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Virtual screening based on the structure of more than $10^5$ compounds against four key proteins of SARS-CoV-2: $M_{\text{Pro}}$, $S_{\text{RBD}}$, RdRp, and $PL_{\text{pro}}$

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**ABSTRACT**

**Background:** SARS-CoV-2 initially originated in Wuhan (China) around December 2019, and spread all over the world. Currently, WHO (Word Health Organization) has licensed several vaccines for this viral infection. However, not everyone can be vaccinated. People with underlying health conditions that weaken their immune systems or those with severe allergies to some vaccine components, may not be able to be vaccinated. Moreover, no vaccination is 100% safe, and the emergence of new SARS-CoV-2 mutations may reduce the efficacy of immunizations. Therefore, it is urgent to develop effective drugs to protect people against this virus.

**Material and method:** We performed structure-based virtual screening (SBVS) of a library that was built from ChemDiv and PubChem databases against four SARS-CoV-2 target proteins: S-protein (spike), main protease ($M_{\text{pro}}$), RNA-dependent RNA polymerase, and $PL_{\text{pro}}$. A virtual screening study was performed using PyRx and AutoDock tools.

**Results:** Our results suggest that twenty-five top-ranked drugs with the highest energy binding as the potential inhibitors against four SARS-CoV-2 targets, relative to the reference molecules. Based on the energy binding, we suggest that these compounds could be used to produce effective anti-viral drugs against SARS-CoV-2.

**Conclusion:** The discovery of novel compounds for COVID-19 using computer-aided drug discovery tools requires knowledge of the structure of coronavirus and various target proteins of the virus. These compounds should be further assessed in experimental assays and clinical trials to validate their actual activity against the disease. These findings may contribute to the drug design studies against COVID-19.

1. Introduction

The coronaviruses (CoVs) belong to genus Coronavirus, the family Coronaviridae, suborder Cornidovirineae, order Nidovirales and the realm Riboviri [1–3]. Cornidovirineae is subdivided into four genera based on their genetic and serologic features; *Alphacoronavirus* and *Betacoronavirus*, which infect mammalian species and humans, *Gammaporonavirus* and *Deltacoronavirus* that have a wider host range, including avian species [4–6].

The first human coronavirus was identified in the 1960s [7,8]. To date, seven coronaviruses were recognized that can infect human, among them CoVs, HCoVNL63, HCoV-229E, HCoV-OC43, and HCoV-HKU1 cause mild clinical symptoms, while SARS-CoV, MERS-CoV, and the newly identified SARS-CoV-2 could cause severe respiratory illness [9,10]. SARS-CoV originated from southern China in 2002, and MERS started in Saudi Arabia in 2012. SARS-CoV-2 was a novel strain of coronavirus that causes coronavirus disease. Due to its extremely infectious nature, COVID-19 first surfaced in Wuhan, China, around December 2019 and has since spread over the whole globe [11–13]. This illness was identified as a pandemic ailment in March 2020 [11]. All three CoVs, SARS-CoV1, MERS-CoV, and SARS-CoV-2 are spread among humans by respiratory droplets or “aerosols” and close

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symptoms of infection [26]. Respiratory difficulties, fatigue, and loss of smell and taste are major genome, is initiated the translation of two polyproteins (P1a/b), which is the biggest known (29.8 kb) RNA virus with 16 open reading frames. It is known as SARS-CoV-2, which has spikes that resemble a crown on the exterior. SARS-CoV-2 can cause disorders including gastrointestinal, liver injury, cardiac and neurological complications, and even stroke [21,22].

COVID-19 is further categorized by the severity of disease: mild, severe, and critical [23,24]. The majority of sufferers solely have moderate signs and get better in few days [23,25]. High fever, dry cough, respiratory difficulties, fatigue, and loss of smell and taste are major symptoms of infection [26].

