Chem. Biol. Lett. 2020, 7(3), 166-175 Article

Chemical Biology
LETTERS

In silico identification of potent FDA approved drugs against Coronavirus COVID-19 main protease: A drug repurposing approach

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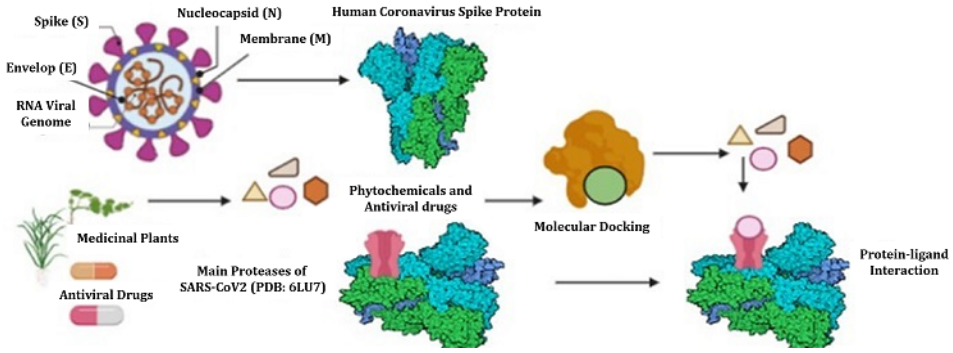
Submitted on: 21-Mar-2020 Accepted and Published on: 27-Mar-2020

ABSTRACT



The recent outbreak of novel coronavirus disease, COVID-19 has created a threat to human population across the world. The unavailability of specific therapeutics and vaccines, demands the sincere efforts in this direction. Main Proteases of this novel Coronavirus (SARS-CoV-2) play critical role during the disease propagation, and hence represents a crucial target for the drug discovery. Herein, we have applied a bioinformatics approach for drug repurposing to identify the possible potent inhibitors of SARS-CoV2 Main Proteases. A library of FDA approved antiviral compounds, and active phytochemicals were screened using PyRx virtual screening tool that led to 19 hits based on their highest binding affinity. Nelfinavir exhibited highest binding affinity -8.4 Kcal/mol and strong and stable interactions with the amino acid residues present on the active site of COVID-19 Main Protease. Besides, drugs including Rhein (-8.1), Withanolide D (-7.8), Withaferin A (-7.7), Enoxacin (-7.4), and Aloe-emodin (-7.4) also showed good binding affinity with favourable ADME properties. Our findings suggest that these small molecules can be used as potential inhibitors against SARS-CoV2 Main Protease. However, further investigation and validation of these inhibitors against SARS-CoV-2 are needed to claim their candidacy for clinical trials.

Novel Coronavirus, SARS-CoV2, COVID-19, Protease, Molecular Docking, Drug Repurposing

INTRODUCTION

Novel Coronavirus (SARS-CoV-2) causing COVID-19 disease was originated from Hubei province of China, and now has been spreading to several other countries. The disease is accountable for a large number of fatal cases. On January 2020, WHO emergency committee declared a global health emergency based on the rate of increasing spread of the infection with fatality rate of about 4%.[1,2] Collaborative efforts from scientists worldwide are underway to understand the rapid spread of the novel coronavirus (CoVs), and to develop effective interventions for control and prevention of the disease. Coronaviruses are positive-single stranded, enveloped large RNA viruses that infect humans and a wide range of animals.[3] Tyrell and Bonne reported the first coronavirus in 1966, who cultivated the viruses from the patients suffering with common cold.[4]

In *Latin*, Corona means "crown" based on their shapes. Coronaviruses have four subfamilies, which includes alpha-,

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Cite as: *Chem. Biol. Lett., 2020, 7(3), 166-175.*
URN:NBN:sciencein.cbl.2020.v7.117

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beta-, gamma- and delta subtypes.[5] Alpha and beta coronaviruses were originated from mammals such as bats, however gamma and delta viruses were derived from pigs and birds.[6] Recombination rates of CoVs are very high due to the ability to develop constant transcription errors and RNA Dependent RNA Polymerase (RdRP) jumps.[7] Most of the RNA content of these viruses encode viral polymerase, RNA synthesis materials, and two large nonstructural polyproteins (ORF1a-ORF1b) that are not involved in host response modulation. The rest one third of the genome portion codes for four structural proteins (spike (S), envelope (E), membrane (M) nucleocapsid (N), and the helper proteins.[8] CoVs have high mutation rates with the capability of causing infections in respiratory, gastrointestinal, hepatic and neurologic systems. The viruses are highly pathogenic in nature as they are also associated with severe acute respiratory syndrome (SARS).

While CoVs infection, the first step is the interaction of spike protein with sensitive human cells.[9] After entering to the cells, CoVs adapt to their human hosts by genome encoding and facilitating the expression of the genes that encode necessary proteins. Genome alteration by CoVs are done through the mechanism of recombination, gene exchange, gene insertion or deletion.[10] SARS-CoV and middle east respiratory syndrome (MERS-CoV) attach to the host cell respectively and binds to the cellular receptor angiotensin converting enzyme 2 (SARS-CoV associated) and cellular receptor of dipeptidyl peptidase 4 (MERS-CoV associated).[11] After infecting the host, the viral RNA manifests itself in the cytoplasm of the cell. Genomic RNA is modified through the process of encapsulation and polyadenylation and encodes several structural and non-structural genes. Proteases exhibiting chymotrypsin-like activity splits these polyproteins which drives the production of (-) RNA through replication as well as transcription.[12] During the replicative machinery the full length (-) RNA copies of the genome are used as a template for full length (+) RNA genomes. During the process of transcription, RNA encoding all structural proteins and a subset of 7-9 sub-genomic RNAs are produced by discontinuous transcription. In the cytoplasm, viral nucleocapsids are combined from genomic RNA and R protein and then are budded into the lumen of the endoplasmic reticulum. Through the process of exocytosis, virions are then released from the infected cell. The released viruses are then capable to infect kidney cells, liver cells, intestines, T lymphocytes, as well as the lower respiratory tract, where they form the main symptoms and signs. In notion of this, three patients having SARS-CoV infection were found to have CDT lymphocytes lower than 200 cells/mm3.[13] MERS-CoV is able to affect human dendritic cells and macrophages *in vitro*. T lymphocytes are also a target for the pathogen due to the characteristic CD26 rosettes. This virus can make the antiviral T-cell response irregular due to the stimulation of T-cell apoptosis, thus causing a collapse of the immune system.

