Acta virologica **65**: 160 – 172, 2021 doi: 10.4149/av_2021_208

Computational screening of dual inhibitors from FDA approved antiviral drugs on SARS-CoV-2 spike protein and the main protease using molecular docking approach

Shanthi Sabarimurugan[1], Indu Purushothaman[2], Rajarajan Swaminathan[2,3], Arun Dharmarajan[4,5,6], Sudha Warrier[7], Sangeetha Kothandan[8]

[1]School of Biomedical Sciences, The University of Western Australia, Perth, Australia; [2]PG & Research Dept. of Microbiology & Biotechnology, Presidence College, Chennai, India; [3]Centre for Drug Design, Discovery and Development of Drug, SRM University, Sonipat, Haryana, New Delhi, India; [4]CHIRI, School of Pharmacy and Biomedical Sciences, Curtin University, Perth, WA; [5]Department of Biomedical Sciences, Faculty of Biomedical Sciences, Technology and Research, Sri Ramachandra Institute of Higher Education and Research, Chennai, India; [6]Department of Human Sciences, Faculty of Life Sciences, The University of Western Australia, Nedlands, WA 6009; [7]School of Regenerative Medicine, Manipal Academy of Higher Education, Bangalore, India; [8] Department of Biotechnology, Saveetha School of Engineering, SIMATS, Chennai, India

Received September 9, 2020; accepted February 2, 2021

Summary. – The deadly disease-causing novel coronavirus has recently swept across the world and endangered many human lives. Although, various research on therapeutic measures to solve this pandemic crisis has been published; no favourable results have been achieved. We propose the use of potential FDA-approved dual inhibitors which can inhibit two targets (either on entry-level or the main protease) for the effective treatment of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). We screened 12 FDA-approved antiviral inhibitors listed in Drug bank and analysed the ADMET properties of each drug of interest to study the bioavailability, safety and toxicity. Two potential targets, the spike protein and the main protease of SARS-CoV-2 obtained from PDB have been used for molecular docking. All the selected drugs were docked with both targets and demonstrated strong hydrogen bond (HB) interactions in multiple active sites. Amongst these, the range of binding energy was from 3–7 kcal/mol for spike protein and 2–8 kcal/mol for the main protease. Upon comparison of all the processed drugs ganciclovir and zanamivir displayed significant binding energy with HB interactions with both, spike (-9.2 and -9 kcal/mol respectively) and the main protease (-9 kcal/mol). Ribavirin and tenofovir showed significant binding energy above -8 kcal/mol with seven HB interactions with the main protease and also spike protein. The novel findings regarding the antiviral properties of these dual inhibitors using a computational approach will be a good starting point for the efficacy determination of these drugs for pre-clinical and clinical studies aimed at developing active antivirals to target SARS-CoV-2.

Keywords: SARS-CoV-2; FDA-approved drugs; viral inhibitors; *in-silico* analysis; molecular docking

Introduction

A newly emerged severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) causing viral pneumonia (coronavirus disease 2019, COVID-19), emerged from wet market in China in December 2019 resulting in pandemic. For the first month, the number of cases reported in China was meagre, with only 42 cases including one

E-mail: Shanthi.Sabarimurugan@uwa.edu.au; phone: +61-410 547-970: ORCID: 0000-0003-2247-3426.

Abbreviations: COVID-19 = coronavirus disease 2019; FDA = U.S. food and drug administration; HB = hydrogen bond; SARS-CoV-2 = severe acute respiratory syndrome coronavirus 2

dead patient and seven in critical care (Elfiky, 2020a). The phylogenetic research proposed its origin from bat that was preceded by pangolin. On the 20th January 2020 the National Health Commission of China has confirmed the human-to-human transmission during the Wuhan outbreak (Yang, 2020). Later, on 30th January of 2020, the WHO declared SARS-CoV-2 as Public Health Emergency of International Concern (PHEIC) and to date, the cases have emerged all over the Africa, America, Europe, the Eastern Mediterranean, the South East Asia and the western specific regions. At the end of June 2020, the total global death toll was estimated at 507,435 with 10,321,689 confirmed cases (WHO, 2020).

Like other species of SARS-related human coronaviruses, SARS-CoV-2 belongs to the *Betacoronavirus* genus and is a positive sense single-stranded RNA virus with 30,000 bp, consisting of structural (spike, M and nucleocapsid) and non-structural proteins (3-chymotrypsin-like protease, helicase, papain-like protease and RNA-dependent RNA polymerase) (Astuti and Ysrafil, 2020). The current study evaluates two susceptible targets, one structural, spike protein, and one non-structural protein, 3-chymotrypsin-like protease (3CL-protease) which is also called M^{pro}. The aim was to predict the docking sites by computational methods with a small number of FDA-approved antivirals. Spike protein is a major structural protein domain which binds ACE receptors for the entry of SARS-CoV-2 to host cells utilising S1 and S2 subunits. Spike resides in the homotrimeric form on the outer membrane of SARS-CoV-2, and is a crucial recognition factor in the interaction with human sensitised receptor (Ibrahim *et al*., 2020). After entering into the host cells, the virus releases the necessary proteins followed by replication and multiplication processes. Amongst the multiple protein associated gene regulation, the main protease acts as one of the predominant non-structural proteins. The SARS-CoV-2 main protease (M^{pro}) helps in further proteolytic maturation and hence the appropriate drug disturbing the protease activity can further inhibit the replication process (Zhang *et al*., 2020). M^{pro} is a key SARS-CoV-2 enzyme which has an essential role in the replication and post replication process (Jin *et al*., 2020). It mainly cleaves polyproteins into replicative proteins of SARS-CoV-2.

SARS-CoV-2 is highly contagious as it causes respiratory syndrome disease, which has a high mutation rate and can lead to multiple complications affecting the respiratory, gastrointestinal, hepatic and neurological systems. Currently, there is no antiviral or vaccine available to address the sudden massive outbreak. However, several *in-silico*, *in-vitro* and clinical studies/trials have been initiated to predict the importance of repurposing of existing FDA-approved drugs on SARS-CoV-2 (Caly *et al*., 2020; Elfiky, 2020a; Kandeel and Al-Nazawi, 2020). Repurposing of approved antivirals is time-consuming as it involves laboratory testing of compounds followed by human trials, usually taking months and years to reach the inclusion in to the medical regimen (Hasan and Mehmet, 2020). In addition, repurposing newly identified drugs may require more time to evaluate their toxicity and adverse effects. Repurposing drugs against SARS-CoV-2 could focus any susceptible target, such as virus receptor, viral structural proteins, non-structural proteins, RBD-ACE2 (receptor binding domain-ACE2) blockers, host cell endocytosis, or replication and maturation process.

The present study identifies potential drugs which might actively control the replication of the virus by blocking either spike and main protease. Before evaluating the experimental studies and human clinical trials some essential preliminary work is needed to choose effective antiviral candidates. For this, computational virtual screening of putative drug candidates is the promising methodology as it is less time-consuming and more cost-effective than random *de novo* drug discovery and randomised pre-clinical studies. Evidently, the U.S Food and Drug Administration (FDA) limits approval of the annual number of newly invented or tested drugs for respective treatments through research experimentation (Greene and Loscalzo, 2017). Herein, we test existing efficacious FDA-approved drugs which have dual antiviral properties for their effectiveness against SARS-CoV-2 by *in-silico* analysis to reach the trial as early as possible.

