Virtual screening and repurposing of FDA approved drugs against COVID-19 main protease

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ABSTRACT

Aims: In December 2019, the Coronavirus disease-2019 (COVID-19) virus has emerged in Wuhan, China. In this research, the first resolved COVID-19 crystal structure (main protease) was targeted in a virtual screening study by of FDA approved drugs dataset. In addition, a knowledge gap in relations of COVID-19 with the previously known fatal Coronaviruses (CoVs) epidemics, SARS and MERS CoVs, was covered by investigation of sequence statistics and phylogenetics.
Materials and methods: Molecular modeling, virtual screening, docking, sequence comparison statistics and phylogenetics of the COVID-19 main protease were investigated.
Key findings: COVID-19 Mpro formed a phylogenetic group with SARS CoV that was distant from MERS CoV. The identity% was 96.061 and 51.61 for COVID-19/SARS and COVID-19/MERS CoV sequence comparisons, respectively. The top 20 drugs in the virtual screening studies comprised a broad-spectrum antiviral (ribavirin), anti-hepatitis B virus (telbivudine), two vitamins (vitamin B12 and nicotinamide) and other miscellaneous systemically acting drugs. Of special interest, ribavirin had been used in treating cases of SARS CoV.
Significance: The present study provided a comprehensive targeting of the first resolved COVID + 19 structure of Mpro and found a suitable save drugs for repurposing against the viral Mpro. Ribavirin, telbivudine, vitamin B12 and nicotinamide can be combined and used for COVID treatment. This initiative relocates already marketed and approved safe drugs for potential use in COVID-treatment.

1. Introduction

Recently, a severe and highly contagious viral disease was evolved in Wuhan, China [1]. The causative agent was detected to be a new coronavirus and termed as Coronavirus disease 2019 (COVID-19).

Coronavirus has been associated with several infectious epidemics with major health hazard concerns. In 2000–2004, the Severe Acute Respiratory Syndrome CoV (SARS CoV) was evolved [2]. The source of the infection was recorded from animal origins, including a bat as an intermediate host [3]. About a decade later, the Middle East Respiratory Syndrome Coronavirus (MERS CoV) was diagnosed in Saudi Arabia [4]. Soon after the emergence of SARS and MERS CoVs, they spread to outside of the countries of its origin, causing major worldwide concerns [5,6]. Lastly, COVID-19 emerged in Wuhan, China. The first isolation was related to an animal market and human-to-human transmission was recorded [7].

The coronavirus polyprotein encodes two proteases, which share in its processing and release of the translated non-structural proteins (NSPs), the main protease is called 3-C-like protease (M-pro) and a papain-like protease (Plpro) [8]. Both Plpro and Mpro were vital targets for drug discovery studies against the recent coronavirus epidemics including SARS and MERS CoV [9–12].

