10	−43.20765873
Chloroquine	−40.46204749
CQD7	−55.4049401
CQD4	−58.3743558
CQD1	−32.47031282
CQD8	−52.27239411
CQD2	−51.74337235
CQD11	−51.76783519
CQD3	−48.48654391

Table 5. ADME properties of all 18 ligands to determine their 'drug-likeness'.

Ligand	mol_MW	donorHB	accptHB	QPlogPo/w	RuleOfFive	QPlogS	%Human Oral Absorption
CQD15	413.946	3	6.45	4.094	0	−4.705	91.032
CQD14	411.973	2	4.75	4.998	0	−4.7	100
CQD13	396.962	3	5	4.313	0	−3.387	92.144
CQD12	397.947	2	5.7	4.235	0	−3.367	100
CQD11	**395.974**	**1**	**4**	**5.569**	**1**	**−4.44**	**100**
CQD10	334.891	3	5	2.496	0	−0.975	78.096
CQD9	337.867	1	4	4.661	0	−4.601	100
CQD8	354.322	1	4	4.991	0	−4.997	100
CQD7	**398.773**	**1**	**4**	**5.066**	**1**	**−5.083**	**100**
CQD6	335.876	2	5.7	3.352	0	−3.13	95.059
CQD5	364.874	1	6	3.505	0	−3.524	92.172
CQD4	334.891	3	5	2.968	0	−2.604	79.338
CQD3	337.867	1	4	4.777	0	−4.824	100
CQD2	354.322	1	4	4.983	0	−4.944	100
CQD1	**398.773**	**1**	**4**	**5.047**	**1**	**−4.798**	**100**
Hydroxy-chloroquine	335.876	2	5.7	3.329	0	−3.335	93.491
Chloroquine	319.876	1	4	4.559	0	−4.582	100
CQD16	412.961	4	5.75	3.619	0	−3.619	76.602

Figure 7. The Protein RMSD trajectory of the (**CQD15-6LU7**) complex during the 100 ns simulation.
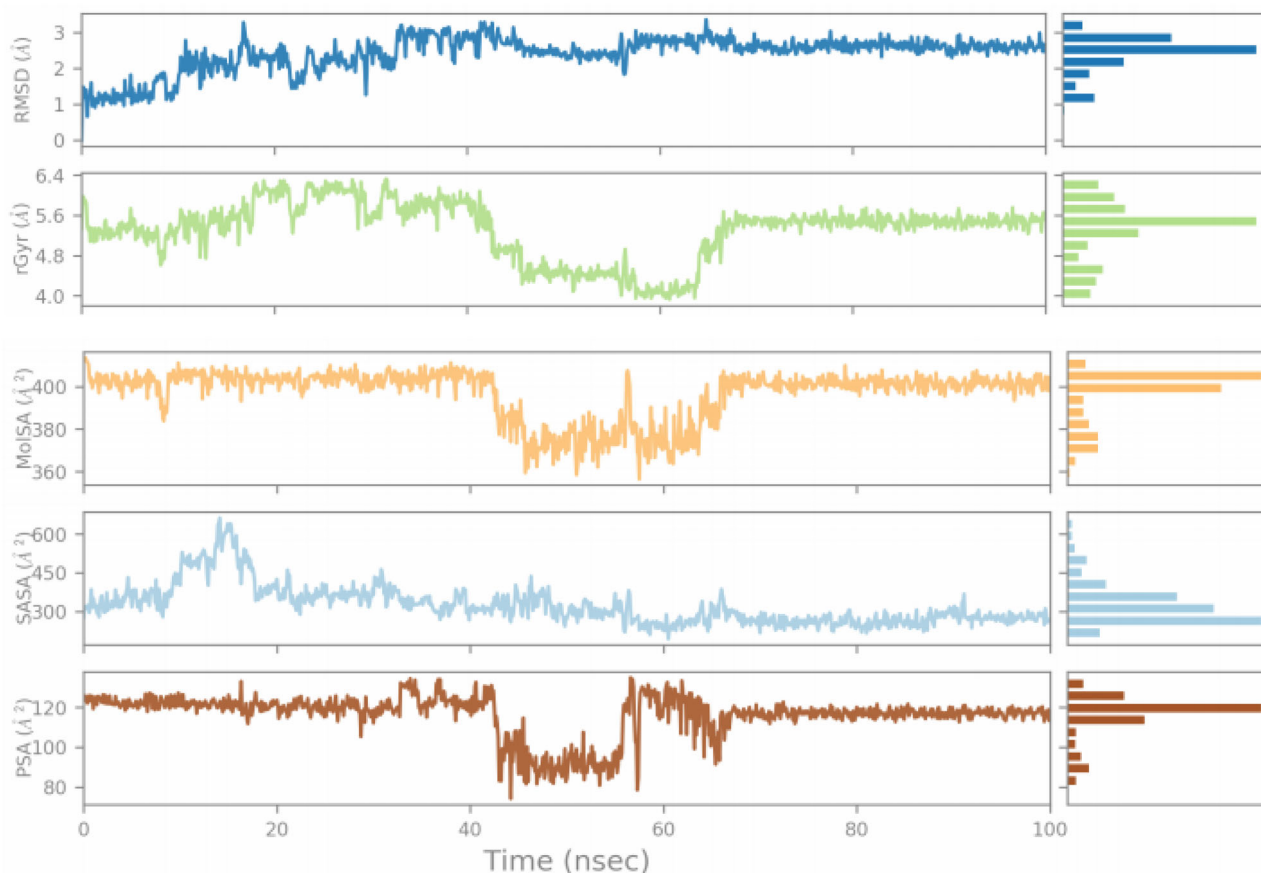