The SARS-CoV-2 virus is an enzanced member of the big family of RNA viruses and has spikes that resemble a crown on the exterior. SARS-CoV-2 is the biggest known (29.8 kb) RNA virus with 16 open reading frames. It has a diameter of 120 nm, non-segmented linear single-stranded positive-sense genomic RNA that is 5' capped and 3' polyadenylated. The (-)-strand genome can directly work as a template for the expression of non-structural and structural proteins. SARS-CoV-2 genome expression of 5'-ORF replicates gene (ORFa/b), that covers two-thirds of the 5' genome, is initiated the translation of two polyproteins (P1a/b), which are hydrolyzed into 16 nonstructural proteins (NSPs). ORF1a encodes nsp1-11 and ORF1b encode nsp12-16. At 3'-end of the viral genome, there are regions encoding structural proteins including the spike protein (S), envelope (E), membrane (M), nucleocapsid (N), and accessory factors [10,27–32]. SARS-CoV-2 genomes have two important lineages (designated L and S), that are well described by two distinct SNPs that show almost complete linkage across the viral strains sequenced to date [33–35]. Researchers reported that L lineage was more common than the S lineage within the limited patient samples [10].

SARS-CoV-2 is a threat to human life and the economy. Researchers are trying to discover effective drugs to cure this epidemic. There are currently several vaccines approved by WHO, but these vaccines are not 100% safe. Scientists globally have suggested using pre-existing drugs (repurposing) against the novel SARS-CoV-2. However, the efficacy of these drugs is limited, and some of them have shown side effects. Previous studies showed that using bioinformatics strategy, plant-derived inhibitors, and antiviral agents can be used to discover potential new therapeutics, especially for viral diseases [36–39].

So far, some drugs have been introduced to treat COVID-19 disease, such as nafamostat [40], lopinavir/ritonavir [41] remdesivir [42,43], favipiravir [44], chloroquine [43,45], galidesivir [44], ribavirin [46] sofosbuvir [47], nitzoxanide [48], fedatrinib, and [49] baricitinib [42, 49].

Most of the drugs for SARS-COV-2 were confirmed by the FDA (Food and Drug Administration) to cure other viral diseases, but new research and clinical trials showed that some of them are ineffective to treat COVID-19 disorder. However, the disadvantage of using therapeutics initially designed for a specific target is the risk of undesired pharmacological effects and detrimental reactions. In many previous studies, effective inhibitors have been discovered based on the virus’s life cycle and integrating with various virtual screening methods [50,51].

The knowledge of the life cycle of virions in human cells has a high critical for controlling virus spread and drug design [52,53]. In COVID-19 life cycle, receptor-binding domain of the spike protein (S_{RBD}), main protease (M_{PRO}), papain-like cysteine protease (PL_{PRO}, NSP3), and RNA-dependent RNA polymerase (RD RP named nsp12) play a vital function in the virus pathogenesis [54]. These proteins are the potential targets for the design of antibody-blocking therapy, small molecule inhibitors, and antibody-mediated vaccinated targets for the treatment of COVID-19 [53,55].

This research is focused on the in silico discovery of small-molecule inhibitors against vital proteins involved in the life cycle of SARS-COV-2. Here, we used the structure-based or target-based virtual screening (SB or TBVS) [56] and molecular docking techniques [57,58]. Virtual Screening is a broadly used method at the initial step of drug discovery from potential drug compounds at high throughput [39,59,60]. In the current study, a large library of over 10^5 compounds were screened to discover novel inhibitors against the SARS-CoV-2 S_{RBD}, M_{PRO}, PL_{PRO}, and RdRP polymerase.

2. Material and method

2.1. Computer programs

The bioinformatics programs; such as Chimera 1.13 [61], Molegro Virtual Docker v 6.0 (MVD) [62] were used for protein target preparation/optimization and residue repair for virtual screening and molecular docking analyses, respectively. BIOVIA Discovery Studio Visualizer was used for 3D and 2D visualization of protein-ligand complexes after molecular docking. AutoDock Vina [63] implicated in PyRx 0.8 [64] and AutoDock tools 1.5.6 [65] were used for receptor-based virtual screening, optimizing ligands, and molecular docking calculation, respectively.

Besides, Open Babel [66] was used to convert structure formats. Online resources like PubChem database [67] and ChemDiv database (www.chemdiv.com), Protein Data Bank (PDB) [68], were used for data collection. Swissadme [69], and ADETab 2.0 [70] were used for ADME/T analysis.

