Selection of active antiviral compounds against Covid-19 disease targeting coronavirus endoribonuclease NendoU/NSP15 via ligand-based virtual screening and molecular docking

Gaurav Joshi,[†] Ramarao Poduri*

Department of Pharmaceutical Sciences and Natural Products, Central University of Punjab, Bathinda, India 151001

*Corresponding Author: ramaraop@yahoo.com, Ph/Fax: 0164-2430586

[†]Co-corresponding Author: garvpharma29@gmail.com

Abstract

SARS-CoV-2 is the seventh coronavirus reported to cause infection in Homo sapiens. Considering its pandemic nature, development of newer and effective therapeutic strategies, drug repurposing in combination with target validation approaches has led to the identification of new antiviral molecules. In current work, we focused on the selection of a library of molecules that includes FDA approved drugs and investigational or experimental drugs. The idea behind drug selection includes repurposing of already available drugs to be made use in the development of medicines for deadly Covid-19 in a short period. We performed virtual screening using 8548 ligands on target protein endoribonuclease NendoU (NSP15) (PDB ID: 6VWW). Virtual screening directed us to identify four drugs that may be repurposed for treatment in Covid-19 disease. The drugs include DB00876 (Eprosartan) (FDA approved), Investigational drugs DB15063 (Inarigivir soproxil), DB12307 (Foretinib), and DB01813 an experimental drug.

Keywords: SARS-CoV-2; Antiviral Agents; Drug Repurposing; Virtual Screening, Molecular Modelling.

1. Introduction

Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) or coronavirus disease or Covid-19[1] was identified first in December 2019. it is seventh virus strain reported to cause infection in *Homo sapiens*.[2] Genomic studies on SARS-CoV-2 confers the virus has not been constructed or manipulated in the laboratory due to the presence of receptor-binding domain (spike protein) and a polybasic cleavage site.[3] The disease was first identified and confined to China and has rapidly spread to more than 213 countries. The disease was categorized to be pandemic by the World Health Organization (WHO) on March 11, 2020. The virus has infected over 28,10,325 people to date and led to mortality of 1,93,825 worldwide as per WHO report updated on April 27, 2020 (https://www.who.int/emergencies/diseases/novel-coronavirus-2019). The fast spread of this disease has caused havoc and panic among individuals, which further worsen with the unavailability of vaccines or some proven drug regime.[4] Various drugs and treatment options were identified with its outbreak, which is still tested for its efficacy against Covid-19 disease. The treatment options under trials include small molecule inhibitors, remdesivir (Gilead Sciences (Phase 3)), and Favilavir (approved in China); Hydrochloroquine. Drug combinations, hydroxychloroquine, and azithromycin. Vaccines that include TJM2, mRNA-1273, engineered RNA vaccine (Arcturus Therapeutics), mRNA vaccine (BioNTech), and human recombinant protein AT-100 (rhSP-D) (Airway therapeutics).[5] Despite controlling viral pathogenesis in the past, re-emerging or new viruses is deprived of specific treatment due to the under-development of newer and effective therapeutic strategies. [6] [7] Currently, drug repurposing has led to the identification of new antiviral molecules and, importantly, newer targets for therapeutic development.[8] Besides, the discovery of antiviral compounds drug repurposing has benefitted in numerous ailments, and successful examples include: Zidovudine (from cancer to HIV AIDS), Minoxidil (from hypertension to hair loss), Sildenafil (from angina to erectile dysfunction), Thalidomide (from morning sickness to leprosy and multiple myeloma), Ketoconazole (from fungal infections to Cushing syndrome); Aspirin (from analgesia to colorectal cancer) and many more.[9]

The current work involves the identification of putative antiviral drug specifically for Covid-19 using computational techniques, involving Computer-assisted drug designing (CADD).[10] CADD has emerged as one of the powerful tools for drug discovery in the recent past.[11] Among various CADD techniques structure-based virtual screening (VS) is an underlying computational technique for high-throughput screening (HTS), that reduces the ligands in larger database or libraries in a quest to identify those selected chemical structures having the highest possibility to bind to a selected drug target.[12]

In the current research, we focused on the crystal structure of NSP15 Endoribonuclease from SARS CoV-2 (6VWW) as a drug target.[13] NSP15 is a mysterious or enigmatic RNA possessing RNA uridylate-specific endoribonuclease (NendoU).[14] Structurally, NSP15 comprise of a C-terminal catalytic domain that catalyzes RNA processing leading to the formation of 2'-3' cyclic phosphodiester and termini 5'-hydroxyl.[14] RNA uridylate-specific endoribonuclease is widespread, including viruses and eukaryotic cells.[15] With their first discovery in *Xenopus laevis* (XendoU), they were associated with maturation of intron-encoded small nucleolar RNAs. Their expansion was tracked in drosophila (DendoU) where they play a pivotal role in their nervous system development, Humans (HendoU) associated with placental development. They are also reported in prokaryotes where they lack uridylate-specific endoribonuclease activity.[16] In viruses (NendoU), these proteins are specifically conserved in coronaviruses, arteriviruses, and toroviruses.[16-17] In recent studies, NSP15 was found to affect innate immune response leading to virus attacks on protein interference, which is devoid of endonuclease activities.[18]

Coronavirus produces minus-strand sub-genomic RNAs (sgRNAs) from subgenomic mRNA(sg mRNA) via discontinuous transcription mechanism.[19] The coronavirus specific genome encodes numerous proteins that are not typically found in positive-strand RNA viruses, including NendoU catalyzing the formation of the negative strand.[20] The NendoU is thought to act by cleaving single and double-stranded RNA catalyzed by Mn^{2+} dependent manner.[14]

The SARS-CoV-2 NendoU oligomer shares higher similarity with those of SARS-CoV, H-CoV-229E, and MERS-CoV enzymes, though it still may display different catalytic properties and potentially altered substrate specificity. The structural comparisons made by Youngchang *et al.*, however, advise that inhibitors of SARS-CoV Nsp15 might have a good chance to inhibit also the SARS-CoV-2 homolog, but inhibitors of MERS-CoV NendoU are unlikely to inhibit the enzyme.[13, 19]

To prove this hypothesis and explore other potential other ligands that may target SARS-CoV-2 NSP15, we performed VS on the protein (6VWW)[13] using 8548 ligands. The study suggested that out of 8548 ligands, four ligands exhibited better affinity (Glide-SP and Glide-XP) with target protein (PDB ID:6VWW)[13] and thus develop a plausibility of possible drug that may be repurposed and tested against Covid-19 in future.

