Repurposing of FDA approved drugs targeting Main protease M^{Pro} for SARS-CoV-2

Suryakant Tiwari

School of Nano Sciences, Central University of Gujarat, Gandhinagar 382030, India

Raghav Jain

Teri-Deakin Nanobiocentre, Gawal Phari, Haryana 122001, India

Indrani Banerjee (✉ indrani.banerjee@cug.ac.in)

School of Nano Sciences, Central University of Gujarat, Gandhinagar 382030, India

Research Article

Keywords: COVID-19, SARS-CoV-2, Drug repurposing, Main protease M^{pro}, Molecular docking

Posted Date: September 9th, 2020

DOI: <https://doi.org/10.21203/rs.3.rs-72576/v1>

License: This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Abstract

SARS-CoV-2 is one of the greatest pandemics in the history. There is no medicine or vaccine yet discovered to control the outbreak. The paper deals with repurposing existing drugs to control the outbreak of SARS-CoV-2 virus.

Ten FDA-approved drugs namely Indinavir, Nelfinavir, Letemovir, Irinotecan, Elbasvir, Saquinavir, Darunavir, Raltegravir, Atazanavir and Amprenavir were studied. *In silico* methods for virtual screening of protein-ligand docking of these drugs against SARS-CoV-2 M^{Pro} was performed. The binding efficiency of the drugs against viral main protease M^{Pro} was significantly high to inhibit SARS-CoV-2.

The results confirmed that Atazanavir, Nelfinavir, and Letemovir not only occupied the active site of M^{Pro} but also showed increased binding affinity (-10.36 kcal/mole, -9.47 kcal/mole and -9.43 kcal/mole) even more than of control drugs of Lopinavir (-8.71 kcal/mole) and Ritonavir (-8.08 kcal/mole). These repurposed drugs can be used in combination or individually as an alternative approach for rapid drug discovery against SARS-CoV-2

Introduction

The outbreak of SARS-CoV-2 started in December 2019 from the Wuhan, China has now created chaos by attacking over 200 countries jeopardize the global public health crisis. It has admonished the scientific community to distil best science. According to the information shared by WHO in the public domain, as of May10, 2020 Covid19 has infected around 3,917,366 confirmed cases in more than 200 countries across the globe including a poignant 274, 361 casualties, with a cumulative death rate of >7% [1]. However, Patients infected with Covid19 having pre-existing co morbidities like cancer, diabetes, cardiovascular and respiratory disease are at risk of mortality [2][3]. The scientific community worldwide is still underway to figure out the outbreak of novel coronavirus 2019. At present, there are no vaccines and therapeutic interventions against this pandemic [4].

Drug repurposing strategies have developed as a competent tool for identification of existing potential drugs with well-known toxicity profiles [5]. Whereas these can be used in combination or individually as an alternative approach for rapid drug discovery against SARS-CoV-2 [6]. In the present study, we have employed an *in silico* methods for virtual screening of protein-ligand docking of FDA approved drugs against SARS-CoV-2 M^{Pro} (PDB ID 7BQY) which have been shown recently in a co-crystallized form with its inhibitor N3 occupying the active binding pocket of the enzyme. It was targeted and screened for selecting the number of FDA approved drugs in order to interact with M^{Pro} (PDB ID 7BQY) and further blocking the active site [7]. The crystal structure of SARS-CoV- 2 M^{Pro} in apo form and (PDB ID: 6Y2E) and N3 bound structure (PDB ID: 7BQY) illustrates that protein is in crystallographic dimer form consisting of two identical monomer units. Every protomer consists of three domains. The interface of domain I and II domain consist of amino acid Cys145-His41 which collectively creates binding pocket of protein, where it's inhibitor N3 is bound in co-crystallised form [7][8].

We have shortlisted the number of commercially available drugs that are already in use for treating humans countering by several viral infections and screened them for M^{Pro} active site binding. Our results have shown that Atazanavir, Nelfinavir, and Letemovir not only occupied the active site of M^{Pro} but also shows increased binding affinity even more than of our control drugs. While the rest of the compounds also hold appreciable binding affinity occupying the important active site determinants. We emphasize that all the repurposed drugs considered and screened in this study for disease COVID-19 should be validated and an *in vitro* inhibitory potential need to be analysed using various robust biophysical and biochemical procedures leading clinical trials.

