

Coronavirus Pandemic

Drug repurposing: identification of SARS-CoV-2 potential inhibitors by virtual screening and pharmacokinetics strategies

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Abstract

Introduction: The coronavirus disease 2019 (COVID-19) pandemic caused global health, economic, and population loss. Variants of the coronavirus contributed to the severity of the disease and persistent rise in infections. This study aimed to identify potential drug candidates from fifteen approved antiviral drugs against SARS-CoV-2 (6LU7), SARS-CoV (5B6O), and SARS-CoV-2 spike protein (6M0J) using virtual screening and pharmacokinetics to gain insights into COVID-19 therapeutics.

Methodology: We employed drug repurposing approach to analyze binding performance of fifteen clinically approved antiviral drugs against the main protease of SARS-CoV-2 (6LU7), SARS-CoV (5B6O), and SARS-CoV-2 spike proteins bound to ACE-2 receptor (6M0J), to provide an insight into the therapeutics of COVID-19. AutoDock Vina was used for docking studies. The binding affinities were calculated, and 2-3D structures of protein-ligand interactions were drawn.

Results: Rutin, hesperidin, and nelfinavir are clinically approved antiviral drugs with high binding affinity to proteins 6LU7, 5B6O, and 6M0J. These ligands have excellent pharmacokinetics, ensuring efficient absorption, metabolism, excretion, and digestibility. Hesperidin showed the most potent interaction with spike protein 6M0J, forming four H-bonds. Nelfinavir had a high human intestinal absorption (HIA) score of 0.93, indicating maximum absorption in the body and promising interactions with 6LU7.

Conclusions: Our results indicated that rutin, hesperidin, and nelfinavir had the highest binding results against the proposed drug targets. The computational approach effectively identified SARS-CoV-2 inhibitors. COVID-19 is still a recurrent threat globally and predictive analysis using natural compounds might serve as a starting point for new drug development against SARS-CoV-2 and related viruses.

Key words: COVID-19; SARS-CoV-2; molecular docking; rutin; hesperidin; nelfinavir.

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Introduction

Coronavirus disease 2019 (COVID-19) was first reported in December 2019 from Wuhan, China. Since then, the disease spread worldwide and emergence of new cases remained uncontrollable. The “Coronaviridae Study Group (CSG) of the International Committee on Taxonomy of Viruses (ICTV)” [1] named the new viral strain causing COVID-19 pneumonia severe acute respiratory syndrome coronavirus 2 (SARS- CoV-2) [2].

The spread of the coronavirus became a significant health crisis worldwide and was declared a pandemic by the World Health Organization (WHO) [3]. As of 7 April 2024, there have been 775,293,630 confirmed cases and 7,044,637 deaths due to COVID-19

worldwide [4]. The first case of COVID-19 was reported in Karachi on 26 February 2020, with an estimated population of 204.65 million in Pakistan. The coronavirus successively spread across the country, leading to an epidemic. As of 10 April 2020, there have been 4,601 confirmed cases, 727 recoveries, and 66 deaths in Pakistan [5].

The symptoms of COVID-19 include upper respiratory tract infection, headache, sore throat, cough, fever, vomiting, muscle pain, shortness of breath, and pneumonia [6]. Anxiety was identified as one of the symptoms of COVID-19 among the teachers in China [7]. The degree of anxiety was influenced by factors such as age, gender, education, type of teacher, school location, information source, levels of worry and fear,

and behavior [8]. Unfortunately, there was currently no effective treatment for COVID-19; and it was essential to explore treatments that target various factors related to the coronavirus [9].

SARS-CoV-2 belongs to the family Coronaviridae and genus Betacoronavirus. It is comparable to previously known SARS-CoV and MERS-CoV with 79% and 50% genome similarity, respectively. Betacoronaviruses are positive-sense, enveloped, single-stranded RNA viruses, ranging in size from 26-32 kb, and are the largest known RNA viruses [10]. The RNA genome of SARS-CoV-2 consists of six functional open reading frames (ORF) arranged in a 5'-3' direction: replicase (ORF1a/b), spike (S), nucleocapsid (N), envelop (E), and membrane (M) [11,12]. The "ORF1a/b" gene encodes a large polyprotein (PP1a/b) and occupies two-thirds of the RNA genome from the 5' end. These polyproteins are cleaved by the main protease M pro (also called 3CL^{pro}) into sixteen non-structural proteins (NSPs). These NSPs are involved in viral replication, suppression of host genes, and formation of multi-domain complexes [13]. When SARS-CoV-2 enters the host, it binds to its receptor angiotensin-converting enzyme 2 (ACE-2) in order to enter cells, and the S proteins mediate the entry of the virus into the host cell. The main protease, Mpro, of SARS-CoV-2 is an attractive target of drugs because of its crucial role in viral replication and transcription [14,15]. The second most commonly targeted protein is the ACE-2 receptor in the kidneys, lungs, nose, and mouth epithelium. It is a promising target for drugs as it allows SARS-CoV-2 to enter into cells. The viral replication process can be disrupted at this stage, before it transcends further into the cell and causes infection.

Main protease of SARS-CoV-2 (6LU7) is a relatively newly identified strain; thus, investigations are being conducted to discover and devise possible therapeutic drugs to treat COVID-19 [16]. In-silico approaches may provide encouraging results for drug repurposing or repositioning strategies. Hesperidin and rutin are flavonoids found in fruits, seeds, and vegetables, and have been used in herbal medicines because of their anti-inflammatory and antiviral properties [17]. A recent study on the diversity of medicinal plants concluded that plant extracts have been used as a source of male contraceptives by indigenous communities and traditional health practitioners in Pakistan [18]. A study on Fabaceae plant extracts demonstrated potential inhibitory effects on acetylcholinesterase, pancreatic lipase, and cancer cell lines, indicating that plant extracts could serve as potential drugs [19].

The plant extracts hesperidin and rutin may be considered for the treatment of SARS-CoV-2 infections because they are less toxic and can synergize with conventional medications. Moreover, they are "pleiotropic" drugs, i.e., their functional groups can interact with diverse cellular targets and block multiple pathways. These properties make them potential candidates for the treatment of SARS-CoV-2 infection [20].