The drugs used in the current study were darunavir, fedratinib, ganciclovir, hydroxychloroquine, imatinib, losartan, oseltamivir, peramivir, ribavirin, ruxolitinib, tenofovir and zanamivir. Amongst these selected drugs, hydroxychloroquine, ribavirin, ganciclovir and darunavir have been studied for SARS-CoV-2 proteins as mono-inhibitors by *in-silico* or *in-vitro* studies (Calligari *et al*., 2020; Dayer, 2020; De Meyer *et al*., 2020; Elfiky, 2020b; Ferron *et al*., 2018; Hasan and Mehmet, 2020; Khaerunnisa *et al*., 2020; Mamidala *et al*., 2020; Shah *et al*., 2020). All these 12 drugs have showed antiviral properties against other lethal viruses. Here, for the first time, we have studied the effect of dual inhibition of two different protein targets of SARS-CoV-2 as there was no study done on FDA-approved drugs on spike and M^{pro} proteins. Therefore SARS-CoV-2 structural and non-structural proteins could be considered attractive target for drug discovery of anti-SARS-CoV-2 drugs. These predicting results may provide an additional starting point for repurposing of approved drugs of choice for further experimental studies to obtain an insight to combat recent dangerous pandemic disease.

Materials and Methods

Preparation of target protein structure. Spike protein and the main protease of SARS-CoV-2 are the two targets chosen for this study. The biochemical structure was retrieved from the RSCB Protein Data Bank (PDB, online database) with enabling crystal structures of biological macromolecules updated with extensive research. Since from the outbreak, only limited amount of protein structures is available in PDB. We have chosen the most studied proteins of SARS-CoV-2 with PDB ID: 6VW1 sequence (spike protein) and PDB ID: 6LU7 (main protease). The 6VW1 spike protein structure formed complex with RBD and ACE which contain A, B, E and F chains https://www.rcsb.org/structure/6VW1 (Shang *et al*., 2020). The PDB published strain, 6LU7 main protease complexed with N3 inhibitors, with bond length of 2.16 Å, and contain A and B chains. By removing all crystal water molecules, the spike protein and the main protease were prepared for molecular docking process.

Pocket identification. The active site of a protein was determined using the Computed Atlas for Surface Topography of Proteins (CASTp) (http://sts.bioe.uic.edu/castp/index.html?201l). The superposition of the target structure was identified by Biovia Discovery Studio 4.5 and Pymol to study the visualisation of the ligands. The active site of amino acids was used to determine the Grid box and docking evaluation results. The preparation of the target enzyme 6LU7 and 6VW1-spike protein with the Auto-Dock Tools software involved the addition of hydrogen atoms. This is the preliminary step essential for accurate fractional atomic charges calculation.

FDA-approved antiviral ligand selection. The ligands (FDA-approved drugs) used in this study were darunavir, fedratinib, ganciclovir, hydroxychloroquine, imatinib, losartan, oseltamivir, peramivir, ribavirin, ruxolitinib, tenofovir and zanamivir. These 12 drugs were studied for antiviral activity, and the details of antiviral properties such as trade name, PubChem ID, FDA-approval information and appropriate molecular formula are shown in Table 1. The 3-dimensional (3D) structures and canonical SMILES were obtained from the chemical drug bank called PubChem database (https://pubchem.ncbi.nlm.nih.gov/), in .sdf format. Meanwhile, the drug bank database was also used to retrieve other additional details. The study identified a list of FDAs permitted drugs from database and studied the docking affinity with key amino acid residues.

ADME analysis. Based on canonical SMILES of the selected ligands, the absorption, distribution, metabolism, and excretion (ADME) properties were calculated using online Swiss ADME program (Daina *et al*., 2017). Few other safety results were obtained from the Drug bank (https://www.drugbank.ca/), which is a unique bioinformatic and cheminformatic resource of comprehensive drug data depository. Though the drugs are FDA-approved, the further intense knowledge on the toxicity and safety were studied aby adopting ADME methodology as it could reflect the adverse effects in SARS-CoV-2 patients. The ADME associated properties such as Lipinski's rule of five, pharmacokinetic properties, the solubility of the drug (log S) and drug likeliness were considered.

Molecular docking and binding energy estimation. Docking experiment was performed using optimised SARS-CoV-2 protein (spike and main protease enzyme) by the aid of Auto-Dock 4.1 software. The preparation of ligand and active site of the target was the first step in the processing of docking experiment. The protein optimisation was done by removing water and the pdbqt format file was adjusted by adding a polar hydrogen group to the protein to generate second step. Grid coordinates such as X, Y and Z were generated to determine the native ligand position to identify the binding site and ligand tethering were determined by Genetic algorithm parameters. The best favourable binding results were assumed by observing less than 1.0 Å in positional root-mean-square deviation (RMSD) through the conformational clustering of poses. The potent and effective drug was considered based on the highest binding energy with most negative value of the ligand taken as maximum binding affinity. The lowest free energy of binding (ΔG) and the most moderate inhibition constant (Ki) are the most favourable binding poses of the compounds analysed (Gurung *et al*., 2020). The energy of interaction of every atom in the ligand formed complexes with binding energy (ΔG_{bind}) values more than or equal to -7 kcal/mol, ten best postures were used for scoring purposes (Park *et al*., 2006) and the predominant poses were evaluated by MOE. The interactive amino-acid active docked sites were described by the estimated hydrogen bond and the bond length.

Suitability of antiviral prediction of selected drugs. The prediction of possible antiviral efficiency of tested drugs in this article has been studied by AVCpred online server. The database is user friendly and consists of several algorithms to evaluate the likelihood percentage of inhibition of several compounds/drugs on various viruses which includes human immunodeficiency virus (HIV), hepatitis C virus (HCV), hepatitis B virus (HBV), human herpesvirus (HHV) and 26 other essential viruses. AVCpred is an extensive ChEMBL bioactivity database clutch with updated antiviral properties and thus helps to identify the percentage of inhibition of these viruses towards the drug of interest. The dataset was updated with 389 compounds for HIV, 124 compounds for HHV, 467 in case of HCV, 112 against HBV, and 1391 AVCs targeting other 26 viruses (Qureshi *et al*., 2017). Thus, this online server was used to study the antiviral properties of 12 FDA-approved drugs to support the experimental docking analysis.

Results

The drug development by computational approaches is the most effective and less time-consuming methodology, to support the pre-clinical validation of unknown antiviral therapeutics regimen. The docking mecha-

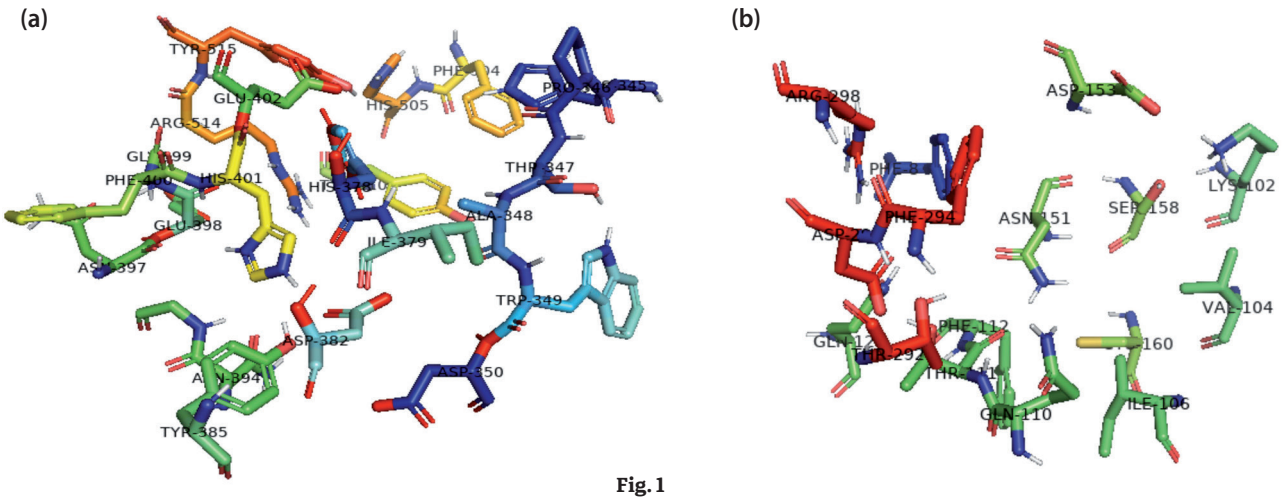

Fig. 1

Active sites of SARS-CoV-2 proteins
Active site of spike protein **(a)** and active site of main protease **(b)**.

