Comparison of Clinically Approved Molecules on SARS-CoV-2 Drug Target Proteins: A Molecular Docking Study

Hasan Cubuk[a] and Mehmet Ozbil[a]*

[a]Department of Molecular Biology and Genetics, Istanbul Arel University, 34537, Buyukcekmece, Istanbul.

*Corresponding author: Mehmet Ozbil, Department of Molecular Biology and Genetics, Istanbul Arel University, 34537, Buyukcekmece, Istanbul.

Phone: +90 850 850 2735. E-mail: mehmetozbil@arel.edu.tr

Abstract

The new type of coronavirus, SARS-CoV-2 has affected more than 1,2 million people worldwide. Since the first day the virus has been spotted in Wuhan, China, there are numerous drug design studies conducted all over the globe. Most of these studies target the receptor-binding domain of spike protein of SASR-CoV-2, which is known to bind human ACE2 receptor and SARS-CoV-2 main protease, vital for the virus' replication. However, there might be a third target, human furin protease, which cleaves the virus' S1-S2 domains taking active role in its entry into the host cell. In this study we docked five clinically used drug molecules, favipiravir, hydroxychloroquine, remdesivir, lopinavir, and ritonavir onto three target proteins, receptor binding domain of SARS-CoV-2 spike protein, SARS-CoV-2 main protease, and human furin protease. Computational results clearly showed that all ligands provided higher binding affinities towards furin protease, except hydroxychloroquine and ritonavir yielding the highest binding affinity. This proves that furin protease might be targeted for drug design studies and must be further explored *in vitro* and *in vivo*.

Keywords: SARS-CoV-2, COVID-19, Furin Protease, Ritonavir, Molecular Docking

Introduction

Coronaviruses are a type of single-stranded RNA viruses that infect mammals and birds. In humans, they cause respiratory diseases ranging from common cold to severe/fatal illnesses.[1] Three types of human-infecting coronaviruses were associated with deadly phenomenia since the early period of 2000s; severe acute respiratory syndrome coronavirus (SARS-CoV), Middle-East respiratory syndrome coronavirus (MERS-CoV) and Severe acute respiratory syndrome coronavirus 2 (SARS-CoV2). In November 2002, SARS-CoV affected 8,098 people and causing 774 deaths in China until the June 2003. In June 2012, MERS-CoV appeared in Middle East, over 2,000 cases and reported by 2017 with about 600 deaths.[2-3]

Lastly, SARS-CoV-2, discovered in China, has affected over 1,276,302 people and killed 69,527 in more than 199 countries as of April 4, 2020. On 11 February 2020. The World Health Organization (WHO) announced “COVID-19” as the name of new disease caused by SARS-CoV-2.[3-4] The ongoing SARS-CoV-2 threat that emerged in China has rapidly spread to other countries and continuing to spread. Thus, many efforts have been directed to the investigation of suitable preventive and control strategies in a few months as neither vaccines nor direct-acting antiviral drugs are available for the treatment of human SARS-CoV-2.

Most of the therapeutic options for COVID-19 were based on anti-viral agents, which are used for treating previous Zika, Ebola, and Nipah viruses, SARS-, and MERS-CoVs.[5] This is due to fact that the time required for drug discovery programs to develop, evaluate, and obtain appropriate new therapeutic agents might take more than 10 years. Thus, researchers are focused on therapeutics, which have proven efficacy against viruses similar to COVID-19 instead of a new potent anti-COVID-19 agent. These available therapeutic agents against SARS-CoV-2 could be either virus-based, involving small molecules targeting viral S protein, viral protease inhibitors and RBD–ACE2 blockers or host cell-based including host cell protease inhibitors and host cell endocytosis inhibitors.[5]

Spike protein directly mediates viral entry with S1 domain, which is responsible for host cell surface binding through ACE2 receptors and S2 domain responsible for membrane fusion. The viral binding to host cell surface is following S1/S2 cleavage

by host proteases such as TMPRSS2, cathepsins B and L. Previous studies have already demonstrated Furin, a kind of proprotein convertases, can mediate S1/S2 cleavage unlike other coronaviruses and contribute to membrane fusion efficiency which explain current strong infectious capacity of SARS-CoV-2 [2-3]. Thus, SARS-CoV-2^{RBD}/ACE2 and Furin could be potential targets for COVID-19 to prevent viral entry. Furthermore, SARS-CoV-2 main protease known as 3CL^{pro} which is essential in processing viral polyproteins and viral replication could be a non-toxic target for managing COVID-19 as humans do not have proteases with a similar cleavage specificity.[1, 3, 6]

