Austin Journal of Proteomics, Bioinformatics & Genomics

Open Access

Austin Publishing Group

Research Article

Screening and Identification of Antiviral Drugs Targeting SARS-CoV-2 Non-Structural Proteins (nsp): A Virtual Screening, and Molecular Docking Study

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Received: August 25, 2021; **Accepted:** September 22, 2021; **Published:** September 29, 2021

Abstract

The ongoing pandemic of Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) is a positive-sense RNA (ss) virus. Mutational change is a typical episode resulting in their emerging regional variants not responding to the vaccine to an equally. Therefore, along with vaccines, antiviral drugs targeting non-structural proteins (nsp) can be a remedy to provide maximum protection. Moreover, in immunocompromised individuals, antiviral drugs can be the only reliable choice to tackle these types of infections. Here, non-structural proteins (nsp) named RNA dependent RNA polymerase (RdRp), Helicase (NSP13), and Papain-like Protease (NSP3) were selected as the target for drugs that can provide protection irrespective of mutational variants. Validated structures of these three essential proteins used to search anti-SARS-CoV-2 drugs (*in silico*). DrugBank database suggests eight drugs, including Remdesivir that can react against these three non-structural proteins. Molecular docking with AutoDock vina tools identified two potential drug-like components such as Nalpha-[(benzyloxy) carbonyl]-N-[(1R)-4-hydroxy-1-methyl-2-oxobutyl]-L-phenylalaninamide (DB08732), and S- [5-(trifluoromethyl L)-4H-1,2,4-triazol-3-YL] 5-(phenylethyl) furan-2-carbothiote (DB07743) showed significant binding energy against these proteins such as -54.39KJ/mole and -52.3KJ/mole, respectively. Also, the ADMET profile showed that DB08732 and DB07743 have no carcinogenicity. In addition, no ortholog of these two proteins was found in the human body, supporting their antiviral drug-like potential for treating SARS-CoV-2. Reflects that DB08732 and DB07743 might be promising candidates for therapeutic intervention to block SARS-CoV-2 replication to the host cell along with vaccines.

Keywords: SARS-CoV-2; Antiviral drug; RdRp; Helicase; PLpro; Molecular docking

Abbreviation

ADMET: Absorption, Distribution, Metabolism, Excretion and Toxicity; PROCHECK: Program to Check the Stereochemical Quality of Protein Structures; BLAST: Basic Local Alignment Search Tool; PROSA: Protein Structure Analysis

Introduction

In December 2019, a newly emerged human coronavirus became the focus of global concern after the dramatic and widespread outbreak of mystic pneumonia-like respiratory illness in the Wuhan region of Hubai, the province in China. On February 11, 2020, The International Virus Classification Commission (ICTV) designated Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2). Later the disease caused by SARS-CoV-2 was named COVID-19 by WHO. In March 2020, COVID-19 declared a Public Health Emergency of International Concern (PHEIC), consequently pandemic by WHO [1].

Based on genomic alikeness and evidence from phylogenetic analysis, SARS-CoV-2 is considered a member of Beta-coronaviruses, including SARS-CoV, MERS-CoV [2]. SARS-CoV-2 showed more than 80% sequence similarity with SARS-CoV and 50% with the MERS-CoV [3]. Also, these viruses might use the same receptor because of the amino acid sequence resemblance of the tentative receptor-binding domain [4].

The SARS-CoV-2 is an enveloped, positive-sense, single-stranded RNA virus with a genome size of 29.7kb [5]. Upon internalization into the cell, genomic RNA is composed of six open reading frames (ORFs). The first ORF (ORF1a/b) is vital, occupying two-thirds of the genome. ORF1a/b was used as a template for translating two polyproteins, pp1a and pp1ab, which encodes the production of 16 non-structural proteins (nsp) [6]. Some of these nsp encode proteins with essential functions, such as Papain-like protease, PLpro (nsp3), Chymotrypsin-like protease, CLpro (nsp5), Helicase (nsp13), and RNA dependent RNA polymerase, RdRP (nsp12). CLpro & PLpro cleave the replicase polyprotein in a sequence-specific manner to produce 15 to16 non-structural proteins (nsp). In contrast, Helicase (nsp13) unwinds duplex oligonucleotides in an NTP-dependent way. RdRp plays a critical role in the viral infection cycle by forming a replication-transcription complex [7].

The rapidly growing cases of COVID-19 lead to the rapid

Citation: Alam MM, Shill DK, Jahan S, Alam M, Hossain ME, Rahman M, et al. Screening and Identification of Antiviral Drugs Targeting SARS-CoV-2 Non-Structural Proteins (nsp): A Virtual Screening, and Molecular Docking Study. Austin J Proteomics Bioinform & Genomics. 2021; 6(1): 1031.

development of new therapeutics and vaccines against SARS-CoV-2. There are multiple phases of vaccine development, including an initial design stage, preclinical studies, phases 13 of clinical trial testing. However, researchers, governments, pharmaceutical companies, and regulatory bodies are tackling the spread of the pandemic virus by coordinating an unprecedented overhaul of the vaccine development process. Vaccines are very effective on stable viruses (Hepatitis B virus, Adenovirus) but are limited in treating already infected patients. Therefore, it becomes difficult to successfully utilize them against rapidly mutating viruses, such as influenza (the vaccine needed to update every year), HIV & HCV [8].