2. Material and Methods

2.1. Selection of Ligands and preparation

The ligands were selected (downloaded or drawn using ChemBio draw), based upon current trends in search for antiviral agents against Covid-19 disease. We importantly focused on

repurposing of putative ligand for Covid-19 disease from an array of all the FDA approved drugs to date. Besides this, we also included investigational or experimental drugs belonging to the anticancer, antiviral, antimicrobial, antimalarial, and antifungal class.

A total of 8548 chemical structures were downloaded from Phase database (Schrödinger, release 2020-1)[21] and drawn using ChemBio draw software. The structures were converted into .sdf format and were further refined using LigPrep.[22]

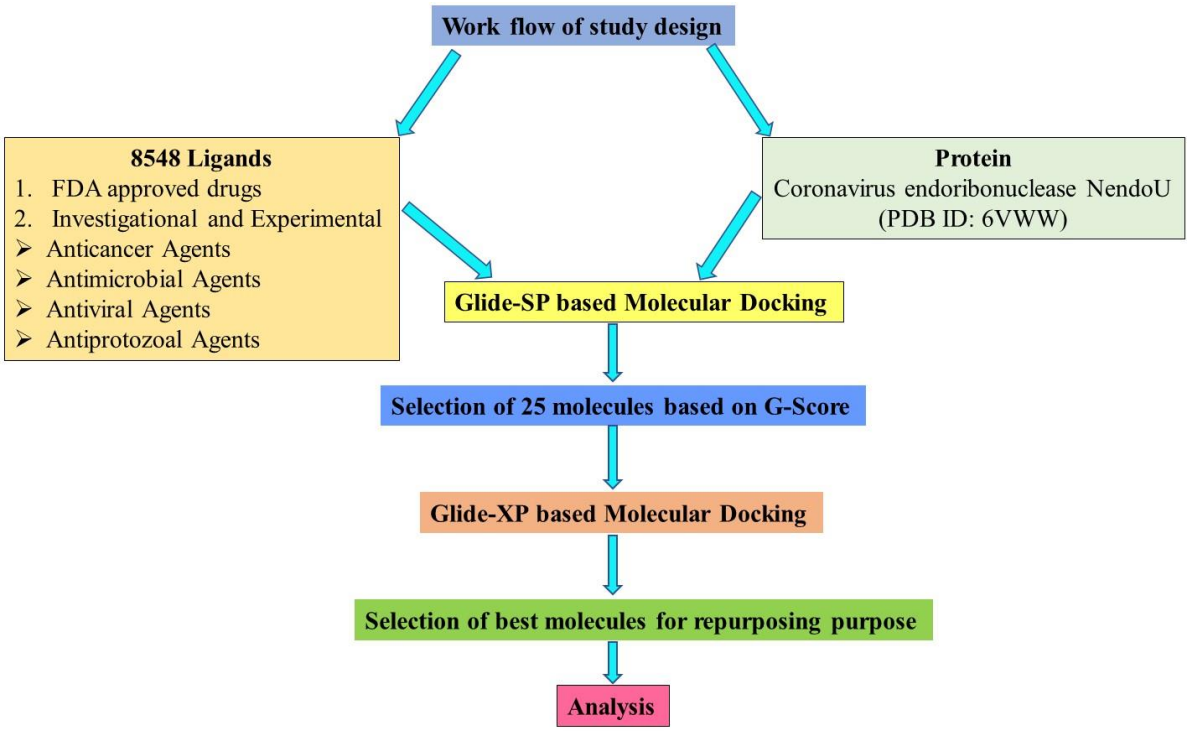
2.2. Protein selection and preparation

The X-ray three-dimensional crystal structure of NSP15 Endoribonuclease from SARS CoV-2 (PDB ID: 6VWW)[13] were retrieved from the Protein Data Bank. The protein was classified to be viral protein (analyzed via X-ray diffraction technique with a resolution of 2.2 Å) expressed in Escherichia coli BL21(DE3) and deposited to the repository on 2020-02-20 and released on 2020-03-04. The protein consisted of 1056 amino acid residues and having two chains (homo-dimer). The protein was further refined for missing sidechains and loops using protein preparation wizard[23] of Schrodinger software (release 2020-1), finally followed with the optimization and minimization. Next, the protein was refined to identify the possible binding sites within its proximity. The Site Map wizard of Schrodinger software (release 2020-1) assisted in the identification of five binding sites in the protein. The binding sites were redefined in the term of energy calculation and one having best site score was selected for molecular docking (Glide)[24] of the identified 8548 ligands.

2.3. GLIDE (Grid-based Ligand Docking with Energetics) and molecular docking

GLIDE is a molecular docking module present in Schrodinger software.[24] Molecular docking was performed by using GLIDE, and outputs were represented as the G score. We initially used the GLIDE-SP module for the filtering of selected ligands using Maestro module Schrodinger software[25] (release 2020-1) molecular docking. For the selection of putative leads, we docked selected compounds into the prepared protein (most favorable binding site with receptor grid generation), and the docking conformation possessing the lowest energy were identified (25 molecules) and selected for further refinement using GLIDE-XP module.[26]

In brief, the work was performed based on workflow depicted in Scheme 1.



