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Research Article

Zoology

In Silico Identification of Clinically Approved Medicines Against the main Protease of Sars-Cov-2 – A Causative Agent of Covid-19

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Abstract: The COVID-19 pandemic triggered by SARS-CoV-2 is a worldwide health disaster. Main protease is an attractive drug target among coronaviruses, due to its vital role in processing the polyproteins that are translated from the viral RNA. There is presently no exact drug or treatment for this disease caused by SARS-CoV-2.Speeding up drug innovation is immediately required. In the present study, we report the potential inhibitory activity of some FDA approved drugs against SARS-CoV-2 main protease by molecular docking study to investigate their binding affinity in protease active sites. Total 47 FDA approved drugs were selected for molecular docking with main COVID-19 protease. The docking of selected drugs to the active site of protein was performed using AutoDock software. Docking was achieved to attain a population of potential conformations and alignments for the ligand at the binding site. Docking study revealed that great inhibitory efficacy of the one anti-H1N1 drug (Oseltamivir), one anti-TB drug (Rifampin), four anti-HIV drugs (Maraviroc, Etravirine, Indinavir, Rilpivirine) and seven anti-malarial drugs (Atovaquone, Quinidine, Halofantrine, Amodiaquine, Tetracylcine, Azithromycin, hydroxycholoroquine) was found since they could launch H2 bonds with different amino acid residues that caused an inhibition of SARS-CoV-2 protease activity with higher binding affinity ranging from (-10.67 to -8.3 kcal/mol). However, the *in-silico* abilities of the drug molecules tested in this study, further needs to be validated by carrying out *in vitro* and *in vivo* studies. Moreover, this study spreads the potential use of current drugs to be considered and used to comprise the fast expanding SARS-CoV-2 infection.

Keywords: *COVID-19, SARS-CoV-2 main protease, Molecular docking, FDA, Coronavirus*

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1. INTRODUCTION

A new occurred human coronavirus (COVID-19) is informed in December 2019 in Wuhan, China.[1,2] Afterward, the COVID-19 underway spreading across the world, making the whole world on high attentive.[3] The World Health Organization (WHO) surveillance draft in January 2020 stated that, any traveller to Wuhan, Hubei Province in China, two weeks earlier the onset of the signs, is supposed to be a COVID-19 case.[4,5] On April 21st, 2020, a total of 2,31,4621 confirmed infections were reported worldwide, with 1,57,847 deaths with a increasing mortality rate of >4.3%.[5] The World Health Organization (WHO) strategy to comprise the distribution includes the decrease of human-to-human dispersal by preventing the interaction between individuals, accordingly avoiding transmission extension events and interactive critical risk evidence to all populations.[5] In India, the first case of the COVID-19 was reported in Kerala on 30 January 2020. As of 21 April, 2020, there are 14,255 cases and 559 deaths as reported by the Ministry of Health and Family Welfare, Government of India.[6] COVID-19 is a member of Beta coronaviruses similar to the Severe Acute Respiratory Syndrome Human coronavirus (SARS HCoV) and the also the Middle-East Respiratory Syndrome Human coronavirus (MERS HCoV).[7] Main protease (Mpro) is one of the greatest-characterized drug targets among coronaviruses.[8] Beside with the papain-like protease(s), this main protease enzyme is vital for processing the polyproteins that are translated from the viral RNA.[8] This crucial function of main protease in virus replication makes this enzyme a capable target for the expansion of inhibitors and possible treatment remedy for infection of novel coronavirus. To date, no precise therapeutic medicine or vaccine has been accepted for the management of human coronavirus due to natural evolution of receptor-binding domain (RBD) portion of the SARS-CoV-2 spike proteins. Some clinical trials, it has been described that anti-HIV drugs and chloroquine phosphate, an anti-malarial drug, has a assured therapeutic effect on the COVID-19.[9] In specific, chloroquine phosphate is suggested to treat COVID-19 related pneumonia in larger inhabitants in the future. Subsequently, other clinical trials suggested that hydroxychloroquine supplementary with azithromycin is very effective in clearing viral naso-pharyngeal carriage of SARS-CoV-2 in COVID-19 patients in only three to six days, in most patients.[10]This encouraged us to accomplish a systematic study on some clinically approved medicines using molecular docking and reinvestigate their biological efficacies and pharmacological properties. However, there is no organized study on the inhibition of the coronavirus by clinically approved drugs to the best of our knowledge. Hence, the present study was aimed at molecular docking studies of clinically approved drugs against the main protease of SARS-CoV-2.

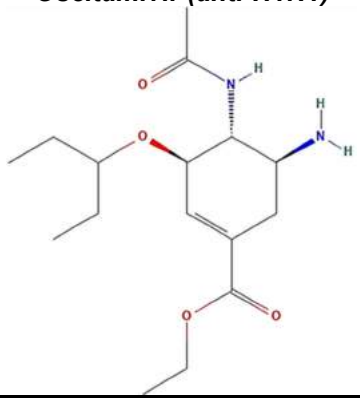
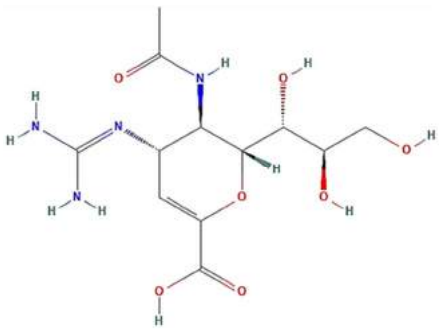
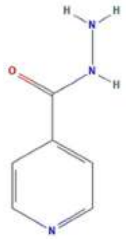
2. MATERIALS AND METHODS

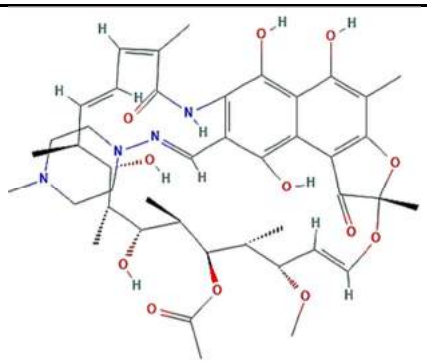
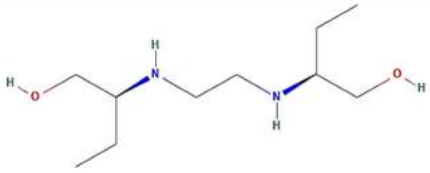
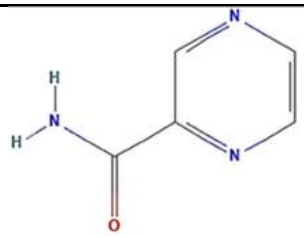
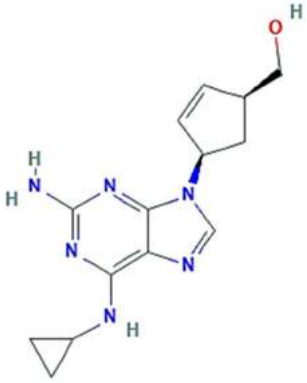
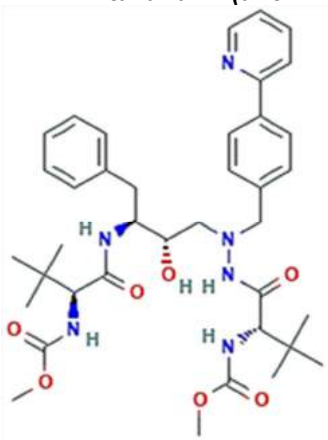
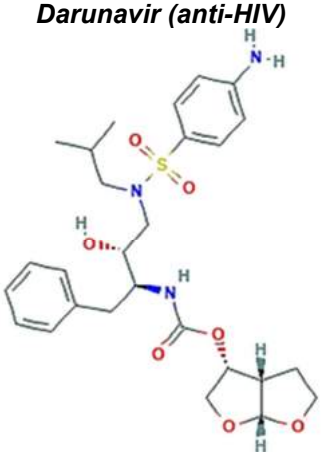
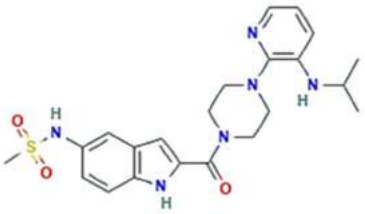
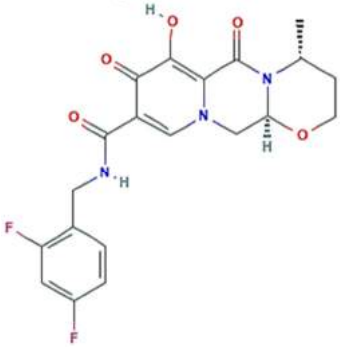
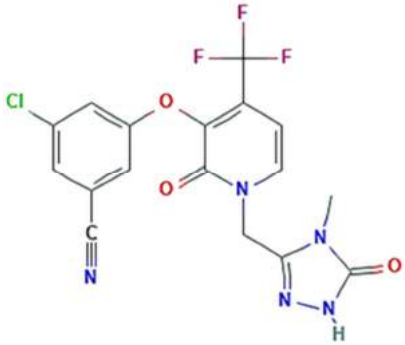
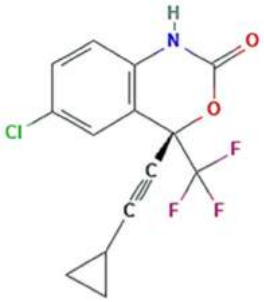
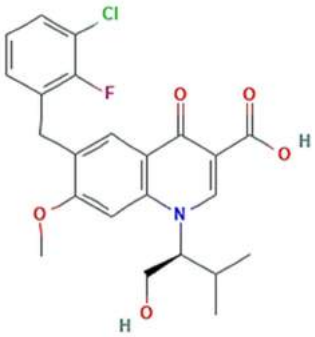
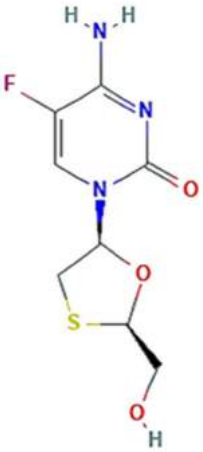
2.1 Molecular Docking Methods

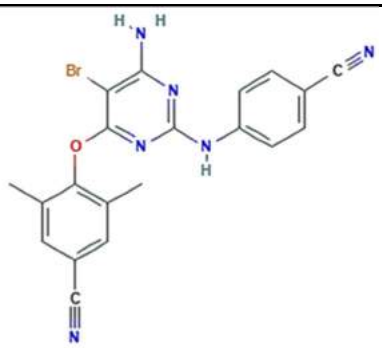
For molecular docking, Auto-Dock 4.2.6 software was used.[11]The free energy (DG) binding of SARS-CoV-2 viral protease with the selected FDA approved drugs was created by means of this molecular docking package. Docking is a computational simulation method of a ligand binding to a receptor or enzyme and expects the favored orientation of binding of one molecule to the second to form a steady complex. To predict the attraction and activity of binding of the minor molecule to their enzyme targets by using scoring functions docking is used. Therefore, docking shows significant role in the rational design of medicines. The sensitivity of docking calculations concerning the geometry of the involvement ligand displays that even minor changes in the ligand structure can lead to big changes in the geometries and scores of the subsequent docked poses.

