In silico Molecular Docking and ADMET Study of Some Potential Antiviral Drug Candidates as Potential Inhibitors of SARS-CoV-2 Protease M^{pro} (6Y2F)

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ABSTRACT: In this present world COVID-19 pandemic is one of the biggest concern. An appealing medication focus among Covids is the fundamental protease; SARS-CoV-2 protease M^{pro} (6Y2F) due to its fundamental role in handling the polyproteins that are interpreted from the viral RNA. The present study showed the interaction of favipiravir, ganciclovir, raltegravir and remdesivir against 6Y2F, using molecular docking were analyzed. Among those ligands' interaction with protein structure, 6Y2F on raltegravir (-7.4 kcal/mol) and remdesivir (-6.9 kcal/mol), respectively displayed maximum binding affinity. The interactions of four ligands were contrasted with each other in that ganciclovir and raltegravir form highest number of hydrogen bond with 6Y2F. The interacting amino acids residues (Gly143, Ser144, Cys145) were studied and all selected ligands were predicted to be non-carcinogens and non-AMES toxic.

Key words: Antiviral drugs, SARS-CoV-2 protease; Molecular docking; Ligand-Protein interactions; ADMET prediction.

INTRODUCTION

In December 2019, another Covid caused a flare-up of pulmonary disease in the city of Wuhan province in China, and has since spread all around the world. As of June 14, 2021, the pandemic COVID-19 has affected around 222 nations with nearly 3,819,727 deaths, 176,725,930 cases of COVID-19 infection had been reported worldwide and 160,781,433 patients who recovered all through the globe. [1-2]

A standout amongst other described medication focuses among coronaviruses is the primary protease (M^{pro}, additionally called $3CL^{pro}$) (PBD ID 6Y2F).[3] Alongside the papain-like protease(s), this enzyme is fundamental for handling the polyproteins that are interpreted from the viral RNA.[4] As indicated by Zhang *et al.* (2020)[5] the M^{pro} works at no less than 11 cleavage destinations on the large polyprotein 1ab (replicase 1ab, ~790 kDa); the recognition sequence at most sites is Leu-Gln↓ (Ser, Ala, Gly) (↓ marks the cleavage site). Inhibiting the activity of this enzyme would block viral replication. Since no human proteases with a comparative cleavage specificity are known, such inhibitors are probably not going to be toxic.[5]

Favipiravir, a purine analogue and a potent RdRp inhibitor that has been endorsed for use in influenza treatment, is likewise being considered for treatment of COVID-19.[6-9] In the literature, scarcely any investigations announced the utilization of ganciclovir in COVID-19 management. Han *et al.* (2020)[10] depicted a total recuperation in one patient who was treated with ganciclovir. Recently, Haiping *et al.* (2020)[11] by the utilization of a deep learning based drug screening, had distinguished ganciclovir as an expected treatment for SARS-CoV-2.[11] According to Jin *et al.* (2020)[12], raltegravir is

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another proposed antiviral medication in treating COVID-19. Remdesivir (GS-5734), an inhibitor of the viral RNA-dependent RNA polymerase with *in vitro* inhibitory action against SARS-CoV-1 and the MERS[13-15] was distinguished ahead of schedule as a promising helpful candidate for Covid-19 in respect of its capacity to restrain SARS-CoV-2 *in vitro*.[16]

Since there is no disease specific vaccine or drug available against COVID-19, there is a prompt need to distinguish antiCOVID-19 specialists to control the episode and spread of viral contamination. Novel ways to deal with drug plan and disclosure are being used to investigate helpful medication contender for COVID-19. Molecular docking is a promising apparatus for drug disclosure and improvement through the investigation of the interaction of ligand (drug) molecules inside the binding pocket of a target protein (receptor).[17]

The potential therapeutic drug candidates such as favipiravir, ganciclovir, raltegravir and remdesivir, which are practiced against COVID-19 worldwide, the present study tried to perform the computer-based techniques to assess the structure of ligand-protein complexes, biochemical pathways and also screen the top hit compounds for adsorption, distribution, metabolism, excretion, and toxicity (ADMET) study through *in silico* methods.