As a possible rapid therapeutics approach against rapidly spreading emerging infections like SARS-CoV-2, Drug purposing has emerged as a substitution of a vaccine. Recently, several studies using clinical drugs against SARS-CoV-2 have been proposed by scientists worldwide.

For a practical therapeutic approach, the active site of RdRp as the most conserved and accessible region constitutes a significant target for inhibition of viral replication. In addition, to block the production of non-structural viral components, followed by a hamper of virus replication, the papain-like protease and Helicase enzyme leads to an attractive drug target.

We used bioinformatics tools to identify SARS-CoV-2 encoded specified proteins and retrieve their sequences for mutational analyses. In addition, performed homology modeling to build targeted protein structures, such as papain-like protease (PLpro), RNA-dependent RNA polymerase (RdRp), Helicase, etc. The main objective was to screen drugs (experimental or approved) from the drug bank targeting non-structural proteins through *in silico* analysis. Thus, our study screens various components that may inhibit SARS-CoV-2 and provide a good starting point for antiviral therapies as an alternative or coadjuvant vaccination strategies. Furthermore, subsequent validation of antiviral effects *in vitro* and *in vivo* will provide helpful information for the clinical treatment of novel coronavirus.

Materials and Methods

Identification of the sole proteins involved in the life cycle of SARS-CoV-2

We identified the sole proteins involved in the life cycle of SARS-CoV-2 through literatures analysis and explored antiviral drugs against those sole proteins [9,10].

Mutation analysis of regional variants among sole proteins

We identified three essential non-structural proteins named RdRp, Helicase (NSP13) and Papain Like Protease (NSP3) were considered sole proteins for drug design. Amino acids sequences of these proteins from different regional variants, for instance, Indian variant (QUE93972), South African Variant (QUA12568.1.), Brazilian variant (QMB22609.1), U.K. variant (QOS14143.1), emerging variants of Bangladesh (QKV26694.1, QWT51802.1) and Wuhan strain (YP_009725308) as a reference, were retrieved from NCBI database. Then, the ClustalW method of Multiple Sequence Alignment (MSA) was used with the bootstrap value of 1000 in conjunction with BioEdit software [11].

Protein modeling

We performed homology modeling of RdRp (NCBI ID QKV26694.1), Helicase (NCBI ID QKV26694.1), and Papain Like protease (PLpro) (NCBI ID QKV26694.1) using the SWISS-MODEL database [12]. A (RdRp), 5rl6.2.A (Helicase), 6wuu.2.A (L-protease) template determines the crystal 3D structure with the sequence identity around 99.89%, 100%, 99%, respectively [13]. For protein 3D structure quality assessment, PROCHECK [14] was used and validated with SAVES-Verify3D [15], PROSA-Web Server [16], Ramachandra Plotting. Finally, we used ExPASy: Protpram tools to determine physicochemical properties [17].

Retrieval of protein like drug components from DrugBank

Protein sequences were used for model, identify protein-like drug components, and determining pharmacological function through DrugBank Database [18]. The sequence of drug-like components (experimental or approved) that can interact and interfere with proteins (sdf or .pdb) were retrieved. Later used to determine binding efficacy analysis with respective proteins. And, ADMET profiling was determined by admetSAR [19].

Molecular docking

For molecular docking, AutoDock Vina 1.1.5 tool [20], used to conduct target protein interaction with ligand drug components. We set proteins dehydration, protonation, and specific grid box with grid point spacing 1 Å within proteins. As per requirement, we formatted the protein and ligand sequences with OpenBabel [21]. Molecular docking was performed with an exhaustiveness value of eight. Based on protein-ligand interaction, binding energy (KJ/mole) was exerted. We used Pymol 3D viewer to visualize protein-ligand three-dimensional interaction.

Broad Spectrum Homology analysis with Homo sapiens

Protein sequences were subjected to pBLAST tools of NCBI [22] to determine whether pathogenic virus contains these proteins or not. Additionally, whether *Homo sapiens* has an ortholog of this protein or not (E value 0.0001).

