Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

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ORIGINAL ARTICLE

Targeting SARS-CoV-2 Main Protease: A Computational Drug Repurposing Study

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Background and Aims. Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2) induced Novel Coronavirus Disease (COVID-19) has currently become pandemic worldwide. Though drugs like remdesivir, favipiravir, and dexamethasone found beneficial for COVID-19 management, they have limitations clinically, and vaccine development takes a long time. The researchers have reported key proteins which could act as druggable targets. Among them, the major protease M^{pro} is first published, plays a prominent role in viral replication and an attractive drug-target for drug discovery. Hence, to target M^{pro} and inhibit it, we accomplished the virtual screening of US-FDA approved drugs using well-known drug repurposing approach by computer-aided tools.

Methods. The protein M^{pro}, PDB-ID 6LU7 was imported to Maestro graphical user interphase of Schrödinger software. The US-FDA approved drug structures are imported from DrugBank and docked after preliminary protein and ligand preparation. The drugs are shortlisted based on the docking scores in the Standard Precision method (SP-docking) and then based on the type of molecular interactions they are studied for molecular dynamics simulations.

Results. The docking and molecular interactions studies, five drugs emerged as potential hits by forming hydrophilic, hydrophobic, electrostatic interactions. The drugs such as arbutin, terbutaline, barnidipine, tipiracil and aprepitant identified as potential hits. Among the drugs, tipiracil and aprepitant interacted with the M^{pro} consistently, and they turned out to be most promising.

Conclusions. This study shows the possible exploration for drug repurposing using computer-aided docking tools and the potential roles of tipiracil and aprepitant, which can be explored further in the treatment of COVID-19.

Key Words: COVID-19, SARS-CoV-2, In silico, Repurposing, M^{pro}, Docking.

Introduction

COVID-19 is a respiratory infection by SARS-CoV-2 originally seen in Wuhan city of China, now become the pandemic worldwide (1). In the past, coronavirus caused epidemics such as SARS and MERS (Middle East Respiratory Syndrome) in 2013 and 2018, respectively in China, and Saudi Arabia (2). COVID-19 was first recognized as intense pneumonia in a medical facility in Wuhan, Hubei Province of China. Later the virus was renamed as SARS-CoV-2. Within a few months, the epidemic spread globally and declared as a pandemic by WHO. The signs and symptoms of COVID-19 include fever, cough, weakness, headache, haemoptysis, diarrhoea, dyspnoea, and lymphopenia. The clinical features identified by chest CT-scans are pneumonia with unusual features such as RNAaemia, acute respiratory distress syndrome, the occurrence of the ground-glass opacity and severe end-organ injury that lead to the death of infected human (3).

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The spread of virus is very rapid, the mortality rate of COVID-19 is low compared to SARS and MERS. Currently, there is no curative therapy available, and the vaccines have not been developed. The main reason being the poorly understood pathology of COVID-19 and the differences across the geography besides the mutations in virus (4). The SARS-CoV-2 spreads mainly through respiratory droplets or droplet nuclei between individuals (5). Hence, social distancing and preventing the spread by respiratory droplets has become the mainstay of COVID-19 management rather than the drug treatment and vaccination (6).

Many drug discovery groups have applied the drug repurposing or repositioning to speed-up and bring out curative therapy (7). The repurposed drugs remdesivir and favipiravir are currently approved by US-FDA for treatment of COVID-19 act by inhibiting the viral RNA-dependent RNA polymerase (RdRp). These drugs have limitation in terms of its cost-effectiveness and adverse drug reactions such as hyperuricaemia, teratogenicity and QT-prolongation (8,9). Similarly, the anti-inflammatory and immunosuppressant class of drugs corticosteroids, such as dexamethasone, is approved by US-FDA. The corticosteroids have limitations in terms of its dose-dependent toxicities and drug-disease interactions.

Target selection and validation are the crucial steps in drug repurposing. One of the recently reported druggable target major protease M^{pro} is the first protein crystallography structure published in PDB on January 26, 2020 (10). M^{pro} is also known as 3-chymotrypsin-like protease ($3CL^{pro}$). Currently, the target M^{pro} is validated, and some studies have reported drug repurposing (11−15). None of these studies is focussed on FDA approved drugs for repurposing. Hence, in this study, we tried drug repurposing using FDA approved drugs. We discovered a new compounds, whose binding could have potentials as SARS-CoV-2 replication inhibitors with less toxic and affordable compared to the currently available drugs.

Materials and Methods

Computational Simulations

The computational simulations were carried out in Maestro graphical user interphase of Schrödinger (www.schrodinger.com) on a desktop workstation with Ubuntu platform, with Intel® Xenon® Gold 6130 CPU @ 2.10 GHz x 64 processors, Quadro P620/PCIe/SSE2 graphics card and 134.8 GB RAM.

Ligand Preparation

The US-FDA approved drugs (2800) were downloaded from DrugBank database (www.drugbank.ca), were optimized using LigPrep (16). By using LigPrep the 3D coordinates for the molecules were generated. The Epik module predicted the ionization state at pH 7.4, the tautomer forms were generated, and the chirality was defined. Finally, drug-molecules were geometry minimized using OPLS3e force-field (17).

Protein Preparation

The protein structure of major protease M^{pro} coordinate with accession code 6LU7 was downloaded from Protein Data Bank (10). The protein structure was optimized using Protein Prep Wizard (PPW) tool in Shrodinger software (18). The missing hydrogens, side chains, and residues were added, waters were deleted. Using PROPKA tool, the correct ionization state was generated at pH 7.4, and the hydrogen bond network was regenerated, and the protein structure was minimized (19,20).