Materials And Methods

Proteins/Macromolecules

The crystal structure of SARS-CoV-2 main protease (M^{Pro}) (PDB ID: 7BQY) structures were retrieved from protein data bank (<https://www.rcsb.org/>), in .pdb format [7]. The structures of the target protein SARS-CoV-2 M^{Pro} with a covalent inhibitor, N3, was available in the PDB and it contains two chains, A and B, which form a homo dimer. Thereafter the chain A of PDB file was optimized for macromolecule preparation.

Protein structure alignment and homology analysis

Crystal structure of main protease M^{Pro} of both SARS-CoV-2 and SARS-CoV were retrieved from Protein Data Bank (<https://www.rcsb.org/>). The M^{Pro} structure file (PDB ID: 7BQY) of SARS-CoV-2 with resolution of 1.7Å and the M^{Pro} structure file (PDB ID: 1Q2W) of SARS-CoV with resolution of 1.86Å [7][9]. The protein sequence were obtained in FASTA format and multiple sequence alignment was performed using Clustal Omega (<https://www.ebi.ac.uk/Tools/msa/clustalo/>) [10]. The DALI server was used for homology analysis and creating superimposed structure of both proteins [11].

Literature survey and drug selection

The list and structures of FDA approved drugs were retrieved from the drug bank (<https://www.drugbank.ca/>) [12]. The 10 FDA-approved drug compounds (**Table 1**) used in the present study were Indinavir, Nelfinavir, Letemovir, Irinotecan, Elbasvir, Saquinavir, Darunavir, Raltegravir, Atazanavir and Amprenavir against viral protease that could inhibit SARS-CoV-2 main protease M^{Pro}. Thereafter, the molecules which were optimized, were saved in the PDB format for further processing.

Table 1 Selected FDA approved drugs and their target

S.No.	Drug ID	Drug	Molecule Type	Target	Indication
1	DB01072	Atazanavir	Peptidomimetic	HIV-1 Protease	HIV infection
2	DB00220	Nelfinavir	Isoquinolines	HIV-1 Protease	HIV infection
3	DB12070	Letermovir	Phenylpiperazines	Tripartite Terminase	Cytomegalovirus
4	DB00224	Indinavir	Amino acid amides	HIV-1 Protease	HIV infection
5	DB00762	Irinotecan	Camptothecins	DNA topoisomerase 1	Colorectal cancer
6	DB00701	Amprenavir	Benzenesulfonamides	HIV-1 Protease	HIV infection
7	DB05355	Raltegravir	Pyrimidinecarboxylic	HIV-1 Integrase	HIV infection
8	DB01232	Saquinavir	Quinolines	HIV-1 Protease	HIV infection
9	DB01264	Darunavir	Amides	HIV-1 Protease	HIV infection
10	DB11574	Elbasvir	Valine and derivatives	Nonstructural Protein 5A	HCV infection

Determination of Active Sites

CASTp 3.0 software (<http://sts.bioe.uic.edu/castp/> server) was used for determining the active sites by using binding pocket analysis tool [13]. The active site residues were used to evaluate the Grid box and docking results. The binding pocket of the ligand in the catalytic site was manually obtained followed by verification by docking analysis.

Molecular Docking

Crystal structure of main protease M^{Pro} and 3D structure of selected FDA was obtained from RCSB protein data bank (PDB ID: 7BQY) and drug bank (<https://www.drugbank.ca/>) respectively. The drug repurposing was done through molecular docking using the 3-D structure of protein PDB and drug compounds. The Molecular docking analysis was done using AutoDock 4.2, which works on the lamrackian genetic algorithm (GA) along with empirical free energy scoring function so as to identify the most favourable binding posture of the chosen FDA approved drugs [14]. The interaction of the ligand with the active site of the main protease M^{Pro} was determined by energy score (S, Kcal/mol). Low energy score indicates good affinity. Pymol version 1.7.4.5 Edu was used for visualization of the docked results [15].