Results

Sole proteins involved in the multiplication of SARS-CoV-2

SARS-CoV-2 has three unique non-structural proteins named RdRp, Helicase, and Papain Like-protease (PLpro). RdRp plays a crucial role in replication and can be an ideal target for antiviral drugs. Whereas, Helicase [23] and Papain Like-protease (PLpro) [24] play a significant role in virus propagation. All three proteins play a vital role in genome replicating and propagating in the host cell [25]. We know that mutation quickly takes place in the structural proteins such as a spike or envelope protein. Therefore, we opt to identify drugs irrespective of mutational resistance as they act on non-structural protein activity. Furthermore, we tried to find out the level of cure they can provide. Therefore, antiviral drugs that can inhibit nsp's function can inhibit virus replication and propagation.

Mutational analysis

We aligned sequences of proteins (RdRp, Heliase, PLpro) from eight regional variants by ClustalW (Figure 1). In Helicase (Figure 1C), amino acid mutation (Proline to Leucine) at 77 positions in emerging Indian Delta variant isolated in Bangladesh (QWT51802.1), and another mutation (Methionine to Isoleucine) at 429 position of

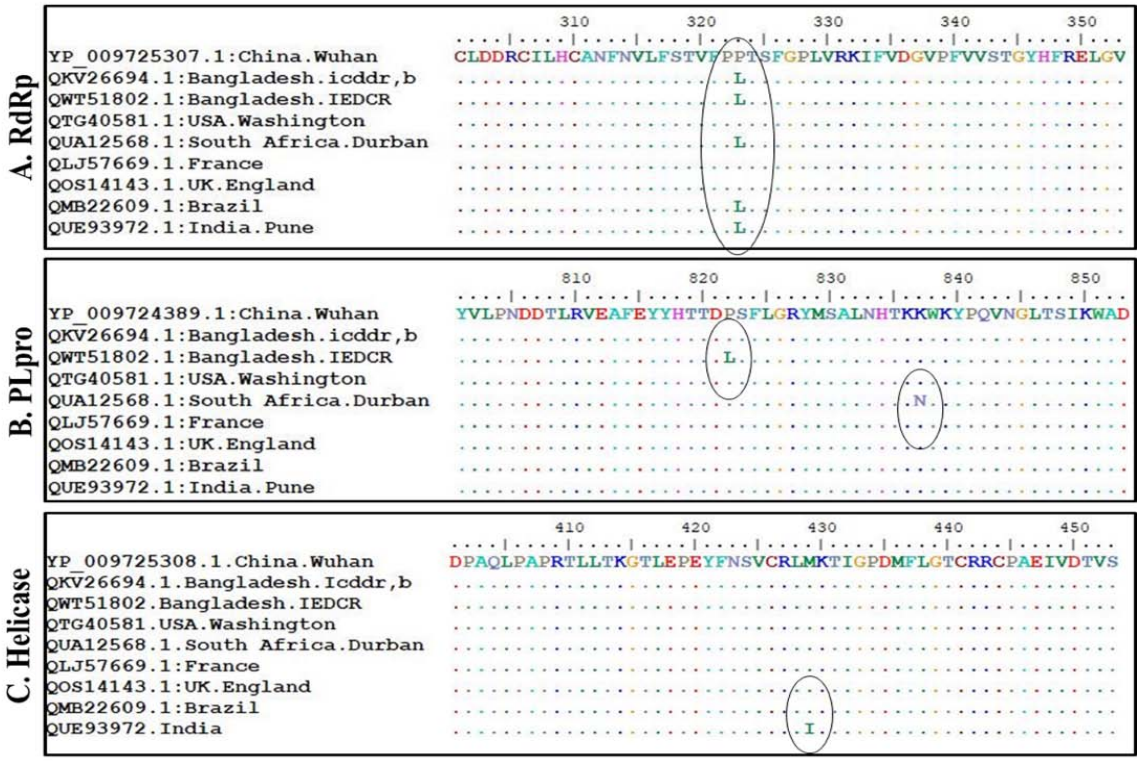

Figure 1: Mutational analysis of three non-structural proteins (nsp). Mutational analysis of amino acid residues of three nsp among eight regional variants was performed considering Wuhan strain (YP_009725307) as reference. Mutation at the amino acid sequence of (A) RdRp, (B) Papain Like Protease (PLpro), (C) Helicase in SARS-CoV-2.