The novel coronavirus (nCoV) emerged in 2019 has been the focus of global attention due to the pneumonia epidemic of unknown cause. The first case of pneumonia was reported on December 12, 2019 where possible pneumonia and coronavirus infection were ruled out by clinicians. Main protease domain (Mpro) has been reported to be a conserved target, in favour to design new inhibitors throughout the entire coronavirinae subfamily. The two-third region of 5' in the coronavirus genome consists of the open reading frame I which encodes two large polypeptides of the replicase machinery: pp1a, and through ribosomal frameshift, pp1ab1. Two proteases encoded in the 5' region of ORF 1: papain-like protease (PLP) and 3C-like protease (3CL or Nsp5) cotranslationally cleaves the two polypetides into mature nonstructural proteins (NSPs).[14] The 3 CL protease is more commonly known as Mpro as it has a dominant role in the posttranslational processing of the replicase protein. Significant homology of Mpros in primary amino acid sequence as well as in 3D architechture has been reported in different human and animal CoVs. Also, they have a similar substrate binding pocket with a requirement for glutamine at P1 position and a preference for leucine/methionine at P2 position. This strong structural basis provides an opportunity to design a wide-spectrum anti CoV inhibitors. In general, there are few or no treatment options for viral diseases that occur suddenly and spread at a higher frequency in the community. In notion of this note, there are no vaccine or effective treatment available to prevent the Novel Coronavirus (COVID-19) infection.

Several measures need to be taken in order to prevent the epidemic at a larger rate, such as early diagnosis, reporting isolation, supportive treatments, avoiding unnecessary panics. Also, basic preventive measures such as regular handwashing, using disinfectant solutions, avoiding contact with patients in order to prevent the spread of viruses by droplets. Healthcare staff should be informed about taking personal protective measures such as the use of gloves, eye masks and N95 masks during the examination of patients with a suspected history of COVID-19.

Table 1 (a): Active site properties of 6LU7 (Interacting chain A-02J)

Index	Residue	Amino Acid	Distance
1	168A	PRO	3.53

Table 1(b): Active site properties of 6LU7 (Interacting chain A PJE-C5)

Index	Residue	Amino Acid	Distance
1	3C	VAL	3,98
2	25A	THR	3.74
3	26A	THR	3.88

Table 1 (c): Active site properties of 6LU7 (Interacting chain C PJE-C5)

Index	Residue	Amino Acid	Distance D-A	Distance H-A	Donor angle	Protein donor	Sidechain
1	143A	GLY	2.87	2.00	145.90	+	-
2	144A	SER	3.99	3.65	104.01	+	+
3	163A	HIS	1.77	1.77	116.24	-	+
4	164A	HIS	1.85	1.85	161.75	-	-
5	166A	GLU	3.48	2.60	144.50	-	+

Table 2: Molecular docking analysis of antiviral compounds against COVID-19 Major Protease (6LU7).

Compound	Binding Affinity (kcal/mol)	Amino acid residues
Aloe-emodin	-7.4	ASP295, PHE294, THR292, GLY109, THR111, ILE106, VAL104, ASN151, ASP153, GLN110, PHE112, ILE152, PHE8, PHE112
Chitranone	-7.0	LYS5, TYR126, GLN127, SER139, GLY138, LYS137, ARG131, ASP197, LEU286, LEU287, GLU288, ASP289, GLU290, SER139
Chrysophanol	-7.0	PHE294, GLN110, THR111, GLN107, ILE106, ARG105, VAL104, LYS102, ASP153, SER158, ASN151, PHE8, ILE152
Diterpene	-7.1	ASP295, PHE294, THR292, THR111, PHE8, GLN110, GLN107, ILE106, ARG105, VAL104, SER158, ASP153, ILE152, ASN151
Elliptinone	-6.9	LYS5, GLN127, GLU290, ALA129, CYS128, TYR126, GLY124, VAL125, SER139
Emetine	-7.0	PHE103, VAL104, SER158, ASN151, THR111, GLN110, ARG105, GLN107, ILE106, ASP153, LYS102
Enoxacin	-7.4	THR292, ASP295, ARG298, GLN127, VAL104, LYS102, THR292, PHE8, GLN107, ILE106, PHE294
+(-)Epicatechin	-7.6	LYS102, VAL104, ARG105, ILE106, GLN107, THR111, THR292, PHE294, ASP285, GLN110, ASN151, ILE152, ASP153, PHE8, GLN127, ASP295
Imatinib	-7.4	ARG131, LYS137, GLN127, TYR126, LYS5, SER284, LEU286, LEU287, GLU288, PHE291, GLU290, ASP289, THR199, ASP197
Nelfinavir	-8.4	TRP207, ILE281, LEU282, PHE3, PHE291, GLN127, ARG4, GLY283, GLU288, LYS5, LYS137, TYR126, GLY138, TYR126, SER139, VAL135
Niclosamide	-7.1	ASP153, PHE8, GLN127, PHE112, THR111, ILE106, ARG105, VAL104, LYS102, SER158, ASN151, ASP295, PHE294, THR292, GLN110
Rhein	-8.1	LYS102, VAL104, ILE106 GLN110, THR29, THR111, PHE294, ASP295, GLN127, PHE8, ASN151, ILE152, ASP153, SER158
Scutellarein 7-rutinoside	-7.3	GLU178, ASP243, GLN116,VAL128, QLQ8, PHE9, TYR156, GLN128, ARG106
Withaferin A	-7.7	PHE294, THR292, ASP295, ASP153, SER158, LYS102, PHE103, GLU178, ARG105, ILE106, GLN110, THR111, GLN178, VAL108
Withanolide D	-7.8	LYS102, PHE103, VAL104, ARG105, ILE106, GLN107, GLN110, PHE294, PHE8, ASN151, TYR154, ASP153
27-Hydroxy withanolide	-7.3	GLU288, LYS137, TYR126, GLN127, VAL125, ALA7, MET6, LYS5, GLU290
24-Methyl cholesta-5,23E-dien-3beta-ol	-7.3	LYS104, THR294, ASP153, ARG105, VAL108, MET7, LYS72, LEU283, TYR124, VAL127, GLU242
17α-Hydroxy withanolide	-7.0	GLU288, GLU290, LYS137, GLY138, SER139, VAL125, TYR126, GLN127, CYS128, LYS5
Aswagandhanolide	-8.1	SER139, GLY138, LYS137, ARG131, GLN290, ASP289, GLU288, LEU286, LYS5, TYR126, VAL125, HIS172, GLY179

Deep understanding of phytochemicals for antiviral activities have assumed greater importance in the last few decades.[15] A wide variety of active phytochemicals, including the flavonoids, terpenoids, organosulfur compounds, limonoids, lignans, sulphides, polyphenolics, coumarins, saponins, chlorophyllins, furyl compounds, alkaloids, polyines, thiophenes, proteins and peptides have been found to have therapeutic applications against different genetically and functionally diverse viruses.

