Revealing Potential Binding Affinity of FDA Approved Therapeutics Targeting Main Protease (3CLpro) in Impairing Novel Coronavirus (SARS-CoV-2) Replication that Causes COVID-19

D. Sivaraman1,*, P.S. Pradeep1, S. Sundar Manoharan2, C. Ramachandra Bhat3, K.V. Leela4 and V. Venugopal5

1Department of Pharmacology and Toxicology, Centre for Laboratory Animal Technology and Research, Sathyabama Institute of Science and Technology, Jeppiaar Nagar, Rajiv Gandhi Road, Chennai, Tamil Nadu 600119, India; 2School of Technology, Pandit Deendayal Petroleum University, Gandhinagar, Gujarat 382007, India; 3Department of Pharmacology, Government Kilpauk Medical College, Chennai, Tamil Nadu 600010, India; 4Department of Microbiology, SRM Medical College and Hospital, Chennai, Tamil Nadu 603211, India; 5Department of Internal Medicine, Sundaram Health Care Centre, Sholingur, Tamil Nadu 632102, India

Abstract: Background: Spread of COVID-19 attains a crucial transition in revealing its pandemic across the boundaries. In combating the infection caused by SARS-CoV-2, there is a spectrum of ideal strategies that have been adopted globally, of which repurposing of approved drugs considerably having high clinical relevance. 3-chymotrypsin-like protease (3CL pro) is considered to be the potential target for the researchers as it is highly essential for cleavage of polyprotein to get 16 nonstructural proteins (called nsp1-nsp16). These proteins are highly essential for viral replication and hence become a primary target for enzyme inhibitors. 3CL pro, having a structural projectile helical chain with biologically active site involved in processing viral polyproteins that are evolved from RNA genome translation.

Objective: The major objective of the present investigation is to evaluate the enzyme inhibition potential of FDA approved therapeutic leads in targeting 3CLpro that mediates the viral replication.

Methods: Docking calculations were carried out for an array of FDA approved molecules which leads to a notable few molecules such as Emtricitabine, Oseltamivir, Ganciclovir, Chloroquine, Baricitinib, Favipiravir, Lopinavir, Ritonavir, Remdesivir, Ribavirin, Tenofovir, Umifenovir, Carbapenam, Ertapenem and Imipenem which have both specificity and selectivity in terms of binding efficiency against 3CL proenzyme.

Results: A combinatorial evaluation employing in-silico screening shows a major lead for remdesivir which possesses a substantial affinity to 3CL pro binding on core amino acid residues, such as Leu 27, His 41, Gly 143, Cys 145, His 164, Met 165, Glu 166, Pro 168 and His 172 which share the biological significance in mediating enzymatic action. Results of docking simulation by Autodock over a host of FDA approved molecules show high degree of selectivity and specificity in the increasing order of binding capacity; Remdesivir> Ertapenem> Imipenem> Tenofovir> Ribavirin> Umifenovir> Lopinavir> Ritonavir> Emtricitabine> Ganciclovir> Baricitinib> Ribavirin> Tenofovir> Chloroquine> Oseltamivir> Favipiravir> Carbapenam.

Conclusion: Till date, there is no known cure attained for treating COVID-19 infection. In conclusion, lead molecules from already approved sources provoke promising potential which grabs the attention of the clinicians in availing potential therapeutic candidate as a drug of choice in the clinical management of COVID-19 time-dependently.

Keywords: COVID-19, Coronavirus, 3-chymotrypsin-like protease, SARS-CoV-2, Drugs repurposing, FDA approved drugs.

1. INTRODUCTION

Proteases group of enzyme operates at different paradigm in viral replication. A similar mechanism may be extended for the prognosis of Severe Acute respiratory syndrome coronavirus (SARS-CoV) and for Middle-East respiratory syndrome coronavirus (MERS-CoV) [1]. Clinical features of COVID-19 demand minimum requirements for new drug entity that includes: minimization on viral load, effective control on cytokine storms, immune-boosting, stabilization of oxidative stress, etc. [2], [3].