2.2. Protein structure preparation for virtual screening

In virtual screening, we used viral proteins named M_{PRO}, PL_{PRO}, RdRP (non-structural or functional), and S_{RBD} (structural protein). These proteins play a key role in the life cycle of SARS-CoV-2 and provide potential targets for the discovery and development of antibodies, vaccines, and new drugs [55]. These proteins play an important role in invading, and entering host cells, proteolysis of polyproteins to produce a mature enzyme, RNA synthesis, or genome replication, and processing of the viral polyprotein, respectively. Also, they have a major role in the pathogenesis of SARS-CoV-2 [71].

At first, the crystal structures of S_{RBD} (PDB ID: 6M0J, 2.45 Å) [72], M_{PRO} (PDB ID: 7AMJ, 1.59 Å) [73], RdRP (PDB ID: 7BD3, 2.80 Å) [74] and PL_{PRO} (PDB ID: 6WX4, 1.66 Å) [75] were collected from PDB.

Using MVD, structures were improved or fixed. Using the MVD and AutoDock 4.2, all water molecules, ligands, superfluous chains, and irregular residues were eliminated, and only polar hydrogen atoms with Kollman charges were added to the protein. The final receptors were constructed, reduced using the AMBER force field in UCSF-Chimera (1.13), saved in PDB format, and imported molecules into the PyRx workspace. AutoDock Vina requires all of inputs in PDBQT format. The created PDBQT file consists of partial charges and atom types. Eventually, the virtual screening of the compounds against the elected chain of S_{RBD} (PDB ID: 6M0J, chain E), M_{PRO} (PDB ID: 7AMJ, Chain A), RdRP (PDB ID: 7BD3, Chain A), and PL_{PRO} (PDB ID: 6WX4, Chain A) was individually performed, using AutoDock Vina in PyRx 8.0 virtual screening tool (Fig. 1).

2.3. Ligand selection and preparation

Around 10^5 of drug-like chemical compounds were harvested from ChemDiv (headquartered in San Diego, CA the USA) database for virtual screening against four SAR-Cov-2 proteins to find the potential antiviral compounds. The compounds were imported into Open Babel program implemented in PyRx 0.8 tool for energy minimization, using Universal Force Field (UFF) [76]. Before performing the docking analysis on the selected ligands, the calculation of Gasteiger charges, the merged non-polar hydrogen, found aromatic carbon, identified rotatable bonds, and AutoDock MGL 1.5.6 tools was performed on the set of TORSDOF, automatically. Several molecules (collected from PubChem) referred to as the reference structure was used for comparison with our selected compounds database.
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of the receptors are presented in Table 1.

8. All of four 3D grids whose information was set to cover the active site of each protein Defined grid box information for four SARS-CoV-2 M

Table 1

| Protein      | Grid box size (Å³) | Binding sites               |
|--------------|--------------------|-----------------------------|
| $S_{RBD}$    | 23.60, 45.00, 21.88; -36, 30, 6 |
| $M_{pro}$   | 34.30, 29.80, 33.60; 95, 92, 100 |
| RdRp         | 34.30, 29.80, 33.60; 95, 92, 100 |
| PLpro        | 35, 29, 48; 5, ~30, ~50 |

2.4. Structure-based virtual screening (SBVS) and molecular docking protocol

Virtual screening and molecular docking analysis were performed on the ligands collected from the ChemDiv database against the active site of four different targets, SARS-CoV-2 to recognize the potential drugs for the treatment of COVID-19. At this step, crystal structures of targets and ligands prepared in the previous step were used for virtual screening. The second stage included creating 3D grid box parameters based on each protein’s binding site cavity using a preset exhaustiveness value of 8. All of four 3D grids whose information was set to cover the active site of the receptors are presented in Table 1.

Afterward, ChemDiv library virtual screening was performed against the selected receptors by PyRx software. AutoDock Vina generated eight various structures for any ligand, which are ranked by binding affinity (kcal/mol). The resulting ligands with a high potential for use as drug candidates were compared to the control molecules. Finally, docked complexes with high binding affinity values based on hydrogen and hydrophobic bond interaction in terms of binding residues, and bond lengths, for each protein binding pocket were analyzed using Discovery Studio Visualizer. Two-dimensional ligand-protein interaction diagrams were analyzed using the Discovery Studio Visualizer program.

