LETTER ARTICLE

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

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Abstract: Background: Spread of COVID-19 attains a crucial transition in reveling 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> Umifenovir> Chloroquine> Lopinavir> Ritonavir> Emtricitabine> Ganciclovir> Baricitinib> Ribavirin> 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

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

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
enzyme 2 (ACE2) receptor. The outcome of several research has clearly implicated that S1 (trimeric protein) portion of the spike glycoprotein categorized as class I viral fusion protein actually initiates the process of ACE2 recognition and binding [4]. Sequential residual amino acids on S1 protein involved in mediating this anchoring procedure are known as receptor binding motif (RBM); either carboxyl or amino-terminal chains of S1 domain manage to bind with the recognition site of the receptor. In the next level, membrane fusion predominantly anchored by the S2 sub-unit present on the ectodomain part of spike glycoprotein [5].

Dissociation of the viral membrane unveils the pathogenic RNA into the host cytoplasm that propagates the sequence of a serious chain of the replication process. SARS-CoV-2 speculatively consists of an open reading frame (ORF) region genetically encoded with the gene responsible for nucleocapsid and spikes formation. Host cell ribosomes start the translation mechanism of converting ORF1a and ORF1ab into the respective polyprotein replicate (pp1a and pp1ab) [6]. Here, it comes to the role of 3-chymotrypsin-like protease (3CL pro), which catalyzes the cleavage of polyprotein to get16 nonstructural proteins (called nsp1-nsp16) [7]. These proteins are highly essential for viral replication and hence become a primary target for enzyme inhibitors. 3CL pro is considered to be the potential target for the researchers as it is responsible for processing the majority of the cleavage sites, proving that it is essential in the replication of coronavirus.

3CLpro shares a common sequential homology with the majority of human coronaviruses with respect to its architectural and functional groups. In perspective, the active site mediating the enzyme activity seems deeply buried inside the S1 pocket [8]. It is construed that the enzyme protein chain having a cohort of active sites, which include His 41, Phe 140, Gly 143, Cys 144, Cys 145, His 163, Glu 166 and His 172 assist the polyprotein that necessitates the replication process [9]. The paramount challenge, therefore, is to engage all the active sites in the helical chain in order to delay the replicating process. In-Silico molecular docking analysis provides reliable information pertaining to the binding tendency of the ligand on the core amino acid residue spread over the protein receptors. Molecular docking plays a vital role in the process of lead identification and optimization [10]. Hence, the present investigation is aimed at evaluating the enzyme inhibition potential of known FDA approved molecules such as Emtricitabine, Oseltamivir, Ganciclovir, Chloroquine, Baricitinib, Favipiravir, Lopinavir, Ritonavir, Remdesivir, Ribavirin, Tenofovir, Umifenovir, Carbapenam, Ertapenem, and Imipenam subjected to molecular docking Investigation against COVID-19 main protease (3-chymotrypsin-like protease 3CL pro) with protein data bank (PDB)-6LU7.

2.2. Protein Preparation

Three dimensional (3D) structure of COVID-19 main protease (3-chymotrypsin-like protease 3CL pro) with protein data bank (PDB)-6LU7 (Fig. 1A) retrieved from Research Collaboratory for Structural Bioinformatics (RCSB). The protein structure was cleaned by removing the existing lead components, water molecules cleaved, Gasteiger charges computed with the inclusion of polar hydrogens, merging of non-polar and rotatable bonds, which were defined using AutoDock4 [11].

2.3. Active Site Prediction on the Target Protein

Biologically active amino acid residues, which are primarily involved in cleavage and production of nonstructural proteins essential for viral replication were predicted using the Ramachandran plot, indicating localization of the residues on the target enzyme. Prediction by MolProbity server and also through literature survey [12] is shown in Fig. (1B).

2.4. Ligand Model Preparation

Structures of the FDA approved molecules such as Emtricitabine, Oseltamivir, Ganciclovir, Chloroquine, Baricitinib, Favipiravir, Lopinavir, Ritonavir, Remdesivir, Ribavirin, Tenofovir, Umifenovir, Carbapenam, Ertapenem, and Imipenam subjected to docking investigation were outlined using ChemDraw sketch software and converted from two-dimension 2D to 3D structures. Figs. (2 and 3) summarize the 2D and 3D structure of approved ligand subjected to molecular docking Investigation against COVID-19 main protease (3-chymotrypsin-like protease 3CL pro with protein data bank (PDB)-6LU7.

2.5. Docking Simulations

Molecular docking analysis was performed using the licensed version of Auto Dock 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 that is 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 programmed 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 ADME (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 (thalamidome) [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 clinical response [29]. Another open labelled randomized trial involving 240 COVID-19 patients to ensure the efficacy of favipiravir (RdRp inhibitor) and umifenovir (antiviral) 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].