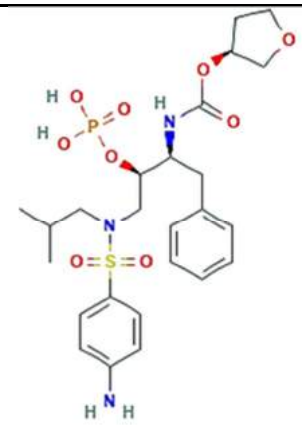
2.2 Selection of Ligand

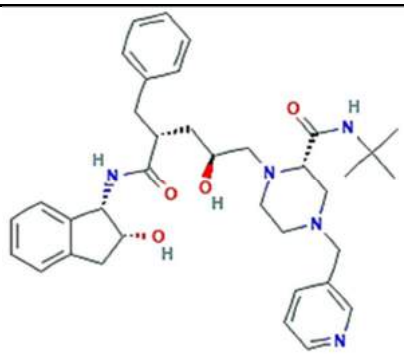
Antiviral and anti-malarial medications were recognized as potential coronavirus inhibitors from diverse literature evaluations. Total 47 FDA approved drugs were selected for molecular docking with main COVID-19 protease. Among the 47 approved drugs, 2 drugs are anti-H1N1 drugs, 4 are anti-TB drugs, 24 are anti-HIV-1 drugs and 17 are anti-malarial drugs selected from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/). The SDF format three-dimensional structure files of the selected FDA approved drugs were downloaded from the PubChem database and were used for molecular docking. Molecular 2D structures of selected FDA approved drugs are shown inFig.1.

Oseltamivir (anti-H1N1) 	***Zanamivir (anti-H1N1)*** 	***Isoniazid (anti-TB)*** 
Rifampin (anti-TB)	***Ethambutol (anti-TB)***	***Pyrazinamide (anti-TB)***

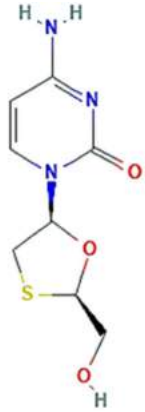
		
Abacavir (anti-HIV)	***Atazanavir (anti-HIV)***	***Darunavir (anti-HIV)***
		
Delavirdine (anti-HIV)	***Dolutegravir (anti-HIV)***	***Doravirine (anti-HIV)***
		
Efavirenz (anti-HIV)	***Elvitegravir (anti-HIV)***	***Emtricitabine (anti-HIV)***
		
Etravirine (anti-HIV)	***Fosamprenavir (anti-HIV)***	***Indinavir (anti-HIV)***



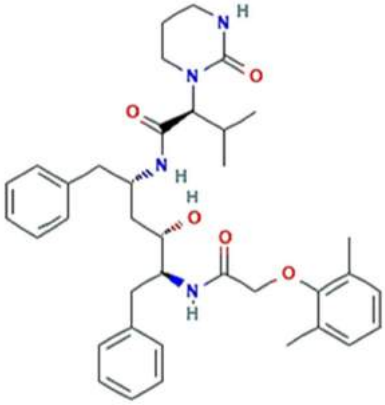




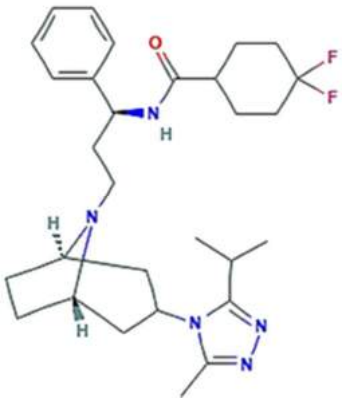
Lamivudine (anti-HIV)



Lopinavir (anti-HIV)



Maraviroc (anti-HIV)



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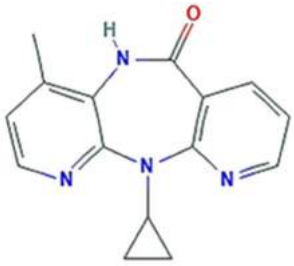
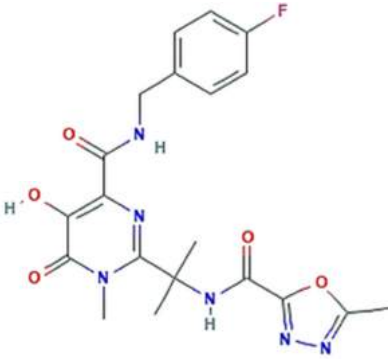


Raltegravir (anti-HIV)



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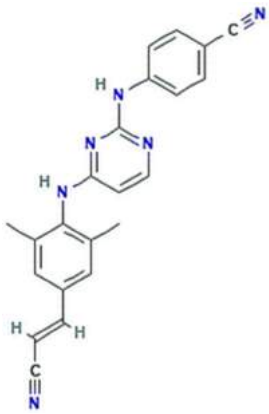

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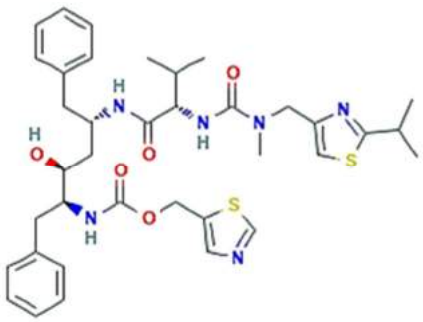

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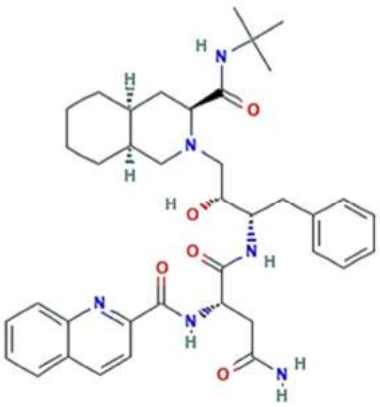

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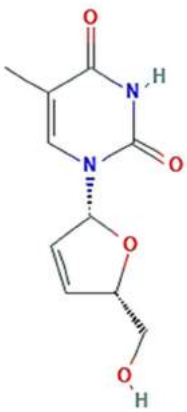
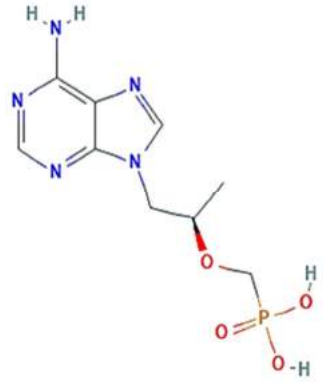
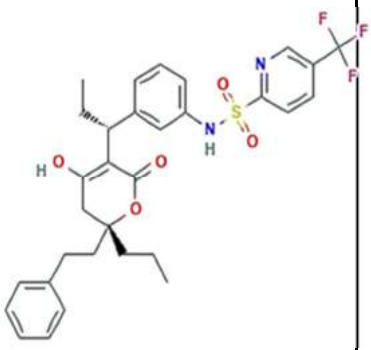
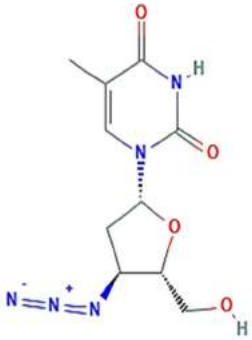
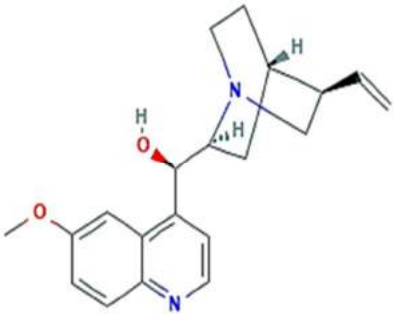
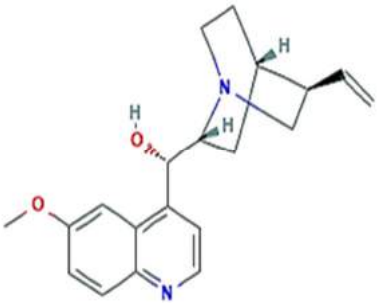
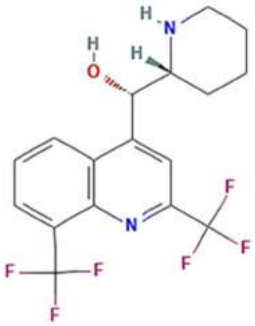
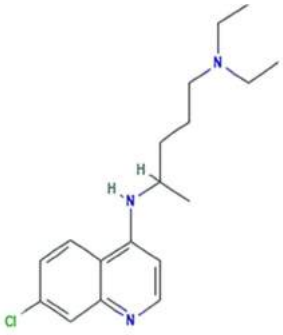
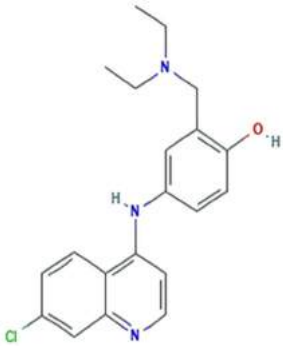
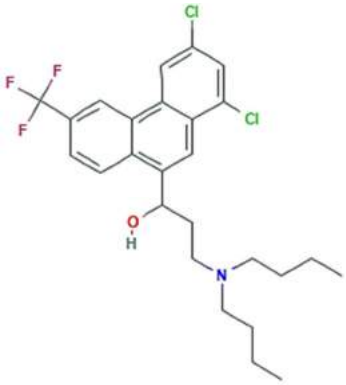
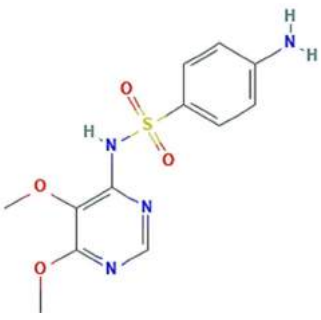
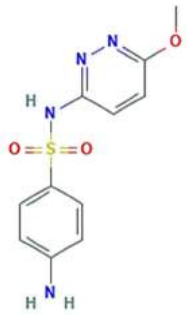