Molecular Docking

For molecular docking, the Glide module in Schrodinger was used (21). The binding site was identified using the Receptor Grid generation module with the default option. The centroid of the bound ligand was considered for Grid generation. The High Throughput Virtual Screening (HTVS) mode was initially followed for 2800 drugs, and top 500 drugs shortlisted based on docking score are screened by Standard Precision mode (SP-docking). Finally, the single best pose was saved for each molecule (22).

Molecular Dynamics Simulation

Based on the molecular interaction and visual analysis of SP-docking results, the top-ranked drugs were taken for the Molecular Dynamics simulation (MD-simulation) study. The MD-simulation was run on Desmond Module (23). The water-soaked solvent system was used to predictions. Using the System Builder tool in Desmond, the water-soaked solvated system was generated. The TIP3P model of water considered for solvating the system. The orthorhombic simulation was the box with periodic boundary condition generated with a buffer distance of minimum 10 Å from the outer surface of the protein. The system was neutralized by adding a suitable number of counter-ions. The isosmotic condition was maintained by adding 0.15 M NaCl to the simulation box. A predefined equilibration protocol was run before the production run of the simulation. The MD simulation was run at 300°K temperature at atmospheric pressure of 1.013 bar. A total of 100 nsec simulation was run during which 1000 frames were saved to the trajectory. The Simulation Interaction Diagram was used for the analysis of the MD-simulation trajectory.

Table 1. Shortlisted drugs by SP-docking for targeting M^{pro} of SARS-CoV-2

S. No	Name of molecule	Current therapeutic indications	Docking score	Molecular interactions	
				H-bond-forming interactions	π-π stacking or π-cation interaction
1	Aprepitant	Chemotherapy-induced emesis, Postoperative nausea and vomiting	−6.892	-	HIE41
2	Barnidipine	Hypertension	−6.421	ASN142, CYS145, GLU166(2)	HIE41
3	Tipiracil	Bioavailability enhancer of Triflurudine in Colorectal cancer	−6.331	HIS164, HIS166	HIE41
4	Arbutin	Skin-lightening agent, Hyperpigmentation	−6.206	ASN142(2)	HIE41
5	Terbutaline	Asthma, Wheezing, COPD	−5.846	GLY143, HIS164, GLN189	HIE41

Residues ASN: Aspargine; CYS: Cysteine; GLU: Glutamic acid; HIS: Histidine; GLY: Glycine; GLN: Glutamine; HIE: Histidine (HISε).

Results

Results of Computational Simulations and Docking

Initial docking of 2800 drugs was to recognize how drugs bind to target major protease M^{pro}. The docking findings provided sufficient knowledge on binding affinity and orientation of ligand-protein interactions to inhibit protein activity. In the SP-docking on 500 top drugs were able to interact via hydrogen bonding with the active site of M^{pro}. Based on docking score and visual interpretations, the