In vitro studies by Liu et al. had already demonstrated that two drugs, chloroquine (CQ) and hydrochloroquine (HCQ) efficiently inhibited SARS-CoV-2 infection in vitro and these findings were supported by preliminary clinical studies as well.[7-10] Several other drugs such as, remdesivir, and favipiravir are currently undergoing clinical studies to test their efficacy and safety in the treatment of COVID-19 in China and other European countries such as Turkey and some promising results have been achieved so far.[11-15] In addition, lopinavir, and ritonavir are widely used as HIV protease inhibitors, and previous in vitro and in vivo studies have also shown their potential activity against other coronaviruses; SARS-, and MERS-CoVs.[16-19]

In the present study, we investigated binding of five active molecules, currently applied as the first line of treatment, favipiravir, hydroxychloroquine, remdesivir, lopinavir, and ritonavir onto three different possible target proteins, receptor binding domain of SARS-CoV-2 spike protein (SARS-CoV-2^{RBD}), SARS-CoV-2 main protease(SARS-CoV-2 M^{pro}), and human furin (hFUR) protease by molecular docking simulations. Our aim was to shed light on the target selection for future drug design studies.

Methods

Preparation of the protein and target molecules

The crystal structure of the proprotein convertase furin (PDB: 1P8J, 2.6 Å), COVID-19 main protease in complex with an inhibitor N3 (PDB: 6LU7, 2.16 Å) and coronavirus spike receptor-binding domain complexed with its receptor ACE2 (PDB: 6LZG, 2.5 Å) were obtained from the Research Collaboratory for Structural

Bioinformatics Protein Data Bank (RCSB PDB).[20-21] Small molecules were removed from crystal structures by using BIOVA Discovery Studio software.[22] Polar hydrogens and Kollman charges were added to the protein and a pdbqt format file was generated by using AutoDockTools 1.5.6 software.[23]

The canonical SMILES of Lopinavir, Remdesivir, Hydrochloroquine, Favipiravir and Ritonavir were obtained from PubChem database. Their structures were built, and structural optimization was carried out with USCF Chimera software.[24] Afterwards, the structures were converted into pdbqt format by using AutoDockTools 1.5.6 software, in use for docking calculations with Vina.

Docking

Autodock Vina 1.1.2 software[25] was used for docking calculations and exhaustiveness parameter was selected as 8, and 10 modes were generated for each ligand. Windows 7 Ultimate operating system (64-bit) installed on a home-built computer, equipped with Intel Core i3-3110M 2.40GHz processor and 8GB memory, was utilized for all computational work. Results were analysed using BIOVA Discovery Studio software and VMD-Visual Molecular Dynamics software.[26]

Results and Discussion

To test inhibition capability of five clinically used molecules disrupting SARS-CoV-2^{RBD}/ACE2 interaction, we designed docking simulations with grid box covering only SARS-CoV-2^{RBD}-ACE2 interface. Binding of five molecules onto SARS-CoV-2^{RBD} yielded binding affinities ranging from -4.2 kcal/mol to -6.9 kcal/mol (Table 1 and Figure 1). These affinities clearly proved among these active molecules only lopinavir and ritonavir had high affinity towards this target. Lopinavir and ritonavir yielded binding affinity of -6.9 kcal/mol and -6.4 kcal/mol, respectively. Favipiravir, hydroxychloroquine, and remdesivir were not good binders of the protein. Relatively small sizes of favipiravir and hydroxychloroquine must be the reason for these small binding affinities. Molecular interactions were provided in Figure 2. These binding affinities suggest lopinavir and ritonavir may have potential activity against COVID-19 through SARS-CoV-2^{RBD}/ACE2 inhibition.

Secondly, we set out docking simulations to examine the structural roles of these drugs on SARS-CoV-2 M^{pro} activity during viral infection. Docking analysis onto

SARS-CoV-2 M^{pro} didn't yield significantly higher binding affinities, they were between from -5.2 kcal/mol to -6.6 kcal/mol. Lopinavir was bound with the highest binding affinity of -6.6 kcal/mol (Table 1 and Figure 1). All the binding sites for all 5 molecules were outside the active site of the protease. Therefore, we repeated simulations with smaller simulations box and this time targeting only the active site, SARS-CoV-2 $M^{pro\text{-}ac}$, comprised of amino acid residues Thr 26, His 41, Met 49, Leu 141, Asn 142, Gly 143, Gly 143, Ser 144, Met 165, Glu 166, and Gln 189. Molecular interactions at this site were provided in Figure 3. This time binding affinities increased for all ligands except for favipiravir. Specifically, for lopinavir and remdesivir binding affinities increased by 0.9 kcal/mol and 2.0 kcal/mol, respectively. Both active molecules produced binding affinities of -7.5 kcal/mol. This binding site was also revealed in x-ray structure (PDB ID: 6LUV),[21] for a peptide derivative inhibitor N3 and was also predicted to be lopinavir binding site in a computational study by Liu et.al.[27] (Figure SI-1). Moreover, binding affinity of lopinavir onto SARS-CoV-2 $M^{pro\text{-}ac}$ yielded the highest binging affinity, equal with the active site of human furin protease ($hFUR^{ac}$). Hydroxychloroquine provided the highest binding affinity among all three targets on this active site. However, it is noteworthy that when ligands were docked onto the whole protein, the molecules did not reach the active site. Although binding to active site yielded higher binding affinities, molecules reached to the active site only with a small grid box with only active site coverage. Overall, our results demonstrated that both lopinavir and remdesivir might have potential activity on preclinical COVID-19 researches as SARS-CoV-2 M^{pro} inhibitors.