Tenofovir (anti-HIV) 	***Tipranavir (anti-HIV)*** 	***Zidovudine (anti-HIV)*** 
Quinine (anti-malarial) 	***Quinidine (anti-malarial)*** 	***Mefloquine (anti-malarial)*** 
Chloroquine (anti-malarial) 	***Amodiaquine (anti-malarial)*** 	***Primaquine (anti-malarial)*** 
Halofantrine (anti-malarial) 	***Sulfadoxine (anti-malarial)*** 	***Sulfamethoxypyridazine (anti-malarial)*** 

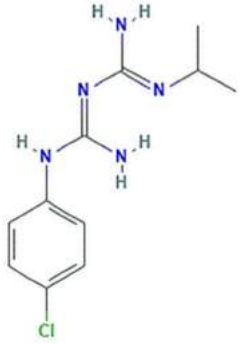
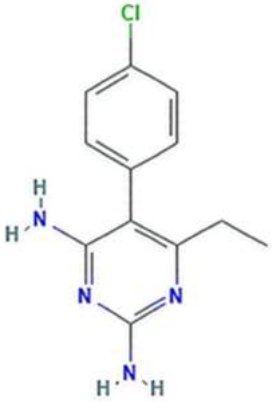
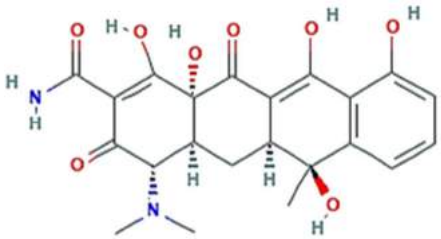
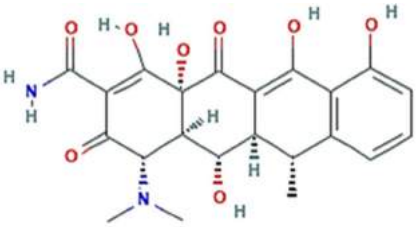
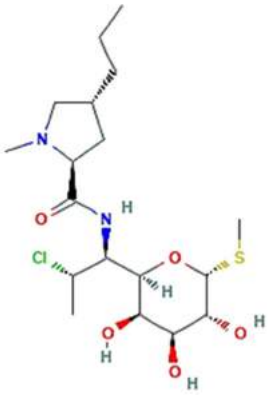
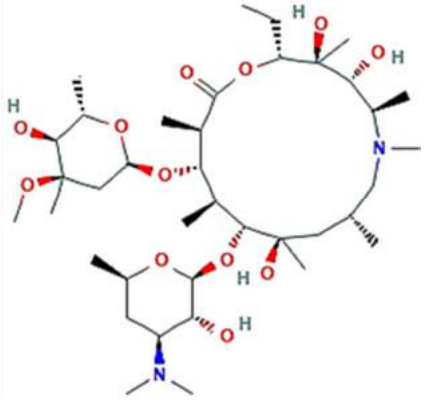
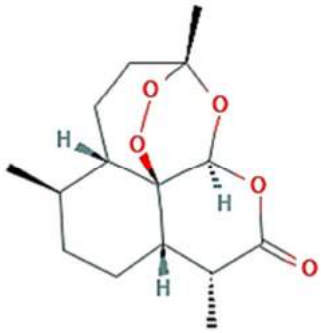
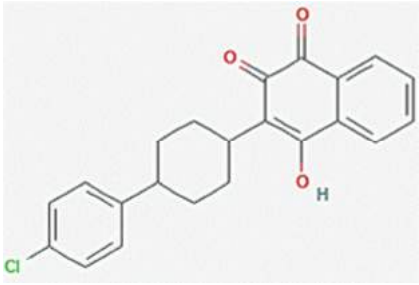
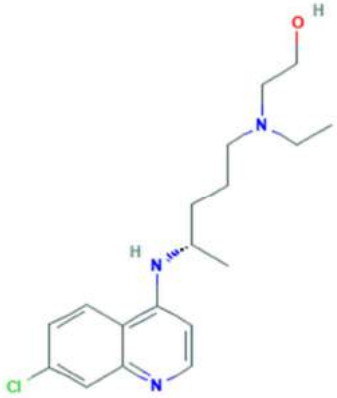
Proguanil (anti-malarial) 	***Pyrimethamine (anti-malarial)*** 	***Tetracycline (anti-malarial)*** 
Doxycycline (anti-malarial) 	***Clindamycin (anti-malarial)*** 	***Azithromycin (anti-malarial)*** 
Artemisinin (anti-malarial) 	***Atovaquone (anti-malarial)*** 	***Hydroxychloroquine (anti-malarial)*** 

Fig 1. Structures of clinically approved drugs

2.3 Selection of Target

The main COVID-19 protease remained used as a target to novelty repurposing candidates over computational selection amongst clinically accepted drugs. The study identified a list of FDA permitted 47 drugs that may form hydrogen bonds to key residues of amino acids within the binding pocket of viral protease and may too have a higher tolerance to conflict mutations. The crystal 3D structure of SARS-CoV-2 protease (PDB ID:6LU7) with hydrophobic loop remained obtained from Protein-Data Bank and showed in fig.2.[12]

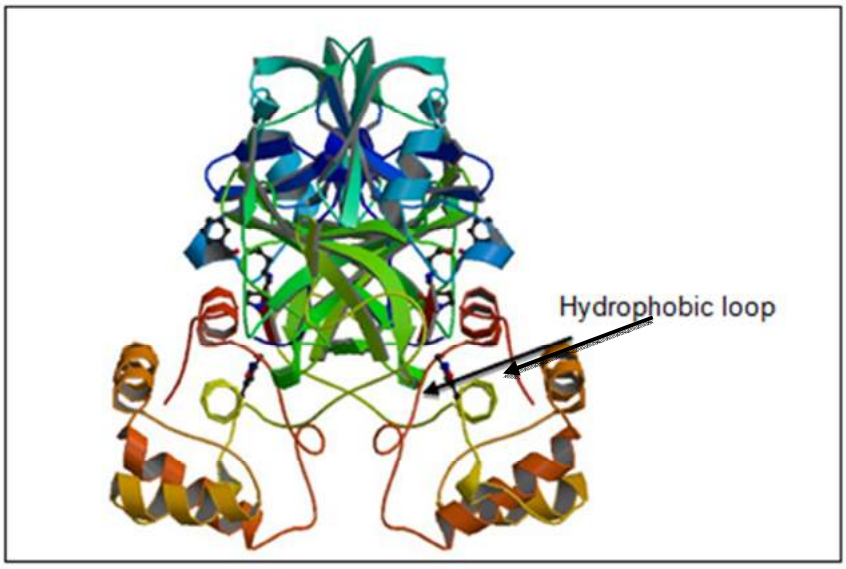


Fig 2.The 3D crystal structure of SARS-CoV-2 protease (PDB ID: 6LU7)

Meanwhile this protease has its crystal structure in a state that signifies the pharmacological target for the progress of new medicines to treat diverse infectious diseases. The preparation of the target enzyme 6LU7 with the Auto-Dock Tools software intricate addition of all H_2 atoms to the enzyme, which is a step essential for accurate calculation of fractional atomic charges. The ligand and all water molecules were detached to make the structure for docking. Gasteiger charges are considered for each atom of the protein in AutoDock 4.2 instead of Kollman charges, which were used in the earlier versions of this package.

2.4 Docking Procedure

For ligand conformational incisive, we take the 'Lamarckian-genetic algorithm (LGA)', which is a mixture of a genetic algorithm and a native search algorithm. This algorithm initially builds a population of entities, being a diverse casual conformation of the docked enzyme. Each distinct protein is then mutated to attain a slightly diverse translation and alternation and the local search algorithm then achieves energy minimizations on a user-specified amount of the population of individuals. The entities with the low subsequent energy are moved to the succeeding generation and the procedure is then repetitive. This algorithm is called Lamarckian while every novel group of entities is allowed to receive the local search variations of their parents.To get many docked structures, Auto-Dock was run numerous times, and used to examine the expected docking energy. Rapid energy assessment was attained by pre-calculating nuclear affinity capacities for every atom in the compound molecule. The binding sites of the target enzyme for these molecules in the AutoGrid process were designated on the patterns of founded ligand-binding pockets.[13] Auto-Dock Tools deliver various approaches to examine the outcomes of docking-simulations such as, structural resemblance, and other limitations like intermolecular energy, visualizing the binding site and its energy and inhibition constant. The energy of interaction of every atom in the ligand was met. For each ligand, 10 best postures were made and scored using Auto-Dock 4.2 scoring purposes.[14]

3. RESULTS

Computational approaches for drug discovery and development are proven to be effective and time efficient, as they are not based on difficult laborious works. The protein-ligand docking elucidates the mechanism of inhibition along with the specificity and efficiency of that ligand as an inhibitor. The association of drug candidate (ligand) to its target receptor is a fundamental binding reaction and the aim of the computer-aided drug discovery is to find small molecules having strong inhibitory or activating action against the biological targets. The strength of inhibition or activation is elucidated through binding affinity.[15]