The first and the only available crystal structure of COVID-19 proteins is Mpro, which was published in February 2020 (PDB ID 6lu7). In this study, the first virtual screening study against the first known COVID-19 was performed. The obtained results will help in the repurposing of already approved drugs to combat the recent dangerous COVID-19.
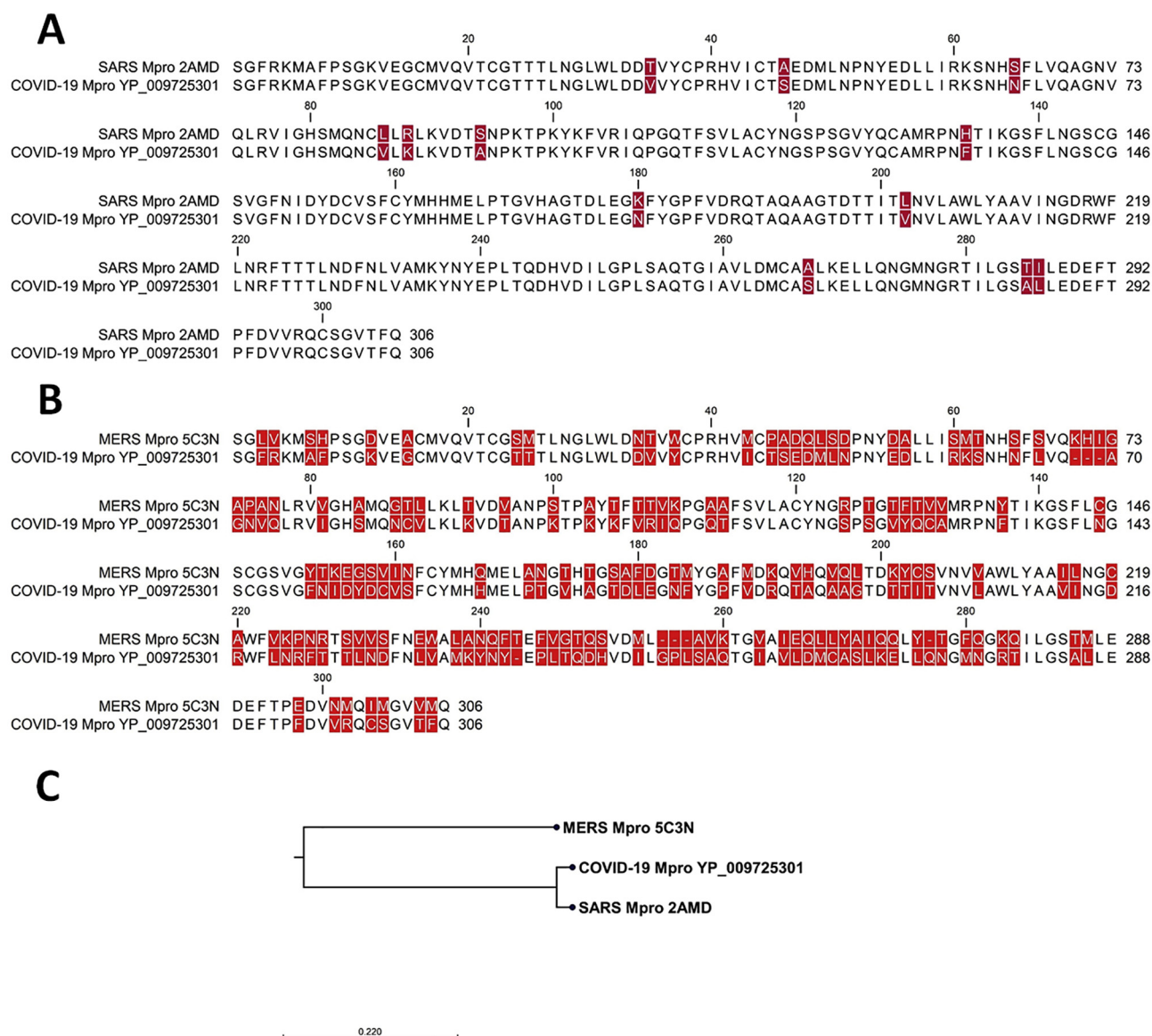
Fig. 1. Sequences alignment and phylogenetic relations of Mpro from SARS CoV, MERS CoV and COVID-19. A) Pairwise sequence alignment of COVID-19 and SARS CoVs Mpro. Conserved residues are not highlighted and displayed in black colour. The different residues are highlighted in by red colour B) pairwise sequence comparison of COVID-19 and MERS CoVs. Conserved residues are not highlighted and displayed in black colour. The different residues are highlighted in by red colour. C) the phylogenetic relations of Mpro, the tree was generated after the maximum likelihood. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

2. Methods

2.1. Retrieval of Mpro sequences

The NCBI GenBank or GISAID (https://www.gisaid.org/) were used to obtain the COVID-19 sequences. SARS CoV and MERS CoV sequences were obtained from the GenBank.

2.2. Sequence alignment and multiple sequence comparisons

Pairwise and multiple sequence comparisons of Mpro were done using CLC genomics software (Qiagen Inc., USA). The sequence comparison matrix was generated, including the number of gaps, number of different residues and identity %.

2.3. Construction of phylogenetic tree

The aligned sequences file was used to generate, a phylogenetic tree

by using the neighbor joining method implemented in CLC genomics workbench software.

2.4. Construction of drugs dataset and ligand preparation

FDA approved drugs dataset was retrieved from Selleckchem Inc. (WA, USA). All compounds were imported to Ligprep software, deslated and 3D optimized using OPLS2005 force field.