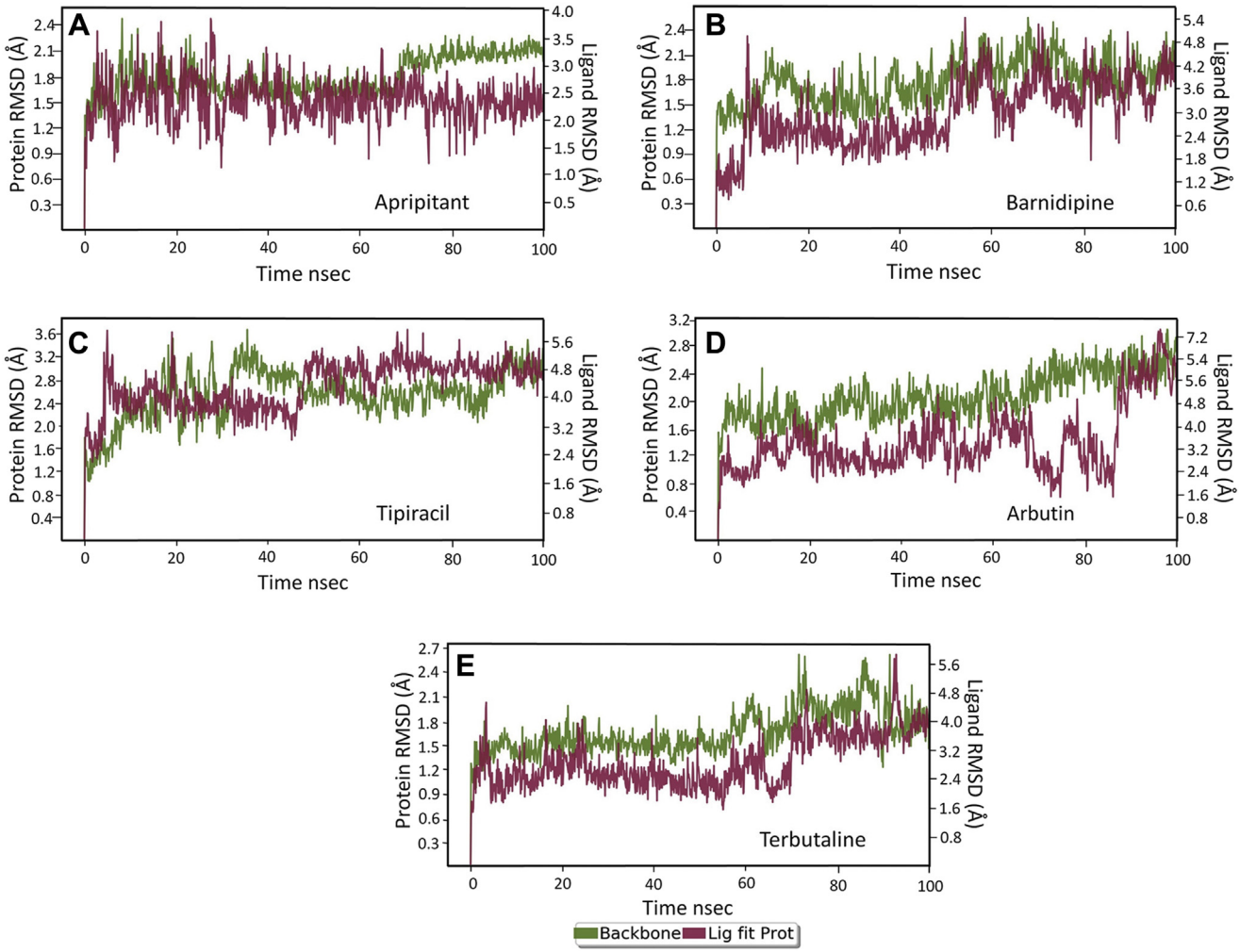

Figure 1. The Root Mean Square Deviations (RMSD) plots. RMSDs for (A) Apripitant; (B) Barnidipine; (C) Tipiracil; (D) Arbutin; (E) Terbutaline; Green colour represents protein backbone fluctuations; red colour represents ligand fluctuations.

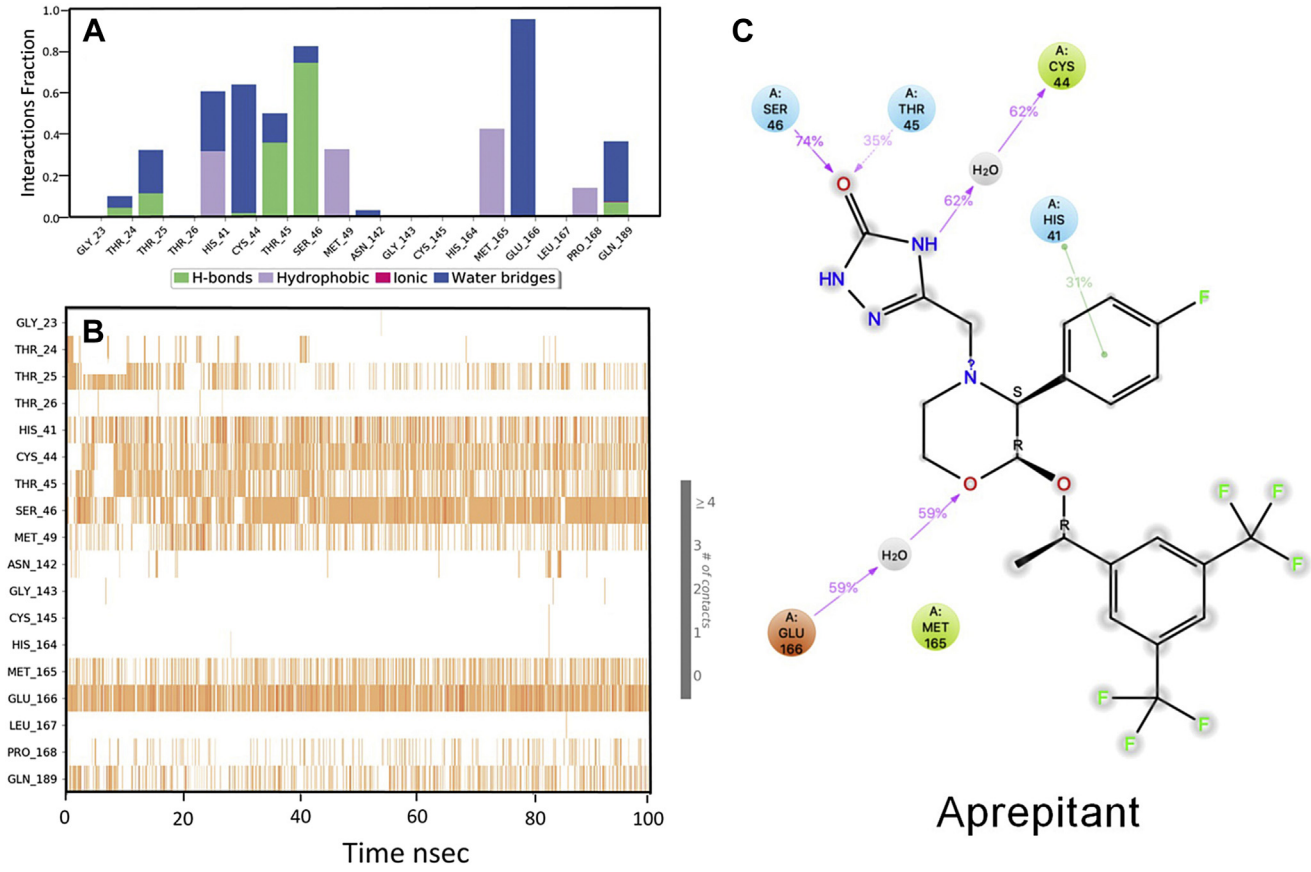

Figure 2. Interaction diagram of aprepitant with M^{pro}. (A) The protein-ligand contacts showing the bonding interactions fraction. (B) Interaction of aprepitant with residues in each trajectory frame. The darker colour higher the interaction or the residues make more contact; (C) Aprepitant interaction with the protein residues during MD simulation. Interactions shown are occurring more than 30% during the simulation time.

drugs aprepitant, barnidipine, tipiracil, arbutin, and terbutaline were found to be significantly interacting with M^{pro} residues compared to all other drugs (Table 1).