3.1 Docking Prediction of anti-H1N1 drugs

Oseltamivir and Zanamivir, two FDA approved drugs docked with SARS-CoV-2 main protease and obtained binding energy, are −7.39 kcal/mol and -3.88 kcal/mol respectively (Table 1). Oseltamivir interacted with Glu:166, Pro:52, 168, Met:49,165, Leu:167, His:164, 41, Tyr:54, Gln:189, Arg:188, Asp:187, Thr:190 and Gln:192 at the binding site of this SARS-CoV-2 protease and Zanamivir interacted with Glu:166, Leu:167, Met:165, Gln:189, 192, Thr:190, Ala:191, Pro:168, Gly:170 at the binding site of this protease (fig.3). The results identified that Oseltamivir is a potential inhibitor of the SARS-CoV-2 main protease. Earlier one study has reported that Oseltamivir is a prodrug of oseltamivir carboxylate, a potent and selective inhibitor of the neuraminidase glycoprotein essential for replication of influenza A and B viruses.[16]

Table 1. Molecular docking analysis of anti-H1N1 drugs against COVID-19 Protease (6LU7)					
Sl. No	Compound Name	Binding energy (kcal/mol)	Residue involving interaction	No. of H bonds	Interaction of residues forming H_2 bonds
1	Oseltamivir	-7.39	GLU:166, PRO:52, 168, MET:49,165, LEU:167, HIS:164, 41, TYR:54, GLN:189, ARG:188, ASP:187, THR:190, GLN:192	1	GLU:166
2	Zanamivir	-3.88	GLU:166, LEU:167, MET:165, GLN:189, 192, THR:190, ALA:191, PRO:168, GLY:170	4	GLU:166, LEU:167

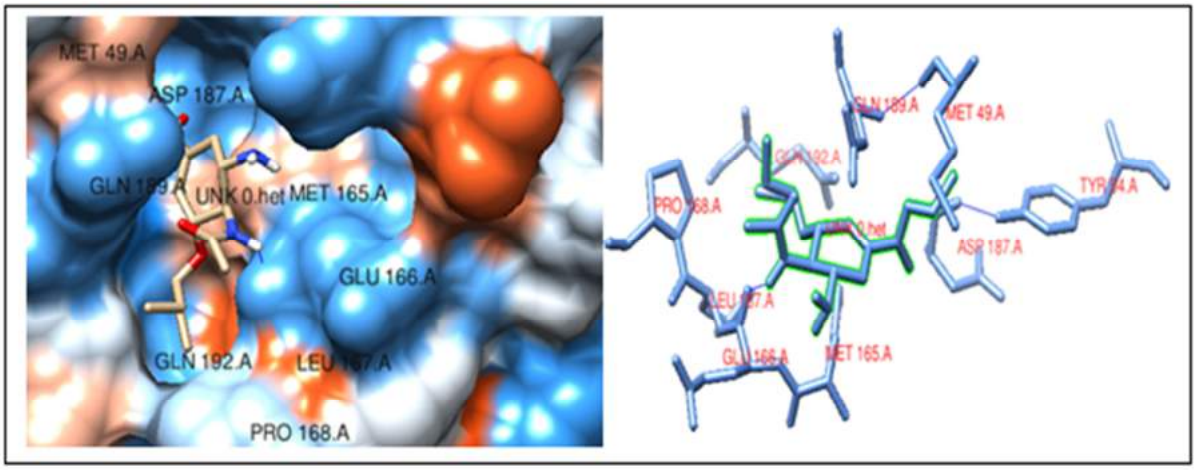


Fig 3.Docking visualisation of COVID-19 protease (6LU7) with Oseltamivir (anti-H1N1 drug)

3.2 Docking Prediction of anti-TB drugs

Isoniazid, Rifampin, Ethambutol and Pyrazinamide are clinically approved drugs were docked with binding energy -4.83, -9.41, -5.02 and -4.05 kcal/mol respectively against SARS-CoV-2 protease (Table 2). Rifampin showing highest binding affinity -9.41 kcal/mol among all the four drugs. The residues involved in the interaction with the Rifampin were Glu:166, Met:165, His:163, 172, 164, 163, Phe:140, Leu:141, 167, Ser:144, Gly:143, Asn;142, Pro:163, Gln:192, Cys:145, Ala:191, Thr:190 and Gln:189 (fig.4).

Table2.Molecular docking analysis of anti-TB drugs against COVID-19 Protease (6LU7)					
Sl. N	Compound Name	Binding energy (kcal/mol)	Residue involving interaction	No. of H bonds	Interaction of residues forming H_2 bonds
1	Isoniazid	-4.83	MET:49, 165, ASP:187, TYR:54, GLN:189, HIS:41, PRO:52, ARG:188	4	MET:49, TYR:54, ASP:187
2	Rifampin	-9.41	GLU:166, MET:165, HIS:163, 172, 164, 163, PHE:140, LEU:141, 167, SER:144, GLY:143, ASN;142, PRO:163, GLN:192, CYS:145, ALA:191, THR:190, GLN:189	1	GLU:166
3	Ethambutol	-5.02	LEU:141, ASN;142, GLU:166, HIS:163,172, MET:165, SER:144, GLY:143, CYS:145, PHE:140, GLY:170	4	LEU:141, ASN:142, GLU:166
4	Pyrazinamide	-4.05	VAL:303, 212, THR:304, 257, GLN:256, GLN:306, ARG:217, ILE:213	3	VAL:303, THR:304, GLN:256

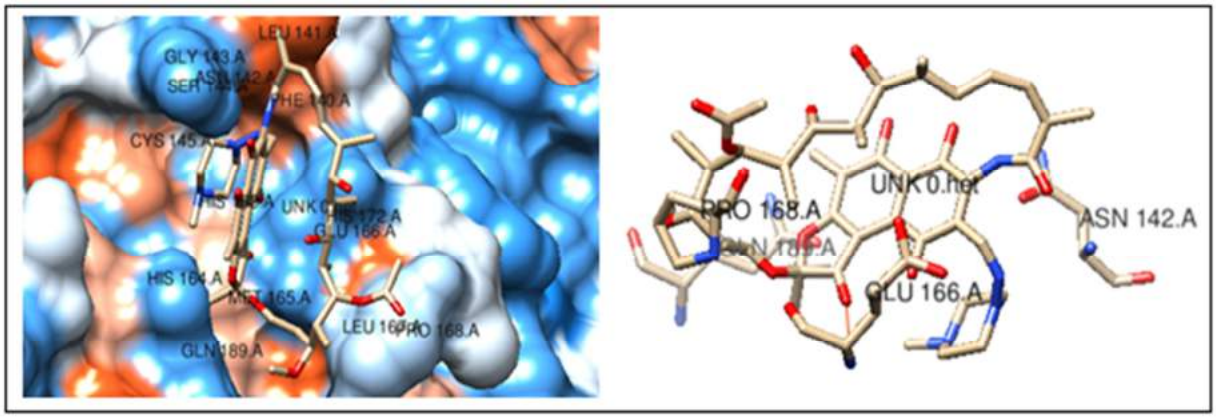


Fig 4. Docking visualisation of COVID-19 protease (6LU7) with anti-TB drug Rifampin

3.3 Docking Prediction of anti-HIV drugs

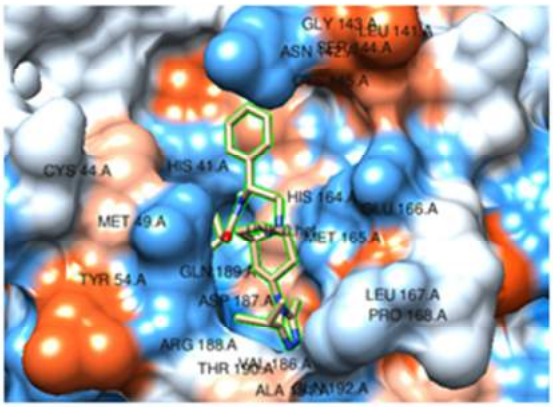
Twenty-four FDA approved anti-HIV drugs were docked with SARS-CoV-2 protease. Among the twenty four drugs, four drugs Maraviroc, Etravirine, Indinavir and Rilpivirine were showed more potential inhibitors of SARS-CoV-2 main protease with binding affinity -10.67, -10.33, -10.00 and -9.66 kcal/mol respectively (Table3). Maraviroc interacted at His:164, 41, Tyr:54, Asp:187, Met:165, 49, Leu:167,141, Pro:168, Cys:44, 145, Arg:188, Gly:143, Asn:142, Ala:191, Gln:192, 189, Thr:190, Etravirine interacted at Gln:83, Lys:88, Thr:175, Met:82, 162, His:164, Cys:85,38, Gly:179, Pro:39, Leu:177,87, Cys:38, Tyr:37, Glu:178, Asn:84, Arg:40, Indinavir interacted at Glu:166, Gln:189, 192, Cys:145, Met:49, 165, Asp:187, His:41, 164, 163, Asn:142, Leu:167, 141, Pro:168, Thr:190, Phe:140, Ser:144, 46, Arg:188 and Rilpivirine interacted at Glu:166, Cys:44, 145, His:164, 41, Met:165, 49, Ala:191, Pro:168,52, Gln:189, Tyr:54, Asp:187, Arg:188, Leu:167, Thr:190 of the SARS-CoV-2 main protease (fig.5). A joint research team of the Shanghai Institute of MateriaMedica and Shanghai Tech University performed drug screening in silicon and an enzyme activity test, and they reported 30 anti-HIV agents with potential antiviral activity against SARS-CoV-2 on January 25, 2020.[17]

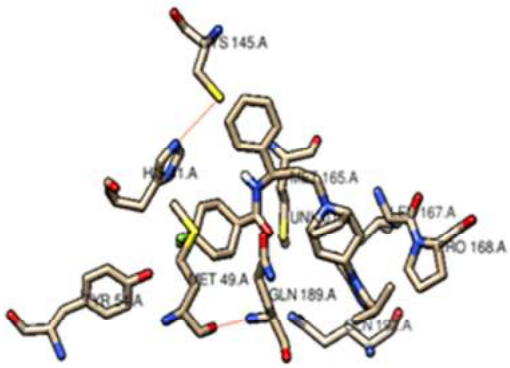
Table3. Molecular docking analysis of anti-HIV drugs against COVID-19 Protease (6LU7)