Scheme 1. The workflow of study design

3. Result and Discussion

3.1. Molecular docking of compounds with coronavirus endoribonuclease NendoU

A total of 8548 drugs were selected. This includes FDA approved and investigational or experimental drugs of anticancer, antiviral, antimicrobial, and antiprotozoal origin. All three categories. The idea behind drug selection includes repurposing[27] of already available drugs to be made use in the development of medicines for deadly Covid-19 in a short period.[28] Due to the sudden outbreak of viral diseases in recent decades and including the Covid-19, controlling these pathogens and infection still is deprived of specific and efficacious treatment. The past decade has witnessed only 12 new antiviral drugs approved by the FDA (8 against hepatitis C virus (2 in combinations for HIV). Thus, it becomes utmost important to propose drugs for new and re-emerging viruses that possess extreme potential for pandemic with higher morbidity.[28] For repurposing we took into account following considerations[28]: a) for anticancer agents, we considered antimetabolite nature of anticancer agents that affect the central dogma of molecular biology along with molecules affecting chromatin molecules, and those targeting the common signaling pathways for both cancer and viral diseases[29]; b) for antimicrobial agents we considered the effect of these molecules on folate synthesis, protein synthesis, the impact on topoisomerases/gyrases, antifungal antibiotics, and anti-helminthic mechanism similar to viral protein[29c]; c) for antiprotozoal agents we considered host-parasite interaction similar to host-virus inter effected by antiprotozoal agents.[30]

The selected ligands were docked into the energetically favorable binding site of NSP15 Endoribonuclease (PDB ID: 6VWW). The favorable binding sites were predicted using sitemap module[31], which suggested five active binding sites (**Figure 1**). Four out of five binding sites were found to be located in the peripheral region of the protein and were energetically less favorable. Site 1, with a site score of 0.978815, was identified (**Table 1**). Initially, 8548 molecules were docked using the GLIDE-SP (standard precision) algorithm, which led to the identification of 25 ligands based on the G score (**see Table 2**). The selected 25 molecules were then re-docked using GLIDE-XP. The crucial underlying difference between Glide SP and XP includes that the former performs thorough sampling requiring roughly 10 sec/compound and allows a balance between speed and accuracy. The Glide XP uses an anchor-and-grow sampling method in which compounds are docked at a rate of approximately 2 min/compound. This leads to higher precision and accuracy.[32]

The analysis of results suggested that among 25 ligands, four ligands with drug bank identification number (DB) (DB15063; DB01813; DB12307; DB00876) were able to bind and exhibit better affinity toward the target protein. Among the four ligands determined, two drugs (DB15063 and DB12307) were found to be investigational, one experimental (DB01813), and one FDA approved (DB00876). Molecular docking result of investigational drugs (DB15063 and DB12307) revealed (**Table 3**; **Figure 2**) the G score of -8.381 and -8.386 Kcal/mol, respectively. Protein-ligands interactions pattern revealed amino acid Thr275, Asn75, Lys 181, and Leu346 in DB15063 and amino acids Ile97, Thr49, Arg91, Ile270, Thr326, Ala95 in DB12307 were involved in a hydrogen bond, pi-cation interactions, and pi-pi interactions. Docking results of experimental drug (DB01813) revealed Gscore of -8.756 Kcal/mol, with Asn75, Asn289, Val276, Leu346, Gln347, Lys277 amino acids participated in significant interactions with ligand. Finally, the docking result of FDA approved drug (DB00876), suggested dock score of -8.05 Kcal/mol with amino acids Asn75, Thr326, Lys345, Lys277, Ile270, Met272, and Asn74 led to significant interaction at the active binding site.