2.5. ADME/T (absorption, distribution, metabolism, excretion, and toxicity) assays

Following the primary virtual screening, ligands with higher EB (Energy Binding) values ($\geq$ 8 kcal/mol) against the targets were selected for evaluation via in silico ADME/T analysis, to predict their pharmacokinetic analysis, drug-likeness, and toxicity studies. At first, we converted the SDF format of selected compounds obtained from the virtual screening of ChemDiv databases to canonical SMILES using Open Babel software. We examined ADME/Tox of hits, such as Lipinski’s rule of five [77], physicochemical property compounds; such as number of the ring (nRing), number of ratable bond (nRot) topological polar surface area (TPSA), water-solubility (LogS), and LogD7.4. The absorption properties, such as caco-2 permeability, P-glycoprotein inhibitor/substrate, human intestinal absorption (HIA), and F30% and distribution properties, such as blood-brain barrier penetration, were examined. Moreover, the metabolism properties; such as CYP 1A2/2C19/2C9/2D6/3A4 inhibitor/substrate and excetration properties including CL, and toxicity properties like hERG Blockers, human hepatotoxicity (H-HT), AMES Toxicity, Carcinogenicity and acute oral toxicity were investigated, too. All of Pharmacokinetic analyses of the selected compounds were performed and calculated, using SwissADME, and ADME/Tox 2.0 (https://admetmesh.scbdd.com/) online servers.

3. Results

SARS-CoV-2 proteins ($M_{pro}$, $PLpro$, RdRp, and $S_{RBD}$) that play a vital role in the SARS-CoV-2 life cycle were selected as drug targets. More than 100,000 compounds were screened to identify new inhibitors against these proteins. After virtual screening, compounds with high dock scoring (above −8 kcal/mol) were used for in silico ADME/T screening. ADMET screening of high-scoring compounds showed that twenty-five of the compounds exhibited pharmacokinetic properties against four key SARS-CoV-2 proteins, as listed in Table S1.

As shown in Table S1, all of selected ligands have higher EB compared to the reference drugs, including remdesivir/RdRp (~6.4 kcal/mol) and Methotrexate/$PLpro$ (~8.2 kcal/mol), Nebivolol/$S_{RBD}$ (~7.4 kcal/mol) and Lapatinib/$M_{pro}$ inhibitors (~7.8 kcal/mol) (Figs. 2–5. A, B, and C). Finally, the ligands that showed the best pharmacokinetic properties in terms of ADME/T, as well as the higher binding affinity against four SARS-CoV-2 proteins, were visualized for three- and two-dimensional protein-ligand interactions using Discovery Studio Visualizer. The interactions among four viral proteins and the successful ligands were obtained from ADME/T screening that are described in detail at the following sections.

3.1. Molecular docking analysis of $M_{pro}$ inhibitors

Main protease (3C-like protease or 3-chymotrypsin-like protease ($3C_{pro}$)), called nsp5, is a homodimer (promoter A and promoter B) which has three domains: domain I, domain II, and domain III. The active site is placed among domains II and I, which features the CYS 145 and HIS 41 (catalytic dyad residues) [52,54]. The $M_{pro}$ enzyme hydrolyzed polyprotein at eleven conserved sites and produced non-structural proteins that are vital in viral replication. Therefore, drugs that target this protease play an important role to prevent the virus from replicating [78–80].
Based on virtual screening and binding analysis, four potential ligand candidates showed a high affinity that inhibits the active site of SARS-CoV-2 Mpro, which may be identified as a candidate drug. EB score values of the top two hits against Mpro showed −9.0 and −9.2 kcal/mol in AutoDock Vina (Table S1c). V011-1641, a top-ranked ligand, was bonded to the Mpro with an AutoDock Vina docking score of −9.0 kcal/mol. It formed H-bonds with Gln189, pi-pi shaping interaction with His41, and pi-sulfur with Cys145 (two bonds) Mpro residues. Moreover, formed Alkyl and pi-Alkyl interaction with His41, Met49 (two bonds) and Met165, and carbon-hydrogen bond with Cys44, make it a promising Mpro inhibitor candidate.