MATERIALS AND METHODS

Protein preparation. The three-dimensional structure of SARS-CoV-2 protease M^{pro} (PDB ID 6Y2F) was recovered from the PDB in RCSB site in PDB Format. Before docking, protein was ready by utilizing BIOVIA Discovery Studio programming. During planning polar hydrogens were added and water molecules, hetero atoms were taken out from the protein for the avoidance of undesirable interaction while docking. Among different ligand presents in the protein, choosing a specific ligand, then, at that point the X, Y and Z ascribes were noted in the protein to track down the binding affinity.

Ligand preparation. Three-dimensional structures of favipiravir (CID: 492405), ganciclovir (CID: 135398740), raltegravir (CID: 54671008) and remdesivir (CID: 121304016) were recovered from PubChem Compound Database (https://pubchem.ncbi.nlm.nih.gov/) in SDF format and changed over into PDB format utilizing Discovery Studio 3.5 suite. Further, structure optimization and protonation condition of all ligands were accomplished utilizing Discovery Studio 3.5 suite.

Molecular docking and ADMET analyses. Molecular docking was performed to foresee the maximum binding affinity between the ligand and protein using Autodock Vina and for discovering the kind of amino acids residues interaction with the ligand utilizing BIOVIA Discovery Studio software. For Autodock Vina, observational scoring capacities assists with figuring the binding affinity utilizing Grid boundaries (X, Y and Z attributes).[18-22] The docking was performed utilizing a comprehensiveness worth of 8. The entirety of the other programming boundaries were the default esteems, and the entirety of the bonds contained in the ligand were permitted to rotate freely while the receptor was rigid. The last perception of the docked structure was performed utilizing Discovery Studio Visualizer 3.5 where different kinds of interactions happen. In view of the quantity of H-bond interaction between ligand-protein, specific ligand mode was chosen. The lower the binding energy, the higher the binding affinity occurs between the ligand-protein.

ADMET properties of favipiravir, ganciclovir, raltegravir and remdesivir were anticipated utilizing the pharmacokinetic parameters of the ADMETSar web server (http://lmmd.ecust.edu.cn/)[23-24] after a successful docking study (Table 2).

RESULTS AND DISCUSSION

The results revealed strong interactions of the compounds against the SARS-CoV-2 protease M^{pro}. After fruitful docking of favipiravir, ganciclovir, raltegravir, and remdesivir to the 6Y2F, modes of interactions are created with a specific docking score. In this study, four drug molecules were evaluated for their inhibitory properties against SARS-CoV-2 protease M^{pro} (6Y2F). Based on H-bond, hydrophobic interactions, Van der Waal interaction and ionic

bonds etc., protein (6Y2F) and the drug molecules (ligand) going through docking try different things with their best docking score considered as the steadiest for the ligand (Table 1). In molecular docking, actual requirement is not always only the RMSD and binding energy values. The molecular interactions like hydrogen bonds, hydrophobic interactions, Van der Waal interaction and ionic bonds etc. are also playing important role.

Table 1. Docking scores and the H-bond formation results obtained between the interactions of ligands (favipiravir, ganciclovir, raltegravir, and remdesivir) and protein (6Y2F).

Sl. No.	Ligands	Protein (receptor)	Affinity (kcal/mol)	H-bond formation
1	Favipiravir	SARS-CoV-2 protease M^{pro} (6Y2F)	-4.5	Gly143, Ser144 and Cys145
2	Ganciclovir		-5.1	Gly143, Ser144, Cys145, Phe140, Glu166 and Leu141
3	Raltegravir		-7.4	Gly143, Ser144, Cys145, Glu166, Gln192 and Leu141
4	Remdesivir		-6.9	Gly143, Ser144, Cys145, Gln189, Cys44

The association of explicit amino acids participating in the drug-protein interactions were likewise recorded. Favipiravir, showed significant binding, yielding a binding affinity of -4.5 kcal/ mol while forming H-bonds with Gly143, Ser144 and Cys145 in the active site of 6Y2F. Some Van der Waals interaction of favipiravir with Leu141, His163, Phe140, Glu166 and Asn142 were observed (Figure 1).