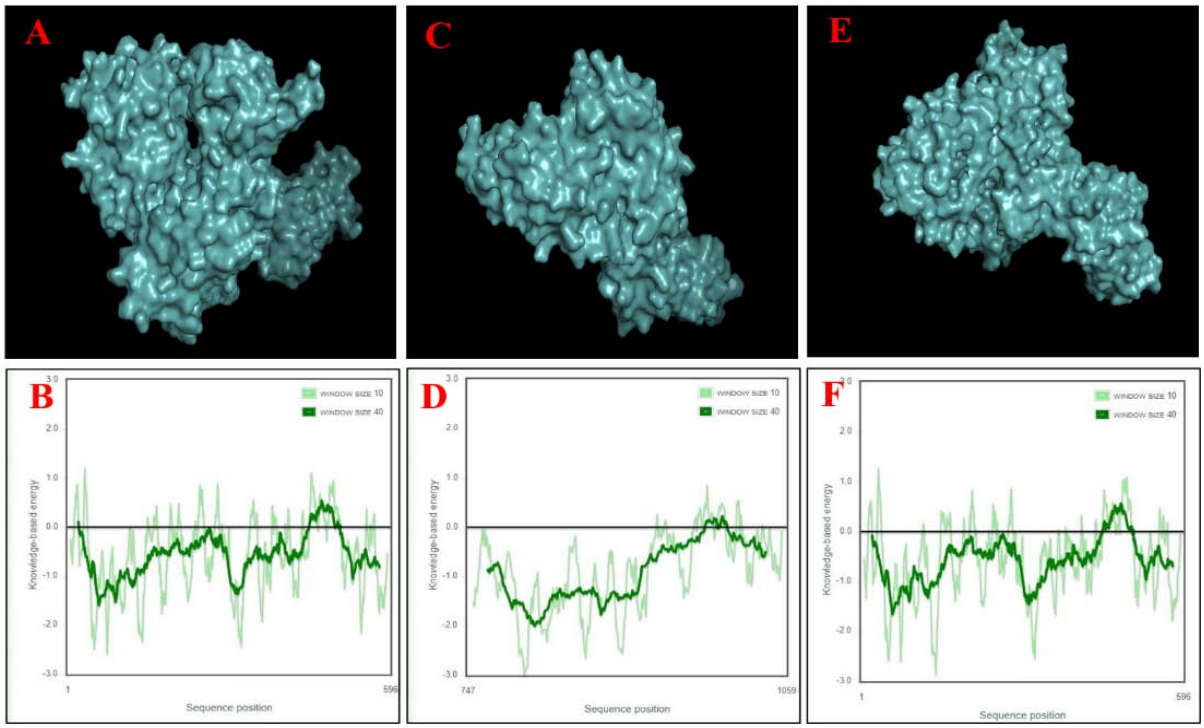

Figure 2: Visualization of 3D surface structure and measurement of structural stability of three non-structural proteins. A) Surface structure of RdRp, B) Structural stability of RdRp by ProSA, C) Surface structure of Papain Like Protease (PLpro, NSP3), D) Structural stability of PLpro by ProSA, E) Surface structure of Helicase, F) Structural stability of Helicase by ProSA.

Indian variant isolated in India was found compared with Wuhan strain. In RdRp (Figure 1A), at 323 position Bangladesh (icddr,b; IEDCR), South African (QUA12568.1), Indian, and Brazilian variant (QMB22609.1) have similar Proline to Leucine mutation. Whereas, Indian variant isolated from Bangladesh (IEDCR) showed mutation at position 671 (Glycine to Serine) and the Indian variant in India at position 675 (Valine to Isoleucine). However, Papain Like Protease (Figure 1B) has only two mutations; one in the Indian variant isolated from Bangladesh (IEDCR) at position 822 (Proline to Isoleucine) and South African variant at position 837 (Lysine to Asparagine).

Protein modeling and validation

We performed protein modeling based on the homology modeling algorithm using the SWISS-MODEL database. First, we visualized the 3D model using .pdb format with PyMOL software (Figure 2). Then, the 3D structure was verified with PROCHECK, SAVES-Verify3D, PROSA-Web, and Ramachandra Plotting. Helicase and PL protease have 85.57% and 89.14% residues (Figure S1), respectively, in the favorable area. Whereas for RdRp, it is 83.72%. Ramachandran Plotting showed RdRp (Figure 3A), Helicase, and PLpro (Figure 3B) have 95.1%, 99%, and 99.6% amino acids residues are resided allowed region (Figure 3), respectively. Including 80.3%, 76.3%, and 95% in the most favored area. Finally, the PROSA-web service determined the overall stability of the protein 3D structure. All three showed stable structure, having Z values -8.9, -8.89, and -8.85 for RdRp (Figure 2B), Helicase (Figure 2F), and PL protease (Figure 2D), respectively.

Inhibition of sole proteins by DrugBank database suggested drugs

Sequence-based *in silico* analysis of DrugBank database suggested eight similar and one (only against PL-protease) chemical compounds (Table 1) that can inhibit the activity of RdRp, Helicase, and Papain Like-protease (PLpro) proteins. Among these experimental drugs, only Remdesivir was found to have Food and Drug Administration (FDA) approval but investigational to treat COVID-19 patients [26].

Molecular docking

Finally, nine drugs were subjected to binding Analysis using the molecular docking method. We used AutoDock tools-1.1.5 to determine the binding affinity and efficiency of drugs with specified protein by observing their binding energy with protein (Table 1) [20].