nism between protein and ligand elucidates inhibition efficiency of the ligand which is a preliminary step to determine the potent antiviral candidate that can be considered for further laboratory experimentation. The association of the drug candidate (ligand) to its target receptor was evaluated as a first binding reaction on aiming of computer-aided drug discovery to predict paradoxically small molecules having potent inhibitory activity against the predicted targets through excellent binding affinity.

Observation of ligands and target site pockets

SARS-CoV-2 spike glycoprotein is an outer envelope protein which consist of 1273 amino-acids and has two main functional subunits: a large N-terminal S1 subunit and a relatively short C-terminal S2 subunit. Before performing the docking study, the protein structure has been stabilised. The missing hydrogen atoms were added to the protein structure while any water molecules or ligands were removed. Figure 1a and 1b shows the active site of the spike protein and the main protease of SARS-CoV-2.

Table 1. Molecular physicochemical descriptors analysis of ligands

Ligand	Trade Name	PubChem ID	Drug Bank number	Molecular formula	FDA approval status
Darunavir	Prezista	213039	DB01264	C27H37N3O7S	Approved for HIV
Fedratinib	Inrebic	16722836	DB12500	C27H36N6O3S	Approved for myelofibrosis
Ganciclovir	Cytovene; Cymevene; Vitrasert	3454	DB01004	$C_9H_{13}N_5O_4$	Approved for herpes simplex keratitis
Hydroxychloroquine	Plaquenil	3652	DB01611	C18H26CIN30_	Approved for COVID-EUA
Imatinib	Gleevec	5291	DB00619	C29H31N7O	Approved for chronic myeloid leukemia
Losartan	Cozaar	3961	DB00678	C22H23ClN6O	Approved for ACE II inhibitors
Oseltamavir	Tamiflu	6508	DB00198	C16H28N2O4	Approved for influenza
Peramivir	Rapivab	154234	DB06614	C15H28N4O4	Approved for influenza
Ribavirin	Copegus	37542	DB00811	C8H12N4O5	Approved for hepatitis C virus
Ruxolitinib	Jakafi	25126798	DB08877	C17H18N6	Approved for high-risk myelofibrosis,
Tenofovir	Viread	464205	DB14126	C9H14N5O4P	Approved for HIV and hepatitis B virus
Zanamivir	Relenza	636424	DB00558	C12H22N4O8	Approved for influenza

Table 2. ADMET and toxicity results of selected ligands

Drug likeness	Darunavir	Fedratinib	Ganciclovir	Hydroxy-chloroquine	Imatinib	Losartan	Oseltamivir	Peramivir	Ribavirin	Ruxolitinib	Tenofovir	Zanamivir
Molecular weight (<500 Da)	547.66	524.68	255.23	335.87	493.6	422.91	312.4	328.41	244.2	306.37	287.21	332.31
Log P (<5)	1.89	4.27	-1.8	3.85	3.47	4.5	1.3	-0.27	-1.9	2.94	-1.5	-2.3
H-bond donor (5)	3	3	4	2	2	2	2	5	4	1	3	7
H-bond acceptor (<10)	8	7	7	4	7	5	4	8	7	4	5	10
Rule of 5 violations	No	No	Yes	No	Yes	Yes	Yes	No	Yes	Yes	No	No
MDDR like rule	Yes	Yes	No	No	Yes	Yes	No	No	No	No	No	No
Ion channel modulator	-0.21	-0.34	-0.10	0.30	-0.09	0.16	0.10	0.11	0.21	0.10	0.73	0.03
pKa (strongest acidic)	13.59	10.21	10.16	15.59	12.45	7.4	14.03	4.09	11.88	13.89	1.35	3.25
pKa (strongest basic)	2.39	9	1.76	9.76	8.27	4.12	9.31	12.46	-1.2	5.51	3.74	11.93
Water solubility mg/ml	0.0668	0.00949	11.5	0.0261	0.0146	0.0047	0.686	0.38	33.2	0.116	1.87	7.31
Kinase inhibitor	-0.24	0.30	0.43	0.44	0.59	0.03	-0.33	-0.31	-0.21	0.91	0.80	-0.24
Blood brain barrier	Low	-	High	High	High	No	Low	High	High	High		No
Protease inhibitor	1.15	0.07	-0.54	0.12	-0.08	0.33	0.29	0.69	-0.20	-0.13	0.29	0.55
Enzyme inhibitor	0.31	-0.06	1.03	0.15	-0.08	0.44	0.47	0.59	0.71	0.24	1.54	0.99
Aromatic heavy atoms	12	18	9	10	24	22	0	0	5	14	9	0
Rotatable bonds	13	11	5	9	8	8	9	8	3	4	5	7
Ames mutagenicity	Nontoxic	Nontoxic	Nontoxic	Toxic	Nontoxic	Nontoxic	Nontoxic	Nontoxic	Nontoxic	Toxic	Nontoxic	Nontoxic
Caco-2 permeability	High		Low	Low	High	No	No	No	No	No	No	No
Carcinogenicity	No		No	No	No	No	No	No	No	No	No	No
ESOL solubility (mg/ml)	1.88E-02	9.86E-04	9.78E+01	4.17E-02	4.20E-03	2.80E-03	4.16E+00	1.49E+01	1.51E+02	1.68E-01	6.68E+01	1.24E+03
GI absorption	Low	Low	Low	High	High	High	High	Low	Low	High	Low	Low
Bioavailability score	0.55	0.55	0.55	0.55	0.55	0.56	0.55	0.55	0.55	0.55	0.56	0.17
Lead likeness violations	2	3	0	2	3	3	1	1	1	0	0	0
Synthetic accessibility	5.67	4.18	2.61	2.82	3.78	3.45	4.44	4.31	3.89	3.16	3.39	4.96
CYP1A2 inhibitor	No	No	No	Yes	No	No	No	No	No	Yes	No	No
CYP2C19 inhibitor	No	Yes	No	No	Yes	Yes	No	No	No	Yes	No	No
BBB permeant	No	No	No	Yes	No	No	No	No	No	No	No	No
Pgp substrate	Yes	No	No	No	Yes	yes	Yes	Yes	No	Yes	No	Yes

Table 3. The interaction energy analysis of ligands against SARS-CoV-2 spike protein