Sl. N	Compound Name	Binding energy (kcal/mol)	Residue involving interaction	No. of H bonds	Interaction of residues forming H_2 bonds
1	Abacavir	-7.77	MET:49, 165, GLN:189,192, HIS:41, THR:190, ASP:189, TYR:54, PRO:52,168 LEU:50,167, ARG:188, GLU:166,	1	MET:49
2	Atazanavir	-5.08	LYS:137,5, GLU:288,290, TYR:126, LEU:287,286, ASP:289, VAL:125, PHE:3, 291, ARG:4, CYS:128, GLY:138, GLN:127, ALA:7,	1	LYS:137
3	Darunavir	-6.08	GLN:189,192, GLU:166, PHE:140, PRO:168, MET:165, LEU:167, THR:190, GLN:192, GLY:143, CYS:145, SER:144, HIS:163,164, 172, LEU:141, ASN:142,	3	GLN:189, GLU:166, PHE:140
4	Delavirdine	-7.89	TYR:54, GLU:166, ALA:191, MET:165,49, LEU:167, 50, GLN:192, 189, PRO:168, HIS:41,164, ARG:188, ASP:187, THR:190,	2	TYR:54, GLU:166
5	Dolutegravir	-7.75	GLU:166, MET:165, 49, GLN:192, THR:190, PRO:168, LEU:167, 27 ALA:191, ARG:188, GLN:189, HIS:164, 41, CYS:145, GLY:143, ASN:142, SER:144	1	GLU:166
6	Doravirine	-8.15	THR:190, 25,ARG:188, TYR:54, MET:165, 49, ASP:187, HIS:41, GLN:189, 192, GLU:166, HIS:164, GLY:143, LEU:27, THR:25,	3	THR:190, TYR:54, ARG:188
7	Efavirenz	-6.61	GLY:143, CYS:145, ASN:142, MET:49, HIS:41,163, 164, THR:26, LEU:27, 141, SER:144, PHE:140, GLU:166, MET:165, GLN:189	1	GLY:143
8	Elvitegravir	-7.98	THR:190,ASN:142, CYS:145, MET:165, 49, GLN:192, 189, PRO:168, ARG:188, GLU:166, LEU:167, 141, GLY:143, SER:144, HIS:163, 164, PHE:140, ASP:187,	1	THR:190
9	Emtricitabine	-4.79	HIS:41, 164, GLU:166, MET:165,49, THR:190, ARG:188, GLN:192, 189, TYR:54, ASP:187,	2	HIS:41, GLU:166
10	Etravirine	-10.33	GLN:83, LYS:88, THR:175, MET:82, 162, HIS:164, CYS:85,38, GLY:179, PRO:39, LEU:177,87,	2	GLN:83

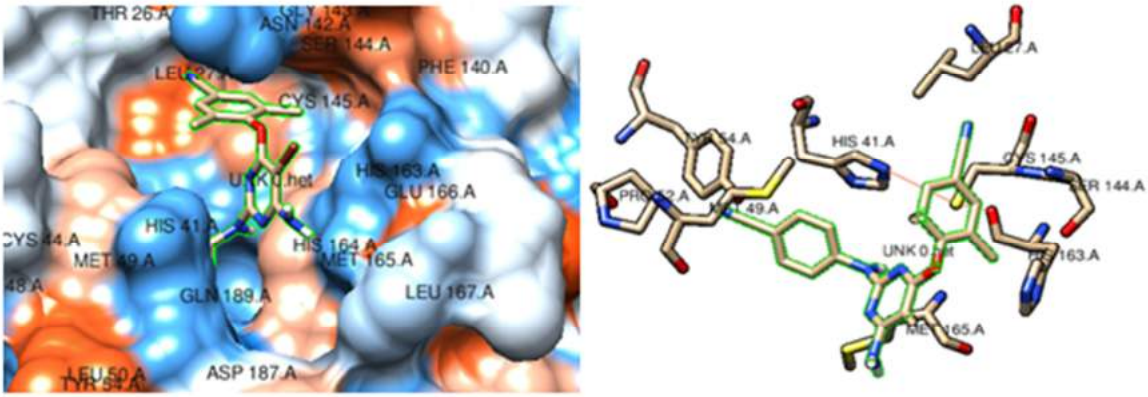
			CYS:38, TYR:37, GLU:178, ASN:84, ARG:40		
11	Fosamprenavir	-4.08	ASN:203, 151, PRO:293, PHE:8,294, ILE:249, 200, 106, VAL:202, 104, GLN:110, THR:292, GLY:109, ASP:153, VAL:104, SER:158,	1	ASN:203
12	Indinavir	-10.00	GLU:166, GLN:189, 192, CYS:145, MET:49, 165, ASP:187, HIS:41, 164, 163, ASN:142, LEU:167, 141, PRO:168, THR:190, PHE:140, SER:144, 46, ARG:188	3	GLU:166, GLN:189
13	lamivudine	-4.75	PHE:140, GLU:166, SER:144, CYS:145, HIS:164, 41, 163, 172, MET:49, 165, ASN:142,LEU:141, GLY:143	5	PHE:140, GLU:166, SER:144, CYS:145, HIS:164
14	Lopinavir	-6.11	GLU:166, ASN:142, SER:144, HIS:163,164 PHE:140, CYS:145, LEU:141,167 ARG:188, MET:165, GLN:189,192, ALA:191, THR:190	1	GLU:166
15	Maraviroc	-10.67	HIS:164, 41, TYR:54, ASP:187, MET:165, 49, LEU:167,141, PRO:168, CYS:44, 145, ARG:188, GLY:143, ASN:142, ALA:191, GLN:192, 189, THR:190,	2	TYR:54, HIS:164,
16	Nevirapine	-6.44	MET:49, 165, HIS:41, 164, SER:144, CYS:145, GLU:166, GLN:189, ASP:187, ARG:188, TYR:54	0	0
17	Raltegravir	-7.81	SER:144, CYS:145, GLU:166, PRO:168,MET:49, 165, HIS:41,163,172, 164, LEU:141, 167, PHE:140, GLY:143, ASN:142, GLN:189, THR:190, ARG:188,	3	SER:144, CYS:145, GLU:166
18	Rilpivirine	-9.66	GLU:166, CYS:44, 145, HIS:164, 41, MET:165, 49, ALA:191, PRO:168,52, GLN:189, TYR:54, ASP:187, ARG:188, LEU:167, THR:190,	3	HIS:164, CYS:44, GLU:166
19	Ritonavir	-8.25	GLY:143,170, GLU:166, LEU:141,27,167, PHE:140, CYS:145, ASN:142, HIS:41, ASP:187, MET:49,163 ARG: 188, GLN:189,192, THR:190, ALA:191, GLY:170, PRO:168,	1	GLY:143
20	Saquinavir	-7.55	GLY:302, 2, ARG:298, SER:301,1, PRO:9, VAL:297,303, MET:6, CYS:300, ALA:7, PHE:8, GLY:2	5	ARG:298, GLY:302, SER:301
21	Stavudine	-5.38	ARG:188, GLN:189, 192, HIS:41, 164, MET:165, THR:190, GLN:192, VAL:186, TYR:54, ASP:187, GLU:166	2	ARG:188
22	Tenofovir	-4.40	THR:190, GLU:166, MET:165, 49, ALA:191, GLN:189, 192, ARG:188, ASP:187, PRO:168, LEU:167, HIS:41	2	THR:190, GLU:166
23	Tipranavir	-8.30	GLN:189, GLU:166, ASN:142, MET:49, CYS:145, MET:165, 49, HIS:41, 164, 163, SER:144, 46, PHE:140, LEU:141, ARG:188, ASP:187, TYR:54, GLY:143,	3	GLN:189, ASNI142, GLU:166
24	Zidovudine	-6.31	ASP:187, GLU:166, ARG:188, TYR:54, MET:165,49, GLN:189, HIS:164,41, PHE:181, PRO:52CYS:44	2	ASP:187, GLU:166

(A) **Maraviroc**

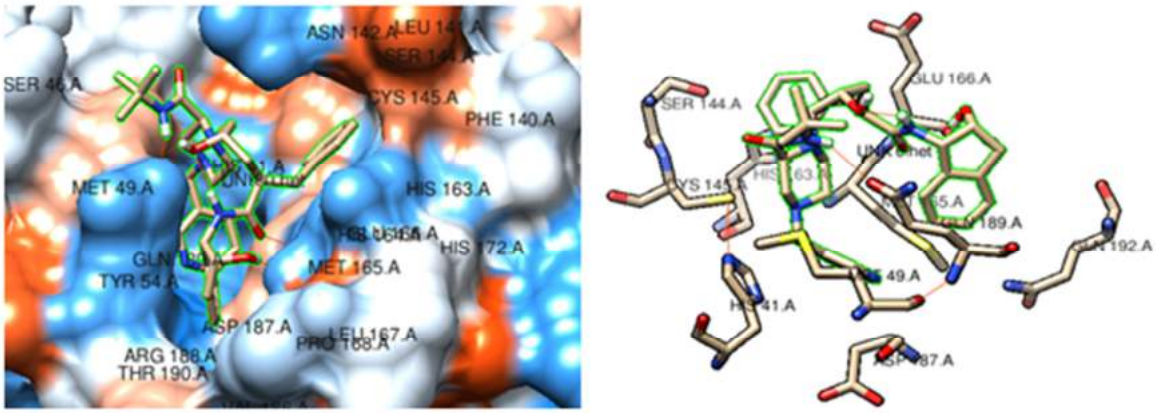




(B) **Etravirine**



(C) **Indinavir**



(D) **Rilpivirine**

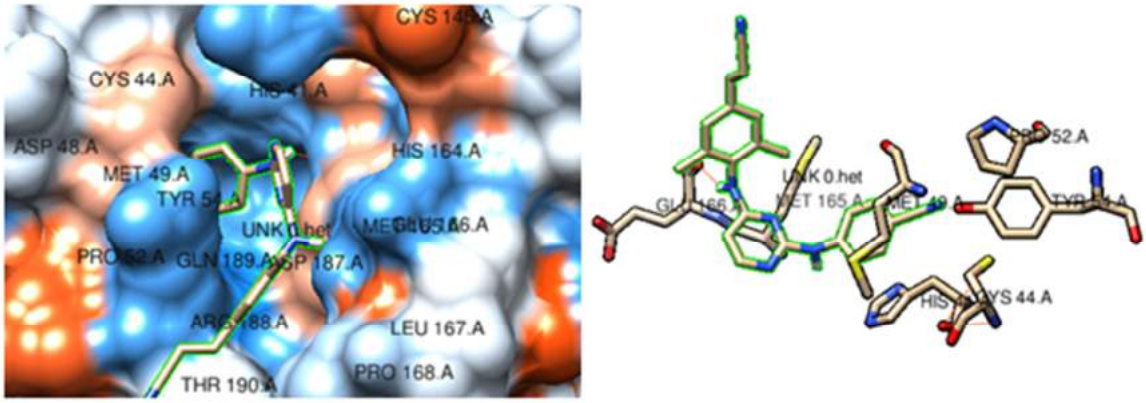


Fig 5. Docking visualisation of COVID-19 protease (6LU7) with anti-HIV drugs, Maraviroc (A), Etravirine (B), Indinavir (C) and Rilpivirine (D).