It is well known that the SARS-CoV-2 virus exerts its pathogenicity by binding with the Angiotensin-converting
The computational molecular investigation was performed using AutoDock version 4 which predicts interactions between FDA approved drug molecules with that of the selected protein target (Novel coronavirus 3-chymotrypsin-like protease (3CL pro)). 3D structure of the main protease (3-chymotrypsin-like protease (3CL pro)) with protein data bank (PDB)-6LU7 retrieved from Research Collaboratory for Structural Bioinformatics (RCSB). 3D componential structure of lead molecules and protein were docked using AutoDock analytical tool version 4. Affinity (grid) maps of 60×60×60 Å grid points and 0.375 Å spacing were generated using the Autogrid program. AutoDock parameter set- and distance-dependent dielectric functions were used in the calculation of the van der Waals and the electrostatic terms, respectively. Docking simulations were performed using the programed algorithm inbuilt with pre automation in the software. Initial position, orientation, and torsions of the ligand molecules were set randomly. All rotatable torsions were released during docking. Each docking experiment was derived from 2 different runs that were set to terminate after a maximum of 250000 energy evaluations. The population size was set to 150. During the search, a translational step of 0.2 Å, and quaternion and torsion steps of 5 were applied [13, 14].
Fig. (1). A. 3D crystalline structure of the target protein main protease of COVID-19 Virus –PDB 6LU7 was retrieved from protein data bank and protein clean-up process was done and essential missing hydrogen atom was added, highlighting the helical chain with the majority of the active site corresponds to the enzymatic action; B. Shows Ramachandran plot indicating a majority of the active site amino acid residues on the target enzyme. Prediction by MolProbity server R = 0.202; Rfree = 0.235. A total of 309 residues is present. The structure was solved at 2.16 Å resolution.

Fig. (2). 2D structure of FDA approved therapeutic ligand subjected to molecular docking Investigation against COVID-19 main protease (3-chymotrypsin-like protease (3CL pro)) -PDB- 26LU7.
2.6. ADME and Drug Likeness

Absorption, distribution, metabolism and elimination properties of all the lead molecules were investigated using Swiss ADMET (absorption, distribution, metabolism, excretion, and toxicity) web tool [15]. Druglikeness properties of all the leads subjected to Lipinski, Ghose rule of druglikeness. (http://www.swissadme.ch/index.php).

3. RESULTS AND DISCUSSION

Repurposing of drugs gains paramount importance in recent times as it accelerates the discovery of novel therapeutic applications of the existing drug molecules. It also reduces the latency of time required in driving the drug to the market since most of the approved drugs satisfies the demanding regulatory safety requirements. Authorities like the FDA already initiated the key process of repurposing programs to elucidate the new clinical significance of already approved drugs [15].

Drugs repositioning have a history of providing suitable ailments some dreadful disease like multiple myeloma (thalidomide) [16], Amantadine for Parkinson's disease [17], Galantamine for Alzheimer's disease [18], Mecamylamine for depression [19], Methotrexate for arthritis [20], Sildenafil for penile erection [21] and Zidovudine for HIV-AIDS [22], Chloroquine for COVID-19 [23] and Remdesivir for SARS-CoV-2 [24].

The computational analysis benefits the researcher in a screening library of compounds with suitable pharmacophore that offers significant interaction with the expected target. Virtual screening improves the understanding of orientation behavior of the ligand over selected protein in a lesser time period. Some of the significant investigational outcome from in-silico screening greatly helps in the transformation of leads to the next level of In-vitro studies and also on subsequent clinical evaluations. SARS and MERS-CoV’s possess most pathogenic RNA that becomes a high epidemic in the recent health care crisis, which causes potential economic instability. These viruses are typically fond of certain non-structural proteins for their survival and replication. 3CLpro is a class of proteases majorly involved in the release of sixteen nonstructural proteins [25]. Interaction sequential analy-
sis proves that the amino acid Glu 166 possesses three potential functional groups, His 41 as a proton acceptor, His 163 and His 172 potentially determine the enzymatic action of 3CLpro, thereby binding of drugs with any of these potential amino acids has higher chances of enzyme inhibition [26].