Results And Discussion

The SARS-COV-2 virus contains around ~30,000 nucleotide long single-stranded positive sense RNA molecule [(+)ssRNA], having 5' capped region along with 3' poly-A tail, which immediately recognized by

eukaryotic machinery and undergoes translation [16]. The Virus genome consists of six open reading frames (ORFs) out of which first ORF encodes by the 5' end of the genome. This first ORF further splits into ORF1a and ORF1b which encodes two polypeptides pp1a and pp1ab. These two candidates are responsible for the synthesis of 16 non-structural proteins (nsP1-16), rest ORFs are utilized in synthesizing remaining four important structural proteins: Spike protein (S), Envelope proteins (E), Membrane proteins (M) and Nucleocapsid protein (N).[17] The virus uses S protein which is heterotrimeric and interacts with the ACE2 (a homolog of angiotensin-converting enzyme 2) which act as a virion receptor.[18] This facilitates host-pathogen interaction enabling viral entry into host cell [19]. Thereafter, genomic RNA acts as a template recognized by host cell machinery to synthesize 2 polyproteins pp1a and pp1ab which undergoes proteolysis [20]. It also gives important non-structural proteins including two proteases; Papain like protease (P^{Pro}) and Chymotrypsin like protease (3CLpro) or main protease (M^{Pro}) [21][22]. These important proteases further process the polyprotein in sequence-specific manner synthesizing 16 different nsPs. These are directly associated with the further replication and transcription of the virus by forming replication transcription complex (RTC) [23]. Therefore, targeting the main protease M^{Pro} represents the attractive drug target for restricting the further production of non-structural viral proteins. This will inhibit replication events of the virus life cycle. Moreover, no evidences have been found of inhibiting human protease activity upon targeting viral proteases, thus, precluding the chances of cellular toxicity even though inhibiting the main protease [24].

As of now, there is no prominent treatment or vaccine that is reported or approved specifically for treating Covid19 patients[25]. However scientific community around the globe putting all efforts in research endeavors towards the advancement of remedial intercessions and researching viral drug targets [26]. Recently scientist has deduced some of the important X-ray crystal structure of viral proteins such as 3CL like protease, papain-like protease, and spike (S) protein [27][28]. From the above finding, it was seen that virus gets attached with angiotensin-converting enzyme 2 (ACE2) receptors which are present in the lower respiratory tract providing entry into the lungs [29].

The understanding of SARS-CoV-2 virus with that of previously studied SARS-CoV virus, was performed through protein structure alignment and sequence similarity and the results are presented in **Fig. 1**. Non-structural proteins (nsPs) of corona viruses are individual functional proteins formed by the proteolysis of its long polypeptide precursor which is processed by chymotrypsin like proteases accompanying papain proteases.[30] Protein sequence similarity analysis of M^{Pro} on comparing SARS-CoV-2 with that of SARS-CoV shows remarkable protein sequence similarity with around 96% of identical protein sequence along with conserved amino acid sequence among both CoVs, while SARS-CoV-2 virus keeps mutating, suggesting M^{Pro} as ideal drug target (**Fig. 1**).

The binding efficiency of the drugs was determined through molecular docking. The crystal structure of COVID-19 main protease in complex with an inhibitor N3 at resolution of 1.7⁰A was extracted from protein data bank (PDB ID- 7BQY) and visualized using Pymol molecular visualization tool presented in

Fig. 2. The amino acid residues interacting with ligand were retrieved by using CASTp 3.0 server by using binding pocket analysis tool and presented in **table 2**.

The molecular docking analysis of the drugs revealed strong interaction with higher energies and binding affinity against the SARS-CoV-2 Mpro. The potential antiviral drugs bind with the unrestrained conformation in the same active groove of protein, where it was co-crystallized with bound native ligand. Intermolecular interaction and ranking based on the molecular docking binding affinity and binding energies of the potential drugs is shown in **table 3**. Re-docking of two reference compounds (lopinavir and ritonavir) were also done in order to check the credibility of the software Autodock 4.2. It was found to be almost same indicating the high fidelity of the docking method. In this study, our focus is on top drug candidates among selected 10 drugs for further analysis as these drugs showing a range of binding affinity from (-10.36 to -5.81). Despite of that among 10 drug compounds, the top 3 drug compounds atazanavir, nelfinavir and letemovir were showing binding affinity even higher than of our reference compounds (**Fig. 2**).