Figure 8. The ligand property trajectory of the (**CQD15-6LU7**) complex during the 100 ns simulation.

MM-GBSA study

All 18 molecules were subjected to ligand-receptor binding energy, MM-GBSA analysis. The MM-GBSA analysis computed the binding free energy of docked ligand-receptor complex which confirms the stability of the ligand after binding to the active site of the enzyme. The generated 'MM-GBSA_Dg_Bind' energy by using the MM-GBSA module shows the energy difference between prime energy (optimized ligand-receptor complex) and the combined energy of optimized free ligand and optimized free receptor. The MM-GBSA_dG_Bind energies for all 18 compounds are shown in Table 4. MM-GBSA study concludes that all the molecules except **CQD1** and **CQD5** shows better binding energy in comparison to Choloroquine and Hydroxychloroquine.

ADME analysis

The drug-likeness properties of the molecules studied by using well-known ADME analysis i.e. Absorption (A),
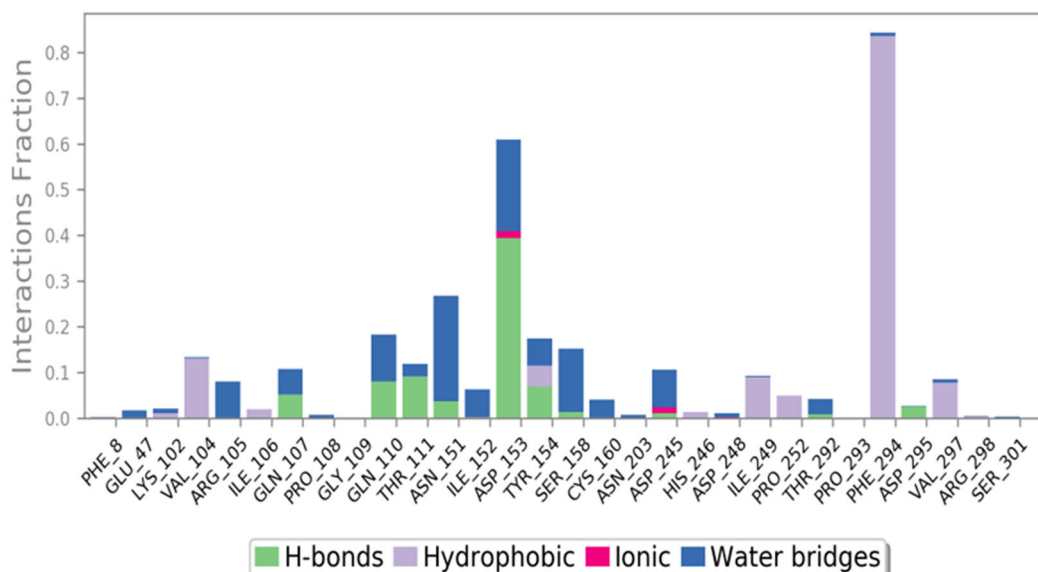
Figure 9. The histogram of protein-ligand contact over the course of the trajectory.