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.

| FDA Approved Molecules | Molecular_weight (g/mol) | Molecular Formula | Docking Score (kcal/mol) | Vital Amino acid Binding Residues |
|------------------------|--------------------------|-------------------|--------------------------|----------------------------------|
| Remdesivir             | 602.6                    | C27H35N6O8P       | -8.38                    | 
|                        |                          |                   | 25 THR 27 LEU 41 HIS 49 MET 41 HIS 49 MET 143 GLY 145 CYS 163 HIS 164 HIS 165 MET 166 GLU 172 HIS |
| Ertapenem              | 475.5                    | C22H25N3O7S       | -8.39                    | 
|                        |                          |                   | 41 HIS 49 MET 142 ASN 145 CYS 163 HIS 165 MET 166 GLU 167 LEU 168 PRO 189 GLN |
| Imipenem               | 299.35                   | C12H17N3O4S       | -7.67                    | 
|                        |                          |                   | 41 HIS 49 MET 54 TYR 140 PHE 145 CYS 163 HIS 165 MET 166 GLU 172 HIS 187 ARG 189 GLN |
| Tenofovir              | 287.21                   | C9H14N5O4P        | -5.98                    | 
|                        |                          |                   | 41 HIS 49 MET 140 PHE 141 LEU 144 SER 145 CYS 163 HIS 165 MET 166 GLU 189 GLN |
| Umifenovir             | 477.4                    | C22H25BrN2O3S     | -7.36                    | 
|                        |                          |                   | 25 THR 27 LEU 41 HIS 49 MET 144 SER 145 CYS 163 HIS 165 MET 166 GLU 167 LEU 168 PRO |
| Chloroquine            | 319.9                    | C18H26CIN3        | -7.75                    | 
|                        |                          |                   | 41 HIS 144 SER 145 CYS 163 HIS 165 MET 166 GLU 167 LEU 168 PRO |
| Lopinavir              | 628.8                    | C37H48N4O5        | -9.14                    | 
|                        |                          |                   | 25 THR 27 LEU 41 HIS 49 MET 54 TYR 142 ASN 145 CYS 165 MET 166 GLU 189 GLN |
| Ritonavir              | 720.9                    | C37H48N6O5S2      | -9.80                    | 
|                        |                          |                   | 25 THR 26 THR 27 LEU 41 HIS 49 MET 144 SER 145 CYS 163 HIS 165 MET 166 GLU 189 GLN |
| Emtricitabine          | 247.25                   | C8H10FN3O3S       | -5.99                    | 
|                        |                          |                   | 41 HIS 49 MET 164 HIS 165 MET 166 GLU 167 LEU 187 ASP 189 GLN |
| Ganciclovir            | 255.23                   | C9H13N5O4         | -6.43                    | 
|                        |                          |                   | 41 HIS 49 MET 54 TYR 145 CYS 165 MET 166 GLU 189 GLU 189 GLN 192 GLN |
| Baricitinib            | 371.4                    | C16H17N7O2S       | -8.17                    | 
|                        |                          |                   | 41 HIS 49 MET 54 TYR 145 CYS 165 MET 167 LEU 168 PRO 189 GLN 190 THR 192 GLN |
| Ribavirin              | 244.2                    | C8H12N4O5         | -6.22                    | 
|                        |                          |                   | 41 HIS 49 MET 54 TYR 165 MET 166 GLU 168 PRO 188 ARG 189 GLN 190 THR 192 GLN |
| Oseltamivir            | 312.4                    | C16H28N2O4        | -6.92                    | 
|                        |                          |                   | 41 HIS 49 MET 54 TYR 145 CYS 165 MET 167 LEU 187 ASP 189 GLN 192 GLN |
| Favipiravir            | 157.1                    | C5H4FN3O2         | -4.60                    | 
|                        |                          |                   | 165 MET 166 GLU 188 ARG 189 GLN 190 THR 192 GLN |
| Carbapenem             | 111.14                   | C6H9NO            | -4.44                    | 
|                        |                          |                   | 41 HIS 49 MET 54 TYR 165 MET 189 GLN |

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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, 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 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 1 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.