In this study, an array of compounds, including anti-retroviral and anti-respiratory agents, which have previously been used to treat the betacoronavirus epidemics, were tested. This study used a drug repurposing approach to identify potential drug candidates from a set of flavonoids, antiviral, and antibacterial compounds, based on their activity against the membrane protein (M pro) and spike protein of SARS-CoV-2. The study is significant because it identified three potential drug candidates (rutin, hesperidin, and nelfinavir) that exhibited high binding affinity and promising protein interaction with their target proteins. The study hypothesized that the computational approach used in this study can effectively be used to successfully identify SARS-CoV-2 inhibitors.

Methodology

Protein structure retrieval

The crystal structures of the main proteases of SARS-CoV-2 (PDB ID: 6LU7; resolution 2.16 Å), SARS-CoV (PDB ID: 5B6O; resolution 2.2 Å), and spike protein (PDB ID: 6M0J; resolution 2.45 Å) were downloaded from protein data bank (<https://www.rcsb.org/>) [21] in .pdb format and used for the docking experiments.

Prediction of active site of protein

The binding sites of proteins (6LU7, 5B6O, and 6M0J) were predicted through literature search and further confirmed using the CASTp 3.0 online web server (<http://sts.bioe.uic.edu/castp/index.html?2011>) [22]. The CASTp server provides detailed, inclusive, and comprehensive topological identification of protein structure and information on residues at the binding site pocket and its volumes, cavities, and channels. The binding pocket size, based on a larger surface area, was designated as an active site, and amino acids in that site were also generated and shown.

Ligand preparation

In order to assign proper bond orders, and create three-dimensional geometries and accessible ionization

and tautomer status, the ligands were prepared prior to docking. All fifteen ligands — rutin (DB01698), hesperidin (DB04703), nelfinavir (DB00220), ritonavir (DB00503), remdesivir (DB14761), lopinavir (DB01601), azithromycin (DB00207), indinavir (DB00224), emetine (DB13393), saquinavir (DB01232), darunavir (DB01264), galidesivir (DB11676), azidothymidine (DB00495), penciclovir (DB00299) and guaifenesin (DB00874) — were obtained from the Drug Bank (<https://go.drugbank.com/>) in .pdb format. This is a web-enabled database that includes wide-ranging molecular and structural data on drugs, their mechanism, pathways, and target interactions [23]. The .pdb files were converted to pdbqt (AutoDock pdbqt format) using PyMOL (v.2.3.4) (Edu Schrödinger, LLC; <https://pymol.org/2/>) software for molecular docking. The ligands were prepared using AutoDock tools through the addition of polar hydrogen and detecting root and torsions.

Molecular docking analysis

Validation of the molecular docking protocol was necessary to ensure accurate conformation of ligands with the binding pocket of proteins, and it was performed by validating the center and size of the grid box's coordinates within the binding pocket. AutoDock Vina (v.1.1.2) (<https://sourceforge.net/projects/autodock-vina-1-1-2-64-bit/>) [24], a well-known virtual screening docking software, was used to examine selected ligands against SARS-CoV-2 proteins. Initially, protein refinement was carried out by removing het atoms, water molecules, and unnecessary chains. Then, polar hydrogen groups and charges to all the structures were added. For site-specific docking, macromolecular files of proteins (6LU7, 5B6O, and 6M0J) and ligands were prepared and docked inside a grid box covering all identified binding residues adjusted to X, Y, and Z dimensions of -13.916 Å, 14.086 Å, 71.213 Å, -12.956 Å, -12.857 Å, -31.937 Å and -36.31 Å, 30.215 Å, -0.304 Å for 6LU7, 5B6O, and ACE-2, respectively. The ligands' binding potential and resulting affinity scores

of the lowest energy output were used to determine the best molecular interactions [25]. The resulting complexes were visualized and analyzed using PyMOL (v.2.3.4) (<https://pymol.org/2/>) [26], and 2-D and 3-D images were processed using BIOVIA Discovery Studio (v20.1.0.19295; San Diego: Dassault Systèmes; <https://discover.3ds.com/discovery-studio-visualizer-download>) [27].

Pharmacokinetics analysis

The pharmacokinetic properties of the top three ligands (lowest binding energy) were achieved through Lipinski's rule of 5 using Molsoft LLC Drug-Likeness and molecular property prediction (www.molsoft.com/mprop/) [28]. Along with this, Lipinski's rule of 5 was evaluated. Molsoft LLC also calculated a drug score for the selected inhibitor molecules. The drug's absorption percentage (ABS%) was calculated using the equation: %ABS = 109 – (0.345 x polar surface area PSA). Molinspiration was conducted to determine molecular properties and bioactivity score calculations for ligands (www.molinspiration.com/cgi-bin/properties) [29], and ADMET properties were determined using the admetSAR prediction tool (<http://lmmd.ecust.edu.cn/admetSar2>) [30].

Results

Prediction of active sites

The active sites of proteins were predicted through literature search [31-33] and the online tool CASTp 3.0 (<http://sts.bioe.uic.edu/castp/index.html?2011>) [34]. The binding pocket functionality and residues were evaluated based on published literature. In this docking study, the residues that superimposed each other were considered active sites of SARS-CoV proteins (Mpro and S) (Table 1).

Molecular docking analysis

In silico analysis was performed by selecting 15 (approved, experimental, and investigational) drugs that interact with Mpro of SARS-CoV-2, SARS-CoV, and the spike protein of SARS-COV-2. Three

Table 1. Summary of predicted binding sites of the main protease of SARS-CoV, SARS-CoV-2, and ACE-2 receptor using CASTp 3.0.