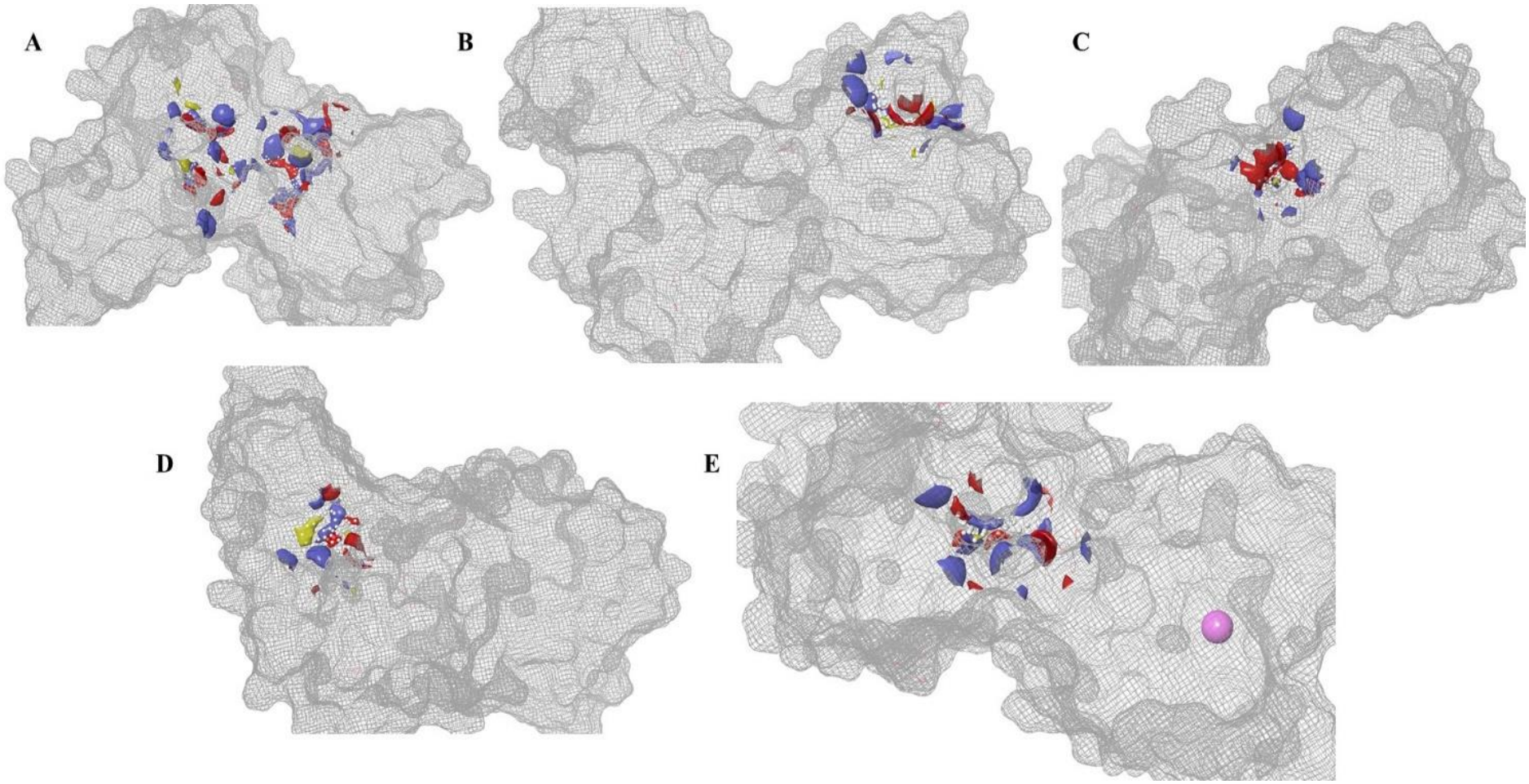


Figure 1. Site map suggesting the five most favorable drug-binding sites; **A.** Site 1; **B.** Site 2; **C.** Site 3; **D.** Site 4; **E.** Site 5. Significance of the different color: Red color: h-Bond acceptor region; Blue color: h-Bond donor region; Yellow color: hydrophobic-hydrophobic interactions region

Table 1. Order of the best to worst site predicted using the Sitemap module considering the described parameters.

Site	SiteScore	size	Dscore	volume	exposure	enclosure	contact	hydrophobic	hydrophilic	balance	donor/acceptor
site_1	0.978815	117	0.988021	368.039	0.602041	0.66632	0.889831	0.409183	1.079054	0.379205	0.596493
site_4	0.74693	39	0.733729	143.717	0.774566	0.642518	0.721278	0.397341	0.732552	0.542406	0.569914
site_2	0.68386	40	0.65073	105.644	0.663866	0.593007	0.890438	0.318832	0.918965	0.346947	1.707859
site_3	0.665956	35	0.539749	95.354	0.639175	0.646245	0.851235	0.09455	1.239601	0.076274	0.412938
site_5	0.632924	33	0.484638	72.03	0.663265	0.615657	0.948606	0.147129	1.3029	0.112924	0.906767

Table 2. Lowest binding energy (G-score) for top 25 ligands out of 8548 ligands identified using Glide-SP

Sr. No.	Drug Bank ID	Molecular Weight	G-score	glide lipo	glide hbond	glide evdw
1.	DB01813	379.28	-8.756	-1.938	-0.602	-33.204
2.	DB03160	418.379	-8.755	-1.801	-0.859	-36.088
3.	DB08322	456.399	-8.721	-2.276	-1.081	-38.076
4.	DB04099	665.418	-8.587	-1.623	-0.16	-44.196
5.	DB02540	482.443	-8.513	-1.371	-0.838	-41.289
6.	DB13027	272.256	-8.493	-1.945	-0.576	-31.378
7.	DB02237	666.579	-8.49	-1.521	-0.64	-30.828
8.	DB04071	663.425	-8.412	-1.021	-0.688	-41.285
9.	DB12307	632.654	-8.386	-3.429	-0.54	-53.209
10.	DB15063	703.62	-8.381	-1.544	-0.868	-46.781
11.	DB08432	338.274	-8.309	-0.851	-1.138	-24.737
12.	DB09219	333.343	-8.305	-2.69	-0.904	-35.771
13.	DB02549	444.225	-8.285	-1.294	-0.581	-35.836
14.	DB13616	429.513	-8.276	-2.045	-1.28	-34.434
15.	DB01643	322.209	-8.275	-0.852	-1.139	-23.514
16.	DB07927	343.332	-8.262	-1.748	-0.37	-33.559
17.	DB03276	688.75	-8.241	-2.045	-0.442	-35.45
18.	DB13755	1253.871	-8.237	-1.501	-0.622	-51.05
19.	DB13490	652.602	-8.168	-2.419	-0.32	-38.899
20.	DB08434	315.216	-8.133	-1.658	-0.32	-28.433
21.	DB04264	482.443	-8.089	-1.892	-0.745	-37.287
22.	DB01690	756.407	-8.086	-0.883	-0.143	-40.186
23.	DB09134	807.115	-8.069	-1.503	-0.96	-39.197
24.	DB00876	424.513	-8.05	-2.696	-0.306	-40.308
25.	DB01830	665.608	-8.043	-1.406	-0.64	-42.71

G-score: *Glide Score (Kcal/mol);* ***glide lipo:*** *Lipophilic term derived from hydrophobic grid potential;* ***glide hbond:*** *Hydrophilic term;* ***glide evdw:*** *protein-ligand steric contact information.*