The last target protein investigated was hFUR and binding affinities were in the range of -5.6 kcal/mol to -7.5 kcal/mol. All three relatively larger molecules, remdesivir, lopinavir, and ritonavir provided similar binding affinities, -7.5 kcal/mol, -7.1 kcal/mol, and -7.3 kcal/mol, respectively (Table 1 and Figure 1). Remdesivir provided the highest binding affinity with -7.5 kcal/mol among all active molecules. On the other hand, favipiravir was bound to this target with the highest binding affinity among all other targets with -6.2 kcal/mol binding affinity. We again repeated docking simulations with a smaller grid box covering only active site of human furin ($hFUR^{ac}$), comprised of amino acid residues Asp 153, Arg193, His 194, Arg 197, Leu 227, Val 231, Ser 253, Asp 258, and Asn 295. Molecular interactions were provided in Figure 4. Binding affinity for remdesivir increased by 0.3 kcal/mol, for lopinavir

increased by 0.4 kcal/mol. The sharpest increase was observed for binding affinity of ritonavir, by 1.5 kcal/mol, which was calculated to be -8.8 kcal/mol. This binding affinity was the highest calculated for the molecules in the study, followed by binding affinity for remdesivir at the same location. Furthermore, binding affinities of remdesivir and ritonavir were strikingly higher than binding affinities at other targets. This might suggest that hFUR could be the main target for remdesivir and ritonavir. We should also mention that when all molecules were docked onto the whole hFUR, three molecules, favipiravir, hydroxychloroquine, and lopinavir hit active site of the protease. This suggested that active site of hFUR was more accessible for these molecules than active site of SARS-CoV-2 M^{pro} and could be used as a potential therapeutic target more specifically than SARS-CoV-2 M^{pro}.

Conclusions

In this study we investigated binding of readily prescribed drug molecules favipiravir, hydroxychloroquine, remdesivir, lopinavir, and ritonavir onto three proteins, which should be targeted for COVID-19 treatment. Among all targets, receptor binding domain of SARS-Cov2 spike protein (SARS-CoV-2^{RBD}), SARS-CoV-2 main protease (SARS-CoV-2 M^{pro}), and human furin (hFUR) protease, binding affinities for all drug molecule were calculated to be the highest for active site of human furin protease, $hFUR^{ac}$. Moreover, ritonavir and remdesivir produced very high binding affinities, -8.8 kcal/mol and -7.8 kcal/mol in this active site. Reason for these high affinities must be hydroxyethylene scaffold that mimics the peptide linkage for ritonavir and the adenosine triphosphate moiety of remdesivir, making strong hydrophobic and polar interactions in the active site, respectively. As reported in our study, clinically used drug molecules have higher binding affinities hFUR than other therapeutic targets in silico. This should be major reason for potential activity of these drugs against COVID-19 in the preclinical studies. Overall, molecular docking calculations performed in the present study, which needs further *in vitro* and *in vivo* experimental proof, clearly highlighted that hFUR might be the novel target for future drug molecule design studies against SARS-CoV-2.