Ganciclovir has a binding energy of -5.1 kcal/ mol while forming conventional H-bonds with Gly143, Ser144, Cys145, Phe140, Glu166 and Leu141 in the active site of 6Y2F (Figure 2). Ganciclovir also formed some Van der Waals interaction with His164, Gln189, Met49, Arg188, Asn142 along with 2 carbon hydrogen bonds with Met165 and His163.

Raltegravir interacts to 6Y2F by forming hydrogen bonds with Gly143, Ser144, Cys145, Glu166, Gln192 and Leu141 and also formed Van der Waals interaction to Thr25, Asn142, His164, Gln189 and Thr190; halogen (fluorine) to Cys44 and Thr45 and pi-sulfar and alkyl bond with Met165 and Pro168, respectively (Figure 3).

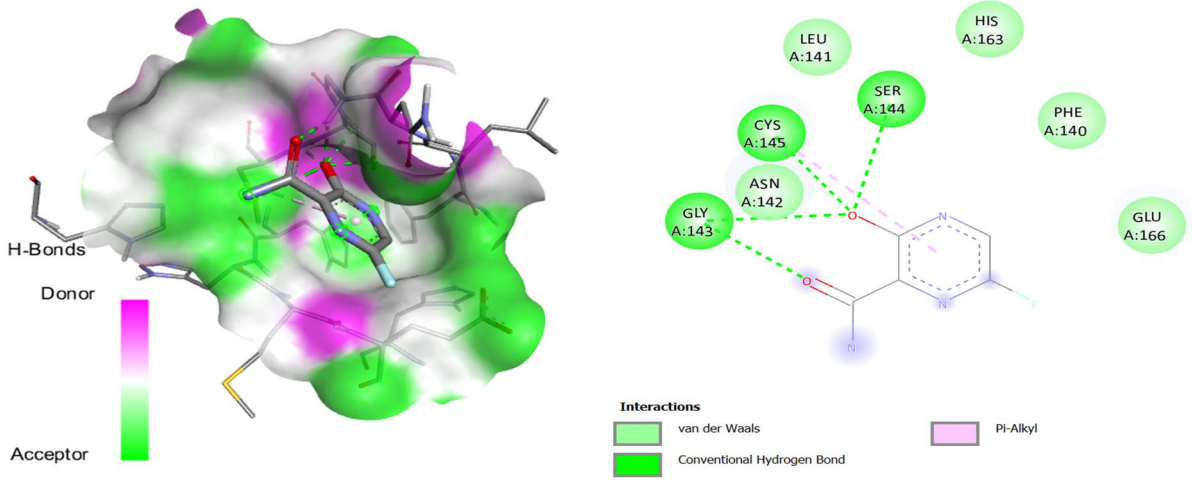

Figure 1. Favipiravir docked in the SARS-CoV-2 protease M^{pro} (6Y2F): **(left)** Pocket view of 6Y2F binding with favipiravir shows the H-bond donor-acceptor residues, **(right)** 2D schematic diagram of docking model of interaction between 6Y2F and favipiravir with amino acid with H-bond (green dashed line).