The antiviral mechanism of these agents may be explained on the basis of their antioxidant activities, scavenging capacities, inhibiting DNA, RNA synthesis, inhibition of the viral entry, or inhibiting the viral reproduction etc. Large number candidate substances such as phytochemicals and their synthetic derivatives have been identified by a combination of *in vitro* and *in vivo* studies in different biological assays.

The preliminary studies done till date are not approved for the therapeutic use against COVID-19 infected patients. Liu et al. (2020)[16] have successfully crystallised the main protease (MPro) of COVID-19, PDB-ID:6LU7, which is now accessible to the globe. 6LU7 represents a potential target for the inhibition of SARS-CoV2 replication. Therefore, in our study we have identified 19 potential inhibitors and 6 best compounds (Nelfinavir, Rhein, Withaferin A, Withanolide D, Enoxacin and Aloe-emodin) as potential inhibitor against COVID-19 major protease. These inhibitors can be repurposed against COVID-19 major protease to control the spread of Coronavirus.

MATERIAL AND METHODS

Data sources

In this study, a dataset of 100 FDA approved antiviral compounds and 1000 active phytochemicals were obtained from FDA and Indian Medicinal Plants, Phytochemistry, and Therapeutics database.[17–19]

Preparation of receptor/membrane transporter

The atomic coordinates of the protein, COVID-19 (PDB ID-6LU7) was downloaded from the RCSB PDB (protein data bank) database. Before analysis or docking, the charge assignment, solvation parameters and fragmental volumes to the protein was done using the Autodock Tool 4 (ADT).[20] The protein molecule was further optimized using Autodock Tool for the molecular docking.

Preparation of ligands

The 3D SDF structure of all the compounds were downloaded from Pubchem database[21] and 2D ligand structures were designed using Chemdraw program (Figure 3 and Table-3). The ligands were optimized using Avogadro and converted into the PDB format with the help of Open Babel. In order to further simplify the analysis, ligands were first optimized and converted to PDBQT format using the graphical user interface version of PyRx virtual screening tool-python prescription 0.8.

Compound screening using PyRx program

Molecular screening of all the compound libraries was performed using PyRx software by autodock wizard as the engine for docking.[22] During the docking period, the ligands were considered to be flexible and the protein was considered to be rigid. The configuration file for the grid parameters was generated using Auto Grid engine in PyRx. The application was also used to know/predict the amino acids in the active site of the protein that interact with the ligands. The results less than 1.0Å in positional root-mean-square deviation (RMSD) were

considered ideal and clustered together for finding the favourable binding. The highest binding energy (most negative) was considered as the ligand with maximum binding affinity.

Analysis and visualization

Visual analysis of the docking site was performed using Pymol version 2.3.4 and the results were validated using Autodock-Vina.[23]

Active site identification

Active site, amino acid residues in 6LU7 was identified using an online program using protein-ligand indentifier profiler (PLIP) BIOTEC Du Dresden.[24]

ADME analysis

On the basis of canonical SMILES of the selected ligands obtained from pubchem, ADME properties of the studied compound were calculated using online SwissADME program.[25] The major parameters for ADME associated properties such as Lipinski's rule of five, the solubility of the drug, pharmacokinetic properties and drug likeliness were considered. The values of the observe properties are presented in Table 3.

RESULTS AND DISCUSSION

Even when the rate of COVID-19 infection in China is currently declining for days, new shocking outbreaks are emerging in Italy, Indonesia, South Korea, India, Middle East, USA, UK and other part of Europe, with a major risk for a pandemic situation. The scientific community is hence called for a collaborative and extraordinary effort for a rapid identification of an effective anti-COVID-19 drug. In this matter, we hope that our contribution through drug repurposing against target COVID-19 major protease of novel coronavirus (COVID-19) can be of great help in such a worldwide endeavour.[26] Coronavirus belongs to a group of viruses which can infect humans and vertebrate animals. It has killed thousands of people around the globe with an increase in death rate every single day. The infection hampers liver, respiratory, central nervous system, and digestive of humans and animals. According to centers for disease control and prevention, human coronaviruses are very common throughout the world and causes mild to severe infections in humans. But this new virus SARS-CoV2 (COVID-19) is a public concern because not so much is known about its mechanism and function also the history of coronaviruses belonging to SARS and MERS have led to major illness to the humans.

Coronaviruses invade cells through so called spike (S) proteins. The S protein is the major surface protein that binds through a receptor that acts as a doorway into a human cell. Once the S protein and the surface of human cell membrane fuse together with the help of ACE-2 receptor, the genome of the virus is able to enter to the human cell and began infection. The virus dissolves its own protein coat and releases its RNA payload inside the cells. Coronavirus hijacks the cell structure to reproduce. The viral RNA takes over the hosts endoplasmic reticulum to replicate itself and manufacture the protein parts to make new viruses and leave the infected cell via the membrane. The completion of viral assembly is marked only when the M protein binds to the nucleocapsid, and these type of interactions mostly take place at the C-terminus of the endodomain of M with CTD3 of the N-protein. Following the assembly, transportation of the virions takes place to the host cell surface in vesicles and is released by exocytosis. Understanding the shape of the S protein and other key proteins such as Mpro is a doorway to generate medicines against these proteins to act against COVID-19 infection. Scientists at NIH, are employing an approach called vaccine rapid response platforms which involes hightech methods that have the potential to save years of development time while still the epidemic is still spreading. Presently there are no FDA approved medications for the novel coronavirus. The primary focus has been on clinical management which includes the prevention of infection, control measures and supportive care. Although an array of drugs approved for other infections as well as several investigational drugs are being studied in sevral clinical trials that are underway across the globe. Currently antimalarial drugs[27–29] like chloroquine, hydroxychloroquine have given positive results in the cell culture systems but has to proceed for the clinical trials to be established as an effective drug. Several other drugs such as remedesivir, lopinavir-ritonavir, favilavir, and many other vaccines are under rapid study to use against coronavirus infection. Our study was focused on the drug repurposing against the main protease in coronavirus (3CLpro/Mpro), (PDB-ID:6LU7), as a potential therapeutic target for the treatment of novel coronavirus (COVID-19). 6LU7

Amino Acid Sequence (FASTA) of Main Protease of SARS-CoV2-PDB-ID: (6LU7)

SGFRKMAFPSGKVEGCMVQVTCGTTTLNGLWLDDVVYCPRHVICT
SEDMLNPNYEDLLIRKSNHNFLVQAGNVQLRVIGHSMQNCVLKLK
VDTANPKTPKYKFVRIQPGQTFSVLACYNGSPSGVYQCAMRPNFTI
KGSFLNGSCGSVGFNIDYDCVSFCYMHHMELPTGVHAGTDLEGNF
YGPFVDRQTAQAAGTDTTITVNVLAWLYAAVINGDRWFLNRFTTT
LNDFNLVAMKYNYEPLTQDHVDILGPLSAQTGIAVLDMCASLKELL
QNGMNGRTILGSALLEDEFTPFDVVRQCSGVTFQ