For RdRp, apart from Remdesivir, the energy, expressed in negative signs to denote that these reactions are exothermic (Table 1). Exerting energy is more than 33KJ/mole recommended as effective against the specific protein (Table 1) [27]. We found 2- [(2,4-Dichloro-5-methyl phenyl) sulfonyl]-1,3-dinitro-5-(trifluoromethyl) benzene, Nalpha-[(benzyloxy) carbonyl]-N-[(1R)-4-hydroxy-1-methyl-2-oxobutyl]-L-phenylalaninamide and S- [5-(trifluoromethyl L)-4H-1,2,4-triazol-3-YL] 5-(phenylethyl) furan-2-carbothiote showed binding energy -54.1KJ/mole (Figure 4), -54.4KJ/mole and -52.3KJ/mole (Figure 4), respectively (Table 1).

For Helicase (NSP13), the S- [5-(trifluoromethyl L)-4H-1,2,4-triazol-3-YL] 5-(phenylethyl) furan-2-carbothiote showed the most effective binding that is -56.06KJ/mole. Although, 2- [(2,4-Dichloro-5-methyl phenyl) sulfonyl]-1,3-dinitro-5-(trifluoromethyl) benzene (-54.4KJ/mole) and Nalpha-[(benzyloxy) carbonyl]-N-[(1R)-4-hydroxy-1-methyl-2-oxobutyl]-L-phenylalaninamide (-52.3KJ/mole) also showed impressive binding response (Figure 4).

In case of PL protease, Nalpha-[(benzyloxy) carbonyl]-N-[(1R)-4-hydroxy-1-methyl-2-oxobutyl]-L-phenylalaninamide showed

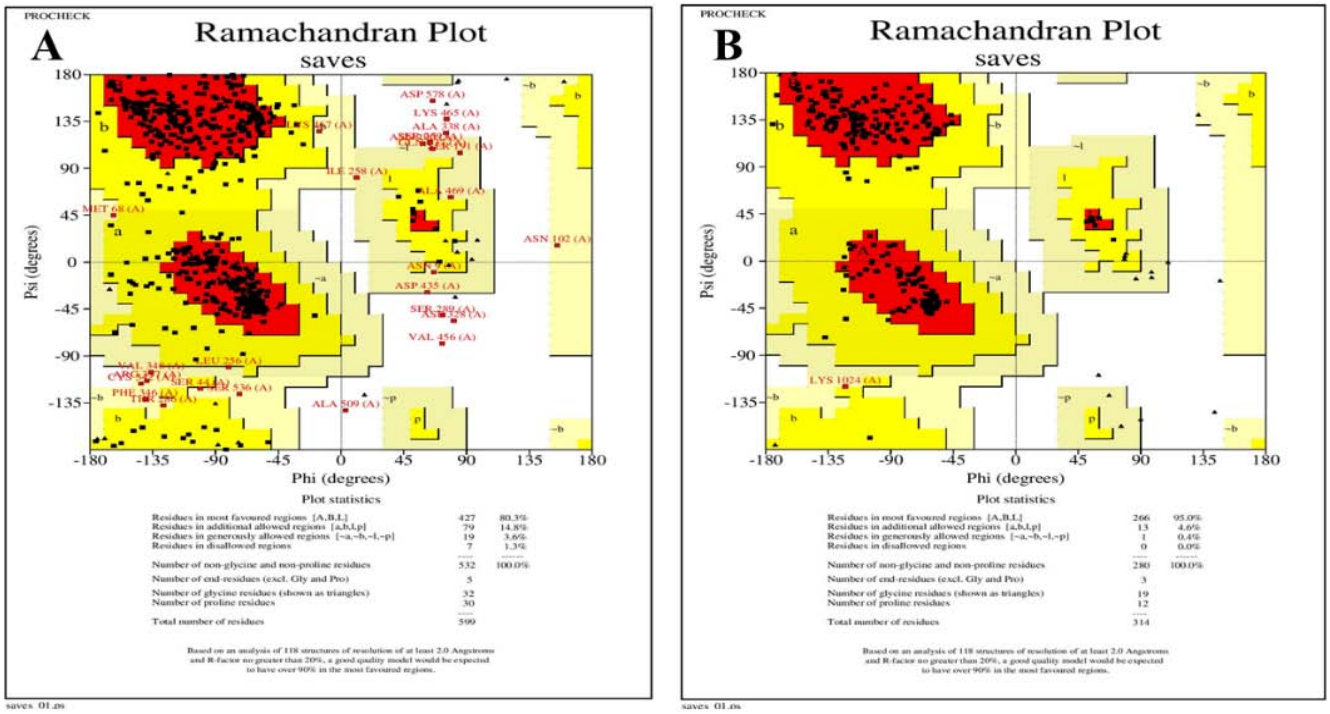

Figure 3: Structural validation of RdRp and PLpro by PROCHECK (SAVES). A) PROCHECK Ramachandran plot of RdRp shows that 94.3% amino acid residues within the allowed region. B) PROCHECK Ramachandran plot of PLpro express that 99.6 % amino acid residues within the allowed region.