2.5. COVID-19 Mpro protein preparation

Virtual screening was implemented by using the published crystal structure of COVID-19 Mpro (PDB ID 6lu7). The protein preparation module in Maestro software package (Schrodinger LLC, NY, USA) was used to optimize the protein structure for docking. Water and other nonspecific molecules were removed, the protein was protonated to add polar hydrogens, the structure was optimized at cellular pH conditions, and the structure energy minimized using OPLS2005 force field. The

Table 1

Sequence comparison matrix of COVID-19, SARS and MERS CoVs Mpro. The upper diagonal panel shows the number of amino acids differences. The lower diagonal panel shows the identity%.

	COVID-19	SARS CoV	MERS CoV
COVID-19		12	150
SARS CoV	96.08		153
MERS CoV	51.61	50.65	

docking grid was generated by using the co-crystalised ligand as the center for docking box of 20 Å size.

2.6. Virtual screening

The Schrodinger glide docking module was used for virtual screening. In order to get accurate results, the standard precision (SP docking) was selected [27,28]. The output results were ordered by docking score. In this set, the curcumin was found to be a strong inhibitor of SARS Mpro [13]. The measured IC50 was 0.0235 μM. The obtained docking results were reanalyzed to include a comparison with curcumin. The relative docking scores were calculated for every compound by dividing its score with the docking score curcumin.

3. Results and discussion

Molecular modeling, virtual screening and other computational techniques are widely used methods to understand the phylogenetics, molecular aspects of proteins and protein-ligand interactions during drug discovery process [14–18]. Virtual screening has been used in drug discovery against emerging and fatal diseases including SARS CoV proteases [19,20], Hepatitis C virus RNA-dependent RNA polymerase [21], dengue virus [22] and Ebola virus [23].

In this work, we cover a knowledge gap about the COVID-19 Mpro and its comparison with the two most dangerous coronavirus epidemics, SARS and MERS CoVs. In addition, we used the first resolved COVID-19 crystal structure of Mpro to find suitable binding agents with proved safety. In this context, the FDA approved drugs were the best starting point in the way of repurposing approved and safe drugs against COVID-19.

At first, the Mpro from COVID-19 was compared with SARS and MERS CoVs (Fig. 1). Comparing COVID-19 Mpro with SARS Mpro (Fig. 1A) and MERS CoV Mpro (Fig. 1B) revealed high degree of

conservation between COVID-19 and SARS CoV Mpros and little number of different residues. However, MERS CoV Mpro showed large number of differences and low conservation with COVID-19 Mpro. This was evidenced by the phylogenetic relations (Fig. 1C), which showed close relation of SARS and COVID-19 Mpros and distant relation to MERS CoV.

Statistics of sequences comparison was generated in a pairwise sequence comparison matrix (Table 1). There was 96.08% identity between COVID-19 and SARS CoV Mpro, while low identity% of 51.61 was detected for COVID-19 and MERS CoV Mpro. The number of COVID-19 amino acid differences was 12 and 153 for SARS CoV and MERS CoV, respectively.

The results of virtual screening of FDA approved drugs against COVID-Mpro is presented in Supplementary Table 1. The presented parameters include the docking scores, ligand efficiency, lipophilic and hydrogen bonding interactions. The top 20 hits showing the highest docking score are provided in Table 2. The data were presented as a relative value to curcumin (a previously approved SARS Mpro inhibitor). Curcumin docking score was ranked at the 334 position, implying the presence of wide range of expected more powerful binding drugs. Within the top 20 drugs there was 2 antivirals, 2 antituberculous agents, 2 vitamins, 1 anticancer and other miscellaneous systemically acting drugs.

Among antivirals, ribavirin and telbivudine were ranked at the second and third positions. They showed 2 folds increase in the docking scores, compared with curcumin. Vitamin B12 and nicotinamide were ranked at the 4th and 6th positions.