Distribution (D), Metabolism (M) and Excretion (E) which explains the disposition of pharmaceutical compound inside an organism and therefore, influences the pharmacological activity of it. The screening was performed based on a violation of 'Lipinski's rule of five', **QPPCaco** and **QPlogS**. In the ADME property analysis out of 18 molecules, 3 were showing violation of 'Lipinski's rule of five' and (**QPlogS**) (shown in the bold in Table 5) whereas remaining 15 molecules were following the standard parameters. All the molecules show percentage of oral absorption more than 70%. ADME property analysis concludes that all molecules except CQD1, CQD7 and CQD11 show drug-likeness properties.

Molecular dynamics simulations

Protein RMSD

MD simulations were performed to study the physical movements of atoms & molecules and the dynamic evolution for the best complex (**6LU7-CQD15**). The RMSD is a quantitative parameter to estimate the stability of the protein system (Figure 7). The protein RMSD trajectory of SARS-CoV-2 (**6LU7**) shows a fluctuation up to 5 ns simulation time then gradually tends to equilibrium (Figure 7). The RMSD average value of SARS-CoV-2 (**6LU7**) after reaching equilibrium was 1.6 Å (Figure 7) as for the small globular protein the deviation within 1-3 Å is acceptable. Based on the stability, **6LU7-CQD15** was considered for further study like Protein-Ligand contact study (Histograms and Graph).

Ligand properties

Ligand property study includes Ligand RMSD, Radius of Gyration (rGyr), Molecular Surface Area (MolSA), Solvent Accessible Surface Area (SASA) and Polar Surface Area (PSA). The root mean squre deviation (RMSD) of the ligand fluctuates up to 40 ns simulation and then gradually comes to equilibrium. The ligand is showing range of RMSD around 1

to 3 Å and the equilibrium around 2.5 Å (Figure 8). The rGyr is the radial distance to a point which would have a moment of inertia the same as the body's (ligand) actual distribution of mass, if the total mass of the body (ligand) were concentrated. rGyr of the ligand also showing a heavy fluctuate up to 65 ns simulation and then gradually comes to equilibrium. The ligand is showing range of rGyr around 4 to 6.4 Å and the equilibrium around 5.5 Å (Figure 8). MolSA is surface calculation with 1.4 Å probe radius. This value is equivalent to a van der Waals surface area. The MolSA of the ligand is constant throughout the simulation but there is some fluctuation in 42 ns to 65 ns simulation period. The ligand is showing range of MolSA around 360 to 400 Å^2 and the equilibrium around 400 Å^2 (Figure 8). SASA is surface area of a molecule accessible by a water molecule. SASA of the ligand also showing a heavy fluctuate up to 23 ns simulation and then gradually comes to equilibrium. The ligand is showing range of SASA around 300 to 600 Å^2 and the equilibrium around 300 Å^2 (Figure 8). PSA is solvent accessible surface area in a molecule contributed only by oxygen and nitrogen atoms. The PSA of the ligand is constant throughout the simulation but there is some fluctuation in 41 ns to 68 ns simulation period. The ligand is showing range of PSA around 80 to 120 Å^2 and the equilibrium around 120 Å^2 (Figure 8). All the ligand properties are showing some fluctuation in the initial simulation period or intermediate simulation period but gradually come to equilibrium at the end of the simulation which conforms that the stability of the ligand in the active site of the protein.