Protein	Binding site residues
SARS-CoV-2 (M)	THR24, THR25, THR26, LEU27, HIS41, THR45, SER46, MET49, PHE140, LEU141, ASN142, GLY143, SER144, CYS145, HIS163, MET165, GLU166, HIS172
SARS-CoV (M)	PRO9, GLY11, LYS12, GLU14, GLY15, CYS16, MET17, VAL18, GLN19, TRP31, GLN69, ALA70, GLY71, ASN72, VAL73, PRO96, LYS97, PRO99, TYR118, ASN119, GLY120, SER121, PRO122
ACE-2	LYS26, THR27, LEU29, ASP30, PHE32, ASN33, HIS34, GLU37, ASP38, PHE40, SER43, TYR50, GLU56, VAL59, MET62, ASN63, SER70, LEU73, LYS74, GLN81, LEU91, THR92, LYS94, LEU95

THR: threonine; LEU: leucine; HIS: histidine; SER: serine; MET: methionine; PHE: phenylalanine; ASN: asparagine; GLY: glycine; CYS: cystine; GLU: glutamic acid; PRO: proline; LYS: lysine; VAL: valine; GLN: glutamine; TRP: tryptophan; ALA: alanine; ASP: aspartate.

categories of drugs were selected for potential treatment of coronavirus, including naturally occurring compounds, antiviral, and antibacterial agents. All ligands were prepared in their optimized form and docked to the target proteins (Table 2). In our docking experiments, the binding affinity of rutin was -10.4 kcal/mol (6M0J), -10.5 kcal/mol (6LU7), and -9.3 kcal/mol (5B6O) (Figure 1 a, b, c). We found that hesperidin showed good interactions with 6M0J, 6LU7, and 5B6O with a binding affinity of -10.5 kcal/mol, -8.5 kcal/mol, and -9.4 kcal/mol, respectively (Figure 1 d, e, f). Nelfinavir, an anti-retroviral drug, was developed synthetically and used against human immunodeficiency virus (HIV). This cyclic protease inhibitor showed good affinity scores compared to other derivatives of the same class. The binding affinities of nelfinavir with the targets' proteins were -9.1 kcal/mol (6M0J), -9.14 kcal/mol (6LU7), and -9.0 kcal/mol (5B6O), respectively (Figure 1 g, h, i).

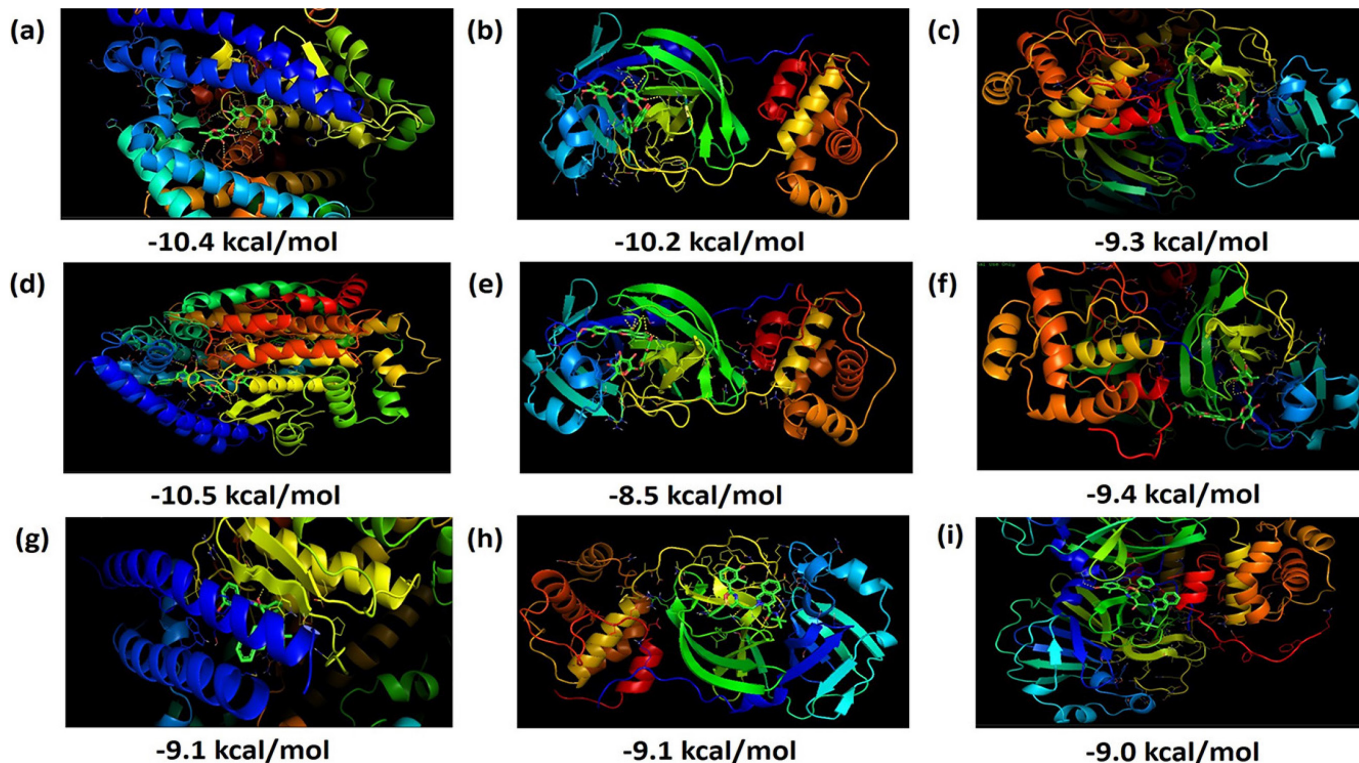
Based on the best-fitting scores, the top three docked complexes were analyzed for protein-ligand interactions through BIOVIA Discovery Studio (<https://discover.3ds.com/discovery-studio-visualizer-download>) [27].

Table 2. Binding energies of selected ligands with targeted proteins (6LU7, 5B6O, and ACE-2).