Ligands	Interacting amino acid residue	Hydrogen bond	RMSD	Bond length (Å)	Binding energy ΔG^ (kcal/mol)
Darunavir	THR276, ARG273	4	0.0	2.5,2.4,2.0,2.9	-7.4
Fedratinib	THR276, GLU406, ARG273, HIS505, GLU402	7	0.0	2.3,2.5,2.5,1.9,2.3,2.5,2.2	-8.3
Ganciclovir	GLU398, ASP350, ASP382, TYR385, ASN394	7	-	3.3,2.2,2.4,2.5,2.9,1.9,2.5	-9.2
Hydroxychloroquine	GLU145, ASP269, PHE274	3	0.0	2.0,2.2,2.3	-5.4
Imatinib	GLU402, GLU406	2	0.0	2.7, 2.1	-6
Losartan	ASN149, TRY515, ARG273, HIS505	5	0.0	2.2, 2.2, 2.1, 2.5, 2.5	-7.2
Oseltamivir	HIS505, ARG273	3	-	2.4, 2.0, 2.1	-5.3
Peramivir	GLU406, ARG518	4	0.0	2.0, 2.0, 2.6, 2.1	-6
Ribavirin	ARG273, ARG518, TYR515, GLU402	7	0.0	2.1, 2.0, 2.6, 2.4, 2.7, 2.3, 2.4	-8.2
Ruxolitinib	GLU402, GLU398, ASN394	5	0.0	2.5,2.5,2.6.,2.4,2.4	-7.1
Tenofovir	ASN394, ALA348, HIS378, ZN	7	0.0	1.9, 2.5, 2.3, 2.2, 2.4, 2.2, 2.7	-8.5
Zanamivir	ALA348, ARG514, ASN397, GLY395, HIS401	7	0.0	2.4,2.1,2.3,2.2,2.7,2.8,2.3	-9

Table 4. The interaction energy analysis of ligands against SARS-CoV-2 main protease

Ligands	Interacting amino acid residue	Hydrogen bond	RMSD	Bond length (Å)	Binding energy ΔG^ (kcal/mol)
Darunavir	LYS5, GLU290, GLY138	5	0.0	2.5, 2.6.2.3, 2.6, 2.2, 2	-7.5
Fedratinib	GLN110	1	0.0	2.7	-5
Ganciclovir	GLU166, LEU141, SER144, GLY143, CSY145, HIS163	7	0.0	2.5, 1.8, 2.2, 2.7, 2.7, 2.6, 2.7	-9
Hydroxychloroquine	THR111, GLN110	2	-	2.0,	-5.4
Imatinib	GLU402, GLU406	2	0.0	2.7, 2.1	-6
Losartan	THR292, THR111	4	0.0	2.4, 2.0,2.8,2.4	-7.2
Oseltamivir	SER158, ASP153	2	-	2.4,2.3	-5.3
Peramivir	THR292, ASP295, THR111, ASN151	5	0.0	1.2,2.4,2.4,2.7,1.9	-6
Ribavirin	LYS102, SER158, ASN151, ASP295, THR111	7	0.0	2.5,2.3,2.7,2.4,2.0,2.3	-8.2
Ruxolitinib	THR111, THR292	3	0.0	2.3,2.5,2.5	-7.1
Tenofovir	ARG105, GLN110, THR111, THR292	5	0.0	2.6, 2.2,2.1,2.0,1.9	-8.5
Zanamivir	GLN110, THR111, SER158, ASP153	8	0.0	2.0,2.3,2.2,2.6,2.9,2.6,2.4	-9

Table 1 shows the molecular properties of 12 selected drugs and their respective antiviral properties with FDA-approval status. Amongst these 12 tested drugs, darunavir (HIV), ganciclovir (herpes simplex keratitis), oseltamivir (influenza), peramivir (influenza), ribavirin (hepatitis C virus), tenofovir (high-risk myelofibrosis) and zanamivir (influenza) are approved as respective antivirals. Interestingly, the drugs fedratinib, hydroxychloroquine and imatinib have shown antiviral properties for COVID-19 by *in-vitro* studies (Coleman *et al*., 2016; FDA, 2020; Wu and Yang, 2020). Losartan has shown the antiviral activity towards cytomegalovirus and HIV by an *in-vitro* and clinical study (Choi *et al*., 2019; Garcia, 2012). For ruxolitinib, the antiviral activity against HIV by *in-vitro* and *in-vivo* studies has been proved (Haile *et al*., 2016). Ruxolitinib and fedratinib are Jak/STAT inhibitors playing an essential role in triggering immune response and substantially mediating innate immune responses and immunity against extracellular pathogens (Wu and Yang, 2020).

ADME and toxicity profiling

Determined ADMET properties for all selected drugs were based on four parameters such as absorption, distribution, metabolism, and excretion. All drugs have demonstrated high suitability upon drug-likeness and

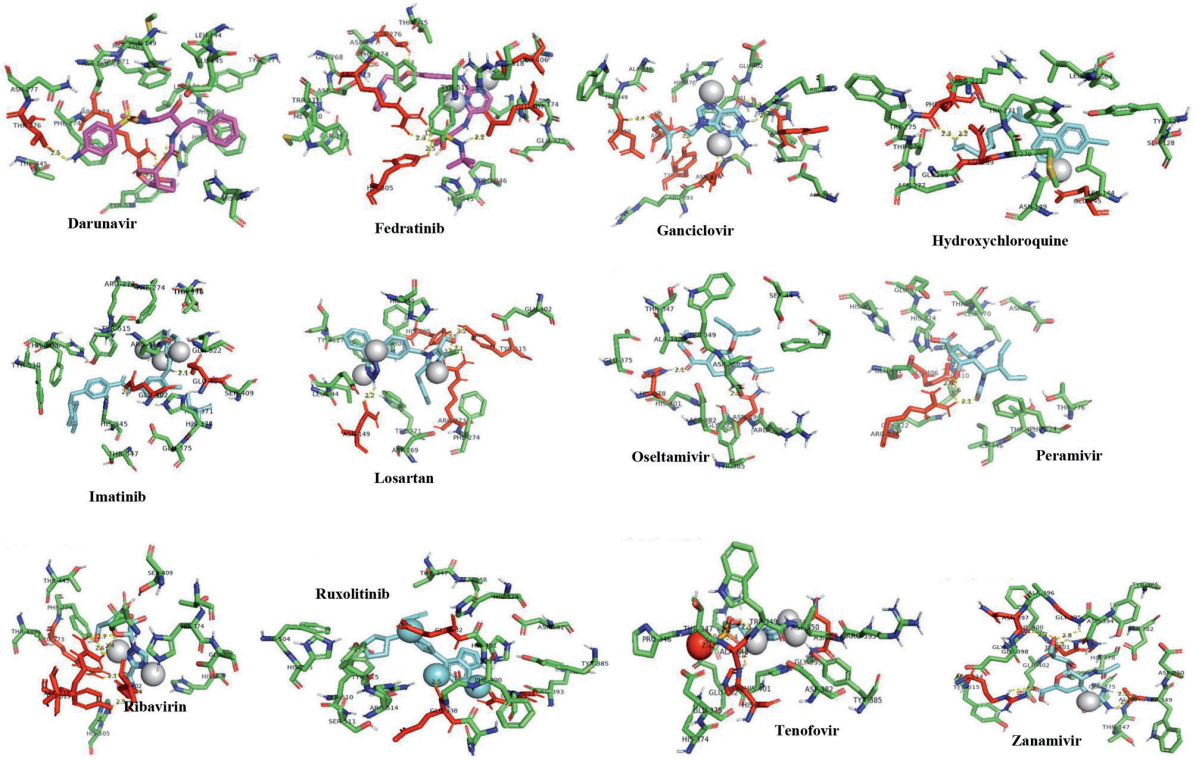

Fig. 2
Molecular docking of selected drugs on SARS-CoV-2 spike protein
The drug of interest docked in the active site of SASR-CoV-2 spike protein with the appropriate amino acids.