References

1. Fung, T. S.; Liu, D. X., Human Coronavirus: Host-Pathogen Interaction. *Annu Rev Microbiol* **2019,** *73*, 529-557.
2. Walls, A. C.; Park, Y. J.; Tortorici, M. A.; Wall, A.; McGuire, A. T.; Veesler, D., Structure, Function, and Antigenicity of the SARS-CoV-2 Spike Glycoprotein. *Cell* **2020**.
3. Canrong WU, Y. Y., Yang LIU, Peng ZHANG, Yali WANG, Qiqi WANG, Yang XU, Mingxue LI, Mengzhu ZHENG, Lixia CHEN, Hua LI Furin, a potential therapeutic target for COVID-19. **2020**.
4. Ajay K. Gautam, S. S., Shubhi Avasthi and Rekha Bhadauria, Diversity, Pathogenicity and Toxicology of A. niger: An Important Spoilage Fungi. *Research Journal of Microbiology* **2011,** *Volume: 6* (Issue: 3), Page No.: 270-280.
5. Dhama, K.; Sharun, K.; Tiwari, R.; Dadar, M.; Malik, Y. S.; Singh, K. P.; Chaicumpa, W., COVID-19, an emerging coronavirus infection: advances and prospects in designing and developing vaccines, immunotherapeutics, and therapeutics. *Hum Vaccin Immunother* **2020**, 1-7.
6. Zhang, L.; Lin, D.; Sun, X.; Curth, U.; Drosten, C.; Sauerhering, L.; Becker, S.; Rox, K.; Hilgenfeld, R., Crystal structure of SARS-CoV-2 main protease provides a basis for design of improved α-ketoamide inhibitors. *Science* **2020**, eabb3405.
7. Liu, J.; Cao, R.; Xu, M.; Wang, X.; Zhang, H.; Hu, H.; Li, Y.; Hu, Z.; Zhong, W.; Wang, M., Hydroxychloroquine, a less toxic derivative of chloroquine, is effective in inhibiting SARS-CoV-2 infection in vitro. *Cell Discov* **2020,** *6*, 16.
8. Gao, J.; Tian, Z.; Yang, X., Breakthrough: Chloroquine phosphate has shown apparent efficacy in treatment of COVID-19 associated pneumonia in clinical studies. *Biosci Trends* **2020,** *14* (1), 72-73.
9. Wang, M.; Cao, R.; Zhang, L.; Yang, X.; Liu, J.; Xu, M.; Shi, Z.; Hu, Z.; Zhong, W.; Xiao, G., Remdesivir and chloroquine effectively inhibit the recently emerged novel coronavirus (2019-nCoV) in vitro. *Cell Res* **2020,** *30* (3), 269-271.
10. Yao, X.; Ye, F.; Zhang, M.; Cui, C.; Huang, B.; Niu, P.; Liu, X.; Zhao, L.; Dong, E.; Song, C.; Zhan, S.; Lu, R.; Li, H.; Tan, W.; Liu, D., In Vitro Antiviral Activity and Projection of Optimized Dosing Design of Hydroxychloroquine for the Treatment of Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2). *Clin Infect Dis* **2020**.
11. Dong, L.; Hu, S.; Gao, J., Discovering drugs to treat coronavirus disease 2019 (COVID-19). *Drug Discov Ther* **2020,** *14* (1), 58-60.
12. https://khgmstokyonetimidb.saglik.gov.tr/TR, f.-.-m.-t.-.-.-c.-.-s.-c.-e.-t.-k.-i.-i.-b. h., Turkey.
13. Agostini, M. L.; Andres, E. L.; Sims, A. C.; Graham, R. L.; Sheahan, T. P.; Lu, X.; Smith, E. C.; Case, J. B.; Feng, J. Y.; Jordan, R.; Ray, A. S.; Cihlar, T.; Siegel, D.; Mackman, R. L.; Clarke, M. O.; Baric, R. S.; Denison, M. R., Coronavirus Susceptibility to the Antiviral Remdesivir (GS-5734) Is Mediated by the Viral Polymerase and the Proofreading Exoribonuclease. *mBio* **2018,** *9* (2).
14. Brown, A. J.; Won, J. J.; Graham, R. L.; Dinnon, K. H., 3rd; Sims, A. C.; Feng, J. Y.; Cihlar, T.; Denison, M. R.; Baric, R. S.; Sheahan, T. P., Broad spectrum antiviral remdesivir inhibits human endemic and zoonotic deltacoronaviruses with a highly divergent RNA dependent RNA polymerase. *Antiviral Res* **2019,** *169*, 104541.
15. Ko, W. C.; Rolain, J. M.; Lee, N. Y.; Chen, P. L.; Huang, C. T.; Lee, P. I.; Hsueh, P. R., Arguments in favour of remdesivir for treating SARS-CoV-2 infections. *Int J Antimicrob Agents* **2020**, 105933.
16. Tim Smith, P., BCPS; Jennifer Bushek, PharmD; Tony Prosser, PharmD, COVID-19 Drug Therapy – Potential Options. *Elsevier* **2020**.
17. Chu, C. M.; Cheng, V. C.; Hung, I. F.; Wong, M. M.; Chan, K. H.; Chan, K. S.; Kao, R. Y.; Poon, L. L.; Wong, C. L.; Guan, Y.; Peiris, J. S.; Yuen, K. Y., Role of lopinavir/ritonavir in the treatment of SARS: initial virological and clinical findings. *Thorax* **2004,** *59* (3), 252-6.
18. Chen, F.; Chan, K. H.; Jiang, Y.; Kao, R. Y.; Lu, H. T.; Fan, K. W.; Cheng, V. C.; Tsui, W. H.; Hung, I. F.; Lee, T. S.; Guan, Y.; Peiris, J. S.; Yuen, K. Y., In vitro susceptibility of 10 clinical isolates of SARS coronavirus to selected antiviral compounds. *J Clin Virol* **2004,** *31* (1), 69-75.