The drug aprepitant made a π-π stacking interaction with histidine (HIE41) residue of M^{pro}. The drug barnidipine made hydrogen bonding interactions with aspargine (ASN142), cysteine (CYS145), glutamic acid (GLU166) residues and a π-π stacking interaction with HIE41. Tipiracil made hydrogen bonding interactions with HIS164 residue and a π-π stacking interaction with HIS41 of M^{pro}. The arbutin made a π-π stacking interaction with HIE41, two hydrogen bonding interaction with ASN142. Terbutaline exhibited hydrogen bonding interactions with glycine (GLY143), HIS164 and glutamine (GLN189) residues of M^{pro} a π-π stacking interaction with HIE41 residue. Further, these five drugs had significant interactions with M^{pro} by visual investigation of their 2D and 3D poses (Supplementary Figure 1−5).

Results of Molecular Dynamics Simulation

MD-simulation was performed to validate the stability of the binding mode predicted by Glide docking of FDA approved drugs to major protease M^{pro}. The data provided adequate information on structural changes in the form of conformations and ligand-protein interactions. The MD simulation conducted for five drugs, aprepitant, barnidipine, tipiracil, arbutin and terbutaline. The Root Mean Square Deviations (RMSD) plot analysis of the structures saved in the trajectory produced during the MD-simulation exhibited strong and stable binding with M^{pro} for the aprepitant and tipiracil where the RMSD-fluctuations for the ligand and protein remained within 2.0 Å. The barnidipine and terbutaline demonstrated strong binding ability and arbutin demonstrated the least stable binding (Figure 1).

The M^{pro}-bound aprepitant exhibited good binding mode during MD-simulation. The plot of the backbone of the protein structures enumerated during the MD-simulation aligned to the initial structure for the RMSD analysis. The protein-ligand complex was stable for the entire simulation period, indicates the binding stability of the ligand with the protein (Figure 1A). During the MD-simulation, the residues CYS44, threonine (THR45) and GLU166 of M^{pro} exhibited H-bond interaction with the ligand aprepitant. Bridged hydrogen bonding interaction was observed with residues

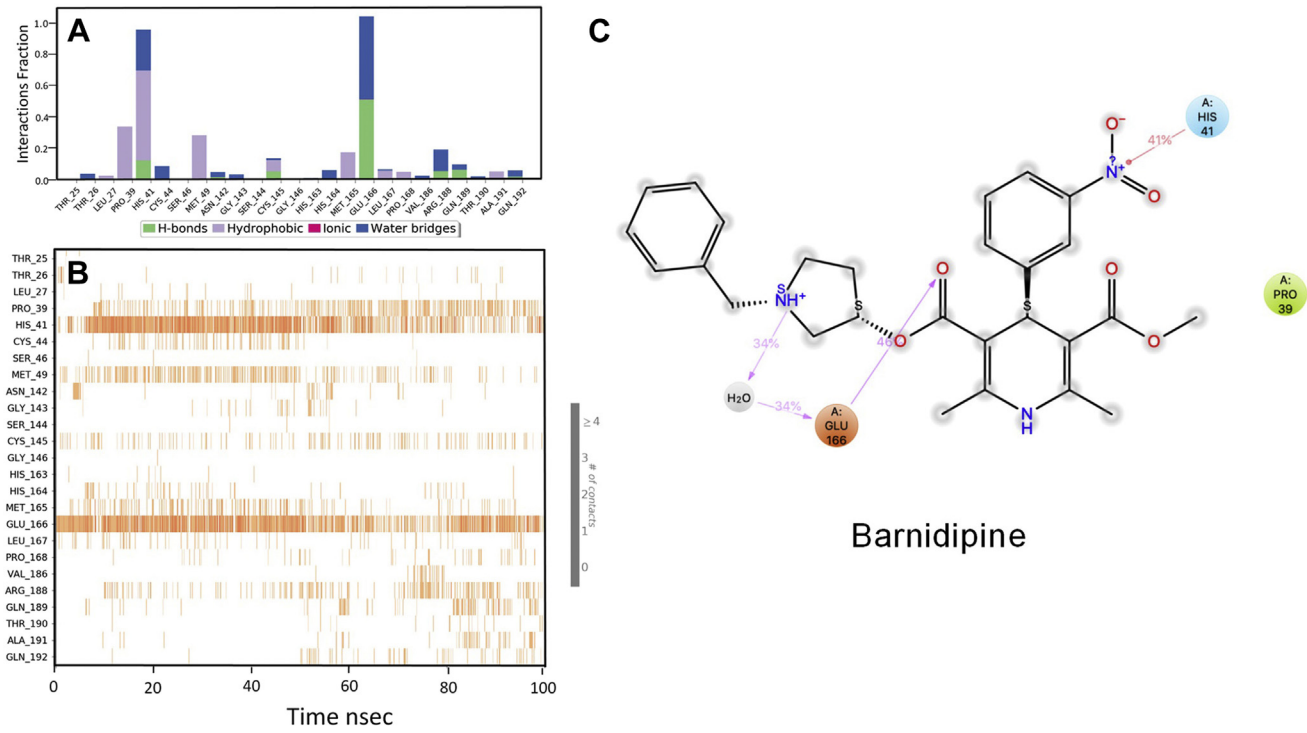

Figure 3. Interaction diagram of barnidipine with M^{pro}. (A) The protein-ligand contacts showing the bonding interactions fraction. (B) Interaction of barnidipine with residues in each trajectory frame. The darker colour higher the interaction or the residues make more contact; (C) Barnidipine interaction with the protein residues during MD simulation. Interactions shown are occurring more than 30% during the simulation time.