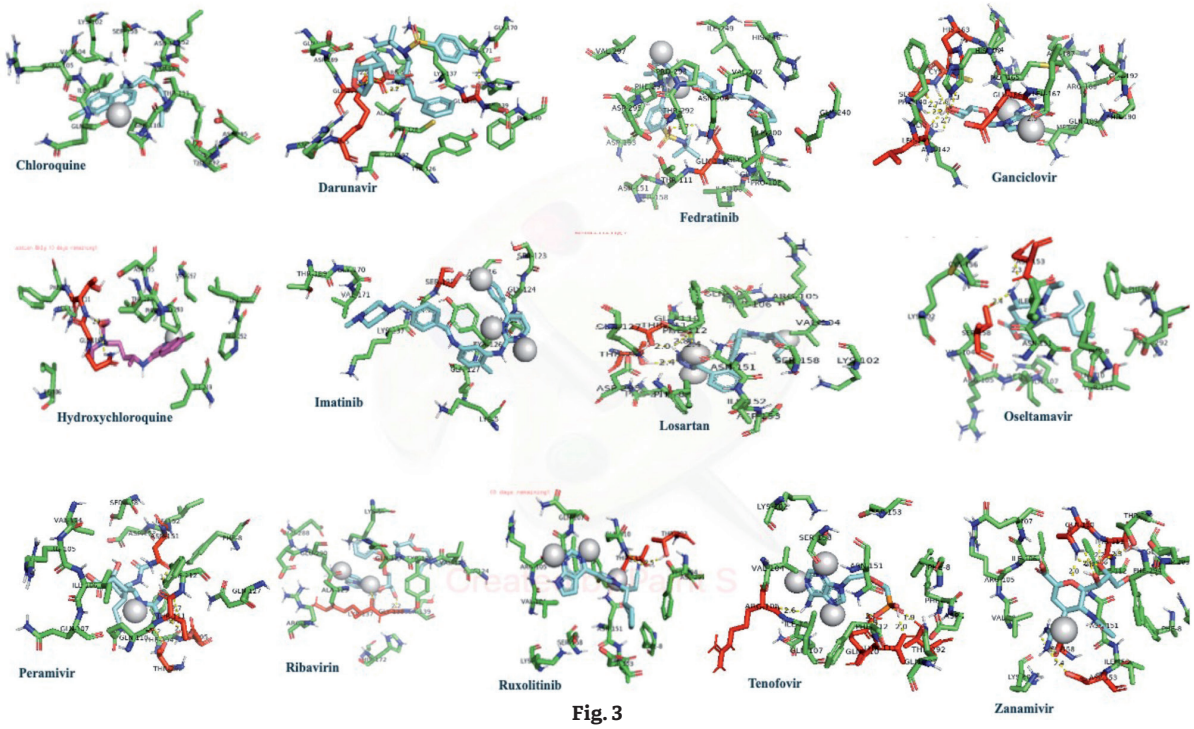

Fig. 3

Molecular docking of selected drugs on SARS-CoV-2 main protease
The drug of interest docked in the active site of SASR-CoV-2 main protease with the appropriate amino acids.

Fig. 4

Binding energy of selected drugs against SARS-CoV-2
Binding energy of selected drugs against SARS-CoV-2 spike protein **(a)** and main protease **(b)**.

ADMET properties which are shown in Table 2. The drug-likeness was performed based on multiple parameters CMC like rules, Lead like rules, MDDR like rules, Lipinski's rule of five and WDI like rules. In ADME study, the values of plasma protein binding, water-solubility, blood-brain barrier penetration, ESOL solubility, skin permeability, gastro-intestinal absorption, and CaCO2 cell permeability were determined for all the drugs of interest. Toxicity of these ligands was predicted using the Ames test and rodent carcinogenicity test. All drugs were nontoxic except ruxolitinib, which showed toxicity in Ames test. The results showed that most of the drugs were non-toxic, non-mutagenic and non-carcinogenic for humans.

Molecular docking results of dual inhibitors

To gain insight into the binding mechanism of the dual inhibitors on SARS-CoV-2, we performed Auto Dock 4.2 to estimate the docking score. The interactive amino acid residues, hydrogen bond, binding energy, RMSD, bonding

length and type of interaction are shown in Table 3 (spike protein) and Table 4 (main protease). The 3D molecular structure with interacting binding site of spike protein and the main protease are represented in Fig. 2 and 3, respectively. In addition, the graphical representation of active site interaction with spike and the main protease of SARS-CoV-2 are shown in Fig. 4a and 4b, respectively.

Docking results of SARS-CoV-2 spike proteins against selected drugs

After evaluating the overall analysis of all the selected approved drugs of SARS-CoV-2 spike protein, ganciclovir and zanamivir have docked with highest binding energy which was above -9 kcal/mol. Exclusively, ganciclovir was binding with affinity of -9.2 kcal/mol as an excellent topmost binding affinity. Both the ligands have bound seven hydrogen bonds with a different amino acid residue, where ganciclovir docked with GLU398, ASP350, ASP382, TYR385 and ASN394, while zanamivir docked with the active sites of ALA348, ARG514, ASN397, GLY395 and HIS401. The strong bond lengths determined for ganciclovir were 3.3, 2.2, 2.4, 2.5, 2.9, 1.9, 2.5 Å and for zanamivir were 2.4, 2.1, 2.3, 2.2, 2.7, 2.8, 2.3 Å. The second topmost binding affinity was noticed towards fedratinib, ribavirin and tenofovir against spike protein targets at above -8 kcal/mol. Individually, fedratinib, ribavirin and tenofovir bound with affinity of -8.3 kcal/mol (THR276, GLU406, ARG273, HIS505, GLU402), -8.2 kcal/mol (ARG273, ARG518, TYR515, GLU402) and -8.5 kcal/mol (ASN394, ALA348, HIS378, ZN), respectively. Among this, ribavirin is well known antiviral agent which was studied on various viruses by *in-vitro* and *in-vivo* studies.

Darunavir, losartan and ruxolitinib are drugs that exhibited next level docking score at above -7kcal/mol with different amino acid active sites. Darunavir interacted with SARS-CoV-2 spike protein by forming four hydrogen bonds with THR276, and ARG273 with the binding energy level of -7.4 kcal/mol. Losartan and ruxolitinib bound with lower affinity than darunavir, -7.2 kcal/mol and -7.1 kcal/mol, respectively. The rest of the drugs, hydroxychloroquine, imatinib, oseltamivir and peramivir exhibited binding affinity of -5.4 kcal/mol, -6 kcal/mol, -5.3 kcal/mol and -6 kcal/mol, respectively. Hydroxychloroquine and oseltamivir bound with three hydrogen bonds with length of 2.0, 2.2, 2.3 Å and 2.4, 2.0, 2.1 Å, respectively. The ligands imatinib and peramivir had length of hydrogen bonds 2.7, 2.1 Å and 2.0, 2.0, 2.6, 2.1 Å for possess 2 and 4, respectively. The interactive amino acids of hydroxychloroquine were GLU145, ASP269 and PHE274, of imatinib GLU402 and GLU406, of oseltamivir HIS505 and ARG273 and of peramivir GLU406 and ARG518.

Docking results of SARS-CoV-2 main protease against selected drugs

In ligands-spike protein docking results, main protease target site has also actively participated in docking towards all the tested approved drugs. Amongst the antiviral efficacy evaluation, ganciclovir and zanamivir have established excellent docking results with spike protein resulting in best possibility to inhibit the SARS-CoV-2 replication. These two drugs have bound with highest affinity of -9kcal/mol at different amino acid active sites, where ganciclovir docked with GLU166, LEU141, SER144, GLY143, CSY145, HIS163 and zanamivir docked with GLN110, THR111, SER158, ASP153 through 7 and 8 hydrogen bonds, respectively. Hence, these drugs are highly efficient to be considered for further pre-clinical validation.