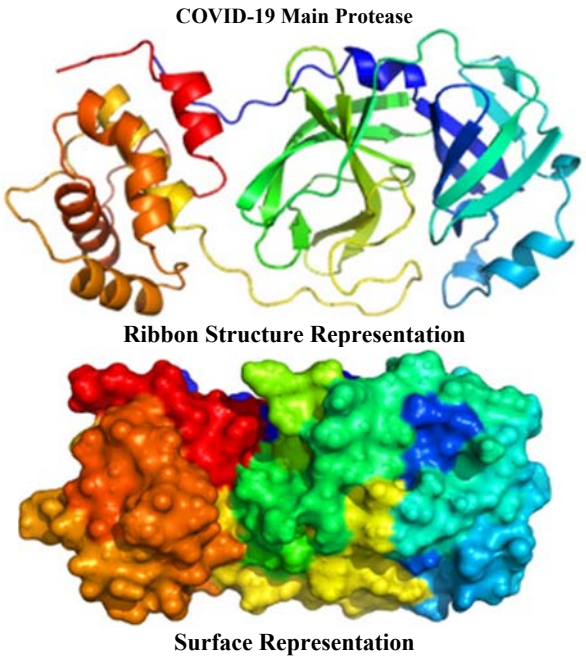


Figure 1 (a): Figure shows the FASTA sequence and crystal structure of COVID-19 main protease (PDB-ID:6LU7).

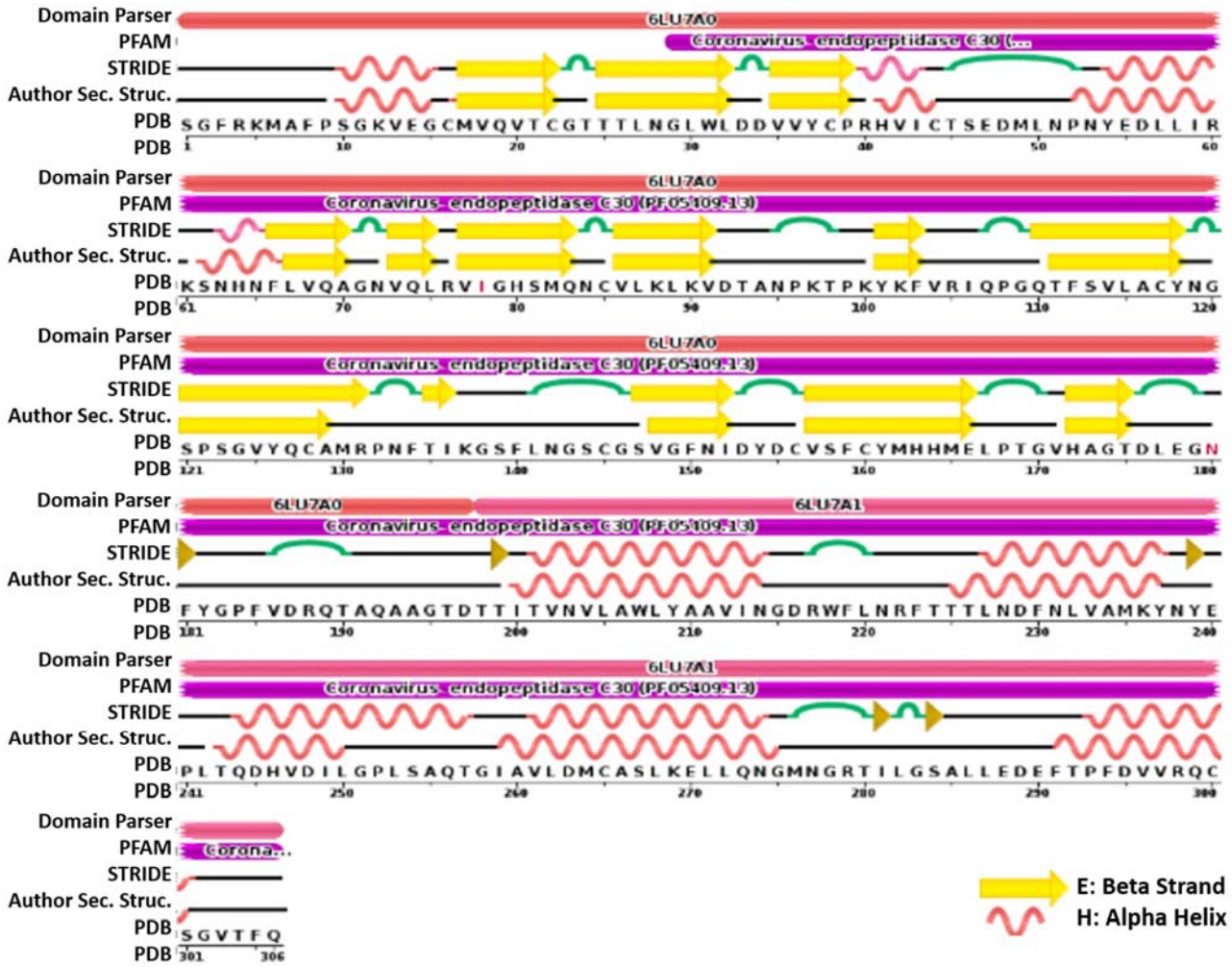


Figure 1 (b). Figure shows the detailed amino acid sequence and structural representation of COVID-19 main protease (6LU7) of functional domain.

is the major protease (Mpro) in COVID-19 that has been repositioned and structured in PDB recently and is accessible online. As shown in Figure 1(a), it represents the amino acid sequence (FASTA) of COVID-19 Main Protease (6LU7); 3D ribbon structure and surface representation. Figure 1(b) shows the detailed amino acid sequence and secondary structure of SARS-CoV2 Main Proteases, where Domain Parser analysis suggest 197 residues in 6LU7A0 chain and 109 residues in 6LU7A1 ; PFAM domain assignment from Sequence analysis suggest that 278 residues in C30 chain. The secondary structure analysis by STRIDE suggest that 26% of 6LU7 structure is helical (9 helices; 82 residues) and 29% is consist of beta sheet structure (19 strands; 90 residues).

Whereas, the Author Secondary Structure analysis suggest that 6LU7 consists of 27% helical structure (9 helices; 84 residues) and 25% beta sheet structure (13 strands; 78 residues). The Mpro in coronavirus is very important for the proteolytic maturation of the virus. Mpro has been examined as a potential target to prevent the spread of infection by inhibiting viral polyprotein cleavage through blocking active sites of the protein (Figure 2).