In the case of compound V009-0308, it showed significant binding affinities towards Mpro with EB of −9.2 kcal/mol. This ligand formed pi-sulfur and pi-cation bonds with Cys145 (catalytic residue), unfavorable positive-positive interaction with catalytic residue, His41, pi-sigma with Thr 25, as well as Alkyl and pi-alkyl interaction with Leu27 and Pro168 residues, but did not form any H-bond (Fig. 2 A and B).

The binding affinity and docking analysis of selected compounds were compared to Rupintrivir, Mefloquine, Lapatinib, Fosaprepitant, Ritonavir, and Lopinavir as the reference molecule inhibitors. Among the reference inhibitors, Lapatinib had higher EB than the Mpro-Lapatinib with an EB of −7.8 kcal/mol, formed two H-bonds with residues Thr26, and Cys44. Also formed carbon-hydrogen bonds with Asn142 and Glu166, and wander walls bond with Gly143 and Met165. On the other, Lapatinib formed Amide-pi stacking and pi-pi stacking with His41 and Asn142, made pi-sulfur and halogen (Fluorine) interaction with Met49 and Glu166, respectively (as well as formed pi-alkyl interaction with Cys145) (Fig. 2C). Moreover, two top-ranked compounds showed higher EB and docking mechanisms to Mpro, compared to the reference prodrug Lapatinib.

### 3.2. Molecular docking analysis of PLpro inhibitors

Viral papain-like cysteine protease (PLpro, NSP3) is essential for SARS-CoV-2 replication and represents a promising target for developing antiviral drugs. One of the attractive targets for discovering antiviral drugs is the cysteine protease-papain-like protease (PLpro) [75]. Through in silico-based screening, six potential inhibitors showing better ADME/T properties towards SARS-CoV-2 PLpro were noted with EB ranging from −8.9 to −9.6 kcal/mol (Table S1b).

Two of the ligands (F477–0645 and V011-1348) showed the highest affinity to PLpro, with EB of −9.6 and −9.3 kcal/mol, respectively.
Complex analysis showed F477-0645 has H-bonds with Gly271 and formed hydrophobic interactions; such as Pi-Sigma with Tyr264, and Pi-Alkyl with residues Leu162 and Pro247, as well as Carbon–Hydrogen bond with residues Gln269 and Pro248.

V011-1348 is the second compound in the list of selected potential PLpro inhibitors with the highest EB, and it formed two H-bonds with Asp164 and Tyr264, pi-pi stacking interactions with Tyr264, as well as alkyl and pi-alkyl interaction with residues Pro248, Leu162 (two bonds), Cys111, and Tyr273 (Fig. 3. A and B). We compared EB and docking mechanism of selected molecules with Chloroquine, Hydroxychloroquine, Mefloquine, Itraconazole, Methotrexate, Pralatrexate, Galidesivir, as the reference molecule inhibitors. Methotrexate had a higher binding affinity among the reference inhibitors to the PLpro.

Methotrexate with an EB of $-8.2$ kcal formed seven H-bonds with residues Thr210, Prp247, Gln260, Tyr251, Lys254, Thr257, and Tyr305. Furthermore, it formed carbon-hydrogen bond with Leu253 and an unfavorable acceptor-acceptor bond with Ser212.

On the other side, Methotrexate formed pi-pi-T shaping bonding and pi-pi stacking with Tyr251 (three bonds) and pi-Alkyl interaction with Ala246, Ala249 (two bonds), Leu211. Moreover, selected molecules showed higher EB and docking scores to PLpro, compared with the reference prodrug Methotrexate. A schematic interaction of PLpro-Methotrexate complex is presented in Fig. 3C.

Fig. 3. 2D and 3D docking interactions of two selected compounds from the chemical library via in silico screening and Methotrexate reference inhibitor with SARS-CoV-2 PLpro (PDB ID: 6W4X). A. Papain-like cysteine protease (PLpro, NSP3) 6WX4-(F477-0645), (EB = $-9.6$ kcal/mol) B. 6WX4-(V011-1348), (EB = $-9.3$ kcal/mol) C. PLpro - Methotrexate (EB = $-8.2$ kcal/mol).
3.3. Molecular docking analysis of RdRp inhibitors

RNA-dependent RNA polymerase (RdRp, also named nsp12) is one of the most important proteins of SARS-CoV-2 that catalyzes the synthesis of viral RNA and plays a pivotal role in the replication and transcription cycle. RdRp is a multimeric protein [74,81,82]. RdRp has minimal activity on its own, but joining of nsp7 and nsp8 cofactors results in enhanced template binding and significantly increases RdRp’s potential to replicate long RNA [83]. The key residues of active site are near aspartates (Asp760, Asp761, and Asp618, which are involved in the actual reaction of RdRp enzyme). These aspartates are highly conserved residues [84,85].