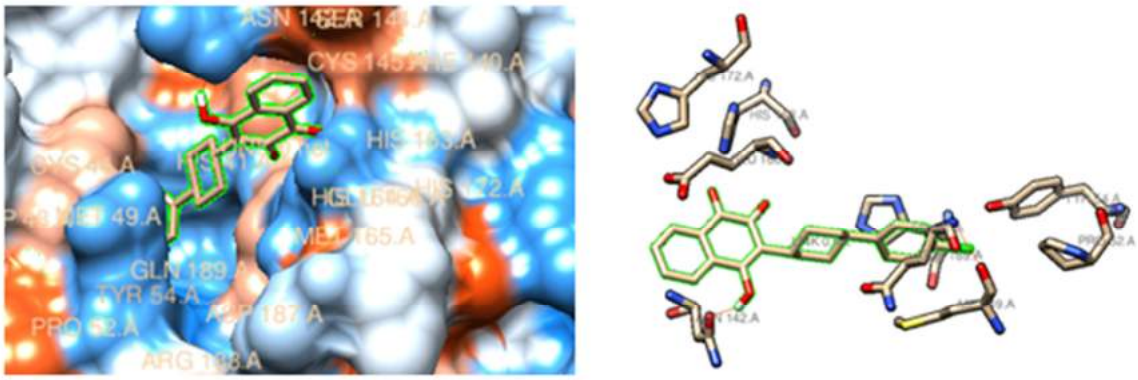
3.4 Docking Prediction of antimalarial drugs

Seventeen clinically approved antimalarial drugs were docked with SARS-CoV-2 protease. Out of seventeen, seven drugs were shown more potential inhibitors of SARS-CoV-2 main protease. Atovaquone, Quinidine, Halofantrine, Amodiaquine, Tetracycline, Azithromycin and hydroxychloroquine docked with binding affinity -8.95, -8.84, -8.68, -8.65, -8.4, -8.32 and -8.30 kcal/mol against SARS-CoV-2 main protease (Table4). Docking visualization of 6LU7 with seven antimalarial drugs was shown in fig.6. Among the seven potent inhibitors Atovaquone was more potent with binding affinity -8.95 kcal/mol. Asn:142, Glu:166, Leu:141, Met:49, 165, Cys:44, Pro:52, Tyr:54, Arg:188, Asp:187, His:41,164, 163, 172, Phe:140 and Gln:189 are the amino acid residues involved in the interaction with Atovaquone (fig.6). As these residues play an important role during protein-ligand interaction, they may serve as a biomarker during the drug discovery process.[18]

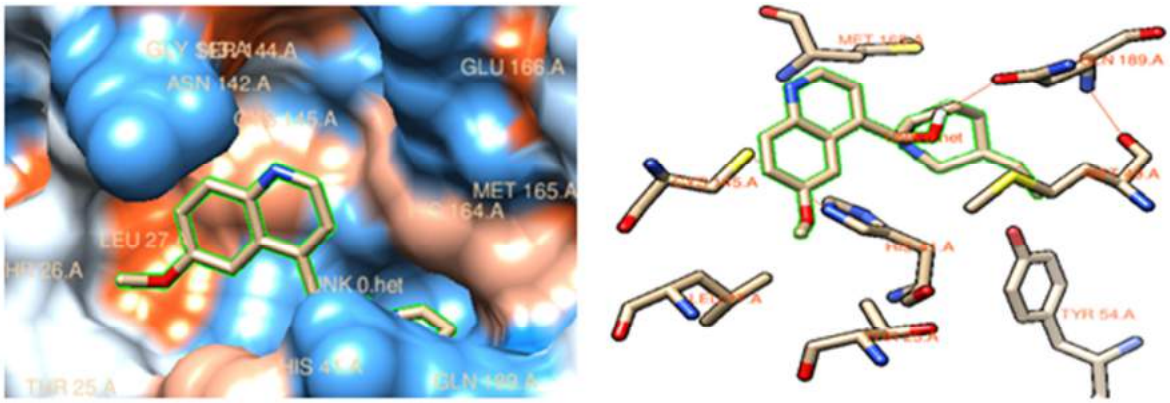
Table4. Molecular docking analysis of anti-malarial drugs against COVID-19 Protease (6LU7)

Sl. N	Compound Name	Binding energy (kcal/mol)	Residue involving interaction	No. of H bonds	Interaction of residues forming H_2 bonds
1	Quinine	-7.86	GLN:189, HIS:172,41, 164, 163 CYS:145, MET:165, 49, GLU:166, ARG:188, TYR:54, ASN:142, PHE:140, SER:144, LEU:141, GLY:143, ASP:187	1	GLN:189
2	Quinidine	-8.84	GLN:189, MET:165, 49, TYR:54, PRO:52, HIS:41, 164, PHE:181, ASP:187, GLU:166, ASN:142, CYS:145, SER:144, GLY:143, THR:26, 25, LEU:27, VAL:186	1	GLN:189
3	Mefloquine	-7.47	GLN:189,192, TYR:54, GLU:166, ARG:188, ASP:187, MET:49, LEU:167, 141, THR:190, MET:165, HIS:163, 172, 164,41, LEU:167, CYS:145, PHE:140, SER:144,	3	GLN:189,192, TYR:54
4	Chloroquine	-7.62	HIS:164,163, 41, 172, ARG:188, PRO:52, TYR:54, MET:49, 165, ASP:187, GLN:189, GLU:166, LEU:141, SER:144, CYS:145, ASN:142, PHE:140	1	HIS:164
5	Amodiaquine	-8.65	LEU:141,SER:144, HIS:41, 172, 163, 164, GLN:189, 192, MET:165,49,PHE:140, ASP:187, ARG:188, THR:190, GLN:192, GLU:166, ASN:142, GLY:143, CYS:145,	2	LEU:141, SER:144
6	Primaquine	-7.15	GLU:166, LEU:167, GLY:170, ASP:187, GLN:189, MET:165, ARG:188, GLN:192, THR:190, PRO:168	4	GLU:166, LEU:167
7	Halofantrine	-8.68	GLN:192, 189, THR:190, ARG:188, GLU:166, CYS:145, MET:49, 165, HIS:164, ASP:187, TYR:54, PRO:168, LEU:167, 141,SER:144, HIS:163, GLY:143,	2	THR:190, GLN:192
8	Sulfadoxine	-6.47	HIS:164,41, 172, 163, SER:144, LEU:141, CYS:145, MET:49, 165 GLU:166, PRO:52, TYR:54, ARG:188, ASP:187, GLN:189, ASN:142	3	SER:144, LEU:141, HIS:164
9	Sulfamethoxypyridazine	-7.40	HIS:163,164, 41,172, SER:144,LEU:141,MET:49, 165, ASP:187,ASN:142, PHE:140, GLU:166, GLN:189, TYR:54, PRO:52, CYS:145, ASN:142, GLY:143,	4	HIS:163,164, SER:144, LEU:141
10	Proguanil	-7.81	HIS:163, 172 GLU:166, LEU:141, PHE:140, MET:165, SER:144, GLY:143, ASN:142, CYS:145	4	HIS:163
11	Pyrimethamine	-6.85	ASN:142, GLU:166, PHE:140, HIS:172, 163, 41, LEU:141, SER:144, MET:165, CYS:145, MET:49, GLY:143,	3	ASN:142, GLU:166, PHE:140
12	Tetracycline	-8.40	GLU:166, ASN:142, LEU:141, SER:144, CYS:145, MET:165,49, GLN:189, ASP:187, ARG:188, PHE:140, HIS:41,163,172, GLY:143	6	GLU:166, LEU:141, SER:144, CYS:145, ASN:142,
13	Doxycycline	-8.30	GLN:189, ASN:142, GLU:166, SER:144, MET:165, HIS:172, 163, PHE:140, LEU:141, CYS:145	6	GLN:189, ASN:142, GLU:166, SER:144
14	Azithromycin	-8.32	ILE:152, PHE:294, 8, ARG:198, PRO:9, VAL:297, SER:301, ASP:153, TYR:154, ASN:151	1	ILE:152
15	Artemisinin	-7.63	MET:165, 49, HIS:41, 164, ARG:188, GLU:166, GLN:189, CYS:145, 44, TYR:54, ASP:187,	0	0
16	Atovaquone	-8.95	ASN:142, GLU:166, LEU:141, MET:49, 165, CYS:44, PRO:52, TYR:54, ARG:188, ASP:187, HIS:41,164, 163, 172, PHE:140, GLN:189	1	ASN:142
17	Hydroxychloroquine	-8.30	GLN:189, ARG:188, MET:165, TYR:54, ASP:187, HIS:41,172,163,164, PHE:140, LEU:141, CYS:145, SER:144, ASN:142, GLY:143, GLU:166	1	GLN:189