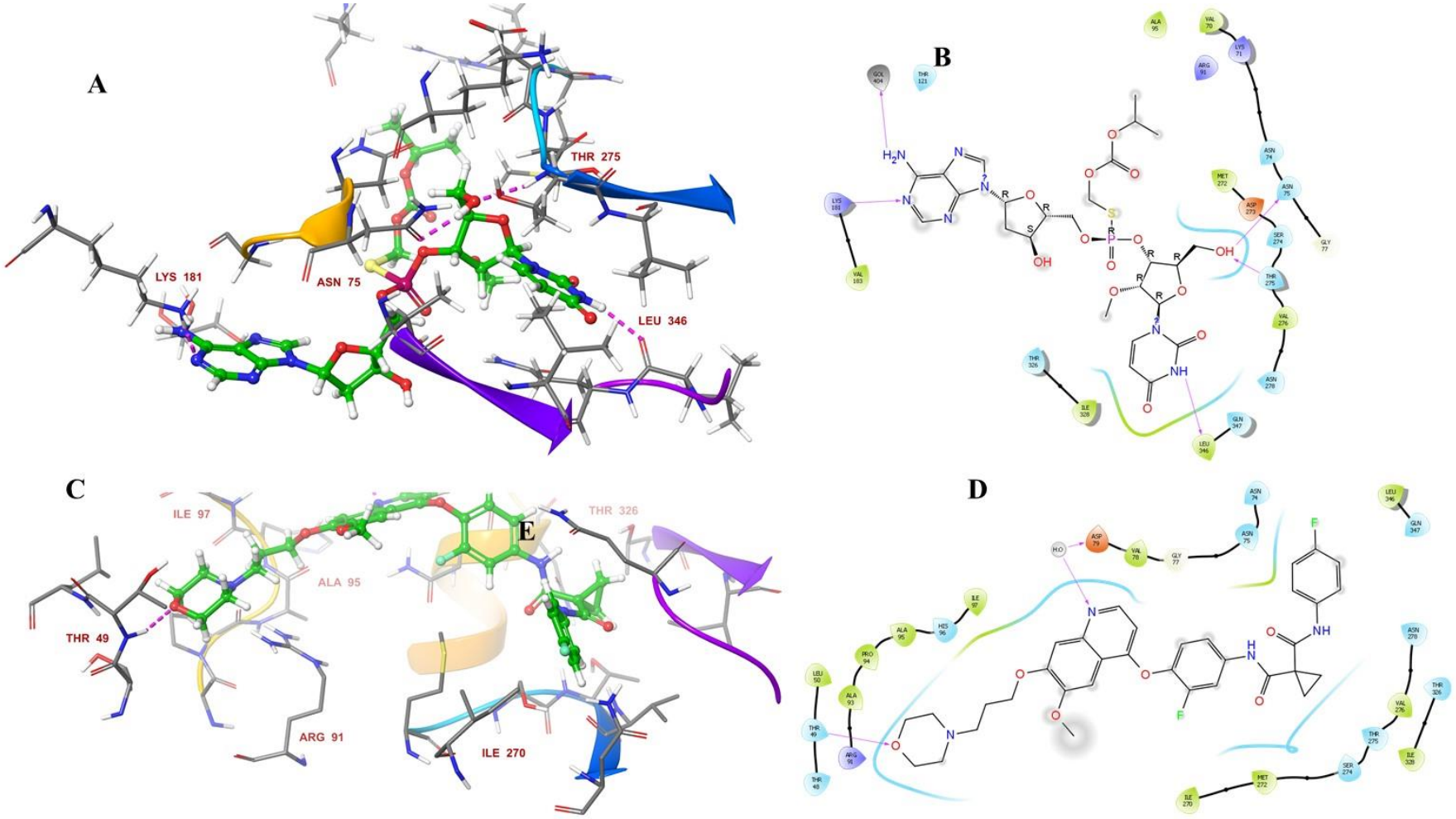


Figure 2. Ligand-protein interaction diagrams of the selected ligand within the active cavity of protein 6VWW. **A.** 3D interaction representation of DB15063; **B.** 2D interaction representation of DB15063; **A.** 3D interaction representation of DB12307; B. 2D interaction representation of and DB12307