The second topmost binding affinity was observed in protease active site with ribavirin and tenofovir with -8.2 kcal/mol and -8.5 kcal/mol, respectively. These two drugs possessed the same second topmost level docking score on spike protein which was also above -8 kcal/mol. The active amino acid sites were LYS102, SER158, ASN151, ASP295, THR111 in ribavirin and ARG105, GLN110, THR111 and THR292 in tenofovir at poses 7 and 5, respectively.

The third most effective drugs with binding energy level above -7 kcal/mol were darunavir, losartan and ruxolitinib with affinity of -7.5 kcal/mol -7.2 kcal/mol and -7.1 kcal/mol, respectively. These are three combinational drugs that exhibited similar results on spike protein active sites except losartan and ruxolitinib, and were better than darunavir possessing leading docking score of the main protease of SARS-CoV-2. The remaining drugs, hydroxychloroquine, imatinib, oseltamivir, and peramivir, possessed the least binding activity among the selected drugs but still an appreciated binding affinity towards both the targets. Imatinib and peramivir exhibited same and similar results with spike protein target with affinity of -6 kcal/mol, while hydroxychloroquine and oseltamivir have docked with affinity of -5.4 and -5.3 kcal/mol, respectively. The amino acids active sites of the main protease of hydroxychloroquine where THR111 and GLN110, imatinib GLU402 and GLU406, oseltamivir SER158 and ASP153, and peramivir THR292, ASP295, THR111 and ASN151.

Antiviral prediction

Antiviral properties prediction of these selected FDA-approved drugs was predicted using the AVCpred server. The supporting evidence of this online server on the prediction of effective antiviral drugs on SARS-CoV-2 was demonstrated as an additional parameter to this study which is shown in Table 5. Losartan has exhibited the

Table 5. Percentage of viral inhibition of FDA approved drugs on general and other viruses

Ligand	Query molecule ID	Binding affinity towards spike protein	Binding affinity towards main protease	General	HBV	HCV	HHV	HIV
Darunavir	213039	-7.4	-7.5	37.421	18.26	49.021	61.295	56.161
Fedratinib	16722836	-8.3	-5	56.903	19.699	69.922	46.087	75.266
Ganciclovir	3454	-9.2	-9	59.675	22.346	61.507	60.06	62.898
Hydroxychloroquine	3652	-5.4	-5.4	37.745	25.356	68.267	61.459	62.153
Imatinib	5291	-6	-6	59.492	27.095	25.825	59.949	56.59
Losartan	3961	-7.2	-7.2	64.579	20.798	31.892	37.442	71.821
Oseltamivir	6508	-5.3	-5.3	24.103	18.121	87.742	51.807	44.163
Peramivir	154234	-6	-6	46.085	23.473	47.804	76.057	48.899
Ribavirin	37542	-8.2	-8.2	33.436	26.621	31.987	49.267	65.564
Ruxolitinib	25126798	-7.1	-7.1	38.694	25.262	37.888	35.003	61.623
Tenofovir	464205	-8.5	-8.5	42.177	23.587	49.11	19.323	59.245
Zanamivir	636424	-9	-9	43.927	18.407	46.955	27.457	51.165

highest percentage of inhibition of 64.579% on general viruses, confirming its third topmost level docking score towards both spike and the main protease targets of SARS-CoV-2 at -7.2 kcal/mol binding affinity. The top-level drugs, ganciclovir and zanamivir showed at 59.675% and 43.927% of inhibition towards general viruses. All other drugs have expressed a certain level of inhibitory activity towards general viruses and showed antiviral properties observed on HIV, HBV, HHV and HCV viruses with a considerable percentage of inhibition as a supportive methodology by online *in-silico* evaluation.

Discussion

During the past decades, the development of antiviral drug research has emerged with several efficacious therapeutics that eventually solved and remained as a remedy for many outbreaks. Among the multiple methodology, *in-silico* drug evaluation has played a vital role in developing new drugs for unknown endangered life-threatening viruses. The computational *in-silico* docking analysis is an excellent method used for a decade to identify new susceptible targets which could match desired binding sites towards the potential drug (Grinter and Zou, 2014). In this study, the computational *in-silico* anti-SARS-CoV-2 drug evaluation has shown that a dozen of FDA-approved antivirals possesses the significant dual inhibitory binding intensity against recent pandemic virus. There are few more studies that have studied the docking ability of FDA-approved drugs on SARS-CoV-2, but none of our selected drugs were published as the dual inhibitors (Arya *et al*., 2020; Contini, 2020; Kandeel and Al-Nazawi, 2020; Kumar *et al*., 2020; Lobo-Galo *et al*., 2020). The computational docking studies have highlighted that all the selected drugs have the ability to bind to the active and allosteric sites of two targets, spike protein and the main protease of SARS-CoV-2 protein structures.

The current study has focussed on ligand binding activity of darunavir, fedratinib, ganciclovir, hydroxychloroquine, imatinib, losartan, oseltamivir, peramivir, ribavirin, ruxolitinib, tenofovir and zanamivir as dual inhibitors of SARS-CoV-2. Amongst these tested drugs, the highest binding energy was displayed by ganciclovir for both the targets, which were above -9 kcal/mol of binding energy to SARS-CoV-2 protein active sites, interacting with the most active amino acid residues. Zanamivir exhibited the appreciable second topmost binding activity against both the targets. Surprisingly, zanamivir shared the same binding affinity towards spike protein and main protease active sites.

Shah *et al*. (2020), has reported that zanamivir showed binding energy of -6.548 kcal/mol for the different active sites of the main protease (5R80) of SASR-CoV-2. The authors have docked zanamivir with few other drugs against the main protease. The study has also reported the docking score of oseltamivir and tenofovir with main protease, which was above -6 kcal/mol. Tenofovir also possessed the ability to dock with the active site of RNA dependent RNA polymerase of SARS-CoV-2 with affinity of -6 kcal/mol (Elfiky, 2020b). Tenofovir and ribavirin have nearly similar binding energy towards spike protein and the main protease. Still, the number of HB interactions were few against RdRp when compared to the main pro-

tease (Elfiky, 2020b). The current study has also analysed oseltamivir and tenofovir which displayed dual inhibitory binding energy above -8.5 kcal/mol and were listed as top-level drugs.

Ribavirin, anti-HCV inhibitor is one among the highly effective drugs for SASR-CoV-2 among the current selected FDA-approved drugs which were proved by *in-vitro*, *in-silico* and clinical studies. The current study has also observed ribavirin as a top-level potent dual inhibitor against spike protein and the main protease of SARS-CoV-2 with the same binding energy of -8.2 kcal/mol and seven hydrogen bonds with appreciable bond length. It is to be noted that ribavirin docked with 2.01 binding energy score with main protease target of SARS-CoV-2, however with Schrodinger glide docking module only with threonine (Kandeel and Al-Nazawi, 2020). The current study has perceived that ribavirin exhibits HB interaction with LYS102, SER158, ASN151, ASP295, THR111 for the main protease active site whereas, ARG273, ARG518, TYR515, GLU402 for the spike protein active sites. RNA dependent RNA polymerase is also a susceptible target of SARS-CoV-2 for ribavirin which docked with affinity of -7.8 kcal/mol (Elfiky, 2020a; Elfiky, 2020b).