The global outbreak of novel coronavirus SARS-CoV-2 rapidly spreading disease named Covid-19, declared as a pandemic by the World Health Organization. This novel virus has affected nearly 206 countries globally after getting hike exponentially in the second week of March 2020.

In the recent study of crystal structure of SARS-CoV-2 main protease M^{Pro} (PDB ID: 7BQY) in complex with an inhibitor N3 at 1.7 Å, it is shown that targeting the main protease M^{Pro} represents the best attractive drug target. It can restrict the production of non-structural viral proteins thus, inhibiting replication events of the virus life cycle.[7] However, testing the already approved drugs against the COVID-19 as drug repurposing strategy facilitates the discovery of potential drug candidates. Ritonavir and Lopinavir are thoroughly researched and established FDA approved protease inhibitors for HIV.[31] [32] Previously these drug candidates were also suggested for treatment of SARS and MERS[33]. Additionally, this combination has been utilized in COVID-19 patients to control the infection.[34] Consequently, we have selected these drugs as a standard reference to compare the efficacy of the binding affinity of our chosen FDA approved drugs. We further visualized main protease M^{Pro} (PDB: 7BQY) in Pymol for the identification of active site residues. Thereafter, we execute the docking interaction of our selected drugs of Atazanavir, Nelfinavir, Letemovir, Indinavir, Irinotecan, Amprenavir, Raltegravir, Saquinavir, Darunavir, and Elbasvir as the potential inhibitor of SARS-CoV-2 main protease M^{Pro}. The binding energies retrieved from the molecular docking with selected FDA approved drugs showed inhibition potential of these candidates in the order of ranked by affinity (ΔG) i.e Atazanavir, Nelfinavir, Letemovir, Indinavir, Irinotecan, Amprenavir, Raltegravir, Saquinavir, Darunavir, and Elbasvir was -10.36, -9.47, -9.43, -8.65, -8.61, -8.38, -7.97, -7.89, -7.44, and -5.81 kcal/mol respectively (shown in **Table 3**). Interestingly, it was found that our top three drugs Atazanavir, Nelfinavir and, Letemovir were interacting with a much more appreciable binding affinity even better than that of our reference drugs.

However, we emphasize that all the repurposed drugs considered and screened in this study for disease COVID-19 should be validated and *in vitro* inhibitory potential needs to be analyzed during various robust

biophysical and biochemical procedures leading clinical trials.

Conclusion

Repurposing of FDA approved drugs could be one of the potential solutions to control the outbreak of SARS-CoV-2 virus in the present scenario. The crystal structure analysis showed that M^{Pro} represents the best attractive drug target for the virus. Atazanavir, Nelfinavir, Letermovir, Indinavir, Irinotecan, Amprenavir, Raltegravir, Saquinavir, Darunavir, and Elbasvir FDA approved drugs were investigated as the potential inhibitor of SARS-CoV-2 main protease M^{Pro}. Molecular docking retrieved that Atazanavir, Nelfinavir and, Letermovir showed significant binding affinity of -10.36 kcal/mole, -9.47 kcal/mole, -9.43 kcal/mole respectively which is even better than the reference drug. The study can help in designing drugs for controlling and preventing the COVID-19 outbreak.

Declarations

Acknowledgement

The authors wish to thank UGC for the financial help towards the execution of the work.

Funding None.

Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest.