Via virtual screening, eleven potential inhibitors showing high affinity for SARS-CoV-2 RdRp with EB ranging from −8.8 to −9.5 kcal/mol (Table S1d) were documented. Among the ligands that interacted with RdRp, we examined two of the ligands that had the highest EB. Ligand 1363-0007 was one of the compounds that had the highest binding affinity for RdRp with an EB of −9.0 kcal/mol. The complex analysis showed that 1363-0007 formed five conventional hydrogen bonds with Asp164, Asp452, Ala554, Thr556, and Lys621 formed pi-anion and cation interactions with Glu167, Lys798, Arg555, and Lys621 and Alkyl and pi-Alkyl interaction with residues Val166, Tyr455, and Asp624 as well as formed unfavorable donor-donor interaction with Cys622. F863-0769, one of the top hits for RdRp with EB of −9.2 kcal/mol, formed five H-bonds with Arg555, Lys621, Cyc622, Tyr619, and Asp623. Furthermore, it formed two pi-Anion interactions with Asp760 (catalytic residues) and Glu811, as well as a carbon-hydrogen bond with Asp618, and pi-Alkyl interaction with Cys622. Moreover, unfavorable acceptor-acceptor interactions were established with Asp761, another catalytic residue (Fig. 4A and B).

Although ligand C073-6209 had the highest EB with the target, but showed fewer pharmacokinetic properties than the 1363-0007 and 1363-0007 ligands (Table S1d).

We compared the EB and docking mechanisms of selected molecules with remdesivir, Ribavirin, Nebivolol, and Fludarabine as the reference inhibitors. Among the reference inhibitors, Remdesivir had the best binding affinity to the RdRp with EB of −6.4 kcal/mol. This compound formed eight conventional H-bonds with residues Lys551, Arg555, Pro620, Lys621, and Arg624 as well as formed unfavorable donor-donor interaction with Cys622. F863-0769, one of the top hits for RdRp with EB of −9.2 kcal/mol, formed five conventional H-bonds with Arg555, Lys621, Cyc622, Tyr619, and Asp623. Furthermore, it formed two pi-Anion interactions with Asp760 (catalytic residues) and Glu811, as well as a carbon-hydrogen bond with Asp618, and pi-Alkyl interaction with Cys622. Moreover, unfavorable acceptor-acceptor interactions were established with Asp761, another catalytic residue (Fig. 4A and B).

Although ligand C073-6209 had the highest EB with the target, but showed fewer pharmacokinetic properties than the 1363-0007 and 1363-0007 ligands (Table S1d).
to Remdesivir, the selected molecules had better EB and binding mechanisms. The interactions of RdRp- Remdesivir complexes are presented in Fig. 4. C.

3.4. Molecular docking analysis of S\textsubscript{RBD} inhibitors

Spike glycoprotein has a crucial function in virus pathogenesis via binding to angiotensin-converting enzyme 2 (ACE2) through RBD \cite{72}. ACE2 is expressed in lower respiratory tract cells \cite{1}. Furthermore, ACE2 receptors are largely expressed in the lung, intestine, heart, kidney, and alveolar epithelial type II cells \cite{86}. The S\textsubscript{RBD} is a homo-trimer that protrudes from the outer surface of the virion and has an important role to identify ACE2 receptor and entering the host cell. SARS-CoV-2 RBD includes two structural domains: the center (S2 subunit) and the outer (S1 RBD domain). RBD domain is used as a target for virtual screening, because this subunit is presented on the virus surface \cite{87}.

Via molecular-docking-based screening and ADME/T properties, four compounds were identified to exhibit favorable pharmaceutical properties (Table S1a). Among these compounds, 8008-2501 and K279-0710 were identified to show remarkable binding to the RBD domain of spike protein with EB of \(-9.0\) and \(-8.8\) kcal/mol, respectively. Compound 8008-2501, showed an H-bond interaction with Arg403. Besides, exhibited pi-pi-T stacking and pi-pi-T shaping bonding with Phe456, Tyr505 (two bonds), pi-sigma interaction with Tyr489, Leu455, and it formed a pi-donor hydrogen bond with Gly496 and pi-Alkyl interaction with Phe456.