Ribavirin is abroad spectrum antiviral agent acting by induction of mutations in the viral genome, especially in RNA viruses [24]. Ribavirin is officially approved for treating respiratory syncytial virus (RSV) infection and, in combination with interferon α2b for hepatitis C virus [25]. In addition, it was used for treating SARS CoV infections [25]. Given the high similarity of SARS and COVID-19 Mpros, ribavirin might be of a value in treating COVID-19.

Telbivudine is approved for treating hepatitis B virus [26]. The drug is nucleoside analogue and best fits the viral polymerase. The high rank of telbivudine in this study suggests its repurposing for COVID-19 treatment by its binding to Mpro.

In comparison with curcumin, the top 10 ranked compounds showed improved hydrogen bonding profile. There was 1.6–9.7 fold increase in hydrogen bonding interactions. In contrast, the hydrophobic interactions were kept constant or lowered.

By inspection of the binding mode of top ranked drugs, hydrogen

Table 2

The top 20 drugs showing the highest docking score.

| Item name	Formula	MW	Relative docking score	Relative ligand efficiency	Relative glide lipo	Relative glide hbond	Clinical uses
Chromocarb	C10H6O4	190	2.08	4.03	0.92	4.80	Vasoprotective
Ribavirin	C8H12N4O5	244	2.01	3.21	0.37	4.36	Antiviral
Telbivudine	C10H14N2O5	242	2.00	3.19	0.44	6.12	Hepatitis B virus
Vitamin B12	C63H88CoN14O14P	1355	1.99	0.60	0.49	9.70	Vitamin
Aminophylline	C16H24N10O4	420	1.92	4.00	0.46	3.20	Bronchodilator
Nicotinamide	C6H6N2O	122	1.91	5.76	0.71	5.76	Vitamin
Triflusal	C10H7F3O4	248	1.87	2.99	1.05	2.53	Cardiovascular
Bemegride	C8H13NO2	155	1.83	4.52	0.68	3.88	CNS stimulant
Aminosalicylate Sodium	C7H6NNaO3	175	1.80	4.44	0.76	1.60	Antituberculosis agents
Pyrazinamide	C5H5N3O	123	1.80	5.42	0.52	4.29	Antituberculosis agents
Temozolomide	C6H6N6O2	194	1.79	3.47	0.12	3.20	Anticancer
Methazolamide	C5H8N4O3S2	236	1.78	3.45	0.00	5.76	Glaucoma
Tioxolone	C7H4O3S	168	1.78	4.38	0.92	3.20	Anti-acne