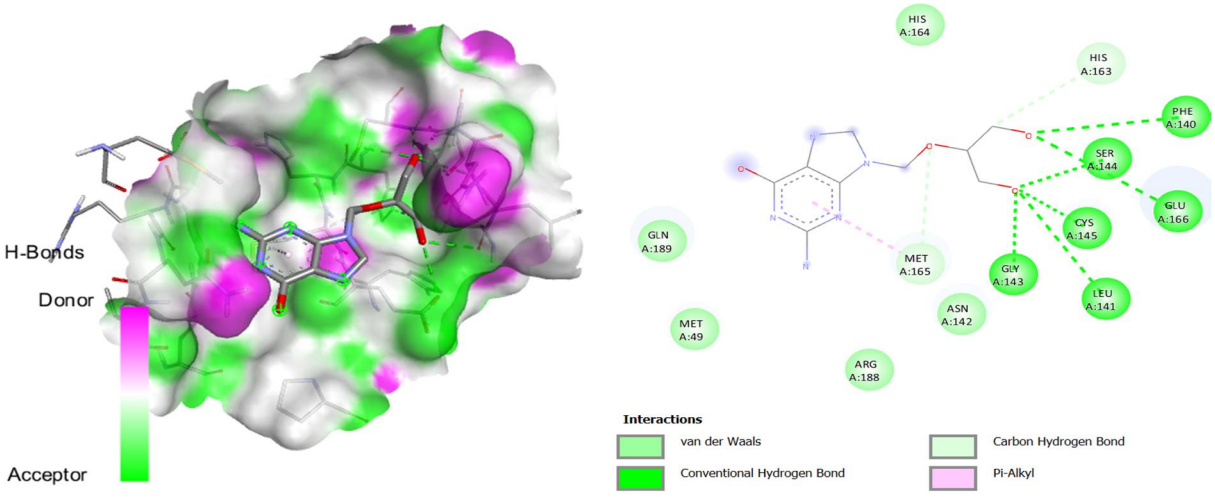

Figure 2. Ganciclovir docked in the SARS-CoV-2 protease M^{pro} (6Y2F): **(left)** Pocket view of 6Y2F binding with ganciclovir shows the H-bond donor-acceptor residues, **(right)** 2D schematic diagram of docking model of interaction between 6Y2F and ganciclovir with amino acid with H-bond (green dashed line).

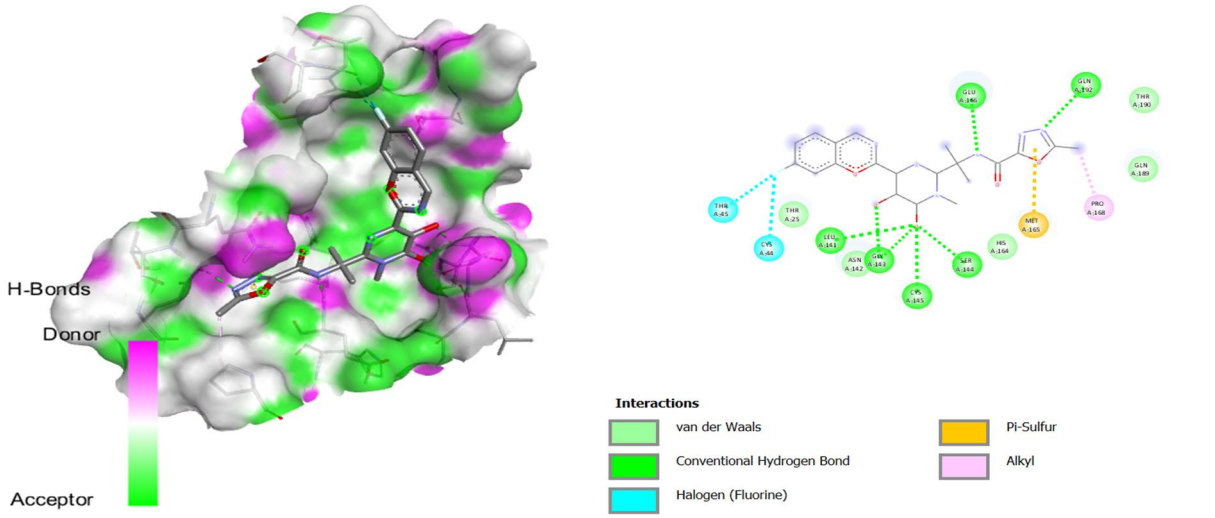

Figure 3. Raltegravir docked in the SARS-CoV-2 protease M^{pro} (6Y2F): **(left)** Pocket view of 6Y2F binding with raltegravir shows the H-bond donor-acceptor residues, **(right)** 2D schematic diagram of docking model of interaction between 6Y2F and raltegravir with amino acid with H-bond (green dashed line).