References

1. WHO. Coronavirus disease. World Heal Organ [Internet]. 2020;2019:2633. Available from: <https://www.who.int/emergencies/diseases/novel-coronavirus-2019>
2. Gupta R, Hussain A, Misra A. Diabetes and COVID-19: evidence, current status and unanswered research questions. Eur J Clin Nutr. 2020;
3. Baud D, Qi X, Nielsen-Saines K, Musso D, Pomar L, Favre G. Real estimates of mortality following COVID-19 infection. Lancet Infect. Dis. 2020.
4. Mehta P, McAuley DF, Brown M, Sanchez E, Tattersall RS, Manson JJ. COVID-19: consider cytokine storm syndromes and immunosuppression. Lancet. 2020.
5. Tripathi V, Pathak Y, Mishra A. Rifampicin may be repurposed for COVID-19 treatment: Insights from an in-silico study. Res Sq. 2020;
6. Yang H, Bartlam M, Rao Z. Drug Design Targeting the Main Protease, the Achilles Heel of Coronaviruses. Curr Pharm Des. 2006;
7. Jin Z, Du X, Xu Y, Deng Y, Liu M, Zhao Y, et al. Structure of Mpro from COVID-19 virus and discovery of its inhibitors. Nature. 2020;

8. Yang H, Yang M, Ding Y, Liu Y, Lou Z, Zhou Z, et al. The crystal structures of severe acute respiratory syndrome virus main protease and its complex with an inhibitor. *Proc Natl Acad Sci U S A*. 2003;
9. RCSB PDB - 1Q2W: X-Ray Crystal Structure of the SARS Coronavirus Main Protease [Internet]. [cited 2020 May 12]. Available from: <https://www.rcsb.org/structure/1Q2W>
10. Daugelaite J, O' Driscoll A, Sleator RD. An Overview of Multiple Sequence Alignments and Cloud Computing in Bioinformatics. *ISRN Biomath*. 2013;
11. Holm L, Elofsson A. Benchmarking fold detection by DaliLite v.5. *Bioinformatics*. 2019;
12. Wishart DS. DrugBank: a comprehensive resource for in silico drug discovery and exploration. *Nucleic Acids Res*. 2006;
13. Tian W, Chen C, Lei X, Zhao J, Liang J. CASTp 3.0: Computed atlas of surface topography of proteins. *Nucleic Acids Res*. 2018;
14. Garrett M. Morris, David S. Goodsell, Michael E. Pique, William "Lindy" Lindstrom, Ruth Huey, Stefano Forli, William E. Hart, Scott Halliday RB and AJO. User Guide: AutoDock Version 4.2. Citeseer. 2012.
15. Yuan S, Chan HCS, Hu Z. Using PyMOL as a platform for computational drug design. *Wiley Interdiscip. Rev. Comput. Mol. Sci*. 2017.
16. Woo PCY, Huang Y, Lau SKP, Yuen KY. Coronavirus genomics and bioinformatics analysis. *Viruses*. 2010.
17. Hussain S, Pan J, Chen Y, Yang Y, Xu J, Peng Y, et al. Identification of Novel Subgenomic RNAs and Noncanonical Transcription Initiation Signals of Severe Acute Respiratory Syndrome Coronavirus. *J Virol*. 2005;79:5288–95.
18. Kumar S, Maurya VK, Prasad AK, Bhatt MLB, Saxena SK. Structural, glycosylation and antigenic variation between 2019 novel coronavirus (2019-nCoV) and SARS coronavirus (SARS-CoV). *VirusDisease*. 2020;
19. Wong SK, Li W, Moore MJ, Choe H, Farzan M. A 193-Amino Acid Fragment of the SARS Coronavirus S Protein Efficiently Binds Angiotensin-converting Enzyme 2. *J Biol Chem*. 2004;
20. Astuti I, Ysrafil. Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2): An overview of viral structure and host response. *Diabetes Metab Syndr Clin Res Rev*. 2020;
21. Hilgenfeld R. From SARS to MERS: crystallographic studies on coronaviral proteases enable antiviral drug design. *FEBS J*. 2014.
22. Lim KP, Ng LFP, Liu DX. Identification of a Novel Cleavage Activity of the First Papain-Like Proteinase Domain Encoded by Open Reading Frame 1a of the Coronavirus Avian Infectious Bronchitis Virus and Characterization of the Cleavage Products. *J Virol*. 2000;
23. Song Z, Xu Y, Bao L, Zhang L, Yu P, Qu Y, et al. From SARS to MERS, thrusting coronaviruses into the spotlight. *Viruses*. 2019.
24. Xue X, Yu H, Yang H, Xue F, Wu Z, Shen W, et al. Structures of Two Coronavirus Main Proteases: Implications for Substrate Binding and Antiviral Drug Design. *J Virol*. 2008;