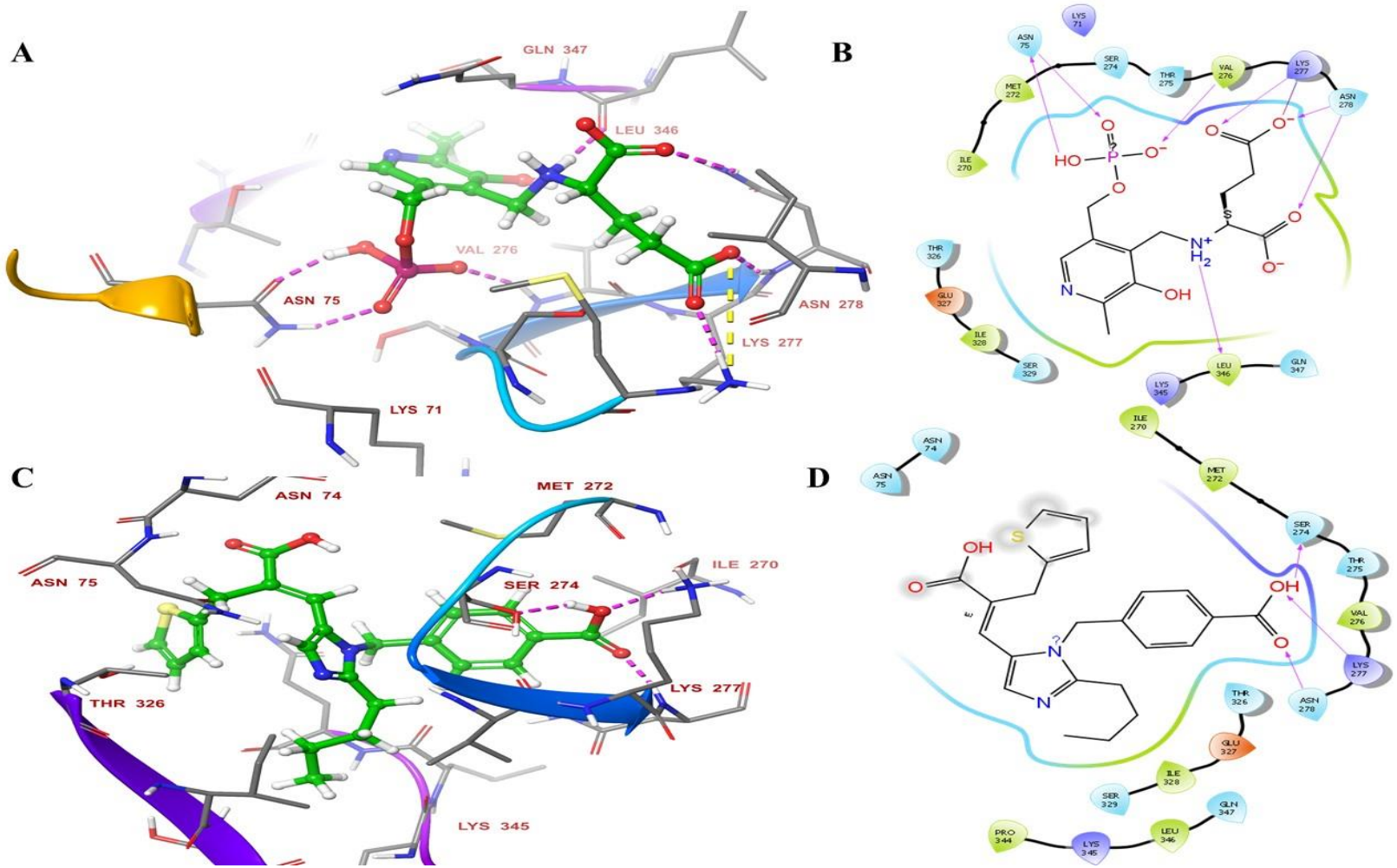


Figure 3. Ligand-protein interaction diagrams of the selected ligand within the activity cavity of protein 6VWW. **A.** 3D interaction representation of DB01813; **B.** 2D interaction representation of DB01813; **A.** 3D interaction representation of DB00876; B. 2D interaction representation of and DB00876

3.2. **Repurposing of the drug candidates and plausible mechanism involved**

The thorough investigation of selected ligands suggested (Figure 4) that FDA approved drug DB00876 (Eprosartan) is an Angiotensin (AT)-II inhibitor.[33] Investigational drugs DB15063 (Inarigivir soproxil) is indicated for antiviral activity against Chronic Hepatitis B (phase trial-II).[34] DB12307 (Foretinib) is investigated for cancer (phase trial-II).[35] Whereas, DB01813 is an experimental drug.

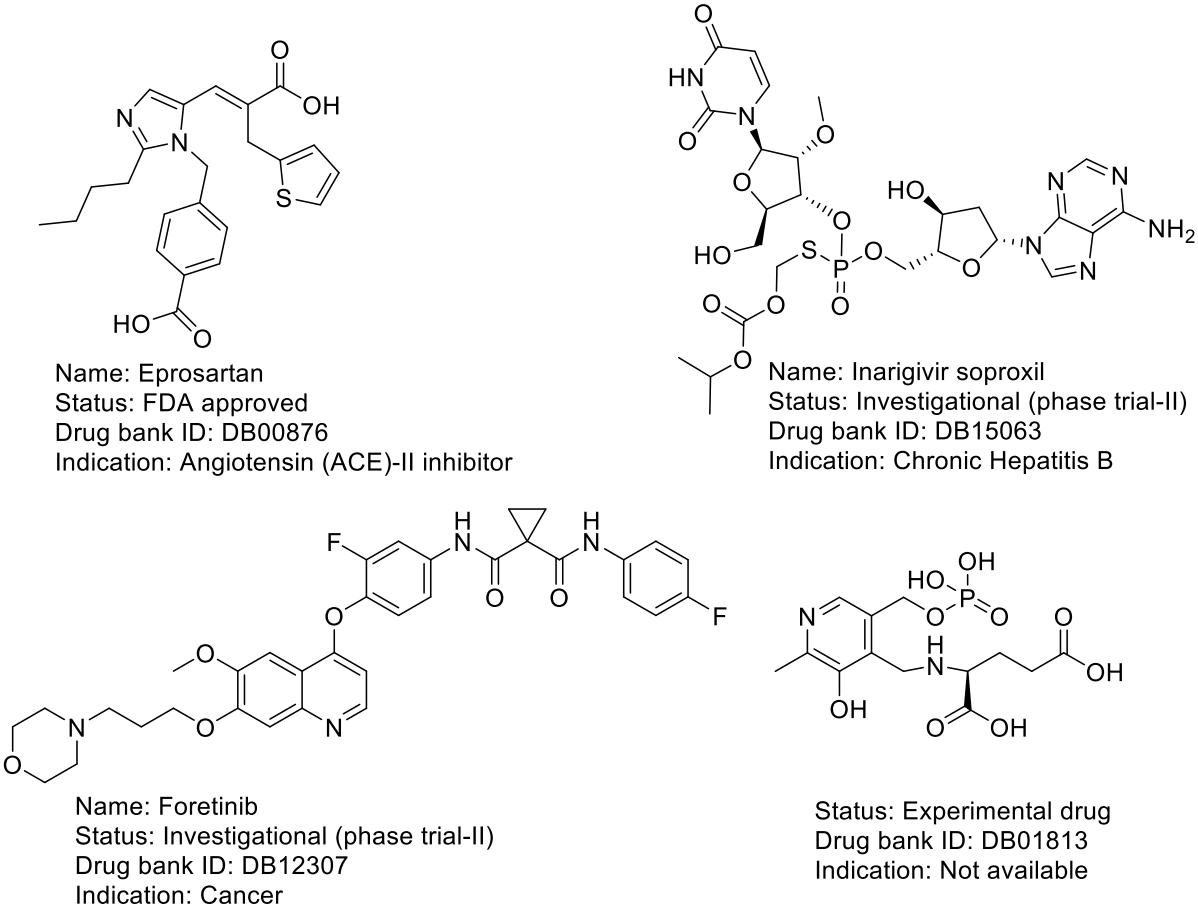


Figure 4. Selected ligands having the best affinity with target protein after applying Glide-XP