Protein-ligand contacts

The protein-ligand contacts of the stable ligand-receptor complex (**CQD15-6LU7**) was studied by using the protein-ligand contact histograms (Figure 9) (Dariya & Nagaraju, 2020). Protein-ligand interactions (or 'contacts') are categorized into four types: *hydrogen honds*, *hydrophobic*, *ionic* and *water bridge*. Hydrogen bonds play a significant role in ligand
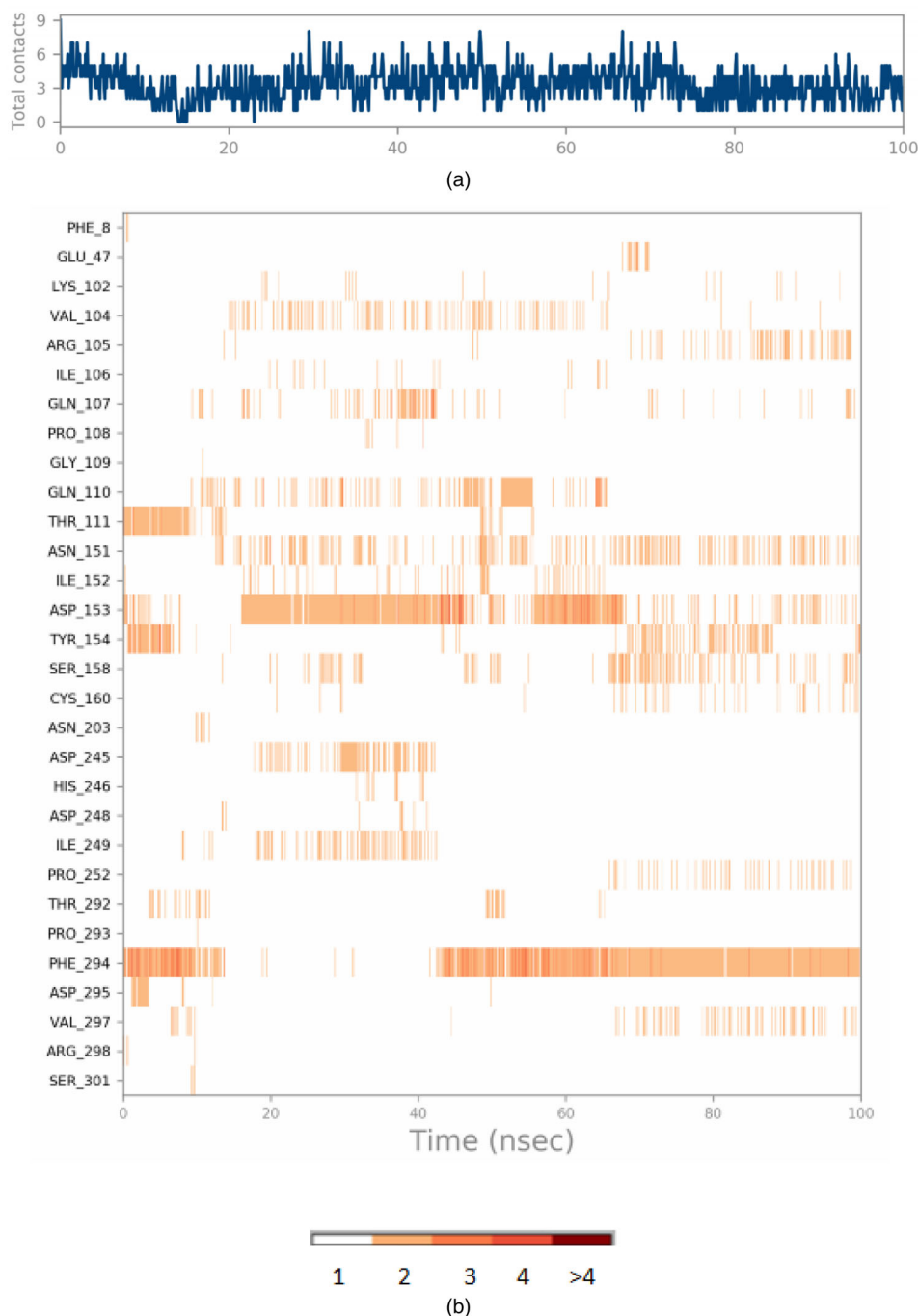
Figure 10. (a) Total number of contacts/interaction in each trajectory frame of **6LU7-CQD15** complex. (b) Interaction shown by the active site amino acids in each trajectory frame of **6LU7-CQD15** complex.