Ligand	Binding Energy (kcal/mol)		
	6LU7	5B6O	ACE-2
Rutin	-10.2	-9.3	-10.4
Hesperidin	-8.5	-9.4	-10.5
Nelfinavir	-9.1	-9.0	-9.1
Ritonavir	-8.4	-8.8	-9.6
Remdesivir	-7.9	-8.2	-9.4
Lopinavir	-7.8	-8.9	-9.8
Azithromycin	-7.2	-8.7	-9.6
Indinavir	-7.7	-8.9	-9.5
Emetine	-7.5	-8.0	-9.4
Saquinavir	-7.5	-8.4	-9.2
Darunavir	-7.3	-8.3	-9.4
Galidesivir	-6.4	-6.9	-6.8
Azidothymidine	-6.2	-6.2	-6.5
Penciclovir	-5.7	-6.4	-5.5
Guaifenesin	-5.1	-5.3	-5.3

The complexes were analyzed to understand binding energy differences and to study protein-ligand profiles. It was found that rutin formed H-bonds with all three protein targets. The docking score of rutin with the spike protein was the highest (Figure 2a), with three H interactions with ARG514, two interactions with GLY395, and one H interaction with TYR515, ASP382, ASP206, and TRP203. The residue HIS401 had a π - π stacked interaction, which occurs when two

Figure 1. PyMOL visualization of best affinity interactions obtained after coupling the drugs with target proteins catalytic site (protein as cartoon and their side chains in thin lines).



Interaction between rutin and ACE-2 (a), 6LU7 (b), and 5B6O (c); Interaction between Hesperidin and ACE-2 (d), 6LU7 (e), and 5B6O (f); Interaction between Nelfinavir and ACE-2 (g), 6LU7 (h) and 5B6O (i). ACE2: angiotensin-converting enzyme 2; Mpro: viral main protease.

aromatic rings interact. The protease structures of Mpro 6LU7 (Figure 2b) and 5B6O (chain-B) (Figure 2c) had two interactions with GLY143 each, and one with SER144 and HIS163. Two π -sulfur bonds with MET49 in the docked complex of 5B6O were observed, which occur when a π -electron cloud of aromatic ring interrelates with a lone pair of the electron cloud of a sulfur atom, and one conventional H bond with GLU166, THR26, and ALA145. However, comparison of the two protease structures revealed that rutin had more interactions in docking with target 6LU7: one H

bond interaction with THR24, LEU141, and two H bond interactions with SER46 and CYS145. Furthermore, carbon-hydrogen and π -alkyl/alkyl bonds were detected.

In the present study, the score for hesperidin was higher with spike protein (Figure 3a). Four H-bond interactions in the docking with spike protein (6M0J), including GLN102, ASP350, and LYS562 residues, showed single H-bond interactions, whereas ASN394 had two interactions. Alkyl and π -alkyl bonds were present; two residues, ARG393 and PHE 390, had carbon-hydrogen interactions, and HIS401 showed an extra π -cation interaction. In addition, hesperidin formed H-bonds with all targeted proteins, with six interactions in 6LU7 and 5B6O (chain B). Both 6LU7 (Figure 3b) and 5B6O (Figure 3c) have four SER144,

Figure 2. 2-D interaction of rutin with (a) angiotensin conversion enzyme 2 (ACE-2), (b) SARS-CoV-2 main protease (6LU7), and (c) SARS-CoV main protease (5B6O) after docking via AutoDock Vina. The 2-D structure was analyzed using BIOVIA discovery studio.

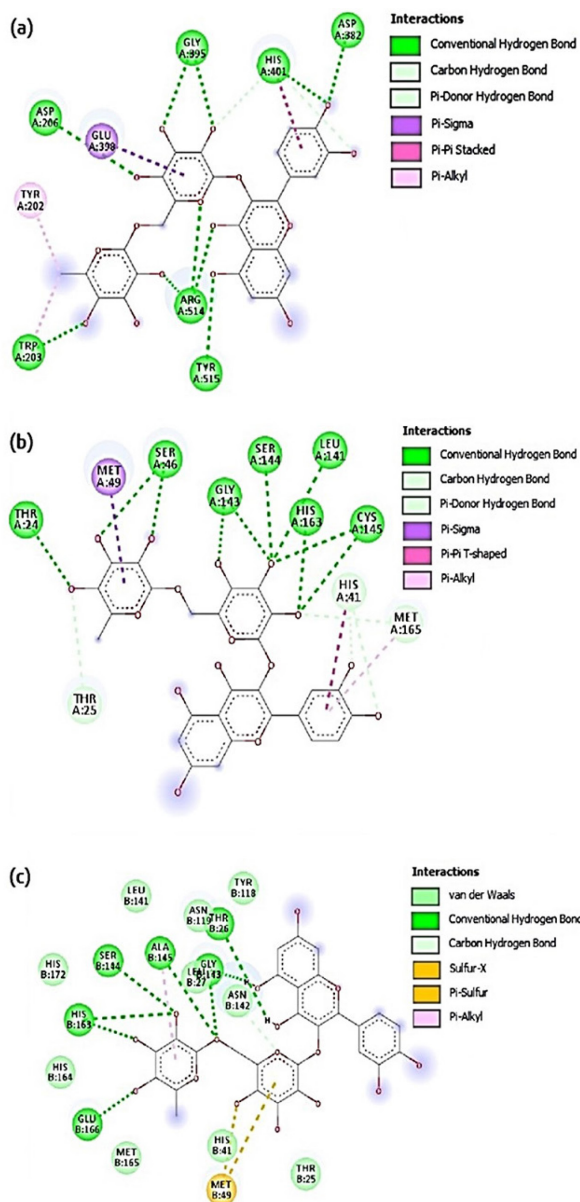
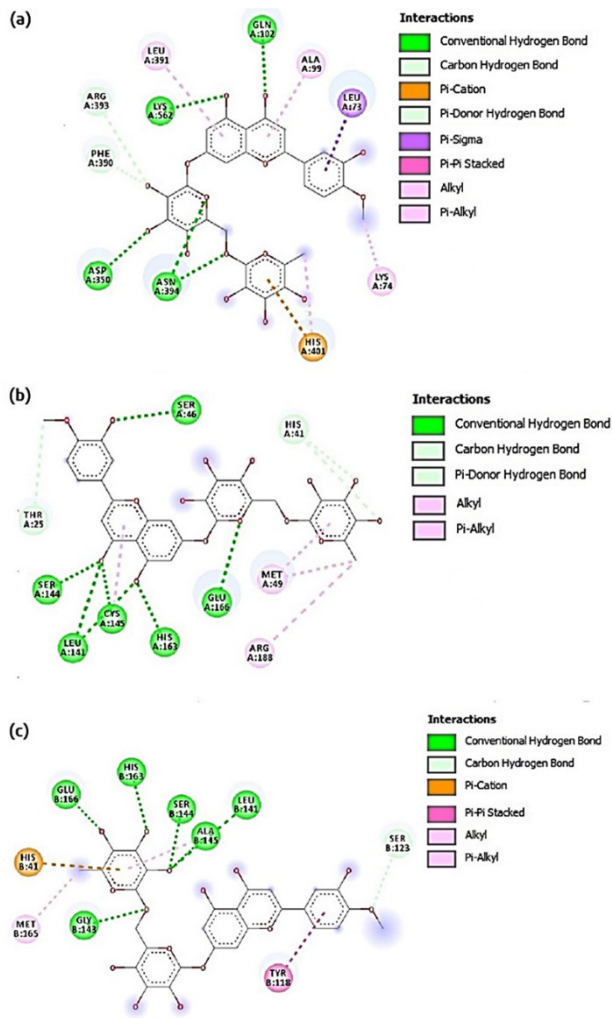