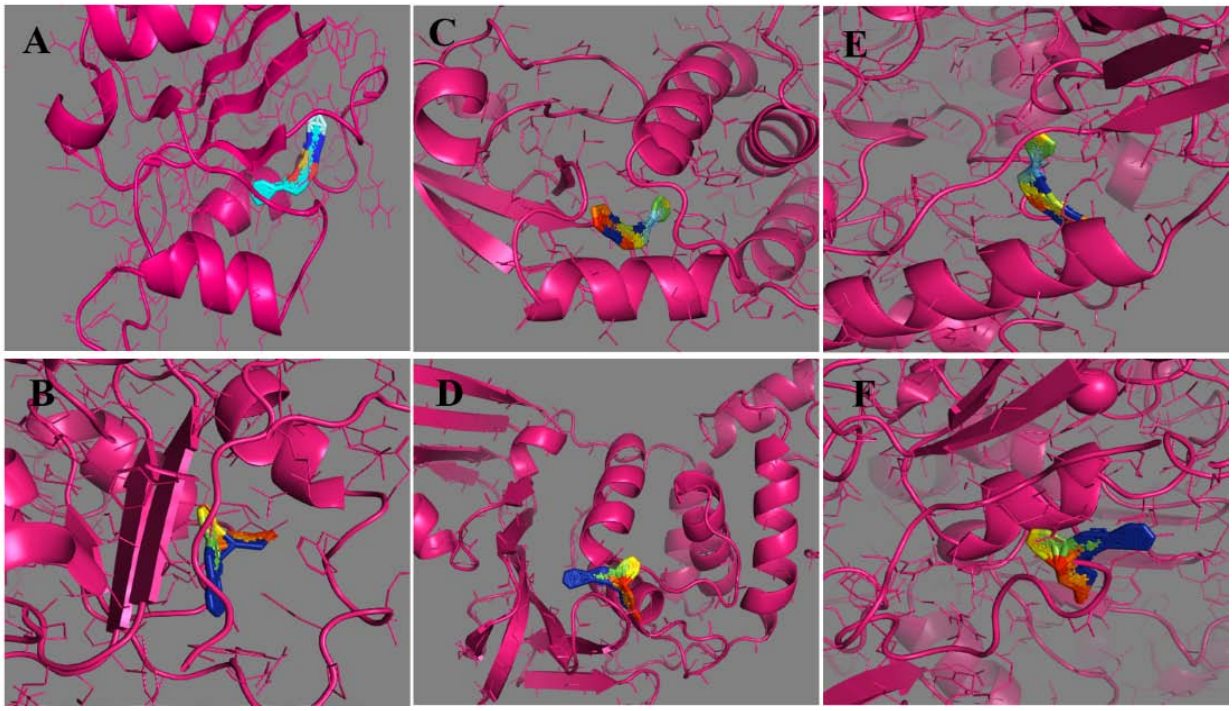

Figure 4: Molecular docking of SARS-CoV-2 non-structural proteins and anti-viral drug-like components. Binding of S- [5-(trifluoromethyl L)-4H-1,2,4-triazol-3-YL] 5-(phenylethyl) furan-2-carbothiote with (A) RdRp (-52.3KJ/mole), (C) PLpro (-47.7KJ/mole), (E) Helicase (-56.06KJ/mole); Binding of Nalpha-[(benzyloxy) carbonyl]-N-[(1R)-4-hydroxy-1-methyl-2-oxobutyl]-L-phenylalaninamide with (B) RdRp (-54.4KJ/mole), (D) PLpro (-49.37KJ/mole), (F) Helicase (-52.3KJ/mole).

Table 1: Molecular docking score or binding energy of nine different antiviral components with their target protein.

Name of Drug	DrugBank ID	Docking/ Binding Energy (KJ/mole) with Specified Protein		
		Papain Like Protease (PLpro)	Helicase	RNA Dependent RNA Polymerase (RdRp)
Benzyl (2-oxopropyl) carbamate	DB07293	-33.89	-32.21	-36.4
2- [(2,4-Dichloro-5-methyl phenyl) sulfonyl]-1,3-dinitro-5-(trifluoromethyl) benzene	DB07620	-54.8	-54.8	-54.8
S- [5-(trifluoromethyl L)-4H-1,2,4-triazol-3-YL] 5-(phenylethyl) furan-2-carbothiote	DB07743	-47.7	-56.06	-52.3
5-Amino-2-methyl-N-[(1R)-1-naphthalen-1-ethyl] benzamide	DB08656	-39.32	-43.93	-46.02
Nalpha-[(benzyloxy) carbonyl]-N-[(1R)-4-hydroxy-1-methyl-2-oxobutyl]-L-phenylalaninamide	DB08732	-49.37	-52.3	-54.4
4-(Dimethylamino)benzoic acid	DB08748	-32.21	-28.87	-28.87
Remdesivir	DB14761	55.64	-64.01	-66.9
GS-441524	DB15686	-42.25	-46.68	-38.9
2-(N-morpholino)ethanesulfonic acid	DB03814	-26.35	N/A	N/A