Significant clinical investigations on repurposed drugs now shifted the COVID-19 therapy to the next level. The open-label trial involves 199 COVID-19 patients, in which, 99 were allocated for treatment with HIV protease inhibitors (lopinavir-ritonavir). Results of the study signify that a combination of lopinavir-ritonavir fails to provide an adequate (lopinavir-ritonavir). Whereas other trial involving 80 SARS-CoV-2 infected patients to compare the efficacy of favipiravir (RdRp inhibitor) and lopinavir-ritonavir reveals higher positive response in favipiravir treated group when compared to lopinavir-ritonavir treatment [28]. A retrospective comparative investigation between monotherapy (lopinavir-ritonavir) and combinational therapy (umifenovir + lopinavir-ritonavir) justifies that 75% of the cases under combinational therapy reveal favorable clinical response when compared to that of the monotherapy group with 35% of clinical response [29]. Another open labelled randomized trial involving 240 COVID-19 patients to ensure the efficacy of favipiravir (RdRp inhibitor) and umifenovir (anti-influenza) reveals a higher level of clinical recovery in favipiravir (71.43%) when compared to that of the monotherapy group with 35% [30]. A versatile comparative in-vitro analysis made between hydroxychloroquine and chloroquine in SARS-CoV-2 infected cell lines witnessed a higher level of clinical recovery in chloroquine (55.86%) treated patients [30].

Table 1. Summarizing docking score and sequential binding behavior of FDA approved lead molecules with that of the target Amino acid residues against COVID-19 main protease (3-chymotrypsin-like protease (3CL pro)) - PDB- 26LU7.

<table>
<thead>
<tr>
<th>FDA Approved Molecules</th>
<th>Mol weight (g/mol)</th>
<th>Molecular Formula</th>
<th>Docking Score (kcal/mol)</th>
<th>Vital Amino acid Binding Residues</th>
</tr>
</thead>
<tbody>
<tr>
<td>Remdesivir</td>
<td>602.6</td>
<td>C27H35N6O8P</td>
<td>-8.38</td>
<td>25 THR 27 LEU 41 HIS 49 MET 143 GLY 145 CYS 163 HIS 164 HIS 165 MET 166 GLU 172 HIS</td>
</tr>
<tr>
<td>Ertapenem</td>
<td>475.5</td>
<td>C22H25N3O7S</td>
<td>-8.39</td>
<td>41 HIS 49 MET 142 ASN 145 CYS 163 HIS 165 MET 166 GLU 167 LEU 168 PRO 189 GLN -</td>
</tr>
<tr>
<td>Imipenem</td>
<td>299.35</td>
<td>C12H17N7O2S</td>
<td>-6.76</td>
<td>41 HIS 49 MET 54 TYR 140 PHE 145 CYS 163 HIS 165 MET 166 GLU 172 HIS 187 ASP 189 GLN -</td>
</tr>
<tr>
<td>Tenofovir</td>
<td>287.21</td>
<td>C9H14N5O4P</td>
<td>-5.98</td>
<td>41 HIS 49 MET 140 PHE 141 LEU 144 SER 145 CYS 163 HIS 165 MET 166 GLU 189 GLN -</td>
</tr>
<tr>
<td>Umifenovir</td>
<td>477.4</td>
<td>C22H25BrN2O3S</td>
<td>-7.36</td>
<td>25 THR 27 LEU 41 HIS 49 MET 144 SER 145 CYS 163 HIS 165 MET 166 GLU 168 LEU 188 ARG 189 GLN -</td>
</tr>
<tr>
<td>Chloroquine</td>
<td>319.9</td>
<td>C18H26ClN3</td>
<td>-7.75</td>
<td>41 HIS 144 SER 145 CYS 163 HIS 165 MET 166 GLU 167 LEU 168 PRO - - -</td>
</tr>
<tr>
<td>Lopinavir</td>
<td>628.8</td>
<td>C37H48N4O5</td>
<td>-9.14</td>
<td>25 THR 27 LEU 41 HIS 49 MET 54 TYR 142 ASN 145 CYS 165 MET 166 GLU 189 GLN -</td>
</tr>
<tr>
<td>Ritonavir</td>
<td>720.9</td>
<td>C37H48N6O5S2</td>
<td>-9.80</td>
<td>25 THR 26 THR 27 LEU 41 HIS 49 MET 144 SER 145 CYS 163 HIS 165 MET 166 GLU 187 ASP 189 GLN - - -</td>
</tr>
<tr>
<td>Emtricitabine</td>
<td>247.25</td>
<td>C8H10FN3O3S</td>
<td>-5.99</td>
<td>41 HIS 49 MET 164 HIS 165 MET 166 GLU 187 ASP 189 GLN - - -</td>
</tr>
<tr>
<td>Ganciclovir</td>
<td>255.23</td>
<td>C9H13N5O4</td>
<td>-6.43</td>
<td>41 HIS 49 MET 54 TYR 145 CYS 165 MET 166 GLU 189 GLU 189 GLN 192 GLN - -</td>
</tr>
<tr>
<td>Baricitinib</td>
<td>371.4</td>
<td>C16H17N7O2S</td>
<td>-8.17</td>
<td>41 HIS 49 MET 54 TYR 145 CYS 165 MET 167 LEU 188 ARG 189 GLN 192 GLN - -</td>
</tr>
<tr>
<td>Ribavirin</td>
<td>244.2</td>
<td>C8H12N4O5</td>
<td>-6.22</td>
<td>41 HIS 49 MET 54 TYR 165 MET 166 GLU 188 ARG 189 GLN 192 GLN - -</td>
</tr>
<tr>
<td>Oseltamivir</td>
<td>312.4</td>
<td>C16H28N2O4</td>
<td>-6.92</td>
<td>41 HIS 49 MET 54 TYR 145 CYS 165 MET 167 LEU 187 ASP 189 GLN 192 GLN - -</td>
</tr>
<tr>
<td>Favipiravir</td>
<td>157.1</td>
<td>C5H4FN3O2</td>
<td>-4.60</td>
<td>165 MET 166 GLU 188 ARG 189 GLN 190 TH 192 GLN - - - -</td>
</tr>
<tr>
<td>Carba-penam</td>
<td>111.14</td>
<td>C6H9NO</td>
<td>-4.44</td>
<td>41 HIS 49 MET 54 TYR 165 MET 189 GLN - - - -</td>
</tr>
</tbody>
</table>