K279-0710, which is one of the top hits for RBD with EB of \(-8.8\) kcal/mol, formed three H-bonds with the residues Asn501, Tyr449, and Ser494. Other interactions of k279-0710 include pi-pi stacking with Tyr505 and Pi-sigma, Pi-sulfur and Pi-Alkyl with Tyr449 and Pi-donor H-bond with Ser494 and Gly496 (Fig. 5 A and B).

To compare the EB of selected compounds with the reference molecules, first, the SDF format of the reference compounds; Celecoxib, Hydroxychloroquine, Mefloquine, and Nebivolol were collected from PubChem database. Docking analysis of reference compounds against S\textsubscript{RBD} SARS-CoV-2 proteins was performed using AutoDock Vina docking tools. The results of the docking analysis showed that Nebivolol as a reference drug had the best EB (\(-7.4\) kcal/mol) with S\textsubscript{RBD}. Therefore, 8008-2501 and k279-0710 showed the best ADMET properties, and higher and more promising EB compared to the Nebivolol, suggesting that they could be the potential inhibitors of S\textsubscript{RBD}.

Nebivolol formed six H-bonds with Tyr453, Gly496 (two H-bonds), Gln498 (two H-bonds), and Asn501, formed Pi-Donor Hydrogen Bond with Gly496, and Pi-Pi stacking and Pi-Alkyl interaction with Tyr505. A schematic interaction of the S\textsubscript{RBD} Nebivolol complexes are presented in...
In the last decade, the advent of bioinformatics and in silico approaches have revolutionized the modern medicine in many aspects. These methods have given rise to several biological assay simulations, which provide predictions that assist to shorten the time and expense needed to conduct these tests in laboratory conditions. Some of the applications of these in silico assays include the prediction of biological structures, molecular docking for predicting receptor and ligand interactions, and designing new therapeutics and vaccines against various pathogens [88–90]. These methods have shown to be useful in practice, when they were used along with laboratory studies, such as those used for cancer drug development [91,92]. The pandemic of COVID-19 has evolved into a global crisis. Scientists have been searching for proper drugs to cure this disorder, and there is an urgent need to recognize effective drugs with lower side effects to fight against SARS-CoV-2. In regards to COVID-19 condition, in silico methods have come helpful in various aspects, including SARS-CoV-2 structure predictions and phylogenetic analysis [93], drug’s virtual screening, natural source-derived chemicals against the virus, and SARS-CoV-2 vaccine design (for more information please refer to the review article by Moradi et al. published earlier in this journal [94]). Moreover, these methods have helped study the efficacy of some natural peptides and even designing them to target different structures of this pathogen. For example, a study by Moradi et al. studied the Inhibiting Papain-like Protease from SARS-CoV-2 by Using Plant-Derived Peptides [95]. In silico methods were useful to design new therapeutics, such as novel aptamers against this virus [96].

Virtual screening is a promising computational tool in drug discovery and identifying possible drug candidates which was used in the time of COVID-19 crisis [97]. In drug discovery and development programs, comprehending the pathogenesis of the disease is a matter of importance. Prevention of infection and spread of SARS-CoV-2 begins when we can block some of the virus’s vital proteins like SRRD, Mpro, RdRp, and PLpro with the potential drug.

SRRD is a homo-trimer protruding from the exterior cover of virus and functions as the main driving force for host cell recognition and entrance. Thus, inhibiting the interaction between the SARS-CoV-2 SRRD and the host cell receptor ECA2 offers the possibility of decreasing the rate of viral infection [72]. Following attachment through SRRD, endocytosis, and uncoating of the virus ensue inside the host cell. The uncoated viral RNA is therefore used as a template to directly translate polyprotein 1a/1b (pp1a/pp1ab) that their role is to encode nsp 1–16 and then PLpro (nsp3) and Mpro (nsp5) proteases, cleave and activate other nsp’s; such as nsp12. Nsp12-RdRp is important in viral replication [98].