binding. Consideration of hydrogen-bonding properties for drug design is important because of their strong influence on drug specificity, metabolization and adsorption. Hydrogen bonds between a protein and a ligand can be further broken down into four sub-types: backbone acceptor, backbone donor, side-chain acceptor and side-chain donor. The geometric criteria for protein-ligand hydrogen bond is of 2.5 Å between the donor and acceptor atoms (D—H···A), a donor angle of $\geq 120°$ between the donor-hydrogen-acceptor atoms (D—H···A) and an acceptor angle of $\geq 90°$ between the hydrogen-acceptor-bonded_atom (H···A—X). The histogram shows the

active site amino acids (mainly GLN-107, GLN-110, THR-111, ASN-151, ASP-153, TYR-154, ASP-295) interacting through hydrogen bonding (Figure 9). *Hydrophobic* contacts fall into three subtypes like π-cation, π-π and other non-specific interactions. These types of interactions involve a hydrophobic amino acid and an aromatic or aliphatic group on the ligand. Some hydrophobic amino acids like VAL-104, ILE-106, TYR-154, ILE-249, PRO-252, PHE-294 and VAL-297 showing hydrophobic interactions with the ligand (Figure 9). *Ionicinteractions* or polar interactions, are between two oppositely charged atoms that are within 3.7 Å of each other and do not involve a hydrogen bond. All *ionic*

interactions are broken down into two subtypes: those mediated by a protein backbone or side chains. ASP-153 and ASP-245 are showing minimal ionic interaction with the ligand (Figure 9). *Water bridges* are hydrogen-bonded protein-ligand interactions mediated by a water molecule. The hydrogen-bond geometry is slightly relaxed from the standard hydrogen bond definition. The current geometric criteria for a protein-water or water-ligand hydrogen bonds are: a distance of 2.8 Å between the donor and acceptor atoms (D—H⋯A); a donor angle of $\geq 110°$ between the donor-hydrogen-acceptor atoms (D—H⋯A); and an acceptor angle of $\geq 90°$ between the hydrogen-acceptor-bonded_atom (H⋯A—X). Almost all the major interacting amino acids are showing water bridges (Figure 9). In this protein-ligand contact histograms some amino acids were showing highly effective interactions like 'ASP-153 and PHE-294' having 62% and 83% time interactions in **6LU7-CQD15** complex of 100 ns simulation.

A timeline representation of the interactions and contacts (Hydrogen bonds, Hydrophobic, Ionic and Water bridges) summarized in the ligand-receptor interaction (histogram) study analysed in the following two panels in Figure 10(a, b). The top panel shows the total number of specific contacts the protein makes with the ligand in each and every trajectory frame. The number of contact varies zero to nine over the course of the trajectory (Figure 10(a)). The contribution of amino acids in each trajectory frame of 100 ns MD simulation was studied from ligand-protein interaction (bottom panel) (Figure 10(b)). The bottom panel shows, which amino acid residues interact with the ligand in each trajectory frame. Some residues make more than one specific contact with the ligand in a particular trajectory frame, which is represented by a darker shade of orange, according to the scale to the bellow the plot. The **6LU7-CQD15** receptor-ligand complex shows two deep bands (PHE-294 and ASP-153 row), which explains that the above amino acid have more interactions with the ligands in almost all possible orientations (geometry) which is exactly similar as histogram results.

Conclusion

A series of computational approaches used to identify more effective drug candidate against SARS-CoV-2. The pharmacophore modelling, molecular docking, MM_GBSA study and ADME property analysis combinedly concluded with 3 ligands (**CQD15**, **CQD14** and **CQD16**) which have good docking score, ligand-receptor interactions, pharmacophore-based structural features and drug likeness property in comparison to chloroquine and hydroxychloroquine. The ligand-receptor MD simulation study validates the molecular docking study by exploring the protein stability (RMSD), various ligand property and protein-ligand contacts. Further, in vitro analysis followed by its in vivo testing may help in proving **CQD15** ligand as a better inhibitor of SARS-CoV-2. The whole study concludes that derivatives of chloroquine may play a prominent role for the treatment of COVID-19.

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