CYS44 and glutamine GLU166 and the ligand. The HIS41 exhibited π-π stacking interaction (31%) during the MD-simulation. There were no ionic, and water bridges were observed throughout the simulation (Figure 2).

Barnidipine bound to M^{pro} exhibited a combination of polar (water bridges), as well as non-polar (hydrophobic) interactions during MD-simulations. The RMSD plot of the backbone of the protein structures enumerated during the MD-simulation aligned to the initial structure for the RMSD analysis. As illustrated in Figure 1B, after 20 nsec initial fluctuations due to the equilibration, the RMSD for the protein-ligand complex varied between 1.0 Å−3.0 Å until the end of the simulation. Initially, the ligand made seldom contact with the protein. But more frequent contacts were made with the ligand after 50 nsec. Hence the binding is weaker compared to aprepitant. The amino acid residue GLU166 made bridged hydrogen bond as well as direct hydrogen bond with the ligand. A π-cation stacking interaction was exhibited with HIS41 amino acid residue (41%) (Figure 3).

Tipiracil bound to M^{Pro} exhibited consistent binding stability during MD-simulation. As illustrated in Figure 1C, after 10 nsec initial fluctuations due to the equilibration, the RMSD for the ligand-protein structures remained within 2.0 Å till the end of simulation which indicates the binding stability of the ligand at the selected protein. The residues THR45 and serine (SER46) made hydrogen bonding with the ligand. A bridged hydrogen bonding was observed with ligand and CYS44 residue. A π- π stacking interaction was exhibited with HIS41 (58%) amino acid residue (Figure 4).

A combination of polar, as well as non-polar interactions, was shown by arbutin bound to M^{pro} during MD-simulation. The plot of the backbone of the protein structures enumerated during the MD-simulation aligned to the initial structure for the RMSD analysis. As illustrated in Figure 1D, after 20 nsec initial fluctuations due to the equilibration, the RMSD for the protein-ligand structures remained between 2 Å until the end of the simulation. The residues CYS44, TYR54, GLU166 and GLN189 made hydrogen bonding different atoms of the ligand. The ligand interacted with the protein backbone by forming bridged hydrogen bonding interactions with CYS44 and GLU166 residues. The hydrogen bonding and hydrophobic interactions with GLU166 accounted to be more than 100%. A π- π stacking interaction (32%) was demonstrated with HIS41 amino acid residue (Figure 5).

Terbutaline bound to M^{pro} exhibited polar as well as non-polar binding during MD-simulation. The plot of the backbone of the protein structures enumerated during the MD-simulation aligned to the initial structure for the RMSD analysis. As illustrated in Figure 1E, after 20-sec initial fluctuations due to the equilibration, the RMSD for the protein structures remained between 0.8 Å−1.7 Å till 70 nsec of simulation which indicates that the association