-49.37KJ/mole (Figure 4), 2- [(2,4-Dichloro-5-methyl phenyl) sulfonyl]-1,3-dinitro-5-(trifluoromethyl) benzene showed -54.4KJ/mole (Figure 4), and S- [5-(trifluoromethyl L)-4H-1,2,4-triazol-3-YL] 5-(phenylethyl) furan-2-carbothiote has -47.7KJ/mole (Figure 4) as binding energy (Table 1).

Properties and pharmacological mode of action of these drug compounds

Remdesivir and GS-441524 were already involved in the inhibitory action; the rest have an unknown pharmacological mode of action (Figure S2). According to the admetSAR 2.0, Nalpha-[(benzyloxy) carbonyl]-N-[(1R)-4-hydroxy-1-methyl-2-oxobutyl]-L-phenylalaninamide, S- [5-(trifluoromethyl L)-4H-1,2,4-triazol-3-YL] 5-(phenylethyl) furan-2-carbothiote and GS-441524 showed good drug-like properties to consider as a lead component for the drug discovery process, with no carcinogenic or eye corrosion or irritation effect. However, 2- [(2,4-Dichloro-5-methyl phenyl) sulfonyl]-1,3-dinitro-5-(trifluoromethyl)benzene has a bit of binary carcinogenic and eye corrosion effect.

Broad spectrum analysis

We conducted pBLAST analysis to determine whether the human genome contains these types of protein or not. If the human genome contains protein like this, it will be great trouble administering this drug to the human body. However, after pBLAST Analysis, we found no protein in the human body like these three target proteins.

Table 2: Physicochemical properties of three non-structural proteins (nsp).

Physicochemical Properties	Helicase	RdRp	PL Protease (NSP3)
Number of amino acids	601	932	1945
Molecular weight	66855	106676.28	217253
Theoritical pI	Hel	6.14	5.56
Total number of negatively charged residues (Glu + Asp)	52	106	222
Total number of Positively charged residues (Lys + Arg)	64	94	185
Extinction coefficient assuming all pairs of Cys residues from cysteines $M^{-1}cm^{-1}$	68785	137670	243675
Extinction coefficient assuming all pairs of Cys residues are reduced $M^{-1}cm^{-1}$	67160	135920	240550
Instability index	33.31	8.12	36.56
Aliphatic index	84.49	78.85	86.22
Grand average of hydropathicity (GRAVY)	-0.096	-0.218	-0.175

pI: Isoelectric Point; RdRp: RNA Dependent RNA Polymerase; NSP: Non Structural Protein.

Cellular location of target proteins

Prediction of CELLO2GO [28] reflects that Helicase is an extracellular protein. For RdRp, it may be extracellular or in the plasma membrane and the host cytoplasm. Furthermore, the PLpro protein position is in the plasma membrane and the cytoplasm of a host. Therefore, these drugs may interact easily for being extracellular.

Discussion

In emerging viral diseases like COVID-19, medical treatment usually starts with approved antiviral compounds, such as an FDA-approved polymerase inhibitor. Here, we tried to explore new antiviral compounds capable of inhibiting multiple essential proteins of the virus. Our study focused on (*in silico*) the identification of components that can interfere with the life cycle of SARS-CoV-2 through interacting with their non-structural proteins (nsp) instead of structural ones [29]. Non-structural proteins such as RNA-dependent RNA polymerase (RdRp), Helicase (NSP13), and Papain Like protease (PLpro) have less sequence variability (Figure 1), solely involved with genome replication and the packaging of the virus into the host cell [30,31]. Among them, Helicase is highly conserved in *Coronaviridae* family members [32].

Multiple Sequence Alignment (MSA) suggests less than 1% amino acid mutation is present among (PLpro 0.05%, RdRp 0.2%, Helicase 0.16%) (Figure 1) these nsp in regional variants. Therefore, antiviral drugs targeting nsp's may protect against SARS-CoV-2 irrespective of regional variants. This conservative nature of nsp (RdRp, Helicase, PLpro) suggests the possibility of long-term protection using drugs.

We performed homology modeling of these sole proteins in conjunction with the SWISS-MODEL database. Based on the homology modeling method and the outputs were saved in protein database (.pdb) format. ProtParam tool, known as ExPASy, is a database to determine a protein model's physicochemical properties (Table 2) of a protein model. ExPASy suggests instability index values for proteins such as 8.12 for RdRp, 33.31 for Helicase, and 36.56 for L protease (Table 2). This index determines the stability of the protein model. Values higher than 40 indicate the instability of the model [33]. As instability index values of three proteins are less than 40, structures of these proteins are highly stable.