Remdesivir interacts to receptor (binding affinity of -6.9 kcal/ mol) by forming H-bonds with Gly143, Ser144, Cys145, Gln189, Cys44, respectively. Besides such strong hydrogen bonding, remdesivir formed hydrophobic interactions with Met165, Pro168 and His41. Some Van der Waals interaction of favipiravir with Gln192, Leu167, His164, Asp187, Arg188, Met49, Thr25, Leu27, Thr26, Leu141, Thr190 and Asn142 were observed (Figure 4). Docking investigation assists with assessing the interacting residues, binding energies and intermolecular distance between the communicating 6Y2F residues to the ligands.

ADMET properties were anticipated utilizing the pharmacokinetic parameters of the ADMETSar web server (http://lmmd.ecust.edu.cn/) for favipiravir,

ganciclovir, raltegravir and remdesivir after an effective docking study (Table 2). Aqueous solubility, Caco-2 permeability, BBB permeability, human intestinal permeability, and P-glycoprotein substrate, P-glycoprotein inhibitor and renal organic transporter were utilized to anticipate the absorption level of the compounds. All drug molecules show good aqueous solubility, which may be because of the quantity of hydroxyl groups in them. Favipiravir, ganciclovir exhibited BBB penetrating potential. All drug molecules anticipated to have a decent absorption rate. Ganciclovir, raltegravir and remdesivir might be effectively exuded from cells by P-glycoprotein as they were all substrates of P-glycoprotein, while all drug molecules were anticipated to be P-glycoprotein and renal organic cation transporter non-inhibitor. Favipiravir and raltegravir reside in mitochondria, whereas ganciclovir and remdesivir is localized in nucleus lysosome, respectively.

Table 2. Predicted ADMET properties of favipiravir, ganciclovir, raltegravir and remdesivir.

Properties	Favipiravir	Ganciclovir	Raltegravir	Remdesivir
		Absorption		
Aqueous solubility	-1.6511	-2.5301	-2.8817	-3.4736
Caco-2 permeability (LogPapp, cm/s)	0.9307	-0.0373	0.5133	-0.1451
BBB permeability	+	+	-	-
Human intestinal permeability (% absorbed)	+	+	+	+
P-glycoprotein substrate	-	+	+	+
P-glycoprotein inhibitor	-	-	-	-
Renal organic cation transporter	-	-	-	-
Distribution				
Subcellular localization	Mitochondria	Nucleus	Mitochondria	Lysosome
		Metabolism		
CYP4502C9 substrate	-	-	-	-
CYP4502D6 substrate	-	-	-	-
CYP4503A4 substrate	-	-	+	+
CYP4501A2 inhibitor	-	-	-	-
CYP4502C9 inhibitor	-	-	-	-
CYP4502D6 inhibitor	-	-	-	-
CYP4502C19 inhibitor	-	-	-	-
CYP4503A4 inhibitor	-	-	-	-
Excretion				
Not available				
Toxicity				
AMES toxicity	-	-	-	-
Carcinogens	-	-	-	-
Acute oral toxicity	III	III	III	III
Rat acute toxicity (LD50, mol/kg)	2.1259	2.0348	2.4448	2.7169

Papp: Apparent permeability coefficient; AMES: Assay of the ability of a chemical compound to induce mutations in DNA; BBB, blood–brain barrier; Acute oral toxicity: Category-III means $LD_{50} \leq 5000$mg/kg.