Vital Amino acid Binding Residues
higher percentage of inhibition potential of hydroxychloroquine with an EC50 value of 0.72 μM in comparison with chloroquine with an EC50 value of 5.47 μM [31].

Docking calculations were carried out for an array of FDA approved molecules such as Emtricitabine, Osel-tamivir, Ganciclovir, Chloroquine, Baricitinib, Favipiravir, Lopinavir, Ritonavir, Remdesivir, Ribavirin, Tenofovir, Umifenovir, Carbapenam, Ertapenem and Imipenam which have both specificity and selectivity in terms of binding efficiency against 3CL proenzyme. Comparatively, the compound Remdesivir ranks first in the series with a total of 8 strong molecular interactions with the amino acids on the active sites, followed by Tenofovir, Umifenovir, Ertapenem and Imipenam with a total of 7 interactions. Docking analysis further exemplifies the binding capacity of other molecules like Chloroquine, Lopinavir and Ritonavir with a total of 6 to 5 active interactions. Reports of present computational analysis clearly signify the efficiency of the selected ligands in the increasing order of binding capacity: Remdesivir > Ertapenem > Imipenam > Tenofovir > Umifenovir > Chloroquine > Lopinavir > Ritonavir > Emtricitabine > Ganciclovir > Baricitinib > Ribavirin > Oseltamivir > Favipiravir > Carbapenam, as shown in Table I and represented in Figs. (4 and 5).