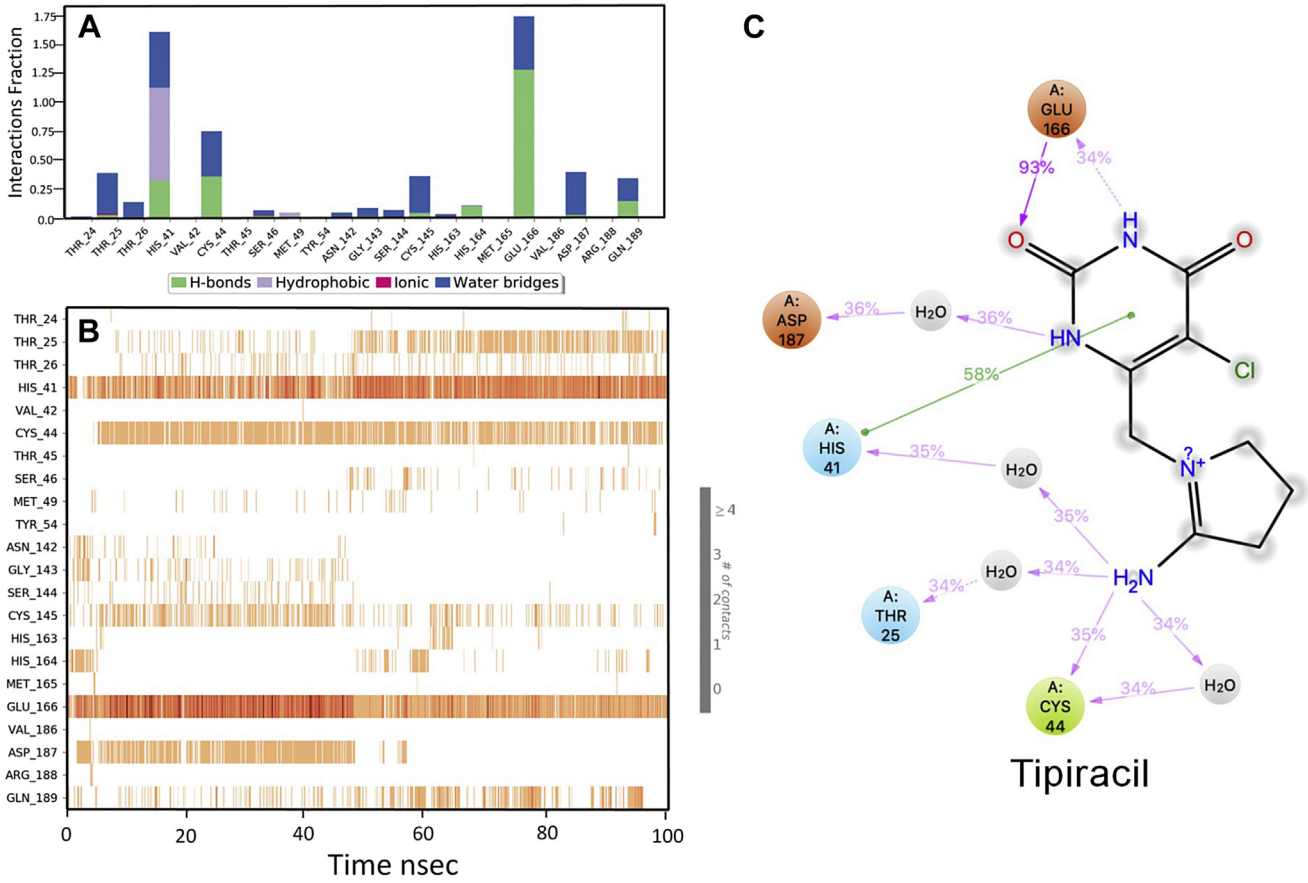

Figure 4. Interaction diagram of tipiracil with M^{pro}. (A) The protein-ligand contacts showing the bonding interactions fraction. (B) Interaction of tipiracil with residues in each trajectory frame. The darker colour higher the interaction or the residues make more contact; (C) Tipiracil interaction with the protein residues during MD simulation. Interactions shown are occurring more than 30% during the simulation time.

with the ligand and protein was good. After 70 nsec, the RMSD of the ligand-protein complex varied considerably. This indicates that the ligand-protein complex was unstable compared to tipiracil and aprepitant. A bridged hydrogen bonding was observed with the residues HIS163 and HIS164 and the ligand. Similarly, the ligand through a water moiety interacted with CYS44 and GLU166 residues. Among them, the interaction with CYS44 seemed to be negligible. A π-π stacking interaction (considered for ~87%) was observed with HIS41 amino acid residue (Figure 6).

Discussion

Repurposing and repositioning of drugs for COVID-19 is the area of interest for many researchers. For drug repurposing, the target selection is an important step. In this study, we have selected major protease M^{pro} which is validated and highly explored for repurposing in COVID-19. The SARS-CoV-2 M^{pro} is 306 amino acid long and functionally inhibit replicase precursor polyproteins whereby prevent viral gene expression and replication. In M^{pro}, there are three domains identified for each protomer. Domain-I with 8−101 residues and domain II with 102−184 residues have six-stranded anti-parallel β-barrel. Domain-III with 201−303 residues encompasses five α-helices grouped into the anti-parallel globular cluster and is linked to Domain-II by a long loop region (residues 185−200). SARS-CoV-2 M^{pro} has a Cys−His catalytic dyad. The M^{pro} substrate-binding site lies in a cleft between Domain-I and II. The inhibitor atoms form an anti-parallel sheet of long strand residues 164−168, 155−168 on one side, and residues 189−191 of the loop links domains-II and III. In the crystal structure of M^{pro} the native compound N3 (also known as peptide-like inhibitor PRD_002214) binds to the substrate-binding pocket by covalent bonding (10). Because the native molecule binding mechanism is covalent bonding it cannot be compared with the molecules of current interest as they are non-covalent ligand binder.

We have carried out the virtual screening of US-FDA approved drugs from the DrugBank database. The virtual screening is a powerful method in drug discovery by computational modelling for hit identification as a starting point for a medicinal chemist to synthesize new chemical entity as well as for drug repurposing. This method reduces