Figure 3. 2-D Interaction of hesperidin with (a) angiotensin conversion enzyme 2 (ACE-2), (b) SARS-CoV-2 main protease (6LU7) and (c) SARS-CoV main protease (5B6O) after docking via AutoDock Vina. The 2-D structure was analyzed using BIOVIA discovery studio.



LEU141, HIS163, and GLU166 common residues. However, hesperidin had double interaction with LEU141 (6LU7), while all other residues had single H-bond interactions. There was a common IS41 residue in 5B6O and 6LU7 for π -cation and carbon-hydrogen bonds, respectively. One alkyl bond with MET165 and one π - π stacked bond with TYR118 in the docked complex of 5B6O were observed. The findings also suggested that better docking scores were attained with natural compounds compared to conventional antiviral drugs.

When docking nelfinavir with spike protein (Figure 4a) and 5B6O (Figure 4c), one π -cation interaction with HIS401 of ACE-2 and two π -cation interactions with ARG298, π - π T shape interaction of HIS378 in ACE-2 and PHE294 in 5B6O, one π -sigma interaction with ILE152 in 5B6O and TRP349 in ACE-2 were observed. A π -donor hydrogen bond interaction with SER47, ASP350 in ACE-2, and carbon-hydrogen interaction with SER123 in 5B6O were also observed. Two π -alkyl interactions were present in all three proteins formed by π -electron cloud interaction between an aromatic and alkyl electron group. Nelfinavir showed three interactions with conventional hydrogen bonds with 6LU7 (Figure 4b), including HIS163, GLU166, and GLY143, two π -alkyl with MET49 and PRO168, and one favorable π -anion bond. The results also suggested that nelfinavir could be used against SARS-CoV-2, as it inhibits the spread of the coronavirus by inhibiting cell-to-cell fusion and disrupting the interaction of the glycol-spike protein with human receptor ACE-2.

Pharmacokinetics analysis

The three leading selected drugs were first evaluated for oral administration using the Lipinski's rule of 5 with Molsoft LLC: drug-likeness and molecular property prediction. Lipinski's rule of 5 is an empirical approach used to predict the drug-likeness of a ligand based on molecular weight (AMU (atomic mass unit) < 500), hydrogen bond donors (HBD; < 5), high lipophilicity LogP (LogP < 5), and hydrogen bond acceptor (HBA; < 10). The molecules with the desired attributes were predicted to have good permeation or absorption across the cell membrane. Nelfinavir exhibited suitable hydrogen bond donor and acceptor scores of 4 and 6, respectively. Lipinski's rule of

molecular weight (< 500) is violated in the top three ligands (rutin, hesperidin, and nelfinavir) (Table 3). The high molecular weight hindered proper absorption of a drug compared to low molecular weight drugs. When a drug's absorption percentage is > 50%, it is a good indicator of its supreme bioavailability, circulation, and

Figure 4. 2-D Interaction of nelfinavir with (a) angiotensin conversion enzyme 2 (ACE-2), (b) SARS-CoV-2 main protease (6LU7) and (c) SARS-CoV main protease (5B6O) after docking via AutoDock Vina. The 2-D structure was analyzed using BIOVIA discovery studio.

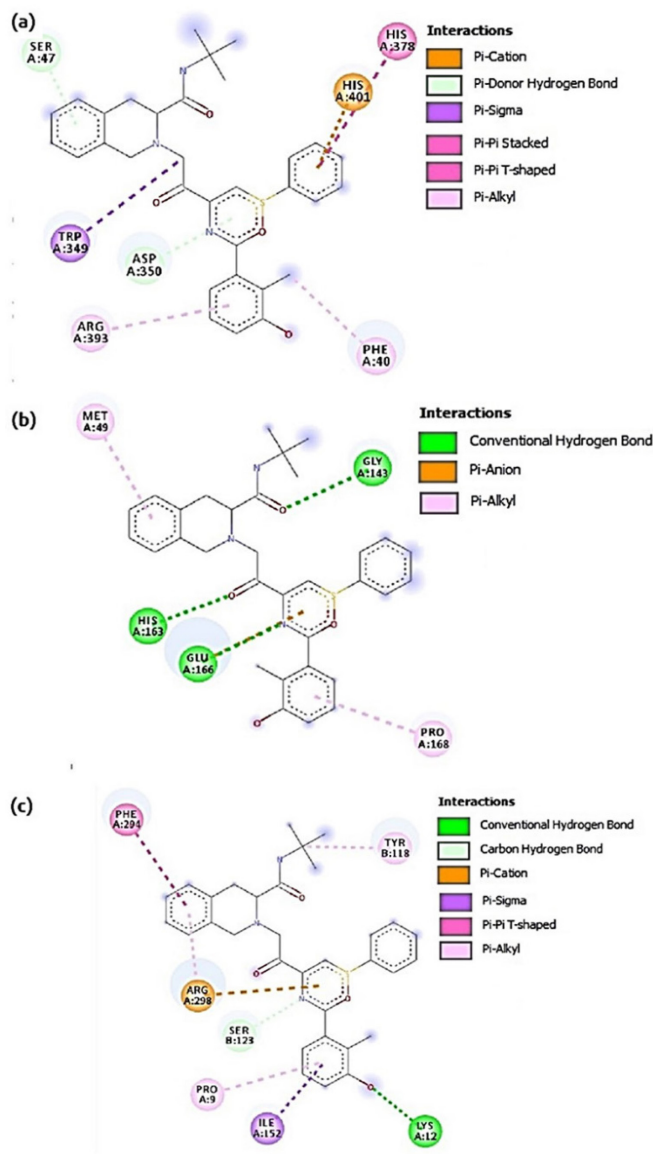


Table 3. Investigation of Lipinski's rule of five selected inhibitors after docking using Molsoft LLC: drug-likeness and prediction.