The hydrophilicity or hydrophobicity of sole proteins was determined with a GRAVY (Grand Average of Hydrophobicity) score. GRAVY score below zero indicates hydrophobic and globular; whereas, score over zero indicates hydrophilic [34]. GRAVY scores of RdRp, PL Pro, Helicase are -0.218, -0.175, and -0.096, respectively (Table 2), meaning all three are hydrophobic, globular [29,35]. In addition, the theoretical isoelectric (pI) points of PL protease, RdRp, and Helicase are 5.56, 6.14, and 8.66, respectively (Table 2). The pI of PL Protease (PLpro) speculates its acidic nature and Helicase slightly alkaline nature [36]. Physicochemical properties show protein's compatibility of making hydrogen bonds promptly [33].

The PROCHECK (Ramachandran Plotting), SAVES-Verify-3D, PROSA databases were used for in-silico validation of protein models. Ramachandran Plotting showed RdRp, PL protease, and Helicase has 95.1%, 99.6%, 99% of amino acid residues in allowed regions, including 80.3%, 95%, and 76.3% are at the most favored area, respectively. Assures high acceptability of protein models. SAVES-Verify-3D suggests that the proteins have valid 3D structures as they passed with a score over 80% (RdRp 83.72%, PLpro 89.14%, and Helicase 85.57%) (Figure S1). PROSA, a web-based tool used to determine the overall quality of the protein model. It provides the folding energy of the 3D structure by the score of Z. Z-score of RdRp, Helicase, and PL protease is -8.9, -8.89, and -8.85, respectively (Figure 2). These results speculate that the structures are native. Negative and values above eight indicate the stability of folding [16]. Together, it reflects the validity of the protein models.

DrugBank server suggested eight drugs (Table 1), including Remdesivir, which can act against all three enzymes with variable binding efficiency (Table 1). Remdesivir is now an approved medication to treat COVID-19 [37]. However, Remdesivir has limitations, such as the low half-life in plasma (0.4h). Moreover, 60% of the Remdesivir medicated patients faced adverse effects, such as renal impairment, hypotension, multiple organ dysfunction, increased hepatic enzyme, diarrhea to some extent (12%), septic shock, as well as kidney injury (approximately 10%) [38]. And 66% adverse events in adults, along with a 1.23 hazard ratio [39]. DrugBank also suggests another drug that reacts only against PLpro protein named 2-(N-morpholino) ethanesulfonic acid (Table 1).

AutoDock Vina tool was used for molecular docking to screen and identify drug-like components with their binding efficacy with specified proteins [27,40]. Molecular docking narrowed the choice

of drug components into two, such Nalpha-[(benzyloxy) carbonyl]-N-[(1R)-4-hydroxy-1-methyl-2-oxobutyl]-L-phenylalaninamide (DB08732), which have binding energy against RdRp -54.4KJ/mole, Helicase -52.3KJ/mole, and PLpro -49.4KJ/mole. And, S- [5-(trifluoromethyl L)-4H-1,2,4-triazol-3-YL] 5-(phenylethyl) furan-2-carbothiote (DB07743), which showed binding energy against RdRp -52.3KJ/mole, Helicase -56.06KJ/mole, and PLpro -47.7KJ/mole. Furthermore, their ADMET profiles represented no carcinogenicity and had intestinal absorption capability with less toxicity [32] and less corrosive. Moreover, pBLAST suggests that RdRp, Helicase (NSP13), and Papain-like protease (PLpro) have non-homologous characteristics with the proteins that are present in the *Homo sapiens*. As a result, these drug-like components will not interfere with any essential cell components. Thus, no harmful reaction will occur by these components. Therefore, both of them can be an excellent choice to incorporate into the drug discovery process.

In the future, *in vitro* experiments with these two components in a cell-line-based bioassay with live virus particle or pseudovirus will be very interesting to observe. In addition, further research on drug discovery prospects, coupled with *in-vivo* experiments, might provide us an alternative to vaccines.

To tackle the current pandemic and any future upsurge by RNA viruses like SARS-CoV-2, we, preemptively, need to develop antiviral drugs that can act irrespective of regional variants. Meanwhile, the vaccination program is ongoing worldwide, including Bangladesh, but different regional variants coupled with mutations in the spike protein remain a matter of concern. Thus, this study explored the possibility and importance of drug-like compounds as antiviral drugs to fight against the pandemic as a supportive drug and backup option for vaccine unavailability or failure.

Acknowledgement

This research study was funded by core donors who provide unrestricted support to icddr,b for its operations and research. Current donors providing unrestricted support include the Governments of Bangladesh, Canada, Sweden and the UK. We gratefully acknowledge our core donors for their support and commitment to icddr,b's research efforts.

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