The active site in the protein 6LU7 consists of two chains; (i)

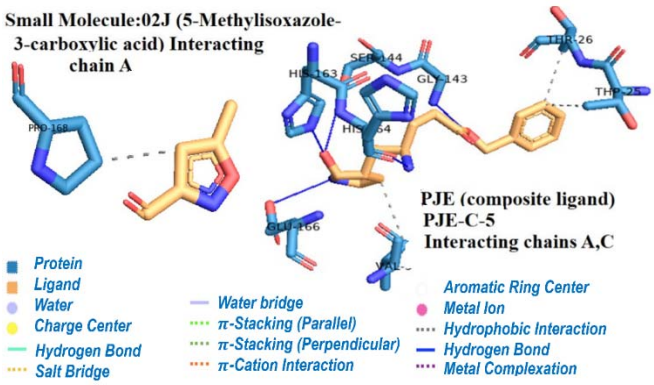


Figure 2. Represents the active sites (Ligand binding) in 6LU7. 6LU7 has 2 interacting chains. (a) Small molecule- 02J (5methylisoxazole-3-carboxylic acid) Interacting chain A (b) PJE (composite ligand) PJE-C-5 Interacting chains A, C.

small molecule- 02J (5-methylisoxasole-3-carboxylic acid) with PRO168 amino acid residue present in the active site. (ii) PJE-C-5-: composite ligand with THR26, VAL3, GLY143, SER144, HIS163, HIS164, GLU166 amino acid residues present in the active side (Table 1). With this new discovery of Mpro structure

in COVID-19, it has provided an immense opportunity to identify potential drug candidates for the treatment of coronavirus.[30] In our study, we have applied a computational approach of drug repurposing in order to identify a specific therapeutic agent against COVID-19. We have created a database of 100 FDA approved antiviral compounds and 1000 active phytochemicals from plants. The compounds were screened using PyRx virtual tool 19 compounds were selected based on their binding affinity with COVID-19 major protease (6LU7). Further, molecular docking was performed with the 19 compounds against COVID-19 (Mpro) structure to validate the findings.[31,32]

In Molecular Docking study, the binding energy suggests the affinity of a specific ligand and strength by which a compound interacts with and binds to the pocket of a target protein. A compound with a lower binding energy is preferred as a possible drug candidate. In order to understand the effect of active antiviral and phytochemicals compounds on COVID-19, molecular docking of 19 active phytochemicals and FDA approved antiviral compounds was performed against COVID-19 (Table-2). Docking results of COVID-19 major protease (6LU7) with selected 6 compounds (Nelfinavir, Rhein, Withanolide D, Withaferin A, Enoxacin, Aloe-emodin) showed favourable binding affinity and were found to be best molecules at the target site of the protein. The chemical structure of the selected 6 lead compounds is shown in Figure 3.

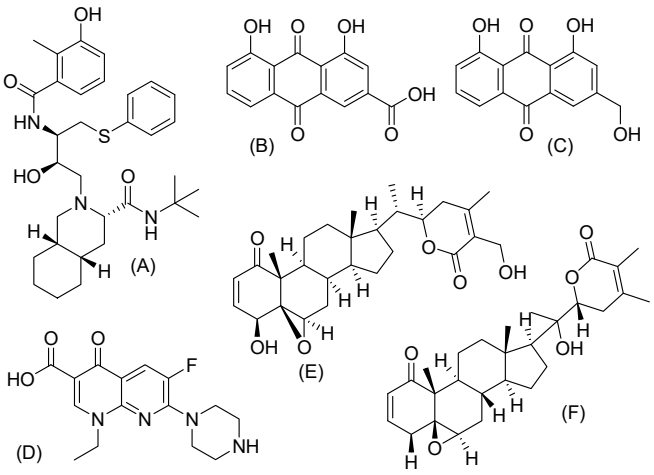


Figure 3. Chemical structures of lead compounds: (A) Nelfinavir; (B) Rhein; (C) Aloe-emodin; (D) Enoxacin; (E) Withaferin A; (F) Withanolide D.

The Molecular Docking analysis and visualisation of 6LU7 binding with Enoxacin (A), Withaferin A (B), Nelfinavir (C), Withanolide D (D), Rhein (E), Aloe-emodin is shown in Fig. 4.

Out of the 6 compounds, Nelfinavir exhibited the best docked score (-8.4 Kcal/mol) with SARS-CoV2 Main Proteases.[33] TRP207, ILE281, LEU282, PHE3, PHE291, GLN127, ARG4, GLY283, GLU288, LYS5, LYS137, TYR126, GLY138, TYR126, SER139 and VAL135 are the amino acid residues participating in the interaction at the binding pocket of COVID-19 (Figure 5).

Nelfinavir is one of the recently identified anti-retroviral drug against HIV. It is a protease inhibitor used to limit the replication of the virus and boost immune function in individuals affected

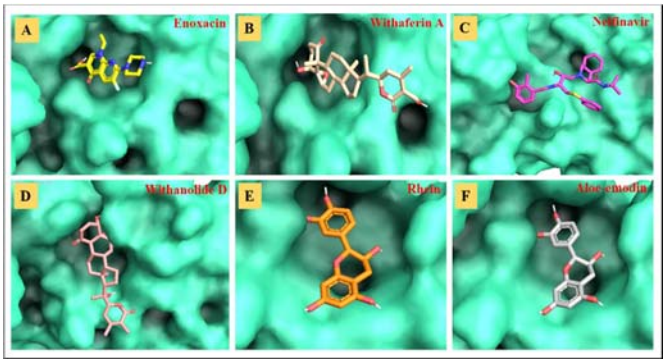


Figure 4. Docking analysis and visualization of 6LU7 binding with Enoxacin (A), Withaferin A (B), Nelfinavir (C), Withanolide D (D), Rhein (E), Aloe-emodin

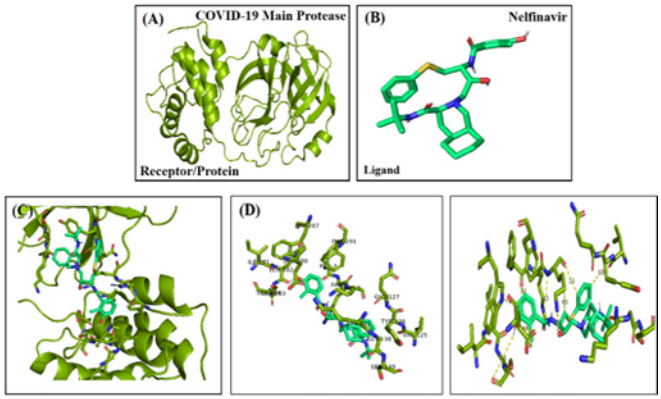


Figure 5. Molecular docking analysis between 6LU7 and Nelfinavir. (A) 3D ribbon structure of Receptor/Protein (6LU7). (B) 3D ligand structure (Nelfinavir). (C) Protein-ligand Interaction. (D) Interaction between the active site residues of the protein and ligand.