Ligand	Molecular mass (< 500)	Hydrogen bond acceptor (< 10)	Hydrogen bond donor (< 5)	LogP (< 5)	MolPSA (< 140)	Absorption percentage (> 50)	Drug likeness score (> 0)
Rutin	610.15	16	10	-1.55	213.63	35	0.91
Hesperidin	610.19	15	8	-0.81	186.7	44	0.94
Nelfinavir	567.31	6	4	5.06	83.94	80	1.41

Log P: partition co-efficient; MolPSA: molecular polar surface area.

Table 4. Prediction of bioactivity of selected inhibitors using molinspiration.

Ligand	GPCR ligand	Ion channel modulator	Kinase inhibitor	Nuclear receptor ligand	Protease inhibitor	Enzyme inhibitor
Rutin	-0.05	-0.52	-0.14	-0.23	-0.07	0.12
Hesperidin	-0.01	-0.59	-0.36	-0.20	-0.00	0.06
Nelfinavir	0.19	-0.25	-0.28	-0.25	0.58	-0.02

distribution by oral route. Rutin and hesperidin had suboptimal percentages of 35% and 44% (or < 50%). This may be due to the high MolPSA (> 140) values of these drugs, 213.63 Å (rutin) and 186.7 Å (hesperidin). Nelfinavir had an AB% of 80, which is an excellent parameter to depict the bioavailability of the drug through an oral route. Moreover, the positive drug score of rutin, hesperidin, and nelfinavir suggested that they were potential drug candidates.

The bioactivity of chosen ligands was evaluated using Molinspiration against G protein coupled receptors (GPCR) ligand, ion channel modulator, protease inhibitor, kinase inhibitor, nuclear receptor ligand, and enzyme inhibitor. The bioactivity of the chosen ligands was evaluated using Molinspiration against the nuclear receptor ligand, GPCR ligand, protease inhibitor, ion channel modulator, kinase inhibitor, and enzyme inhibitor. The bioactivity of a ligand is interpreted based on bioactivity score as: > 0 (active), - 5.0 - 0 (moderately active), and < -5.0 (inactive). Rutin and Hesperidin were active enzyme inhibitors with scores of 0.12 and 0.06, respectively. Nelfinavir had better protease inhibitor activity and GPCR ligand activity of 0.58 and 0.19, respectively. All three ligands possess moderate kinase inhibitor activity. The values of bioactivity of ligands generated through Molinspiration are shown in Table 4.

Despite the mild symptoms rutin, nelfinavir, and hesperidin that cause headache, diarrhea, and sleepiness, respectively, the pharmacokinetics properties of selected ligands were estimated using admetSAR, which determined the reaction of a drug inside the host. The pharmacokinetics parameters

considered in this study were absorption, digestibility, metabolism, excretion, toxicity, and water solubility. Nelfinavir and Hesperidin showed better human intestinal absorption (HIA) and human epithelial cell line (CaCo-2) permeability scores indicating better absorption in the intestine when administered orally. Nelfinavir had the highest HIA of 0.93, which indicated its maximal absorption in the host body. Moreover, all three ligands were non-inhibitor of CYP450, demonstrating proper metabolism of ligands by CYP450. All the selected ligands were non-carcinogenic and non-Ames toxic, except rutin which was Ames toxic.

Oral rat acute toxicity (LD50) is used to determine the lethality of a drug. The lower the LD50 value, the more lethal the drug will be. However, these selected ligands showed optimal LD50 scores. The values of admetSAR are presented in Table 5.

Sequence analysis

A comparative study of one indigenously isolated Pakistani strain with the Wuhan strain (GenBank accession numbers: MN-908947 and MT-500122), was performed to find similarities between the two SARS-CoV-2 strains. This was carried out to gain insight and help predict the effect of a novel variant of the Pakistan strain on the severity of COVID-19 and suggest if known drugs could inhibit the spread of the coronavirus. The ORF 1a/b sequences of both strains were followed by translation into an amino acid sequence using Trans-Seq (EMBL-EBI) (<https://www.ebi.ac.uk/Tools/st/>) [35] The alignment of multiple sequences was carried out using Clustal

Table 5. Pharmacokinetic properties of selected inhibitor molecules predicted using admetSAR tool.

Ligands	Water solubility (log mol/L)	Human intestinal absorption (HIA)	CaCo-2 permeability (log Papp in 10-6 cm/s)	Blood-brain barrier (BBB) (log BB)	CYP substrate / inhibitor	Ames toxicity	Carcinogenicity	LD50 (oral rat acute toxicity) (mol/kg)
Rutin	-2.77	0.73	0.92	0.93	Non-substrate/non-inhibitor	Yes	Non-carcinogen	2.60
Hesperidin	-2.64	0.81	0.88	0.95	Non-substrate/non-inhibitor	No	Non-carcinogen	2.03
Nelfinavir	-3.49	0.95	0.83	0.5	Substrate/non-inhibitor	No	Non-carcinogen	3.00

HIA: human intestinal absorption; CaCo-2: Human epithelial cell line; CYP: cytochrome P450; LD50: the lethal dose for rodent oral acute toxicity: measured in milligrams per kilogram of body weight. It indicates the amount of a substance needed to cause death in half of a group of test animals within 14 days after a single oral dose.