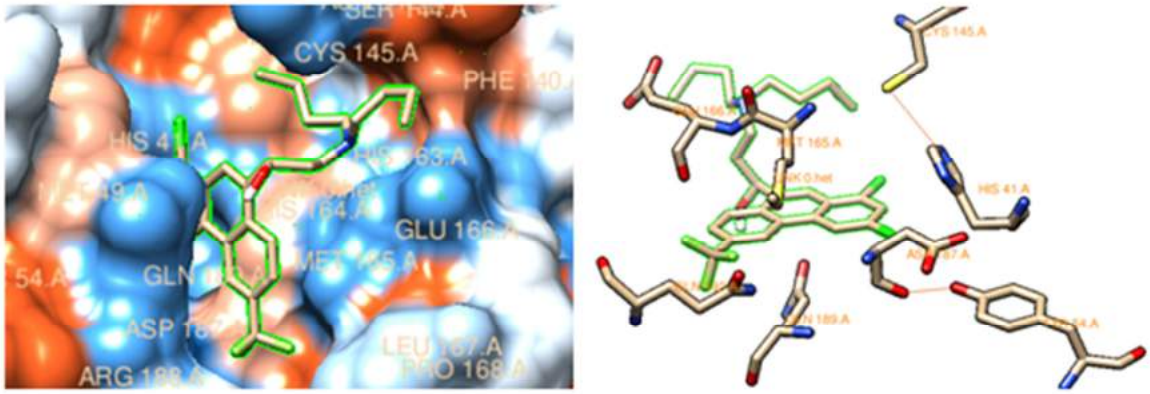
(A) **Atovaquone**



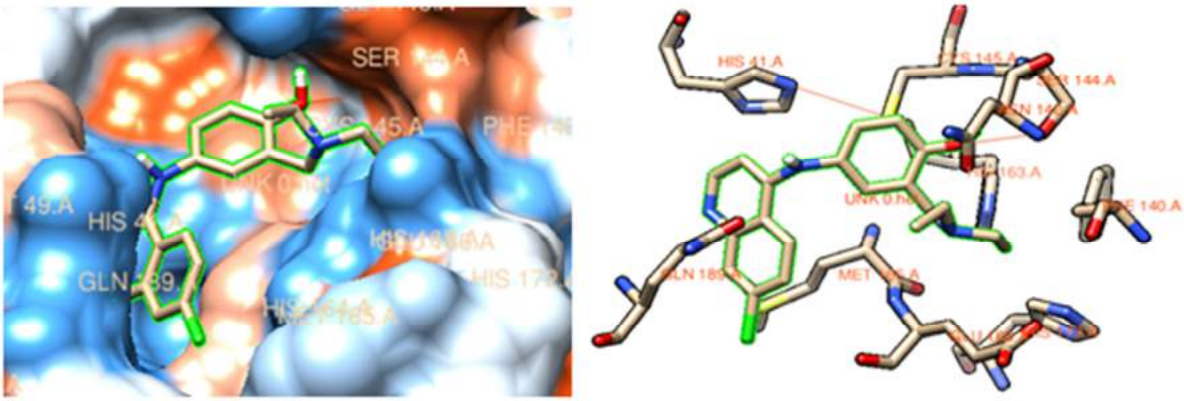
(B) **Quinidine**



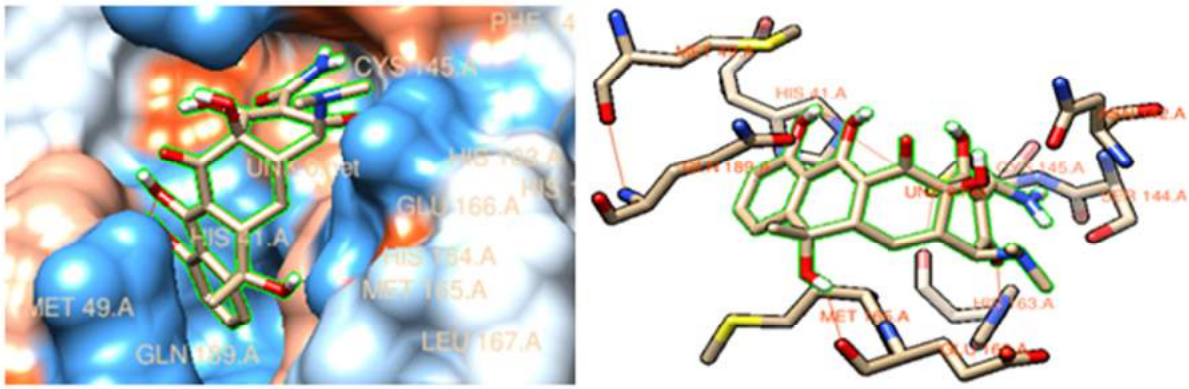
(C) **Halofantrine**



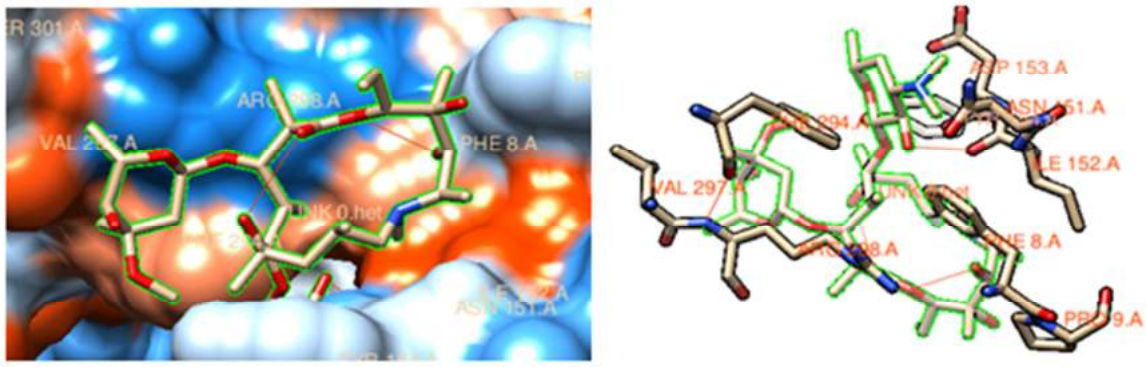
(D) **Amodiaquine**



(E) **Tetracycline**



(F) **Azithromycin**



(G) **Hydroxychloroquine**

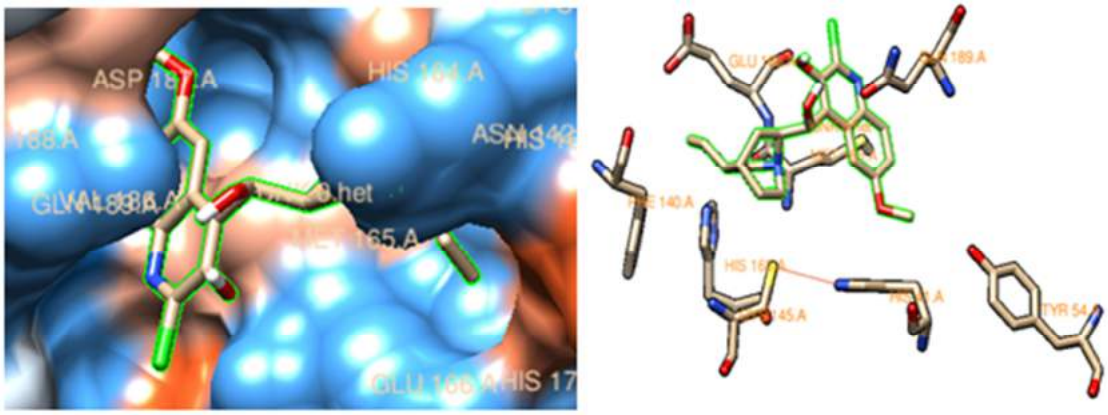


Fig 6. Docking visualisation of COVID-19 protease (6LU7) with anti-malarial drugs, Atovaquone (A), Quinidine (B), Halofantrine (C), Amodiaquine (D), Tetracycline (E), Azithromycin (F) and hydroxychloroquine (G)

This study focused on identification of potential inhibitors against SARS-CoV-2 from corona virus to control the viral replication. Outcomes from the *In silico* molecular docking study maintained the great inhibitory efficacy of the one anti-H1N1 drug (Oseltamivir), one anti-TB drug (Rifampin), four anti-HIV drugs (Maraviroc, Etravirine, Indinavir, Rilpivirine) and seven antimalarial drugs (Atovaquone, Quinidine, Halofantrine, Amodiaquine, Tetracycline, Azithromycin, hydroxychloroquine) since they could launch H_2 bonds with different amino acid residues that caused in an inhibition of SARS-CoV-2 protease activity with higher binding affinity ranging from (-10.67 to -8.3 kcal/mol). Thus, the projected binding interactions of the dynamic molecules with the protease by the docking study evidently established their inhibitory strength towards catalytic response of the protease. This study provides the support of the repurposed drugs, which may be helpful for the treatment of novel coronavirus disease and can serve as potential drug candidates to curb the ongoing and ever enlarging COVID-19 pandemic. Since all the drugs used in this study are of known pharmacokinetics standards and approved by FDA for human use they do not need to undergo specific long term clinical trials and therefore can fasten up the process of therapeutics development.

4. DISCUSSION

Coronavirus fits a set of viruses which can contaminate vertebrate animals and humans. It has slaughtered thousands of individuals around the world with growth in mortality rate