19. Liu, X.; Wang, X. J., Potential inhibitors against 2019-nCoV coronavirus M protease from clinically approved medicines. *J Genet Genomics* **2020**.
20. Henrich, S.; Cameron, A.; Bourenkov, G. P.; Kiefersauer, R.; Huber, R.; Lindberg, I.; Bode, W.; Than, M. E., The crystal structure of the proprotein processing proteinase furin explains its stringent specificity. *Nat Struct Biol* **2003,** *10* (7), 520-6.
21. Jin, Z.; Du, X.; Xu, Y.; Deng, Y.; Liu, M.; Zhao, Y.; Zhang, B.; Li, X.; Zhang, L.; Peng, C.; Duan, Y.; Yu, J.; Wang, L.; Yang, K.; Liu, F.; Jiang, R.; Yang, X.; You, T.; Liu, X.; Yang, X.; Bai, F.; Liu, H.; Liu, X.; Guddat, L. W.; Xu, W.; Xiao, G.; Qin, C.; Shi, Z.; Jiang, H.; Rao, Z.; Yang, H., Structure of M^{pro} from COVID-19 virus and discovery of its inhibitors. *bioRxiv* **2020**, 2020.02.26.964882.
22. Dassault Systèmes BIOVIA, Discovery Studio Modeling Environment, Release 2017, San Diego: Dassault Systèmes, 2016.
23. Morris, G. M.; Huey, R.; Lindstrom, W.; Sanner, M. F.; Belew, R. K.; Goodsell, D. S.; Olson, A. J., AutoDock4 and AutoDockTools4: Automated docking with selective receptor flexibility. *J Comput Chem* **2009,** *30* (16), 2785-2791.
24. Pettersen, E. F.; Goddard, T. D.; Huang, C. C.; Couch, G. S.; Greenblatt, D. M.; Meng, E. C.; Ferrin, T. E., UCSF Chimera--a visualization system for exploratory research and analysis. *J Comput Chem* **2004,** *25* (13), 1605-12.
25. Trott, O.; Olson, A. J., AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. *J Comput Chem* **2010,** *31* (2), 455-461.
26. Humphrey, W.; Dalke, A.; Schulten, K., VMD: Visual molecular dynamics. *Journal of Molecular Graphics* **1996,** *14* (1), 33-38.
27. Liu, X.; Wang, X.-J., Potential inhibitors for 2019-nCoV coronavirus M protease from clinically approved medicines. *bioRxiv* **2020**, 2020.01.29.924100.

Tables:

Table 1. Binding affinities in kcal/mol of five molecules on three different target proteins.

Binding Affinites (kcal/mol)	Favipiravir	Hydrochloroquine	Remdesivir	Lopinavir	Ritonavir
SARS-CoV-2^{RBD}	-5.1	-4.2	-4.9	-6.9	-6.4
SARS-CoV-2 M^{pro}	-5.7	-5.2	-5.5	-6.6	-6.4
SARS-CoV-2 M$^{pro\text{-}ac}$	-5.4	-5.9	-7.5	-7.5	-6.8
hFUR	-6.2	-5.6	-7.5	-7.1	-7.3
hFUR^{ac}	-6.2	-5.6	**-7.8**	**-7.5**	**-8.8**

Figures:

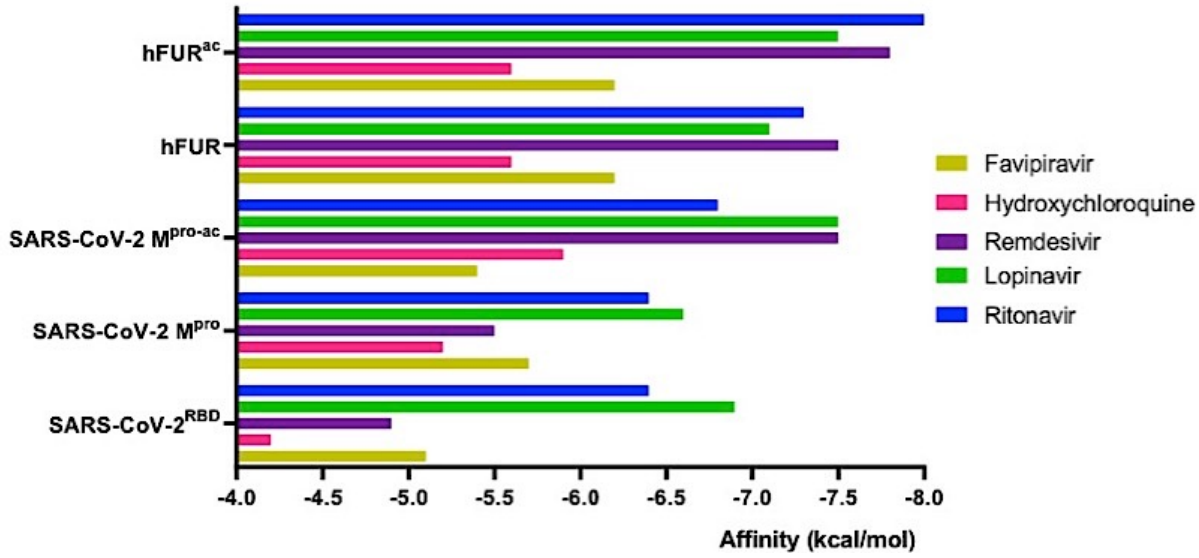
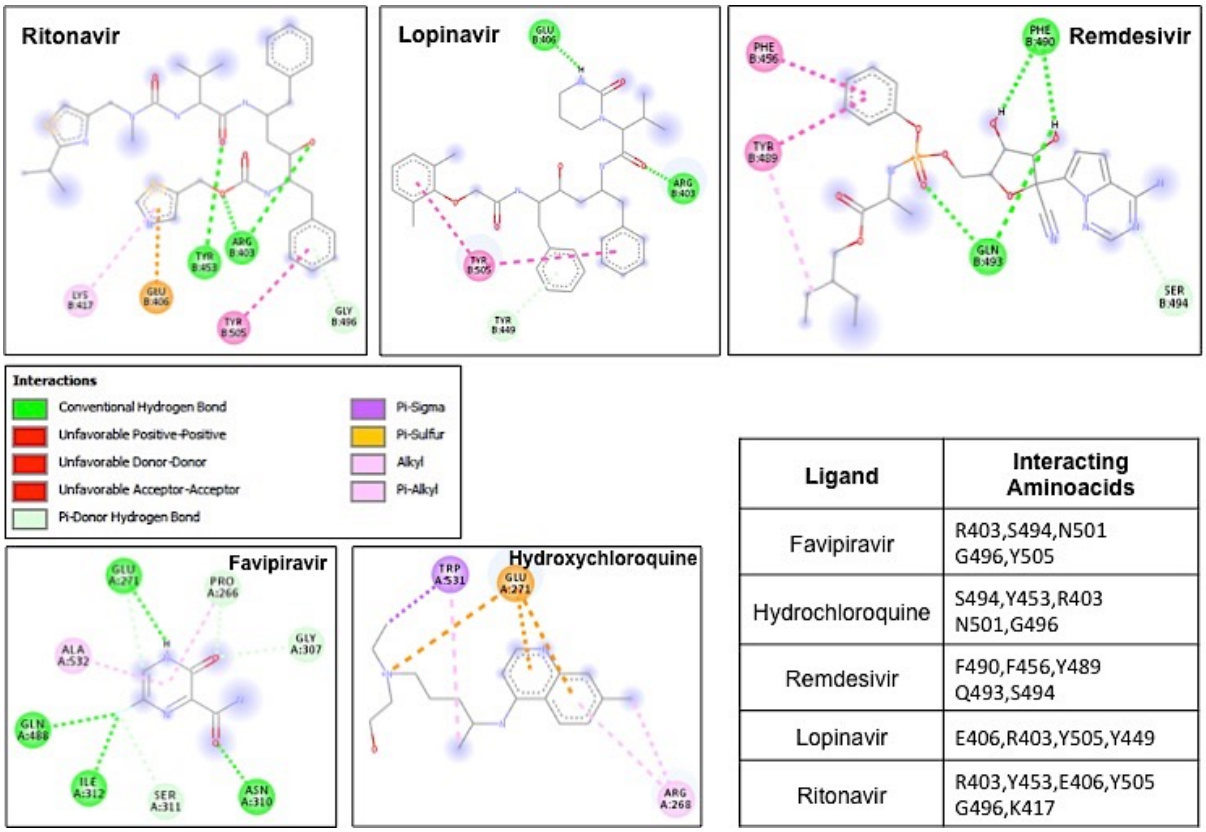
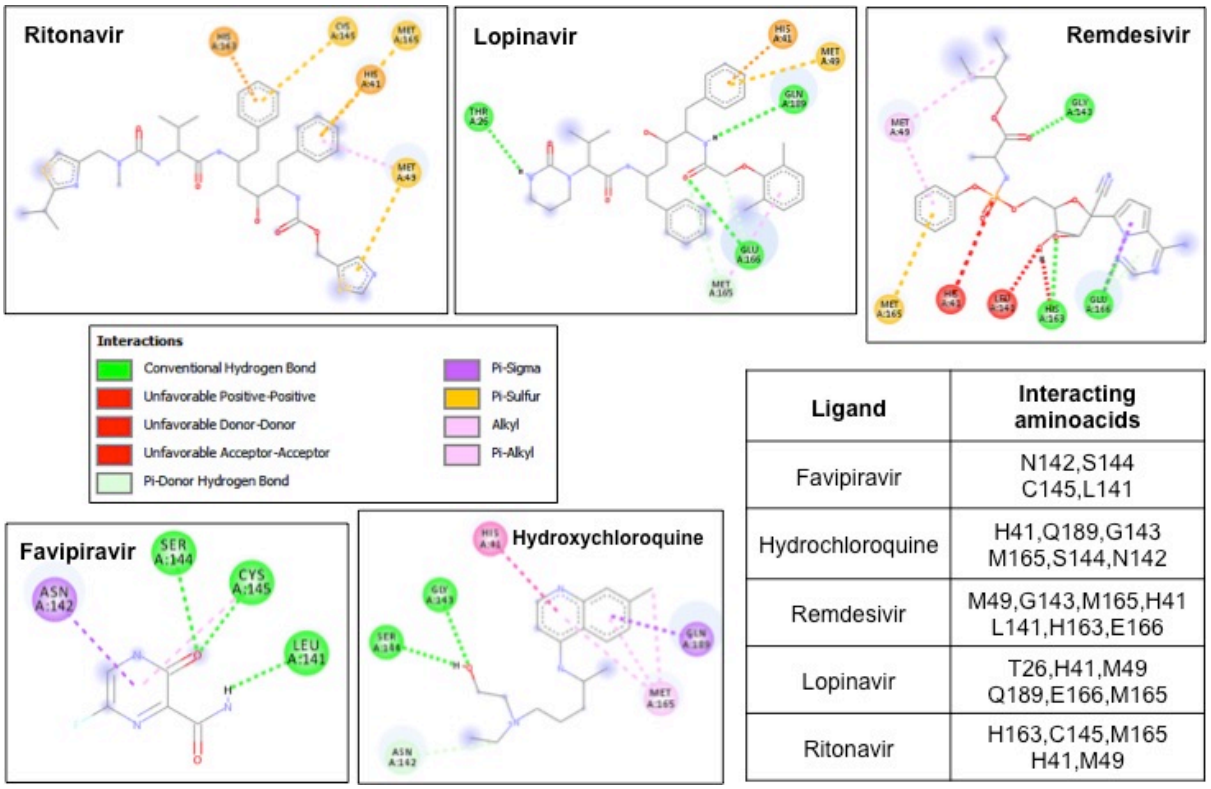