| FDA Approved Molecules | Pharmacokinetic and drug-likeness Property |
|------------------------|-------------------------------------------|
|                        | GI Absorption | BBB Permeant | Skin permeation Cm/s | Lipinski violations | Ghose violations |
| Remdesivir             | Low           | No           | -8.62                 | 2                  | 3                |
| Ertapenem              | Low           | No           | -10.24                | 0                  | 0                |
| Imipenam               | Low           | No           | -8.62                 | 0                  | 1                |
| Tenofovir              | Low           | No           | -9.19                 | 0                  | 0                |
| Umifenovir             | High          | No           | -6.07                 | 0                  | 0                |
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| FDA Approved Molecules | Pharmacokinetic and drug-likeness Property |
|------------------------|-------------------------------------------|
|                        | GI Absorption | BBB Permeant | Skin permeation Cm/s | Lipinski violations | Ghose violations |
| Chloroquine            | High          | Yes          | -4.96                | 0                  | 0               |
| Lopinavir              | High          | No           | -5.93                | 1                  | 3               |
| Ritonavir              | Low           | No           | -6.40                | 2                  | 4               |
| Emtricitabine          | High          | No           | -8.25                | 0                  | 0               |
| Ganciclovir            | Low           | No           | -9.04                | 0                  | 1               |
| Baricitinib            | High          | No           | -8.89                | 0                  | 0               |
| Ribavirin              | Low           | No           | -9.10                | 0                  | 1               |
| Oseltamivir            | High          | No           | -7.42                | 0                  | 0               |
| Favipiravir            | High          | No           | -7.66                | 0                  | 4               |
| Carbapenam             | Low           | No           | -6.99                | 0                  | 3               |

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, Tenfovir 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

[1] Yi Y, Lagnotto PNP, Ye S, Li E, Xu RH. COVID-19: what has been learned and to be learned about the novel coronavirus disease. Int J Biol Sci 2020; 16(10): 1753-66.
[2] Lu G, Hu Y, Wang Q, et al. Molecular basis of binding between novel human coronavirus MERS-CoV and its receptor CD26. Nature 2013; 500(7461): 227-31.
[3] Li F, Li W, Farzan M, Harrison SC. Structure of SARS coronavirus spike receptor-binding domain complexed with receptor. Science 2005; 309(5742): 1864-8.
[4] Kuba K, Imai Y, Rao S, et al. A crucial role of angiotensin converting enzyme 2 (ACE2) in SARS coronavirus-induced lung injury. Nat Med 2005; 11(8): 875-9.
[5] Shereen MA, Khan S, Kazmi A, Bashir N, Siddique R. COVID-19 infection: Origin, transmission, and characteristics of human coronaviruses. J Adv Res 2020; 24: 91-8.
[6] Ou X, Liu Y, Lei X, et al. Characterization of spike glycoprotein of SARS-CoV-2 on virus entry and its immune cross-reactivity with SARS-CoV. Nat Commun 2020; 11(1): 1620.
[7] Cyranoski D. Did pangolins spread the China coronavirus to people? Nature 2020.
[8] Berry M, Fielding BC, Gamicielid J. Potential broad spectrum inhibitors of the Coronavirus 3CLpro: A virtual screening and structure-based drug design study. Viruses 2015; 7(12): 6642-60.
[9] Yang H, Yang M, Ding Y, et al. The crystal structures of severe acute respiratory syndrome virus main protease and its complex with an inhibitor. Proc Natl Acad Sci USA 2003; 100(23): 13190-5.
[10] Muhammad SA, Fatima N. In silico analysis and molecular docking studies of potential angiotensin-converting enzyme inhibitor using quercetin glycosides. Pharmacog Mag 2015; 11(Suppl. 1): S123-6.
[11] Forli S, Huey R, Pique ME, Sanner MF, Goodsell DS, Olson AJ. Computational protein-ligand docking and virtual drug screening with an inhibitor. Proc Natl Acad Sci USA 2003; 100(23): 13190-5.
[12] Williams CJ, Headd JJ, Moriarty NW, et al. MolProbity: More and better reference data for improved all-atom structure validation. Protein Sci 2018; 27(1): 293-315.
[13] Osterberg F, Morris GM, Sanner MF, Olson AJ, Goodsell DS. Automated docking to multiple target structures: incorporation of protein mobility and structural water heterogeneity in AutoDock. Proteins 2002; 46(1): 34-40.
[14] Morris GM. Automated docking using a Lamarckian genetic algorithm and an empirical binding free energy function. J Comput Chem 1998; 19: 1639-62.
[30] Chang C, Yi Z, Jianying H, Ping Y, Zhenshun C, Jianyuan W, et al. Favipiravir versus Arbidol for COVID-19: A randomized clinical trial. MedRxiv 2020.

[31] Liu J, Cao R, Xu M, et al. Hydroxychloroquine, a less toxic derivative of chloroquine, is effective in inhibiting SARS-CoV-2 infection in vitro. Cell Discov 2020; 6: 16.
http://dx.doi.org/10.1038/s41421-020-0156-0 PMID: 32194981