with HIV. Nelfinavir is a nucleoside reverse transcriptase inhibitors (NRTIs) which is evaluated as first-line therapy in HIV patients.[34,35] Withanolides are a group of naturally occurring steroids which are oxygenated and are present in medicinal plants of solanaceae family. Formulations of withanolides have been explored in various pharmacological activities including immunomodulatory, antioxidant, antibacterial, antiviral, antitumor, angiogenesis inhibitor, hypnosedative and antiarthritic,[36] Withanolide D exhibited (-7.8Kcal/mol) binding affinity with 6LU7. LYS102, PHE103, VAL104, ARG105, ILE106, GLN107, GLN110, PHE294, PHE8, ASN151, TYR154, ASP153 are the amino acid residues participating in the interaction at the binding pocket of 6LU7 (Figure 6).

Rhein showed (-8.1Kcal/mol) binding affinity with 6LU7. LYS102, VAL104, ILE106 GLN110, THR29, THR111, PHE294, ASP295, GLN127, PHE8, ASN151, ILE152, ASP153, SER158 are the amino acid residues participating in the interaction at the binding pocket of 6LU7. Rhein (4, 5-dihydroxyanthraquinone-2-carboxylic acid) is extensively found in several medical herbs, such as *Cassia tora* L., *Rheum palmatum* L., *Aloe barbadensis* Miller., and *Polygonum multiflorum* Thunb, which have been used medically in China for over a decade. It is a lipophilic anthraquinone and has many anti-inflammatory effects, including anticancer, hepatoprotective,

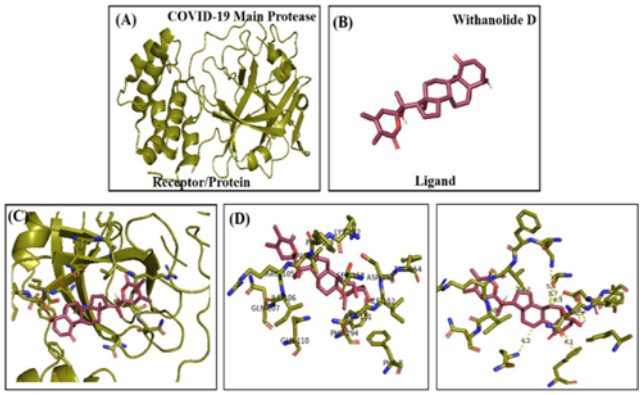


Figure 6. Molecular docking analysis between 6LU7 and Withanolide D. (A) 3D ribbon structure of Receptor/Protein (6LU7). (B) 3D ligand structure (Withanolide D). (C) Protein-ligand Interaction. (D) Interaction between the active site residues of the protein and ligand.

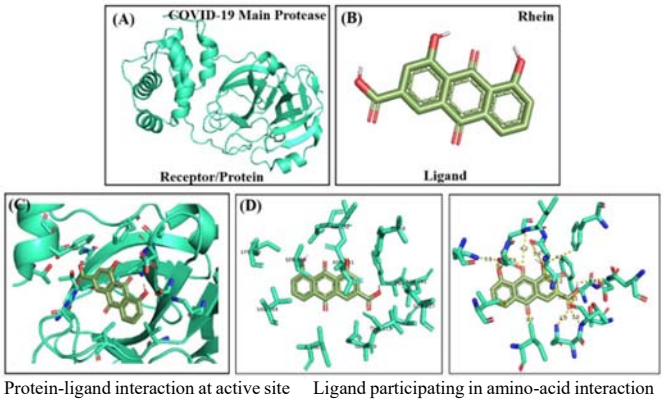


Figure 7: Molecular docking analysis between 6LU7 and Rhein. (A) 3D ribbon structure of Receptor/Protein (6LU7). (B) 3D ligand structure (Rhein). (C) Protein-ligand Interaction. (D) Interaction between the active site residues of the protein and ligand.

antioxidant, nephroprotective, pharmacological effects, and antimicrobial activities.[37] (Figure 7).

Withaferin A exhibited (-7.7Kcal/mol) binding affinity with 6LU7. PHE294, THR292, ASP295, ASP153, SER158, LYS102, PHE103, GLU178, ARG105, ILE106, GLN110, THR111, GLN178, VAL108 are the amino acid residues participating in the interaction at the binding pocket of 6LU7. Withaferin A is an active component and phytoconstituent of *Withania somnifera*. It has been explored as a therapeutic potential and is significantly validated for various pharmacological activities including neurological, cardioprotective, immunomodulatory, anti-cancer, anti-stress, neuroprotective activities (Figure 8).

Enoxacin exhibited (-7.4Kcal/mol) binding affinity with 6LU7. ASP295, PHE294, THR292, GLY109, THR111, ILE106, VAL104, ASN151, ASP153, GLN110, PHE112, ILE152, PHE8, PHE112 are the amino acid residues participating in the interaction at the binding pocket of 6LU7. Enoxacin belongs to the class of 4-quinolone. It has been recently identified as anti-cancer, anti-inflammatory and anti-bacterial agent[38] (Figure 9).

Aloe-emodin exhibited (-7.4Kcal/mol) binding affinity with 6LU7. Aloe-emodin belongs to the class of anthraquinones and

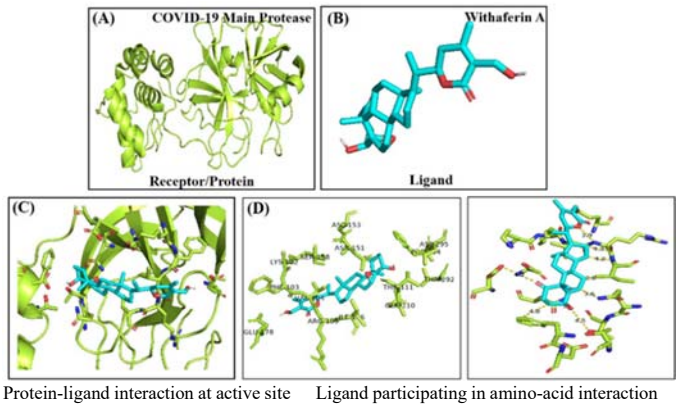


Figure 8. Molecular docking analysis between 6LU7 and Withaferin A. (A) 3D ribbon structure of Receptor/Protein (6LU7). (B) 3D ligand structure (Withaferin A). (C) Protein-ligand Interaction. (D) Interaction between the active site residues of the protein and ligand.