each single day. Digestive, central nervous system, liver, respiratory systems of humans and animals hampered by this virus infection.[19] Our study was focused on the FDA approved drugs against the main protease in coronavirus, as a possible beneficial target for the management of coronavirus. 6LU7 (PDB ID) is the major protease in COVID-19 that has been relocated and structured in PDB recently and is available to everybody in the world (Figure 1). For the proteolytic maturation of a virus, the protease is precisely significant. Protease has been studied as a possible target to avoid the extent of contamination by inhibiting viral polyprotein cleavage via blocking active sites of the protein. This new finding of protease assembly in COVID-19, has provided an enormous chance to recognize possible drug candidates for the management of coronavirus.[20] In this study, we have applied a computational approach of FDA approved drugs in order to find a specific therapeutic possible agent against COVID-19. We have selected 47 FDA approved antiviral, anti-H1N1, anti-TB and antiviral drugs and retrieved directly from the PubChem (National Library of Medicine). Molecular docking was accomplished with the 47 drugs against COVID-19 structure. Molecular docking is a computational technique which aims to find non-Covalent binding among protein (receptor) and a ligand/inhibitor (small molecule). For recognized binding sites, the docking expects the method of interaction among a target protein and a ligand. Binding energy proposes the attraction of an exact ligand and asset by which a ligand interacts with and binds to the pocket of a target protein. A drug with a lesser binding energy (ΔG) is chosen as a probable drug candidate. In order to recognize the effect of active antiviral drugs on COVID-19, 47 FDA approved antiviral compounds were selected and performed molecular docking against COVID-19. Docking results of SARS-CoV-2 protease with selected 47 drugs out of the selected 13 showed the best docking score and were found to be the best molecules at the target site of the protein. Out of the 13 drugs, Maraviroc exhibited the best docked score (-10.67 kcal/mol) with SARS-CoV-2 protease.HIS:164, 41, TYR:54, ASP:187, MET:165, 49, LEU:167,141, PRO:168, CYS:44, 145, ARG:188, GLY:143, ASN:142, ALA:191, GLN:192, 189 and THR:190 are the amino acid residues participating in the interaction at the binding pocket of SARS-CoV-2 protease (fig.5A).Maraviroc is an effective antiretroviral agent permitted for the treatment of HIV-1 infection that blocks interaction among the virus and the CCR5 co-receptor, a critical step in the HIV-1 replication. Earlier clinical trials of this drug have established the efficacy, tolerability, and safety of maraviroc in both treatment-naive and treatment-experienced patients.[21,22] Among the 17 anti-malarial approved drugs, Atovaquone presented best docking score (-8.95 kcal/mol) and ASN:142, GLU:166, LEU:141, MET:49, 165, CYS:44, PRO:52, TYR:54, ARG:188, ASP:187, HIS:41,164, 163, 172, PHE:140, GLN:189 are the amino acid residues participating in the interaction at the binding pocket of SARS-CoV-2 protease (Figure 5A). Recent studies also explores that, Atovaquone significantly inhibited ZIKV (Zika virus) in human placental JEG3 cells *in vitro*.[23]

5. CONCLUSION

The present study concludes that thirteen clinically approved drugs were identified as potent inhibitors against SARS-CoV-2 protease activity. These outcomes afford a strong foundation for the use of these drugs for CORONA management. Moreover, the dynamic ligands inhibit the catalytic response of protease by blocking the residues of amino acids intricate in the processing and strand transmission reactions. The interactions by the structural model at the protease active site can afford a valuable guide for additional strategies for structure-based medicines and development of new operative inhibitors of SARS-CoV-2 protease. Therefore, the effect of these inhibitors can be further revealed through *in vitro* and *in vivo* analysis in the termination of intracellular replication of coronavirus, prior to the use as drugs in humans.

6. ACKNOWLEDGEMENTS

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7. AUTHORS CONTRIBUTION STATEMENT

Rakesh Davella gathered the data and carried out the molecular docking regard to this work. Dr. Swapna Gurrapu contributed to the interpretation of the results and data analysis. Pujala Shivakrishna wrote the manuscript. Dr. Estari Mamidala conceived the original idea and supervised the research work. All authors discussed the methodology and results to the finalizing the manuscript.

8. CONFLICT OF INTEREST

Conflict of interest declared none.

9. REFERENCES

1. Huang C, Wang Y, Li X, Ren L, Zhao J, Hu Y, Zhang L, Fan G, Xu J, Gu X, Cheng Z. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. The Lancet. 2020 Feb 15;395(10223):497-506. doi: 10.1016/S0140-6736(20)30183-5
2. Chan JF, Kok KH, Zhu Z, Chu H, To KK, Yuan S, Yuen KY. Genomic characterization of the 2019 novel human-pathogenic coronavirus isolated from a patient with atypical pneumonia after visiting Wuhan. Emerging microbes & infections. 2020 Jan 1;9(1):221-36.doi: 10.1080/22221751.2020.1719902
3. Chan JF, Yuan S, Kok KH, To KK, Chu H, Yang J, Xing F, Liu J, Yip CC, Poon RW, Tsoi HW. A familial cluster of pneumonia associated with the 2019 novel coronavirus indicating person-to-person transmission: a study of a family cluster. The Lancet. 2020 Feb 15;395(10223):514-23. doi: 10.1016/ S0140-6736(20)30154-9
4. Chen N, Zhou M, Dong X, Qu J, Gong F, Han Y, Qiu Y, Wang J, Liu Y, Wei Y, Yu T. Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. The Lancet. 2020 Feb 15;395(10223):507-13. doi: 10.1016/ S0140-6736(20)30211-7
5. World Health Organization. Coronavirus Disease 2019 (COVID-19) Situation Report –91. 2020. Available online:

https://www.who.int/emergencies/diseases/novel-coronavirus-2019/situation-reports (accessed on 21 April 2020).
6. Ministry of Health and Human Welfare, Government of India. COVID-19 cases. Available online: https://www.mohfw.gov.in/ (Accessed on 21 April 2020)
7. Hilgenfeld R. From SARS to MERS: crystallographic studies on coronaviral proteases enable antiviral drug design. The FEBS journal. 2014 Sep 1;281(18):4085-96. doi: 10.1111/febs.12936
8. John SE, Tomar S, Stauffer SR, Mesecar AD. Targeting zoonotic viruses: Structure-based inhibition of the 3C-like protease from bat coronavirus HKU4—The likely reservoir host to the human coronavirus that causes Middle East Respiratory Syndrome (MERS). Bioorganic & medicinal chemistry. 2015 Sep 1;23(17):6036-48. doi: 10.1016/j.bmc.2015.06.039
9. Pastick KA, Okafor EC, Wang F, Lofgren SM, Skipper CP, Nicol MR, Pullen MF, Rajasingham R, McDonald EG, Lee TC, Schwartz IS. Hydroxychloroquine and Chloroquine for Treatment of SARS-CoV-2 (COVID-19). InOpen Forum Infectious Diseases 2020 Apr 15. doi: 10.1093/ofid/ofaa130
10. Colson P, Rolain JM, Lagier JC, Brouqui P, Raoult D. Chloroquine and hydroxychloroquine as available weapons to fight COVID-19. Int J Antimicrob Agents. 2020 Mar 4;105932(10.1016). doi: 10.1016/j.ijantimicag.2020.105932
11. Kouatly O, Eleftheriou P, Petrou A, Hadjipavlou-Litina D, Geronika A. Docking assisted design of novel 4-adamantanyl-2-thiazolylimino-5-arylidene-4-thiazolidinones as potent NSAIDs. SAR and QSAR in Environmental Research. 2018 Feb 1;29(2):83-101 doi: 10.1080/1062936X.2017.1410220
12. Available online: https://www.rcsb.org/structure/6LU7 (accessed on 23 March 2020).
13. Chang MW, Ayeni C, Breuer S, Torbett BE. Virtual screening for HIV protease inhibitors: a comparison of AutoDock 4 and Vina. PloS one. 2010;5(8):e11955. doi: 10.1371/journal.pone.0011955
14. Park H, Lee J, Lee S. Critical assessment of the automated AutoDock as a new docking tool for virtual screening. Proteins: Structure, Function, and Bioinformatics. 2006 Nov 15;65(3):549-54. doi: 10.1002/prot.21183
15. Geronikaki AA, Dearden JC, Filimonov D, Galaeva I, Garibova TL, Gloriozova T, Krajneva V, Lagunin A, Macaev FZ, Molodkin G, Poroikov VV. Design of new cognition enhancers: from computer prediction to synthesis and biological evaluation. Journal of medicinal chemistry. 2004 May 20;47(11):2870-6. doi: 10/1021/jm031086k
16. Gubareva LV, Kaiser L, Hayden FG. Influenza virus neuraminidase inhibitors. The Lancet. 2000 Mar 4;355(9206):827-35. doi: 10.1016/S0140-6736(99)11433-8
17. Shanghai Institute of Materia Medica website, Chinese Academy of Sciences. A joint research team of the Shanghai Institute of Materia Medica and Shanghai Tech University discovered a group of old and traditional Chinese medicines that may be efficacious in treating the novel form of pneumonia. Available at: http://www.simm.ac.cn/xwzx/kydt/202001/t20200125_5494417.html (accessed February 22, 2020).
18. Donde R, Gupta MK, Gouda G, Kumar J, Vadde R, Sahoo KK, Dash SK, Behera L. Computational characterization of structural and functional roles of DREB1A, DREB1B and EB1C in enhancing cold tolerance in rice plants. Amino acids. 2019 May 1;51(5):839-53. doi: 10.1007/s00726-019-02727-0
19. Khaerunnisa S, Kurniawan H, Awaluddin R, Suhartati S, Soetjipto S. Potential Inhibitor of COVID-19 Main Protease (Mpro) From Several Medicinal Plant Compounds by Molecular Docking Study. Preprints 2020; Mar 13:1-4:2020030226. doi: 10.20944/preprints202003.0226.v1.2020
20. Gupta MK, Vemula S, Donde R, Gouda G, Behera L, Vadde R. *In-silico* approaches to detect inhibitors of the human severe acute respiratory syndrome coronavirus envelope protein ion channel. Journal of Biomolecular Structure and Dynamics. 2020 Apr 2(just-accepted):1-11.doi: 10.1080/07391102.2020.1751300
21. Dorr P, Westby M, Dobbs S, Griffin P, Irvine B, Macartney M, Mori J, Rickett G, Smith-Burchnell C, Napier C, Webster R. Maraviroc (UK-427,857), a potent, orally bioavailable, and selective small-molecule inhibitor of chemokine receptor CCR5 with broad-spectrum anti-human immunodeficiency virus type 1 activity. Antimicrobial agents and chemotherapy. 2005 Nov 1;49(11):4721-32. doi: 10.1128/AAC.49.11.4721-4732.2005
22. Cooper DA, Heera J, Heera J, Goodrich J, Tawadrous M, Saag M, DeJesus E, Clumeck N, Walmsley S, Ting N, Coakley E. Maraviroc versus efavirenz, both in combination with zidovudine-lamivudine, for the treatment of antiretroviral-naive subjects with CCR5-tropic HIV-1 infection. The Journal of infectious diseases. 2010 Mar 15;201(6):803-13. doi: 10.1086/650697
23. Gulick RM, Lalezari J, Goodrich J, Clumeck N, DeJesus E, Horban A, Nadler J, Clotet B, Karlsson A, Wohlfeiler M, Montana JB. Maraviroc for previously treated patients with R5 HIV-1 infection. New England Journal of Medicine. 2008 Oct 2;359(14):1429-41. doi: 10.1056/NEJMoa0803152