After the outbreak of SARS-CoV-2, Angiotensin Converting Enzymes (ACE 1 and 2) proteins have been correlated with coronavirus infection.[36] It was observed that ACE2 protein consists of binding domain favourable for spike protein of SARS-CoV-2. All the epithelial cell, blood vessels along with lung and intestine possesses ACE2 protein and thus blocking ACE2 for treating SARS-CoV-2 becomes an attractive drug target. The current high morbidity in the patient with SARS-CoV-2 is noted with cases suffering from diabetes or hypertension.[1] Both types of patients are treated with ACE-1 inhibitors or angiotensin-receptor antagonists. This leads to overexpression of ACE2, making the more favourable situation for the virus to invade the body system.[36-37] However, considering the major side effects of ACE inhibitors, which

include mild to chronic cough[38], which is the major pathway for viral transmission[39], the selection of Angiotensin (AT)-II inhibitor Eprosartan may be recommended.[40] Importantly, the spike (S) glycoprotein of SARS-CoV-2 consists of a furin protease site required for the entry of virus particles into the cell.[41] The S protein includes two functional domains that include a receptor binding and a fusion mediating domain, which importantly interacts with ACE2, as evidenced so far.[42] There is growing recognition that barrier integrity of the epithelial cell and endothelial dysfunction plays a vital role in bacterial sepsis and acute lung injury.[43] In past influenza, Hantavirus, pulmonary syndrome, led to the epithelial cell and endothelial dysfunction. Statins and angiotensin receptor blockers have been effectively used in the past for the treatment of mentioned viral diseases. A combination of statin and angiotensin receptor blockers dramatically reduced mortality during the recent Ebola outbreak. Other than treating heart diseases and hypertension, these drugs are known to possess broad anti-inflammatory and immunomodulatory activities that could additionally complement in diseases including Covid-19.[44]

The second drug, Inarigivir, is an investigational drug for Chronic Hepatitis B (phase trial-II). It possesses immune-modulatory activity and enhances host innate antiviral response.[45] Mechanistically, inarigivir binds to the cytosolic site of recognition receptor, which is capable of recognizing molecules found in pathogens. Recognition receptor then induces the RIG-1 gene (retinoic-acid-inducible gene), which activates transcription factors, including IFN regulatory factor 3 (IFR3) and nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB).[46] These actions directly induce antiviral gene products and lead to the production of type I and III interferons maintaining an antiviral state.[47] Thus, it is therefore presumed based on the fact that inarigivir might exhibit its potency against Covid-19.

The third drug, foretinib, is an anticancer agent. It works by inhibiting hepatocyte growth factor receptor (HEGFR or MET) receptor and Vascular Endothelial Growth Factor (VEGFRs).[35]

MET is a class IV receptor tyrosine kinase expressed on the surface of epithelial cells. Modulation of MET has been noted in the case of Influenza A Viruses, where the MET receptor found to enhance the entry of viruses by inducing virus binding signaling molecules.[48] The viruses bind to sialic-acids at host site cellular surfaces and enter cells via endocytosis routes, which require specific cellular signals, including the MET receptor. Many pathogens are known to employ host MET receptors to achieve a comfortable environment for their habitat inside the host cell and also for infection prognosis.[49] The broad inhibition of kinases, including MET by small-molecule inhibitors (nibs), has claimed to reduce virus uptake and consequently leading to reduced progeny virus titers in host cells.[50]

Next, VEGFR, which is known to plays a vital role in angiogenesis in humans. The levels of it are found to be overexpressed in cancers (Kaposi's sarcoma herpesvirus (KSHV) and Epstein-Barr virus (EBV)) or non-oncogenic viruses (herpes simplex virus (HSV-1) and dengue virus). These hijacks cellular signaling machinery and upregulate VEGF expression for their physiological functionality and pathogenesis.[51] The viruses directly affect VEGF expression by directly targeting key mediators that include HIF-1α, COX-2, and AP1 or indirectly by activating promoter region of VEGFR gene to enhance its expression by their own effector proteins. Therefore, it becomes vital to delve deeper into the mechanism and understand the interconnection between viruses and VEGF upregulation for more targeted design or test the existing VEGFR inhibitor for their efficacy in various viral diseases including Covid-19.[51a] Further research also exemplify the role of Angiotensin II in upregulating VEGF that leads to vascular inflammation a common insight during viral pathogenesis.[52] Thus, authors are in the opinion that co-treatment of angiotensin-II inhibitors and VEGFR-MET inhibitors may lead to some wonderful expedition in antiviral therapy.

The fourth drug, DB01813 (Pyridoxyl-Glutamic Acid-5'-Monophosphate), is an experimental drug, which has not been explored much so far. Considering the highest dock score of -8.756 kcal/mol, it exhibited the highest affinity with the target protein, i.e., NSP15 Endoribonuclease from SARS CoV-2. The molecules might affect NendoU, which is involved in catalyzing the formation of negative strand.[20]

3.3 Scope of current work in consideration to tentative molecules proposed by Chemical and Engineering News (C&EN)

During the compilation of this work, Chemical and Engineering News (C&EN) has disclosed some of the small molecules that may be explored for Covid-19 disease. The findings were published entitled "Can old drugs take down a new coronavirus ?".[5b] The exploration of the reported leads (**Figure 5**) suggested that although the molecules were able to interact with the target protein (6VWW) but were not found to possess affinity as compared to current study leads.