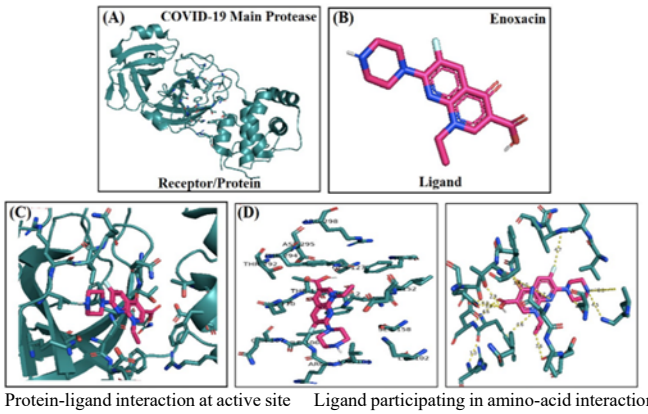


Figure 9: Molecular docking analysis between 6LU7 and Enoxacin. (A) 3D ribbon structure of Receptor/Protein (6LU7). (B) 3D ligand structure (Enoxacin). (C) Protein-ligand Interaction. (D) Interaction between the active site residues of the protein and ligand.

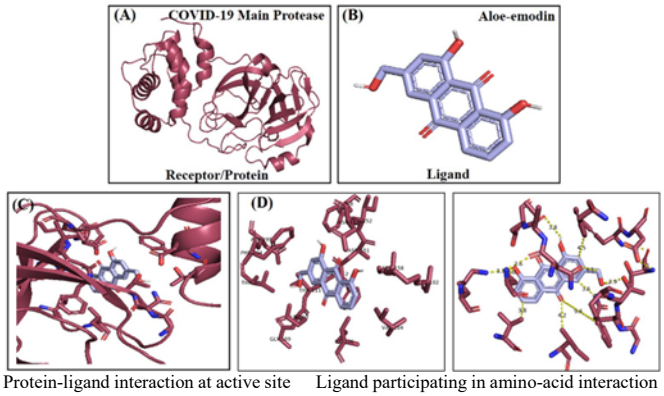
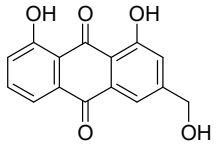
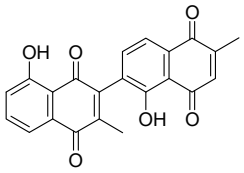
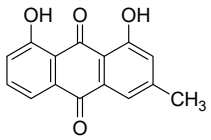
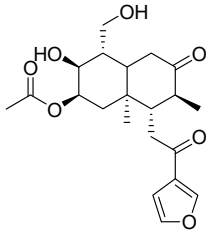
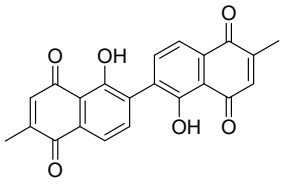
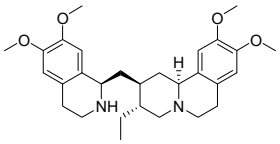
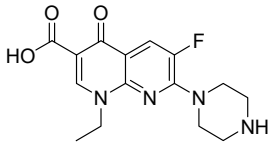
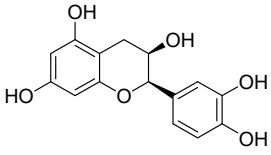
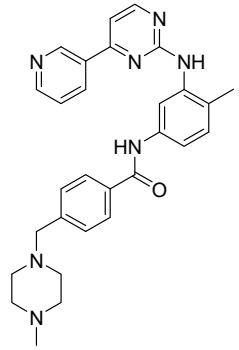
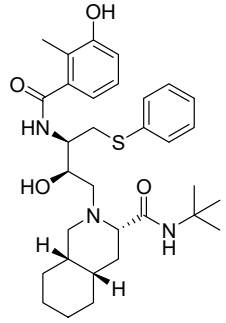
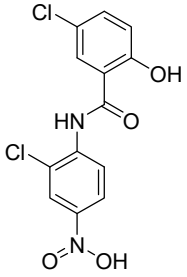
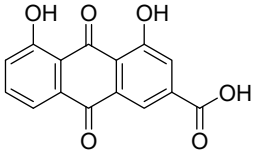
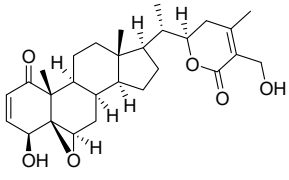
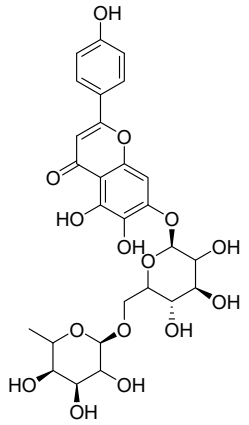
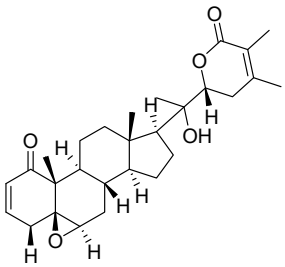
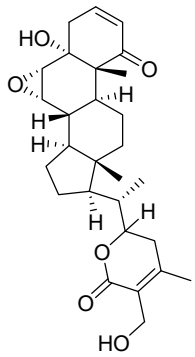
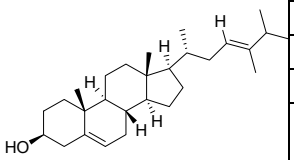
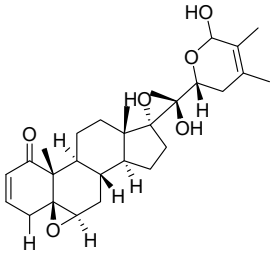


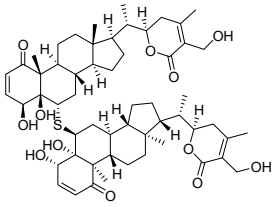
Figure 10. Molecular docking analysis between 6LU7 and Aloe-emodin. (A) 3D ribbon structure of Receptor/Protein (6LU7). (B) 3D ligand structure (Aloe-emodin). (C) Protein-ligand Interaction. (D) Interaction between the active site residues of the protein and ligand

possesses multiple anti-carcinogenic, anti-proliferative and anti-viral actions on humans[39] (Figure 10). The molecular docking analysis in the present study showed the inhibitiory potential of 6 compounds, ranked by affinity (ΔG); Nelfinavir > Rhein > Withanolide D> Withaferin A> Enoxacin>Aloe-Emodin.