Omega at EMBL-EBI (<https://www.ebi.ac.uk/Tools/msa/clustalo/>) [36]. After analyzing the results, we concluded that the Mpro of both strains were approximately similar and had low dissimilarity values of $\leq 1\%$, which was highlighted using the BoxShade (v 3.2) tool (<https://boxshade.soft112.com/>) [37] (Figure 5).

Discussion

This study provides insight into the drug repurposing approach for using existing drugs against the Mpro present in SARS-CoV-2 and SARS-CoV, and the spike protein of SARS-CoV-2, to decipher their antiviral potential against COVID-19. We concluded that the three best-fitted drugs (rutin, hesperidin, and nelfinavir) showed a good binding affinity with Mpro of SARS-CoV-2 (6LU7) and spike protein (6M0J). Rutin was a promising candidate for antiviral therapies, with a high binding affinity to the ACE2 receptor and with the potential to modulate inflammation and innate antiviral immunity [38]. Another notable candidate, nelfinavir, can treat COVID-19, especially in asymptomatic and mild cases, offering practical implications for disease management [39]. We identified hesperidin as a promising compound that can hinder the entry of SARS-CoV-2 by preventing the interaction between the viral S protein and ACE2. It also reduces the expression of ACE2 and TMPRSS2. Hesperidin showcases its potential as a prophylactic measure against COVID-19 [40]. These findings emphasize that the considered drugs (rutin, nelfinavir, and hesperidin) are valuable elements and can be used globally and within national healthcare strategies.

Recently, the inhibitory effects of 80 flavonoid compounds on the crystal structure of Mpro SARS-CoV-2 were investigated and found to be more effective than HIV protease inhibitors [41]. Their findings supported that flavonoids employ their antiviral effects by blockage of cellular receptors, losses in enzymatic functions, and inhibiting viral antigenic determinants [41]. In addition, docking of 51 phytochemicals of *Juniperus procera* Hochst against Mpro of SARS-CoV-

2 demonstrated that Rutin has the highest binding score amongst all the plant chemicals and other commercial antiviral agents used [42]. The favorable use of hesperidin in the prophylaxis and management of COVID-19 prevents the entry of SARS-CoV-2 in host cells through receptor ACE-2 and can potentially inhibit the spread of infection. This antiviral property of hesperidin is an excellent choice for treating COVID-19 through improved immunity and effective anti-inflammatory action to control cytokine storms. A mixture of diosmin co-administered with heparin could prevent disease progression [43].

Similarly, this study also noticed a good binding affinity score of hesperidin with ACE-2, indicating that hesperidin can be used as a prophylactic and favorable adjuvant treatment choice for SARS-CoV-2 infection. Hesperidin is an ancient herbal medicine that is well-known and present in lemons, oranges, and other citrus fruits. Hesperidin exhibited antiviral effects on the influenza virus by significantly minimizing replication of the virus. Hesperidin substantially enhanced cell-autonomous resistance processes by treating infected cells through p38 and JNK activation and up-regulation because it is vital for cell defense mechanisms in fighting the influenza virus [17]. Numerous molecular docking studies reported the highest binding affinity of Hesperidin with spike protein (S), the main protease (3CL^{pro}) of SARS-CoV-2, SARS-CoV, MERS-CoV, and receptor binding domain (RBD) of ACE-2 complex. In a recent study from China, hesperidin was the only drug among a variety of natural compounds tested to target the binding interface between ACE-2 and spike protein (S); thus, it may disrupt the binding of RBD with ACE-2 and prevent virus entry into the cell [44].

A study by Upreti *et al.* demonstrated that *Urtica dioica* has anti-SARS-CoV-2 activity by examining interaction with its spike protein via a molecular repurposing approach. The phytoconstituents of *U. dioica* revealed the highest ligand binding affinity with the S1 subunit of the SARS-CoV-2 spike protein. In addition, computational screening of *Urtica* further

Figure 5. Multiple sequence alignment of ORF 1a/b of SARS-CoV-2 strain of Wuhan (QJX57851) with Pakistan (QHD43415) depicting amino acid sequence similarity. The alignment was generated with Clustal Omega and shaded with box shade.

QHD43415.1	1	MESLVPGFNEKTHVQLSLPVLQVRDVLVRGFGDSVEEVLSEARQHCLKDGTCTGLVEVEKGV
QJX57851.1	1	MESLVPGFNEKTHVQLSLPVLQVRDVLVRGFGDSVEEVLSEARQHCLKDGTCTGLVEVEKGV
QHD43415.1	421	AYWVPRASANIGCNHTGVVGESEGLNDNLLLEILQKEKVNINIVGDFKLNELIATILASE
QJX57851.1	421	AYWVPRASANIGCNHTGVVGESEGLN- NLLLEILQKEKVNINIVGDFKLNELIATILASE
QHD43415.1	601	ITGGVVQLTSQWL TNIFGTVYEKLPVLDWLEEKFKEGVEFLRDGWEIVKFI STCACEIV
QJX57851.1	600	ITGGVVQLTSQWL TNIFGTVYEKLPVLDWLEEKFKEGVEFLRDGWEIVKFI STCACEIV

highlighted that out of 40 selected potential ligand compounds from the plant, almost all the compounds showed the highest binding affinity compared to synthetic drugs. It advocated the exploration of herbal compounds as effective inhibitors of COVID-19. [45].

Another study showed that ursolic acid is a biologically active compound that is known as a potent antiviral drug and has a higher docking score (-8.7 kcal/mol) than hyperoside (-8.6 kcal/mol) and α -Hederin (-8.5 kcal/mol) with high binding affinity for Mpro. [46]. Recent studies on molecular docking showed that some phyto-compounds such as oleanolic acid and ursolic acid form strong complexes with papain-like proteases (PL^{pro}) of SARS CoV-2. These compounds have higher docking scores than approved antiviral and antibacterial drugs. The higher docking score shows strong binding affinity with target ligands and helps identify potential therapeutic ways to combat viral infections. The proteases need to be inhibited to treat SARS-COV-2 by damaging viral polyproteins. [47]. A similar study conducted in 2022 by Bhattacharya *et al.* identified three bio-active derivatives of quercetin phyto-compound using the molecular docking method. Among those derivatives were some that showed the highest binding affinity, including quercetin 3-O-arabinoside 7-O-rhamnoside with papain-like protease, and another derivative, quercetin 3-rhamnosyl-(1- > 2)- α -L-arabinopyranoside that showed the highest affinity with spike protein and receptor-binding domain [48]. Similarly, biologically active phyto-compounds in Kencur have antiviral properties. These bioactive molecules and quercetin have shown a strong affinity for Mpro on SARS-COV-2 through molecular simulation and docking analysis [49].