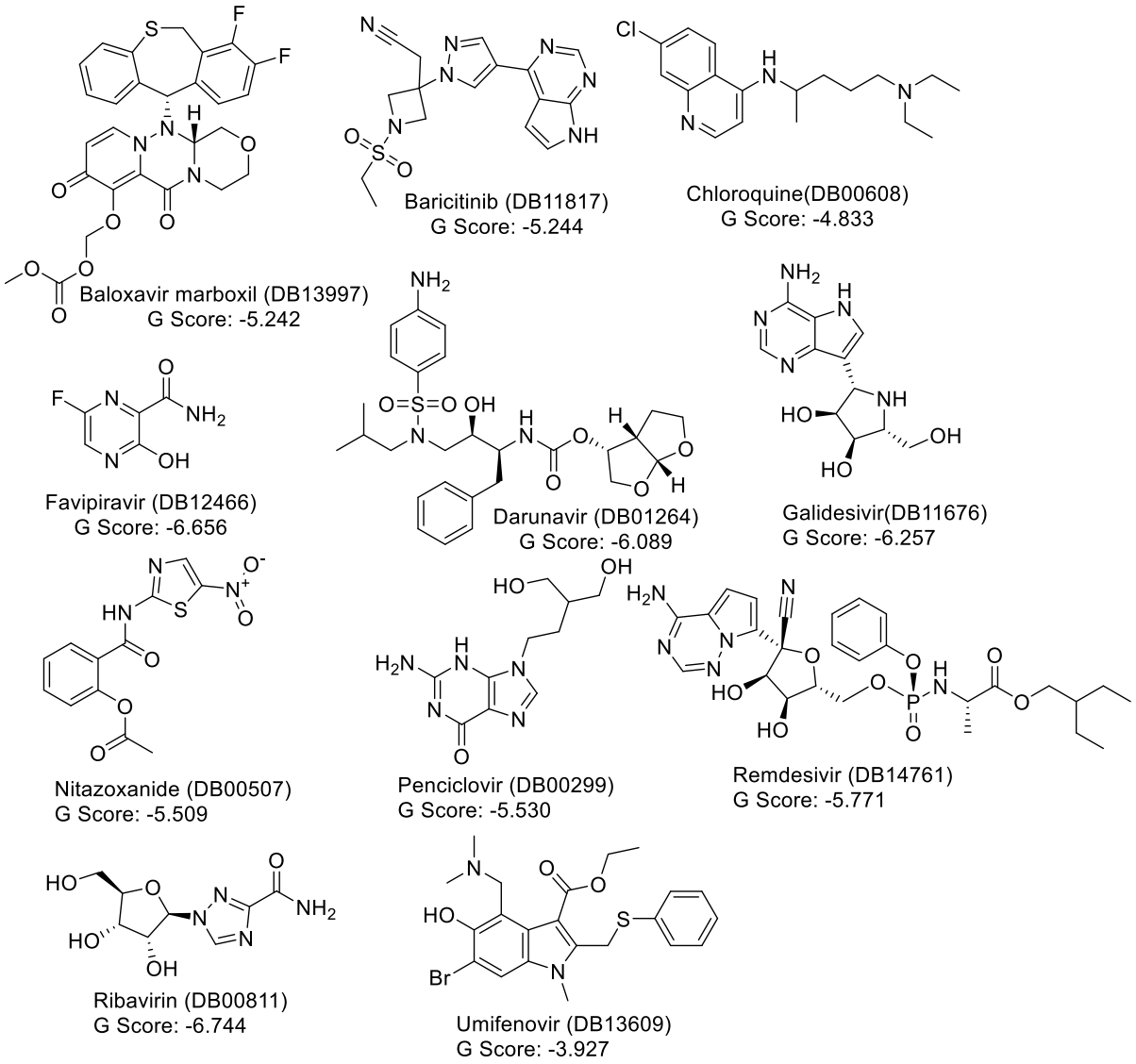


Figure 5. Tentative molecules proposed by Chemical and Engineering News (C&EN) and their outcome in investigated VS

4. Conclusion and Future Implication in development of drug regime for Covid-19

Due to the sudden outbreak of viral diseases in recent decades, including the Covid-19, controlling these pathogens and infection is still deprived of specific and efficacious treatment. The past decade has witnessed only 12 new antiviral drugs approved by the FDA (8 against hepatitis C virus (2 in combinations for HIV). Thus, it becomes of utmost importance to propose drugs for new and re-emerging viruses that possess the potential for pandemic with higher morbidity. Re-investigating existing drugs in pandemic diseases such as Covid-19 is considered to be an economical and time-saving method to repurpose already established medications for a particular need.

In the current work, we disclosed drug repurposing methodology using a virtual screening technique. We performed virtual screening and molecular docking of 8548 ligands on target protein coronavirus endoribonuclease NendoU (6VWW). To obtain a known drug for repurposing, we included FDA approved drugs along with investigational or experimental drugs for anticancer agents, antimicrobial agents, and antiprotozoal agents. We considered host-parasite interaction similar to host-virus inter effected by antiprotozoal agents. Various parameters like, antimetabolite nature of anticancer agents that affect the central dogma of molecular biology along with molecules affecting chromatin molecules, signaling pathways common to both cancer and viral diseases and effect of antimicrobial and antiprotozoal molecules on inhibition of DNA, RNA and protein synthesis along with their impact in treating plasmodial and helminthic disease sharing similar type of mechanisms with the viral infection were considered. The thorough selection based on virtual screening led us to propose that FDA approved drug DB00876 (Eprosartan), Investigational drugs DB15063 (Inarigivir soproxil), DB12307 (Foretinib) and DB01813 an experimental drug may be repurposed for treatment of Covid-19 disease.

The current work thus offers the scope of generation to test and corroborate the findings from virtual screening via *in vitro* and *in vivo* techniques against Covid-19 in the future. This may help in tracing their molecular mechanism(s) in addition to their development at the clinical level in the future for the treatment of ever rapidly increasing problems of Covid-19 disease, and similar strategies may be applied to other re-emerging or new viral diseases in future.

Conflicts of interest

There are no conflicts to declare.

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Author Contribution

RRP developed the idea and set the study plan, GJ executed the plan and wrote the manuscript,

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