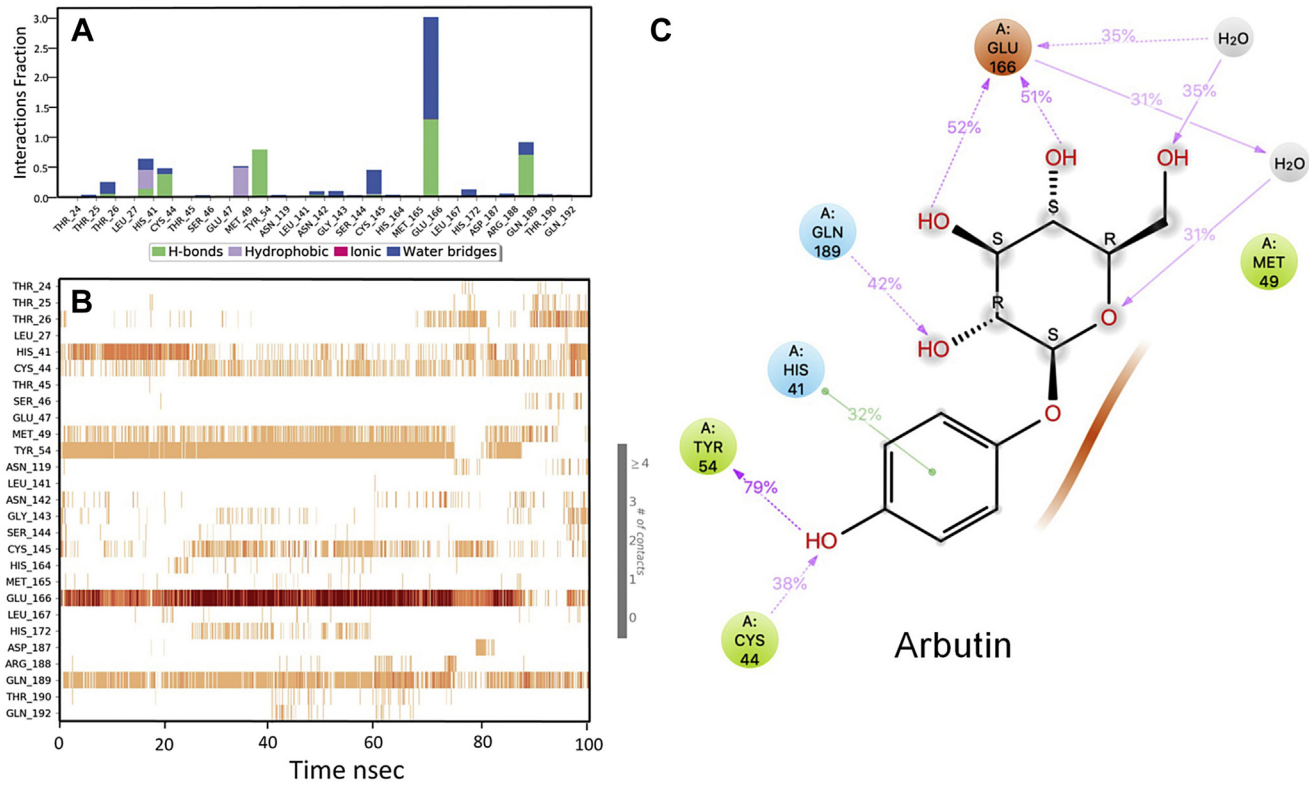

Figure 5. Interaction diagram of arbutin with M^{pro}. (A) The protein-ligand contacts showing the bonding interactions fraction. (B) Interaction of arbutin with residues in each trajectory frame. The darker colour higher the interaction or the residues make more contact; (C) Arbutin interaction with the protein residues during MD simulation. Interactions shown are occurring more than 30% during the simulation time.

the time and cost of drug discovery (24). Researchers have followed virtual screening by targeting M^{pro} and shortlisted 50 top-drugs based on docking scores (13). In our study, after the initial virtual screening of 2800 drugs, five drugs are shortlisted based on docking score and visual analysis of SP-docking and MD-simulation. These drugs are first time reported by us for targeting M^{pro}. The drug aprepitant is used as an antiemetic in chemotherapy-induced emesis and post operatives emesis (25). Aprepitant is an antagonist of Neurokinin 1 receptor (NK-1R), it was proposed to be beneficial as adjuvant therapy with anti-HIV drugs (26). In the current studies, aprepitant is found a better drug based on its results of interactions and molecular simulations studies.

The drug barnidipine, a calcium channel blocker and an antihypertensive agent was short listed because of its significant interaction with M^{pro} (27). In the current study, it was a hit-drug can be screened for inhibiting replication of SARS-CoV-2 in COVID-19. The drug tipiracil is currently used along with an anticancer agent trifluridine for treatment of advanced or recurrent colorectal cancer. Tipiracil prevents the degradation of trifluridine by inhibiting the enzyme thymidine phosphorylase, whereby, increases the concentration anticancer agent trifluridine at the cite of action. At therapeutic doses, tipiracil has no reported adverse events (28). In this study, the data infers that the tipiracil interaction with M^{pro} is much more durable and stable in both SP-docking and molecular dynamics simulations. Thus, the repurposing of tipiracil will have advantages compared to other selected molecules.

The drug arbutin is a prodrug of hydroquinone. Chemically arbutin is glycosylated hydroquinone, an inhibitor of melanin formation and a skin-lightening agent. Hydroquinones are known tyrosinase inhibitors, and hence arbutin is repurposed to treat melanogenesis or hyperpigmentation (29). Arbutin is officially used therapeutically for hyperpigmentation and other skin related problems (30). The data from this work suggests that arbutin can be repurposed to treat COVID-19 if explored further. The drug terbutaline sometimes used to prevent premature labour, is also the medicine for asthma, wheezing and COPD (31). Because of its lung specificity, terbutaline will have advantages if it is found useful in further in vitro enzyme inhibition studies.

In the recent literature on COVID-19 drug discovery, there are many reports with the thought of repurposing. Researchers have reported repurposing possibilities of many existing antiviral drugs like remdesivir and favipiravir have been officially approved for COVID-19 treatment. Similarly, dexamethasone is an immunosuppressant is