Figure 1. Graphical representation of binding affinities for five molecules on three proteins including the active site of proteases SARS-CoV-2-Mpro and hFUR.



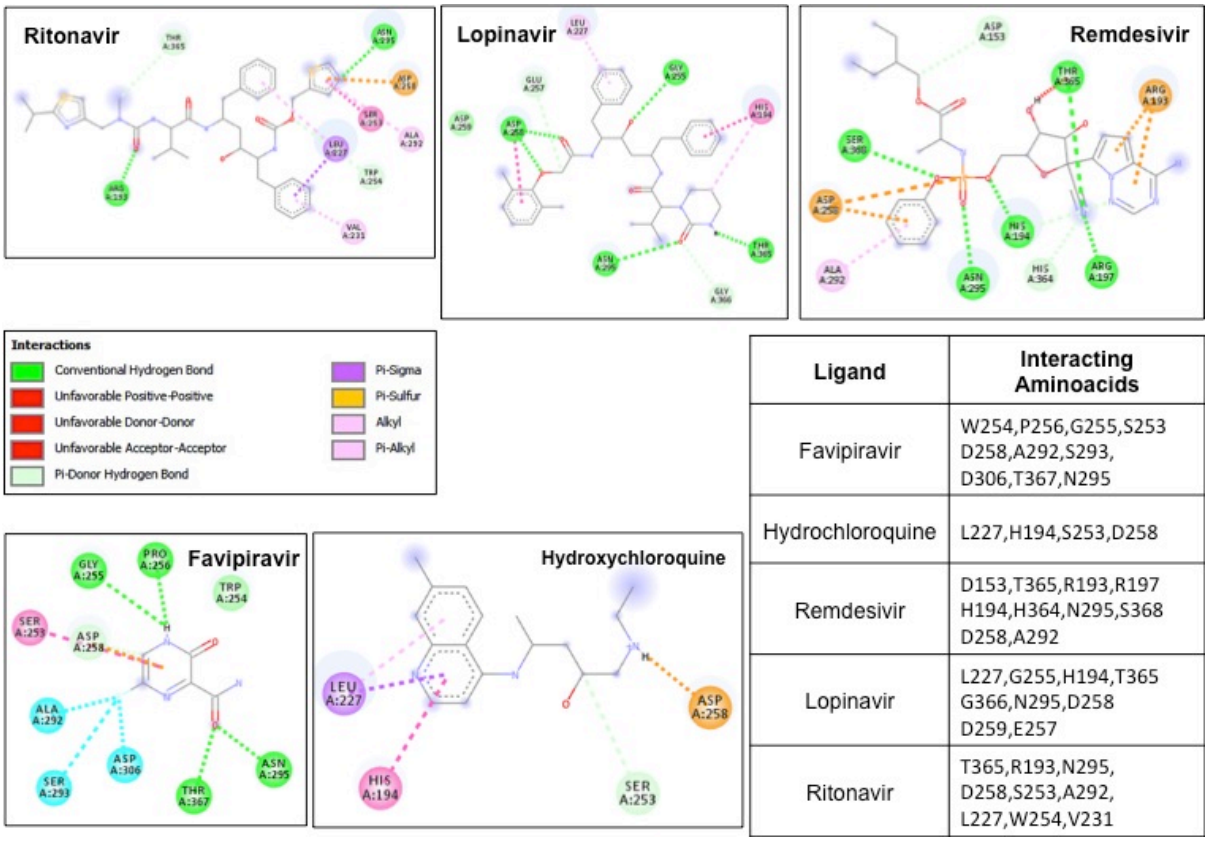
Ligand	Interacting Aminoacids
Favipiravir	R403,S494,N501 G496,Y505
Hydrochloroquine	S494,Y453,R403 N501,G496
Remdesivir	F490,F456,Y489 Q493,S494
Lopinavir	E406,R403,Y505,Y449
Ritonavir	R403,Y453,E406,Y505 G496,K417