Nelfinavir is known for its antiviral properties. Nelfinavir mesylate, a Food Drug Authority (FDA)-approved HIV protease inhibitor, has been found to inhibit cell fusion caused by drugs containing cardiac glycoside and kinase-inhibiting agents at a concentration of 10 μ M [50]. Previous studies have indicated the possibility that nelfinavir would bind inside S trimmer structures, directly inhibiting S-o- and S-n-mediated membrane fusion once proximally bound to S2 amino terminals. This study found that nelfinavir could be used as an anti-SARS CoV-2 drug, especially when individuals displayed early signs of infection. It may inhibit the entry of glycol-spike protein by inhibiting cells from cell fusion. Nelfinavir may prevent S-proteolytic processing within cells through its actions.

An essential herbal medicine, triphala, has various potential derivatives that have been shown to act against Mpro of SARS-CoV-2 using molecular docking. In this case, the highest docking score was -14,521 kcal/mol. Terafflavin A, chebulagic acid, and corilagin can inhibit viruses by binding through catalytic domains of Mpro. This study identified potent biologically active compounds from triphala medicine as favorable inhibitors of Mpro of SARS-CoV-2 [51].

Oroxylum indicum, a medicinal plant with antiviral properties, was reported recently by Shah *et al.* in the intervention of novel COVID-19 disease [52]. Computational and algorithmic screening of the natural constituents of this plant shed light on its strong interactions with Mpro of the SARS-CoV-2 virus. Furthermore, docking analysis indicated that the three components, baicalein-7-O-glucoside, chrysin-7-O-glucuronide, and oroxindin, showed more significant interactions and binding energy consistent with remdesivir. This means that *O. indicum* and its natural compounds can be efficacious inhibitors against the main protease of SARS-CoV-2 [52].

In 2022, Sherwani *et al.* studied the *N. sativa* plant and extracted its seeds to experiment on bioactive compounds. They confirmed the interaction of the five most essential plant ligands with the spike proteins of SARS-CoV-2. They further elaborated their work, explaining that seed extracts of this plant have a strong affinity for angiotensin-binding polyproteins of SARS-CoV-2. The most important bioactive compounds of *N. sativa* seed extracts were thymoquinone, P-cymene, 4-terpineol, and dithymoquinone, and they were found to be capable of inhibiting ACE2 receptor binding. Thus, they can prevent vascular and inflammatory impairments [53].

A study by Mujwar and Harwansh identified the taraxerol compound as a potential natural candidate to display its therapeutic effects against SARS-CoV 2. Binding site analysis indicated that the compound targets the Mpro enzyme of the coronavirus by making contact with its residues, namely: Cys145, Pro168, Met165, Leu167, Gln192, Thr190, and Met49, for active ligand binding. With a high binding energy of -10.17 kcal/mol, the compound could target viral proteins, including spike, main protease, and RNA-dependent RNA polymerase. This confirms that this compound, like other flavonoids, can control COVID-19 [54].

Several studies have confirmed that phytochemicals can be a potential therapeutic tool to prevent and treat COVID-19 infection by blocking the interaction of proteins and their receptors. The S-protein can mutate

within short periods, and targeting these proteins is quite challenging to prevent such infections. Three phyto-compounds, deoxyartemisinin, deoxypodophyllotoxin, and dictamnine, were studied by Irfan *et al.* [45]. They observed low binding energies of these compounds with the S-protein of SARS-CoV-2. They suggested that high negative values of binding energies of these phyto-compounds can become an effective tool to control infections caused by SARS-CoV-2 [55].

After experiencing COVID-19 for more than two years, researchers are trying to find proper treatment methods for the novel variants of this deadly virus. In 2023, Pirolli *et al.* used virtual screening and molecular docking simulations on various antihistaminic drugs such as fexofenadine and checked their affinity for Delta spike RBD. They discovered that these antihistaminic drugs contain antiviral capacity for SARS-CoV-2 [56]. Many antiviral medications have been used to restrain COVID-19, but the emergence of new strains make it challenging to manage treatment for all its new variants. Adding to this, Rabie and Wafa found that preclinical drugs ChloViD2022, CoViTris2022, and Taroxaz-26 can potentially cause SARS-CoV-2 to mutate and thus inhibit replication [57].

Conclusions

COVID-19 is still a worldwide health concern. Developing therapeutic strategies against this viral disease is challenging. We used molecular docking to identify inhibitory drugs that show promising binding features with SARS-CoV-2 enzymes (Mpro and ACE-2). The results of this study indicate that rutin, hesperidin, and nelfinavir showed the highest binding results against the proposed drug targets. As a result of this similarity, we concluded that since our drug targets give approximately the exact binding affinities when docked with these ligands, they depicted excellent pharmacokinetic properties. Our drug targets demonstrate similar binding affinities when docked with these ligands, which have favorable pharmacokinetic properties. Thus, the computational approach can be effectively used to identify the inhibitors of SARS-CoV-2. The study included fewer samples due to limited resources, time constraints, complexity, and a focused approach to specific candidates. However, it still provides a basis for future research on the identified compounds and drug repurposing for COVID-19 treatment. Further *in vitro* and *in vivo* studies are needed to validate their efficacy

and safety. The compounds can serve as a starting point for new drug development.

Authors' contributions

SJK conceived the idea. AF, AK, JM, TNH, MUR, and SSN analyzed the data and drafted the initial manuscript. MZY and NN helped with manuscript writing. ZR and SJK finalized the manuscript and supervised the study. All authors read and approved the final manuscript.

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