Table-3: ADME Properties of selected COVID-19 major protease inhibitors

Compound	ADME Properties (Lipinki's Rule of Five)	
	Properties	**Value**
Aloe-emodin $C_{15}H_{10}O_5$ 	Molecular weight (<500Da)	270.24
	LogP (<5)	1.5
	H-Bond donor (5)	3
	H-bond acceptor (<10)	5
	Violations	0
Chitranone $C_{22}H_{14}O_6$ 	Molecular weight (<500Da)	374.34
	LogP (<5)	2.8
	H-Bond donor (5)	2
	H-bond acceptor (<10)	6
	Violations	0
Chrysophanol $C_5H_{10}O_4$ 	Molecular weight (<500Da)	254.24
	LogP (<5)	2.3
	H-Bond donor (5)	2
	H-bond acceptor (<10)	4
	Violations	0
Diterpene $C_{21}H_{28}O_7$ 	Molecular weight (<500Da)	392.44
	LogP (<5)	1.6
	H-Bond donor (5)	2
	H-bond acceptor (<10)	7
	Violations	0
Elliptinone $C_{22}H_{14}O_6$ 	Molecular weight (<500Da)	374.34
	LogP (<5)	2.9
	H-Bond donor (5)	2
	H-bond acceptor (<10)	6
	Violations	0
Emetine $C_{29}H_{40}N_2O_4$ 	Molecular weight (<500Da)	480.64
	LogP (<5)	4.1
	H-Bond donor (5)	1
	H-bond acceptor (<10)	6
	Violations	0
Enoxacin $C_{15}H_{17}FN_4O_3$ 	Molecular weight (<500Da)	320.32
	LogP (<5)	0.9
	H-Bond donor (5)	2
	H-bond acceptor (<10)	6
	Violations	0
+(-)Epicatechin $C_{15}H_{14}O_6$ 	Molecular weight (<500Da)	290.27
	LogP (<5)	0.8
	H-Bond donor (5)	5
	H-bond acceptor (<10)	6
	Violations	0
Imatinib $C_{29}H_{31}N_7O$ 	Molecular weight (<500Da)	493.60
	LogP (<5)	3.38
	H-Bond donor (5)	2
	H-bond acceptor (<10)	8
	Violations	0
Nelfinavir $C_{32}H_{45}N_3O_4S$ 	Molecular weight (<500Da)	567.78
	LogP (<5)	4.3
	H-Bond donor (5)	4
	H-bond acceptor (<10)	5
	Violations	1
Niclosamide $C_{13}H_8Cl_2N_2O_4$ 	Molecular weight (<500Da)	327.12
	LogP (<5)	2.9
	H-Bond donor (5)	2
	H-bond acceptor (<10)	4
	Violations	0
Rhein $C_{15}H_8O_6$ 	Molecular weight (<500Da)	284.22
	LogP (<5)	1.4
	H-Bond donor (5)	3
	H-bond acceptor (<10)	6
	Violations	0
Withaferin A $C_{28}H_{38}O_6$ 	Molecular weight (<500Da)	470.60
	LogP (<5)	3.4
	H-Bond donor (5)	2
	H-bond acceptor (<10)	6
	Violations	0

Scutellarein 7- rutinoside $C_{27}H_{30}O_{15}$ 	Molecular weight (<500Da)	594.52
	LogP (<5)	-1.10
	H-Bond donor (5)	9
	H-bond acceptor (<10)	15
	Violations	3
Withanolide D $C_{28}H_4O_7$ 	Molecular weight (<500Da)	488.61
	LogP (<5)	2.1
	H-Bond donor (5)	4
	H-bond acceptor (<10)	7
	Violations	0
27-hydroxywithanolide $C_{28}H_{38}O_6$ 	Molecular weight (<500Da)	470.60
	LogP (<5)	3.4
	H-Bond donor (5)	2
	H-bond acceptor (<10)	6
	Violations	0
24-Methylcholesta-5,23E-dien-3beta-ol $C_{28}H_{46}O$ 	Molecular weight (<500Da)	398.66
	LogP (<5)	6.7
	H-Bond donor (5)	1
	H-bond acceptor (<10)	1
	Violations	1
17-alpha-hydroxywithanolide D $C_{28}H_{40}O_7$ 	Molecular weight (<500Da)	480.64
	LogP (<5)	4.1
	H-Bond donor (5)	1
	H-bond acceptor (<10)	6
	Violations	0

Ashwagandhanolide $C_{56}H_{78}O_{12}S$ 	Molecular weight (<500Da)	975.28
	LogP (<5)	5.8
	H-Bond donor (5)	6
	H-bond acceptor (<10)	12
	Violations	3

Lipinski's rule of five is a major criterion to evaluate drug likeliness and if a particular chemical compound with a certain biological and pharmacological activity has physical and chemical properties that would make it a likely orally active drug in humans. Lipinski's rule determines the molecular properties which are important for a drug's pharmacokinetics in the human body such as absorption, distribution, metabolism, and excretion (ADME). Lipinski's rule of five criteria for an ideal drug are (i) a molecular mass less than 500 daltons, (ii) no more than 5 hydrogen bond donors, (iii) no more than 10 hydrogen bond acceptors, (iv) an octanol–water partition coeffcient log P not greater than 5. Three or more than 3 violations do not fit into the criteria of drug likeliness and it is not considered in order to proceed with drug discovery. ADME studies of selected 19 compounds showed that out of 19, 15 virtual hits were successful at passing through these ADME test filters (Table 3).

This preliminary screening of potential molecules (Anti-viral, natural products, anti-malarials) would help in providing the fast in-silico analysis towards development of therapeutics for SARS-CoV2 (COVID-19).

CONCLUSION

The drug repurposing approach would be the fast and most approapriate option to find therapeutic solutions for the SARS-CoV2, the Novel Coronavirus (COVID-19). The bioinformatics approach could be a very useful tool to identify potent inhibitors against the Novel Coronavirus. In this study, we have used Bioinformatics tools, PyRx and Autodock-Vina to identify potent FDA approved inhibitors against COVID-19 Main Proteases, which play crucial role in Coronavirus propagation. We have identified 19 potent inhibitors from the library of thousands of compounds and found Nelfinavir, Rhein, Withanolide D, Withaferin A, Enoxacin and Aloe-emodin as significantly appropriate inhibitors against COVID-19 Main Proteases. Our findings suggest that the Nelfinavir, Withanolide D and Withaferin A can be used as protential inhibitors against COVID-19 Main Proteases, which can be further explored to test against Coronavirus (COVID-19) in pre-clinical and clinical settings.

ACKNOWLEDGMENTS

Authors acknowledge Department of Science and Technology-Science and Engineering Research Board (DST-SERB), Government of India for partial financial support.

Conflict of Interest: Authors declare no conflict of interest.

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