Chloroquine and hydroxychloroquine have suddenly emerged as a potent inhibitors of SARS-CoV-2 in various *in-vitro* and *in-silico* studies where eventually FDA declared the approval for use as therapeutics to treat COVID-19 patients (FDA, 2020). Due to the risk of large randomised clinical trial in COVID-19 patients, FDA has withdrawn EUA against SARS-CoV-2. However, the FDA approval status of the drug for malaria, lupus and Rheumatoid arthritis will not be affected (Food and Administration, 2020). Though these drugs are not used, this study has checked their dual inhibition efficiency. Surprisingly the hydroxychloroquine alone has possessed significant docking score of -5.4 kcal/mol, but chloroquine did not exhibit dual inhibition against SARS-CoV-2. Hydroxychloroquine was reported as a dual inhibitor in an earlier study as it is docking with the spike protein and the main protease of SARS-CoV-2 with -8.05 and -8.86 kcal/mol, respectively (Gurjar, 2020). Srivastava *et al*. has also reported the strong affinity of hydroxychloroquine to the same main protease target of SARS-CoV-2 with -7.62 kcal/mol by binding PHE140 amino acid residue with 2.501 Å (Srivastava *et al*., 2020). In contrast, the present study has identified two different amino acid residues THR111 and GLN110 intended for the binding affinity.

Narkhede *et al*. (2020) has studied the same targets for docking studies and stated that the binding energy of oseltamivir and ribavirin against the main protease was -4.7 kcal/mol and -5.6 kcal/mol, respectively. In addition, hydroxychloroquine had expressed significant binding affinity towards spike protein associated with ACE receptor complex with -5.3 kcal/mol (Narkhede *et al*., 2020). Nevertheless, the current study has found that the FDA drug oseltamivir and hydroxychloroquine has showed the binding energy of -5.3 kcal/mol with two HB interactions. Also, it is to be noted that these two drugs were the ones among the least observed binding energies on spike protein and the main protease. Nonetheless, in our study, ribavirin was one of the potential dual inhibitors with significant HB interactions. Oseltamivir also docked with 11 amino acid residues with a single hydrogen bond interaction with the binding affinity of -7.39 kcal/mol (Mamidala *et al*., 2020). Also, the authors have studied the binding affinity of darunavir, tenofovir, and hydroxychloroquine against the main protease of SARS-CoV-2 with -6.8, -4.0, and -8.3 kcal/mol, respectively (Mamidala *et al*., 2020).

Antiretroviral drug darunavir possessed the significant binding energy against various main proteases, which was confirmed by AutoDock Vina and SMINA tools (Sekhar, 2020). It binds with multiple bonds with multi amino acid residues and acts as a significant inhibitor of SARS-CoV-2 by residue energetic hydrophobic index of -1.45 with 45% similarity among other tested drugs (Dayer, 2020). Darunavir has also been reported to block papain-like viral protease (PLVP) and coronavirus endopeptidase C30 (CEP_C30) of SARS-CoV-2 which was studied using Discovery Studio by the Libdock Scores (Lin *et al*., 2020). Perhaps, unfavourable results were exhibited on anti-SARS-CoV-2 activity for darunavir in *in-vitro* study with EC50 >100 μM despite several docked poses during *in-silico* docking studies. The authors suggested combinational therapy with ritonavir or cobicistat for solely therapeutic measures for COVID-19 (De Meyer *et al*., 2020). Likewise, as observed from our results, the considerable dual binding energy of darunavir, could be considered for pre-clinical studies.

Conclusion

In conclusion, we studied FDA-approved antiviral drugs for repurposing in the treatment of recent viral outbreak calamity caused by SARS-CoV-2. The present study has hypothesised with potent dual inhibitors that can act against SARS-CoV-2 by *in-silico* validation. The binding affinity and docking interaction displays that all the ligands tested in this analysis express dual inhibition of the active sites of spike protein and the main protease. Since the virus mutates rapidly, this multi-targeted approach could be an effective strategy for promising therapeutic regimen. The drugs ganciclovir and zanamivir were the most effective ligands with the highest binding affinity among all the tested drugs with binding affinity above -9 kcal/mol with multi hydrogen bonds interactions of the

SARS-CoV-2 targets. Ribavirin and tenofovir are second top-level ligands which exhibited strong interaction with similar binding energy range towards both the targets. Remaining tested drugs expressed substantial affinity towards the selected targets. Since the antivirals used in the study are FDA-approved drugs, it could certainly be considered for treatment measures soon after the pre-clinical testing and clinical trials. Hence the selected drugs used in the current study could be taken to the next step with our findings for the further level for experimentation to overcome the anti-SARS-CoV-2 non-available therapeutic catastrophe.

References

Arya, R, Das, A, Prashar, V, Kumar, M (2020): Potential inhibitors against papain-like protease of novel coronavirus (SARS-CoV-2) from FDA approved drugs. ChemRxiv. https://doi.org/10.26434/chemrxiv.11860011.v2

Astuti, I, Ysrafil (2020): Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2): An overview of viral structure and host response. Diab. Metab. Syndrome 14, 407–412. https://doi.org/10.1016/j.dsx.2020.04.020

Calligari, P, Bobone, S, Ricci, G, Bocedi, A (2020): Molecular Investigation of SARS-CoV-2 Proteins and Their Interactions with Antiviral Drugs. Viruses 12, 445. https://doi.org/10.3390/v12040445

Caly, L, Druce, JD, Catton, MG, Jans, DA, Wagstaff, KM (2020): The FDA-approved Drug Ivermectin inhibits the replication of SARS-CoV-2 in vitro. Antiviral. Res. 104787. https://doi.org/10.1016/j.antiviral.2020.104787

Choi, JA, Kim, J-E, Ju, H-h, Lee, J, Jee, D, Park, CK, Paik, S-y (2019): The effects of losartan on cytomegalovirus infection in human trabecular meshwork cells. PLoS One 14, e0218471. https://doi.org/10.1371/journal.pone.0218471

Coleman, CM, Sisk, JM, Mingo, RM, Nelson, EA, White, JM, Frieman, MB (2016): Abelson Kinase Inhibitors Are Potent Inhibitors of Severe Acute Respiratory Syndrome Coronavirus and Middle East Respiratory Syndrome Coronavirus Fusion. J. Virol. 90, 8924–8933. https://doi.org/10.1128/JVI.01429-16

Contini, A (2020): Virtual screening of an FDA approved drugs database on two COVID-19 coronavirus proteins. ChemRxiv. https://doi.org/10.26434/chemrxiv.11847381.v1

Daina, A, Michielin, O, Zoete, V (2017): SwissADME: a free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules. Sci. Reports 7, 42717. https://doi.org/10.1038/srep42717

Dayer, MR (2020): Old drugs for newly emerging viral disease, COVID-19: Bioinformatic Prospective. arXiv preprint arXiv:2003.04524.

De Meyer, S, Bojkova, D, Cinatl, J, Van Damme, E, Meng, CB, Van Loock, M, Woodfall, B, Ciesek, S (2020): Lack of antiviral activity of darunavir against SARS-CoV-2. Intect. J. Inf. Dis. 97, 7–10. https://doi.org/10.1016/j.ijid.2020.05.085

Elfiky, AA (2020a): Anti-HCV, nucleotide inhibitors, repurposing against COVID-19. Life Sci. 248, 117477. https://doi.org/10.1016/j.lfs.2020.117477

Elfiky, AA (2020b): Ribavirin, Remdesivir, Sofosbuvir, Galidesivir, and Tenofovir against SARS-CoV-2 RNA dependent RNA polymerase (RdRp): A molecular docking study. Life Sci. 117592. https://doi.org/10.1016/j.lfs.2020.117592

FDA (2020): Hydroxychloroquine.