Figure 2. Molecular interactions for five molecules at the SARS-CoV-2^{RBD} site. Legend for interactions was provided in the middle and interacting amino acid residues were provided in the table.



Ligand	Interacting aminoacids
Favipiravir	N142,S144 C145,L141
Hydrochloroquine	H41,Q189,G143 M165,S144,N142
Remdesivir	M49,G143,M165,H41 L141,H163,E166
Lopinavir	T26,H41,M49 Q189,E166,M165
Ritonavir	H163,C145,M165 H41,M49

Figure 3. Molecular interactions for five molecules at the SARS-CoV-2 $M^{pro\text{-}ac}$ site. Legend for interactions was provided in the middle and interacting amino acid residues were provided in the table.



Ligand	Interacting Aminoacids
Favipiravir	W254,P256,G255,S253 D258,A292,S293, D306,T367,N295
Hydrochloroquine	L227,H194,S253,D258
Remdesivir	D153,T365,R193,R197 H194,H364,N295,S368 D258,A292
Lopinavir	L227,G255,H194,T365 G366,N295,D258 D259,E257
Ritonavir	T365,R193,N295, D258,S253,A292, L227,W254,V231

Figure 4. Molecular interactions for five molecules at the hFUR^{ac} site. Legend for interactions was provided in the middle and interacting amino acid residues were provided in the table.

Supporting Information Figure:

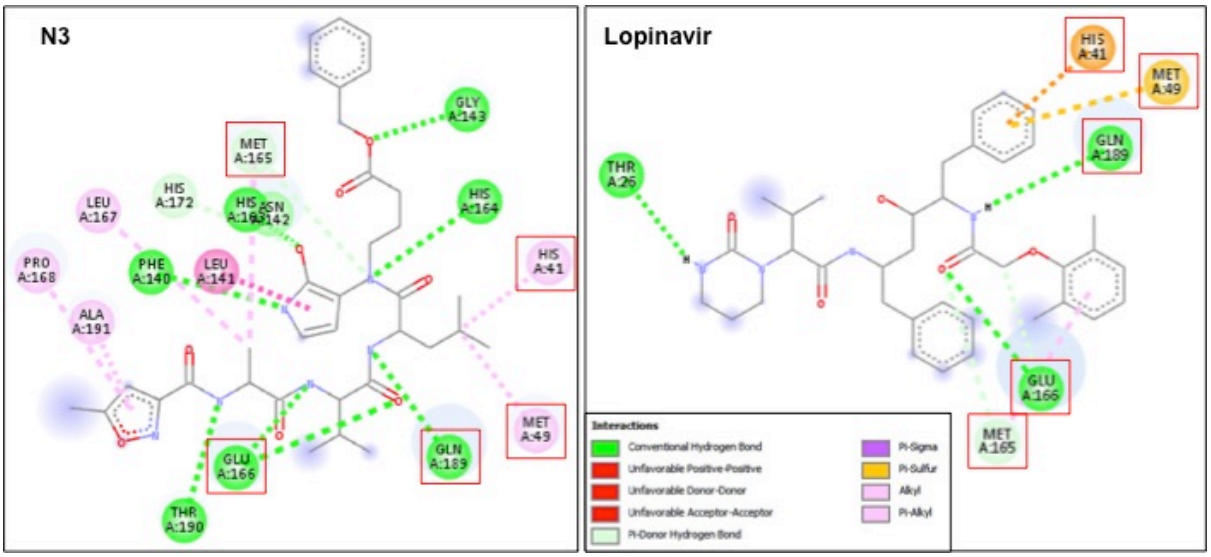


Figure SI-1. Comparison of N3 binding from crystal structure, PDB ID: 6LUV, and Lopinavir binding from the current study at SARS-CoV-2 $M^{pro\text{-}ac}$. Same interacting amino acid residues were shown in red rectangular boxes.