Propylthiouracil	C7H10N2OS	170	1.77	4.38	0.56	3.20	Antithyroid agent
Cysteamine HCl	C2H8ClNS	114	1.77	12.01	0.00	2.00	Nephropathic cystinosis
methoxamine hydrochloride	C11H18ClNO3	248	1.77	3.20	0.10	5.09	Alpha-adrenergic agonist
Zonisamide	C8H8N2O3S	212	1.76	3.42	0.47	3.20	Anticonvulsant
(+,-)-Octopamine HCl	C8H12ClNO2	190	1.76	4.33	0.09	5.09	Adrenergic agonist
Amiloride hydrochloride	C6H9Cl2N7O	266	1.76	3.18	0.30	6.69	Diuretic
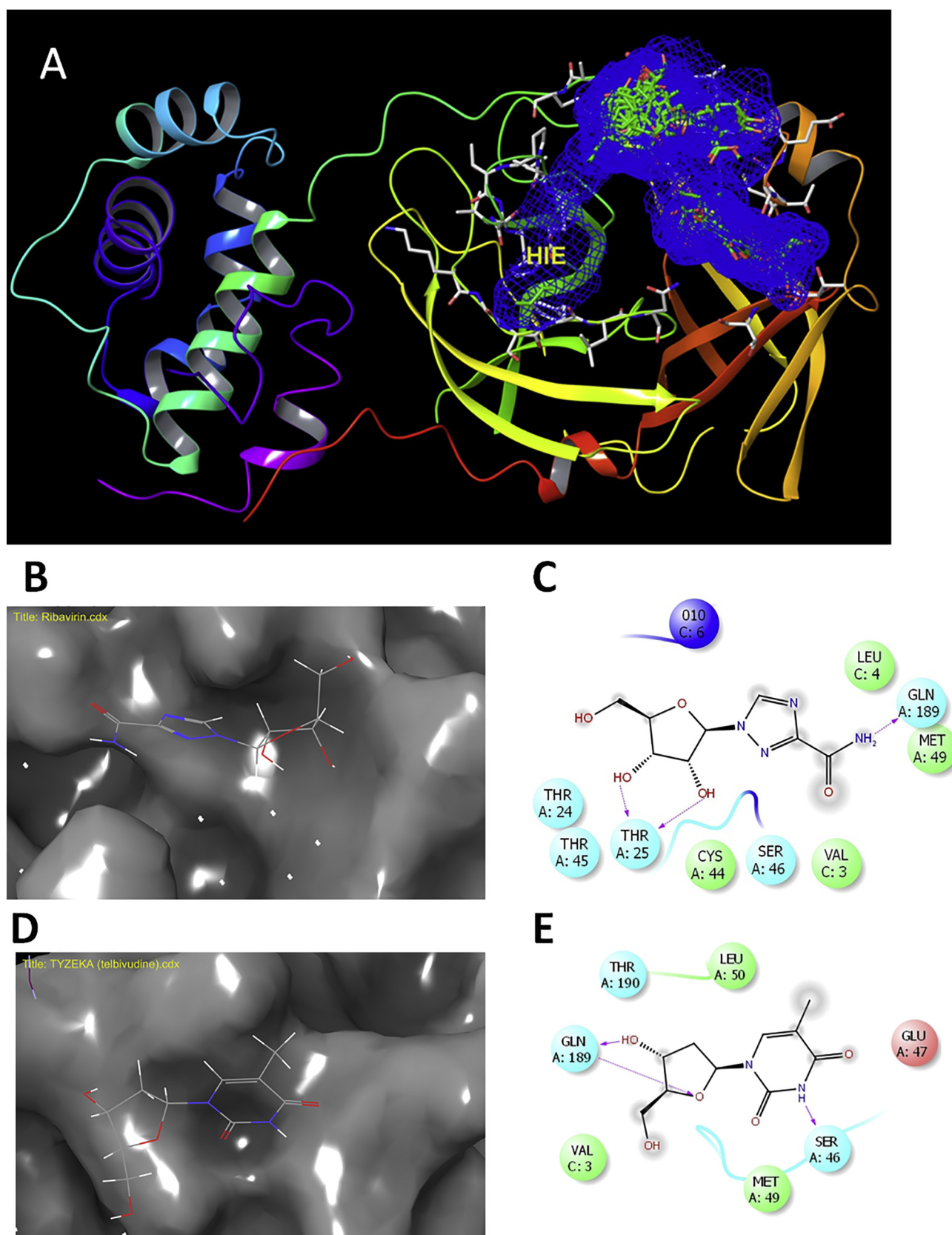
Fig. 2. Virtual screening and docking of FDA drugs with COVID-19 Mpro. A) The top 40 compounds docked into the Mpro binding site. B) The docking site of ribavirin C) The ligand interactions of ribavirin with Mpro D) The site of docking of telbivudine E) The ligand interactions of telbivudine with Mpro. Hydrogen bonds are shown in purple arrows, hydrophobic interactions in grey circles. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

bonding and hydrophobic interactions were the driving force for binding (Fig. 2). Ribavirin formed two hydrogen bonds with the backbone of THR25 and side chain of GLN189 (Fig. 2B and C). Telbivudine formed two hydrogen bonds with SER49 and GLV189 (Fig. 2D and E).

In conclusion, molecular modeling tools were used to compare the Mpro of the recently evolved COVID-19 and the previous two coronavirus epidemics SARS and MERS CoV. COVID-19 Mpro was more phylogenetically related to SARS Mpro. After a virtual screening cap-main against COVID-19 Mpro, a set of antivirals, vitamins, antimicrobials, and other systemically acting drugs were more potent binding with COVID-19 Mpro, compared with curcumin (known Mpro inhibitor). The repurposing of ribavirin, telbivudine, vitamin B12 and nicotinamide is suggested.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.lfs.2020.117627.

Data availability

All data are within the manuscript and the supplementary materials.

Declaration of competing interest

The authors declare no conflict of interest.

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