Ferron, F, Subissi, L, Silveira De Morais, AT, Le, NTT, Sevajol, M, Gluais, L, Decroly, E, Vonrhein, C, Bricogne, G, Canard, B, Imbert, I (2018): Structural and molecular basis of mismatch correction and ribavirin excision from coronavirus RNA. Proc. Natl. Acad. Sci. USA 115, E162. https://doi.org/10.1073/pnas.1718806115

Food, Administration, D (2020): FDA cautions against use of hydroxychloroquine or chloroquine for COVID-19 outside of the hospital setting or a clinical trial due to risk of heart rhythm problems. Food and Drug Administration. Available from: https://www. fda.gov/drugs/drug-safety-and-availability/fda-cautions-against-use-hydroxychloroquine-or-chloroquine-covid-19-outside-hospital-setting-or. Accessed April 29.

Garcia, F (2012): Effects of losartan and antiretroviral regimen containing raltegravir in fibrosis inflammation. . In: ClinicalTrials.gov. Bethesda (MD): National Library of Medicine (US).

Greene, JA, Loscalzo, J (2017): Putting the Patient Back Together-Social Medicine, Network Medicine, and the Limits of Reductionism. New England J. Med. 377, 2493. https://doi.org/10.1056/NEJMms1706744

Grinter SZ, Zou X (2014): Challenges, applications, and recent advances of protein-ligand docking in structure-based drug design. Molecules 19, 10150–10176. https://doi.org/10.3390/molecules190710150

Gurjar, G (2020): Screening of anticancer drugs as potential candidates to target COVID-19 disease. ChemRxiv. https://doi.org/10.26434/chemrxiv.12277046.v1

Gurung, AB, Ali, MA, Lee, J, Farah, MA, Al-Anazi, KM (2020): Unravelling lead antiviral phytochemicals for the inhibition of SARS-CoV-2 Mpro enzyme through in silico approach. Life Sci. 117831. https://doi.org/10.1016/j.lfs.2020.117831

Haile, WB, Gavegnano, C, Tao, S, Jiang, Y, Schinazi, RF, Tyor, WR (2016): The Janus kinase inhibitor ruxolitinib reduces HIV replication in human macrophages and ameliorates HIV encephalitis in a murine model. Neurobiol. Dis. 92, 137–143. https://doi.org/10.1016/j.nbd.2016.02.007

Hasan, C, Mehmet, O (2020): Comparison of Clinically Approved Molecules on SARS-CoV-2 Drug Target Proteins: A Molecular Docking Study. ChemRxiv. https://doi.org/10.26434/chemrxiv.12277046.v1

Ibrahim, IM, Abdelmalek, DH, Elshahat, ME, Elfiky, AA (2020): COVID-19 spike-host cell receptor GRP78 binding site prediction. J. Infect. 80, 554–562. https://doi.org/10.1016/j.jinf.2020.02.026

Jin, Z, Du, X, Xu, Y, Deng, Y, Liu, M, Zhao, Y, Zhang, B, Li, X, Zhang, L, Peng, C, Duan, Y, Yu, J, Wang, L, Yang, K, Liu, F, Jiang,

R, Yang, X, You, T, Liu, X, Yang, X, Bai, F, Liu, H, Liu, X, Guddat, LW, Xu, W, Xiao, G, Qin, C, Shi, Z, Jiang, H, Rao, Z, Yang, H (2020): Structure of M(pro) from SARS-CoV-2 and discovery of its inhibitors. Nature 582, 89–293. https://doi.org/10.1038/s41586-020-2223-y

Kandeel, M, Al-Nazawi, M (2020): Virtual screening and repurposing of FDA approved drugs against COVID-19 main protease. Life Sci. 251, 117627. https://doi.org/10.1016/j.lfs.2020.117627

Khaerunnisa, S, Kurniawan, H, Awaluddin, R, Suhartati, S, Soetjipto, S (2020): Potential inhibitor of COVID-19 main protease (Mpro) from several medicinal plant compounds by molecular docking study. Prepr. https://doi.org/10.20944/preprints202003.0226.v1

Kumar, D, Chandel, V, Raj, S, Rathi, B (2020): In silico identification of potent FDA approved drugs against Coronavirus COVID-19 main protease: A drug repurposing approach. Chem. Biol. Letters 7, 166–175.

Lin, S, Shen, R, He, J, Li, X, Guo, X (2020): Molecular modeling evaluation of the binding effect of ritonavir, lopinavir and darunavir to severe acute respiratory syndrome coronavirus 2 proteases. BioRxiv. https://doi.org/10.1101/2020.01.31.929695

Lobo-Galo, N, Terrazas-López, M, Martínez-Martínez, A, Díaz-Sánchez, ÁG (2020): FDA-approved thiol-reacting drugs that potentially bind into the SARS-CoV-2 main protease, essential for viral replication. J. Biomol. Structure Dynam. 1–12. https://doi.org/10.1080/07391102.2020.1764393

Mamidala, E, Davella, R, Gurrapu, S, Shivakrishna, P (2020): In silico identification of clinically approved medicines against the main protease of SARS-CoV-2, causative agent of covid-19. arXiv preprint arXiv:2004.12055. https://doi.org/10.22376/ijpbs/lpr.2021.11.1.L107-122

Narkhede, RR, Cheke, RS, Ambhore, JP, Shinde, SD The Molecular Docking Study of Potential Drug Candidates Showing Anti-COVID-19 Activity by Exploring of Therapeutic Targets of SARS-CoV-2. Screening 5, 8.

Park, H, Lee, J, Lee, S (2006): Critical assessment of the automated AutoDock as a new docking tool for virtual screening. Proteins 65, 549–554. https://doi.org/10.1002/prot.21183

Qureshi, A, Kaur, G, Kumar, M (2017): AVC pred: an integrated web server for prediction and design of antiviral compounds. Chem. Biol. Drug Design 89, 74–83. https://doi.org/10.1111/cbdd.12834

Sekhar, T (2020): Virtual Screening based prediction of potential drugs for COVID-19. Preprints.

Shah, B, Modi, P, Sagar, SR (2020): In silico studies on therapeutic agents for COVID-19: Drug repurposing approach. Life Sci. 252, 117652. https://doi.org/10.1016/j.lfs.2020.117652

Shang, J, Ye, G, Shi, K, Wan, Y, Luo, C, Aihara, H, Geng, Q, Auerbach, A, Li, F (2020): Structural basis of receptor recognition by SARS-CoV-2. Nature 581, 221–224. https://doi.org/10.1038/s41586-020-2179-y

Srivastava, AK, Kumar, A, Tiwari, G, Kumar, R, Misra, N (2020): In Silico Investigations on the Potential Inhibitors for COVID-19 Protease. arXiv preprint arXiv:2003.10642.

WHO (2020): Coronavirus death report - WHO.

Wu, D, Yang, XO (2020): TH17 responses in cytokine storm of COVID-19: An emerging target of JAK2 inhibitor Fedratinib. J. Microbiol. Immunol. Infect. 53, 368–370. https://doi.org/10.1016/j.jmii.2020.03.005

Yang, L (2020): China confirms human-to-human transmission of coronavirus.

Zhang, L, Lin, D, Sun, X, Curth, U, Drosten, C, Sauerhering, L, Becker, S, Rox, K, Hilgenfeld, R (2020): Crystal structure of SARS-CoV-2 main protease provides a basis for design of improved α-ketoamide inhibitors. Science 368, 409–412. https://doi.org/10.1126/science.abb3405