Hence, we conducted molecular docking and virtual screening of ligands downloaded from the ChemDiv against four proteins of SARS-CoV-2, using the AutoDock Vina tool. As a result of the virtual screening, we selected twenty-five ligands that had good pharmacokinetic properties and passed ADMET filter, and had the best EB, identified by molecular docking studies as potential inhibitors against the four SARS-CoV-2 targets. After ADME/Tox analysis, the major parameters for ADME/T associated with several elected compounds showed more favorable results, which are listed in Table 5 (a, b, c, and d). Eventually, three- and twodimensional protein-ligand interactions of the top two compounds showed the best pharmacokinetic properties due to ADME/T, as well as the higher binding affinity (most negative Gibbs ‘free energy of binding) against four SARS-CoV-2 proteins.

Our study suggested that candidate ligands V011–1941 and V009-0308/Mpro, F477–0645 and V011-1348/PLpro, 8008-2501, and K279-0710/SRRD, and 1363-0007 and F863-0769/RdRp as the best inhibitors. These eight ligands showed the highest binding affinities to PLpro, Mpro, RdRp, and SRRD and can be considered as potential candidate drugs to further clinical studies. The eight ligands selected in our study showed higher EB, compared to Lapatinib/Mpro, Remdesivir/RdRp, Methotrexate/PLpro, and Nebivolol/SRRD as the control ligands (see above). We plotted the interaction of our suggested potential drugs with SARS-CoV-2 proteins using Discovery studio visualizer to further assist in choosing drugs.

Pharmacokinetics parameters of selected compounds: (F477-0645 & V011-1348)-PLpro, (V011-1641 & V009-0308) - Mpro, (1363-0007 & F863-0769)-RdRp, and (8008-2501 & K279-0710)-SRRD were predicted via the elucidation of their ADME/T profiles via in silico studies. Notably, the selected ligands showed high drug-likeness, as per toxicity prediction. The selected ligands did not show any form of toxicity.

All of the studied compounds are non-inhibitors of CYP1A2 and CYP2C19, except ligand V009-0308/Mpro, which inhibits the structure, while K279–0710, F477–0645, and F863-0769 are non-substrates for all CYP. 8008-2501/SRRD and K279-0710/SRRD are inhibitors of CYP2C9, CYP3A4, and 8008-2501/SRRD only substrates for CYP2C9. F477–0645/PLpro are the non-substrates and non-inhibitors of all the CYP450, while V011-1348/PLpro is non-inhibitor of all the CYP450 substrates for CYP2A219 and CYP3A4. V009-0308/Mpro, an inhibitor of CYP1A2 and CYP3A4 and substrate for CYP2D6 and CYP3A4, while V011-1641/Mpro is the only inhibitor of CYP2D6 and substrate for CYP2C19 and CYP3A4. 1363-0007/RdRp inhibited CYP3A4, and is a substrate for CYP2C9, while, F863-0769/RdRp is an inhibitor for CYP2D6 and non-substrates of all the CYP450.

All studied compounds satisfied Lipinski’s rule except for 8004-8704/SPRRD, while it had the best interactions with SRRD. Our results showed that all selected ligands showed good ADMET properties. We believe that computational docking analysis has its limits and additional laboratory and clinical investigations are required to verify the inhibitory effects of these candidates against SARS-CoV-2, as the potential drugs for COVID-19.

5. Conclusion

In this in silico study, SBVS workflow of over 100,000 ligands were screened against four key proteins; SARS-CoV-2 Mpro, SRRD, RdRp, and PLpro. Candidate ligands V011–1641 and V009-0308/Mpro, F477–0645 and V011-1348/PLpro, 8008-2501, and K279-0710/SRRD, and 1363-0007 and F863-0769/RdRp were suggested as the potential inhibitor agents for the four key protein in SARS-CoV-2. These ligands with the highest EB as the potential inhibitors against SARS-CoV-2 targets could be considered for further experimental investigation and clinical studies.

Ethics approval

NA.

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Consent to participate

All authors have seen the manuscript and approved to submit the manuscript.

Consent to publish

All authors consent to the publication of the manuscript.

Availability of data and material

The data sets used and/or analyzed during the current study are available from the corresponding author on reasonable request.
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