25. Maurya VK, Kumar S, Prasad AK, Bhatt MLB, Saxena SK. Structure-based drug designing for potential antiviral activity of selected natural products from Ayurveda against SARS-CoV-2 spike glycoprotein and its cellular receptor. *VirusDisease* [Internet]. 2020; Available from: <https://doi.org/10.1007/s13337-020-00598-8>
26. Protein based biomarkers for Non-invasive Covid-19 detection. Available from: <https://osf.io/qn256/>
27. Xia S, Liu M, Wang C, Xu W, Lan Q, Feng S, et al. Inhibition of SARS-CoV-2 (previously 2019-nCoV) infection by a highly potent pan-coronavirus fusion inhibitor targeting its spike protein that harbors a high capacity to mediate membrane fusion. *Cell Res*. 2020;
28. RCSB PDB - 6W9C: The crystal structure of papain-like protease of SARS CoV-2 [Internet]. [cited 2020 May 11]. Available from: <https://www.rcsb.org/structure/6w9c>
29. Singh AK, Gupta R, Misra A. Comorbidities in COVID-19: Outcomes in hypertensive cohort and controversies with renin angiotensin system blockers. *Diabetes Metab Syndr Clin Res Rev*. 2020;
30. Dai W, Zhang B, Su H, Li J, Zhao Y, Xie X, et al. Structure-based design of antiviral drug candidates targeting the SARS-CoV-2 main protease. *Science* (80-). 2020;
31. Chandwani A, Shuter J. Lopinavir/ritonavir in the treatment of HIV-1 infection: a review. *Ther Clin Risk Manag* [Internet]. Dove Medical Press; 2008;4:1023–33. Available from: <https://pubmed.ncbi.nlm.nih.gov/19209283>
32. Rajendran DK, Rajagopal V, Alagumanian S, Santhosh Kumar T, Sathiya Prabhakaran SP, Kasilingam D. Systematic literature review on novel corona virus SARS-CoV-2: a threat to human era. *VirusDisease* [Internet]. 2020; Available from: <https://doi.org/10.1007/s13337-020-00604-z>
33. Chu CM, Cheng VCC, Hung IFN, Wong MML, Chan KH, Chan KS, et al. Role of lopinavir/ritonavir in the treatment of SARS: Initial virological and clinical findings. *Thorax*. 2004;
34. Lim J, Jeon S, Shin HY, Kim MJ, Seong YM, Lee WJ, et al. Case of the index patient who caused tertiary transmission of coronavirus disease 2019 in Korea: The application of lopinavir/ritonavir for the treatment of COVID-19 pneumonia monitored by quantitative RT-PCR. *J Korean Med Sci*. 2020;

Tables

Due to technical limitations, tables 2 and 3 are only available as a download in the supplemental files section.

Figures

Figure 1

(A) Superimpose protein structure of main protease MPro. SARS-CoV-2 is shown as yellow while SARS-CoV as green and conserved amino acid residues highlighted with blue. (B) Multiple sequence alignment of amino acid sequence of SARS-CoV-2 and SARS CoV. Identical amino acid sequence of each protein is highlighted as green along with residues marked underneath with * show catalytic residues and amino acids marked with # shows ligand binding residues of different subsites.

Figure 2

Docking interaction of top 3 antiviral drugs based on their binding affinity with SARS CoV-2 main protease MPro. A. Atazanavir as top candidate with binding affinity -10.36 kcal/mol B. Nelfinavir with -9.47

kcal/mol and C. Letetmovir with -9.43 kcal/mol

Figure 3

Docking poses of all COVID-19 virus Mpro protease inhibitors. Mpro is shown in grey background, inhibitors are in green colour.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [Tables2and3.docx](#)