Docking calculations were carried out for an array of FDA approved molecules such as Emtricitabine, Oseltamivir, Ganciclovir, Chloroquine, Baricitinib, Favipiravir, Lopinavir, Ritonavir, Remdesivir, Ribavirin, Tenofovir, Umifenovir, Carbapenam, Ertapenem and Imipenam, which have both specificity and selectivity in terms of binding efficiency against 3CL proenzyme. Comparatively, the compound Remdesivir ranks first in the series with a total of 9 strong molecular interactions with the amino acids on the active sites, followed by Tenofovir, Umifenovir, Ertapenem and Imipenam with a total of 7 interactions. Docking analysis further exemplifies the binding capacity of other molecules like Chloroquine, Lopinavir and Ritonavir with a total of 6 to 5 active interactions.

Results of the kinetic predictions clearly signify that most of the leads are not permeant through BBB, which denotes the level of safety index and also obeys the Lipinski rule of drug-likeness with not more than 2 violations. Further, most of the molecules are indicated with high GI absorption in elaborating the kinetic property of approved molecules, as shown in Table 2.
Fig. (5). Representing interaction analysis plot of FDA approved lead molecules against COVID-19 main protease (3-chymotrypsin-like protease (3CL pro)) -PDB- 6LU7.

Table 2. Summarizing Pharmacokinetic and drug-likeness Property of FDA approved lead molecules.

<table>
<thead>
<tr>
<th>FDA Approved Molecules</th>
<th>Pharmacokinetic and drug-likeness Property</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>GI Absorption</td>
</tr>
<tr>
<td>Remdesivir</td>
<td>Low</td>
</tr>
<tr>
<td>Ertapenem</td>
<td>Low</td>
</tr>
<tr>
<td>Imipenem</td>
<td>Low</td>
</tr>
<tr>
<td>Tenofovir</td>
<td>Low</td>
</tr>
<tr>
<td>Umifenovir</td>
<td>High</td>
</tr>
</tbody>
</table>

(Table 2) contd....
CONCLUSION

Emerging SARS-CoV-2 infection rates urge the need for a dynamic therapeutic strategy that has a tendency to halt the progression and adequately lowers the viral replication at the cellular level. Virtual screening offers a tremendous opportunity for the researcher in the process of lead identification and optimization. Molecular dynamic simulation models attain greater importance due to a high degree of reliability and confidence in revealing affinity on selective target. Further, simulation models reduce the actual time involved in the event of drug discovery. Results of the present investigation clearly depict that the FDA approved lead molecules such as Remdesivir, Ertapenem, Imipenam, Tenofovir Umifenovir and Chloroquine occupies a high priority in the scale of increasing binding affinity against the target enzyme 3CLpro. In conclusion, lead molecules from already approved sources provoke promising potential, which grabs the attention of the clinicians in availing potential therapeutic candidates as a drug of choice in the clinical management of COVID-19 time-dependently.

LIST OF ABBREVIATIONS

ACE2 = Angiotensin-converting enzyme 2
ADME = Absorption,distribution,metabolism and elimination
AIDS = Acquired immunodeficiency syndrome
CLpro = Chymotrypsin-like protease
CoV = Coronavirus
COVID-19 = Coronavirus Disease 2019
Cys = Cysteine
FDA = Food and Drug Administration
Glu = Glutamate
Gly = Glycine
His = Histidine
HIV = Human immunodeficiency viruses
ICMR = Indian Council of Medical Research
MERS = Middle East respiratory syndrome
ORF = Open reading frame
Phe = Phenylalanine
pp = polyprotein
PDB = Protein data bank
RCSB = Research collaboratory for structural bioinformatics
RNA = Ribonucleic acid
SARS = Severe acute respiratory syndrome
S1 = Spike protein
2D = Two dimensional
3D = Three dimensional
ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

HUMAN AND ANIMAL RIGHTS

No Animals/Humans were used for studies that are basis of this research.

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF DATA AND MATERIALS

Not applicable.

FUNDING

Indian Council of Medical Research (ICMR), Government of India, New Delhi. Project Ref No: 35/2/2019-Nano/BMS.
CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

ACKNOWLEDGEMENTS

The authors would like to thank the Indian Council of Medical Research (ICMR), Government of India, New Delhi.

REFERENCES


http://dx.doi.org/10.1038/s41421-020-0156-0 PMID: 32194981