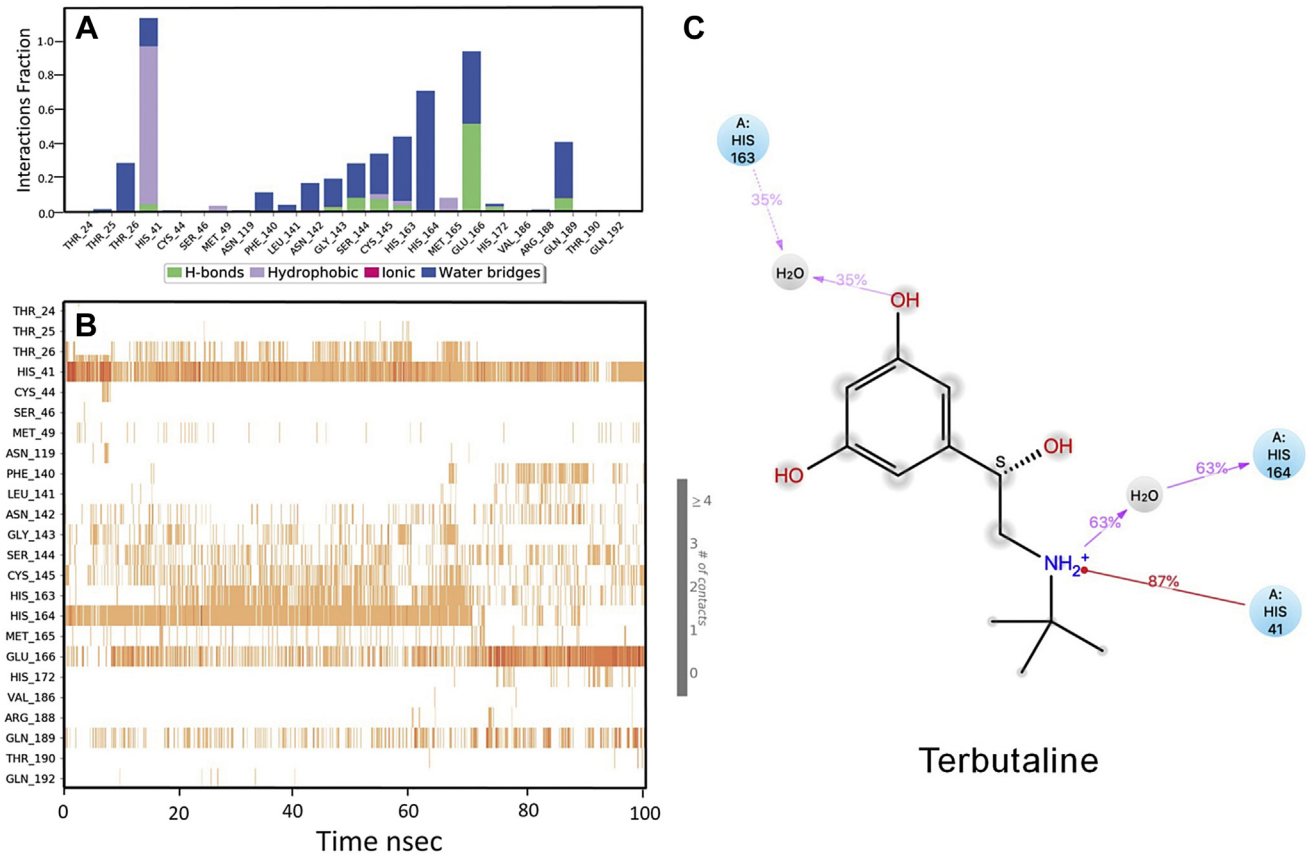

Figure 6. Interaction diagram of terbutaline with M^{pro}. (A) The protein-ligand contacts showing the bonding interactions fraction. (B) Interaction of terbutaline with residues in each trajectory frame. The darker colour higher the interaction or the residues make more contact; (C) Terbutaline interaction with the protein residues during MD simulation. Interactions shown are occurring more than 30% during the simulation time.

repurposed for COVID-19 to reduce the cytokine storm and whereby reduce the mortality. The anti-HIV drugs such as ritonavir and nelfinavir, have been proposed for repurposing in COVID-19 by targeting to M^{pro} (32). The antiviral drugs such as solutegravir, raltegravir, paritaprevir, bictegravir and dolutegravir were also suggested in another report (33). Similarly, anti-hepatitis-C virus drugs paritaprevir and simeprevir was suggested in another report as a covalent inhibitor of M^{pro} (34). Limitation of these drugs being high adverse events and many drug-drug/drug-disease interactions. In another study, drugs such as simeprevir, ergotamine, bromocriptine and tadalafil are proposed by the researcher (35). There was a virtual screening report on peptides drugs such as nafarelin and icatibant for repurposing to COVD-19 (36). Similarly, the drugs leupeptin, hemisulphate, pepstatin A, nelfinavir, birinapant, lypression and octreotide have been reported by targeting M^{pro} in virtual screening (37). These studies strengthen and support our hypothesis of drug repurposing from an entirely new class of drugs which are not screened for treating any infections. One of our ambition is to give avenues for repurposing rather than the novelty of selected drugs in comparison with the other reports.

The limitations of current management of COVID-19 are many, but the adverse events and the cost-effectiveness is most important (38). Any drug that was suggested had a lot of economic and managerial impact on supply and demand apart from the therapeutic efficacy potency and toxicity. In this view, we propose that the shortlisted drugs in this study will have a higher ratio of benefits and can be further studied and repurposed in clinical trials.

Conclusion

The SP-docking of USFDA approved drugs resulted in five potential SARS-CoV-2 M^{pro} inhibitors. These drugs include aprepitant, barnidipine, tipiracil, arbutin and terbutaline. Among these five drug-hits, aprepitant and tipiracil were found strongest in binding M^{pro} by MD-simulation studies. Further, these drugs are cost-effective, least toxic and currently used in therapy for other diseases. Thus, the present study provides an insight into the inhibition of major protease M^{pro} of SARS-CoV-2 using computation tools and shortlisted five drugs. Further, testing theses drugs,

especially aprepitant and tipiracil, by in vitro M^{pro} inhibitory activity and in vitro antiviral activity can be fast-tracked for repurposing in COVID-19 clinical trials.

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Disclosures

The authors declare that they do not have any conflict of interest in publishing these data.

Supplementary Data

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