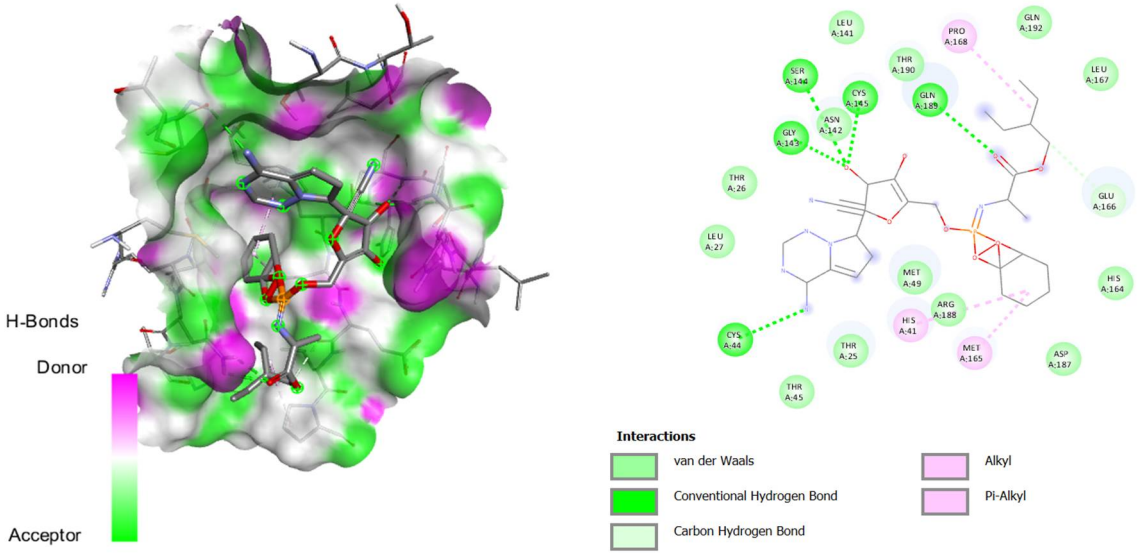

Figure 4. Remdesivir docked in the SARS-CoV-2 protease M^{pro} (6Y2F): **(left)** Pocket view of 6Y2F binding with remdesivir shows the H-bond donor-acceptor residues, **(right)** 2D schematic diagram of docking model of interaction between 6Y2F and remdesivir with amino acid with H-bond (green dashed line).

Cytochrome P450s is a significant enzyme system for drug metabolism in liver. Table 2 showed that favipiravir, ganciclovir, raltegravir and remdesivir were not substrates for the CYP4502C9, CYP4502D6. Raltegravir and remdesivir were anticipated to be CYP4503A4 substrates. All drug molecules were not anticipated to be CYP4501A2, CYP4502C9, CYP4502D6, CYP4502C19, and CYP4503A4 inhibitors. Such reason it may be mention that the compounds having CYP450 non-inhibiting property might not be metabolized in the liver. The results also suggested that favipiravir, ganciclovir, raltegravir and remdesivir may be non-toxic in AMES test and none were predicted to be carcinogenic. All drug molecules fall in class III (LD50 ≤ 5000mg/kg) (Table 2).

CONCLUSION

The results of the docking showed the various kinds of binding interactions of the drugs with proteins and some were favorable. The antiviral drugs favipiravir, ganciclovir, raltegravir and remdesivir showed high affinity interaction with the SARS-CoV-2 protease M^{pro} (6Y2F). Overall, these antiviral drug candidates would be helpful to prevent the SARS-CoV-2 protease M^{pro} (6Y2F) and to reduce the risk of infection in the host body but *in vitro* and *in vivo* studies are required to verify their potential to inhibit viral replication.

CONFLICT OF INTEREST

We announce that we have no conflict of interests.

REFERENCES

1. Zhou, P., Yang, X-L., Wang, X-G., Hu, B., Zhang, L., Zhang, W., Si, H-R., Zhu, Y., Li, B., Huang, C-L., Chen, H-D., Chen, J., Luo, Y., Guo, H., Jiang, R-D., Liu, M-Q., Chen, Y., Shen, X-R., Wang, X., Zheng, X-S., Zhao, K., Chen, Q-J., Deng, F., Liu, L-L., Yan, B., Zhan, F-X., Wang, Y-Y., Xiao, G-F. and Shi, Z-L. 2020. A pneumonia outbreak associated with a new coronavirus of probable bat origin. *Nature*. **579**, 270–273.
2. Wu, F., Zhao, S., Yu, B., Chen, Y-M., Wang, W., Song, Z-G., Hu Y., Tao, Z-W., Tian J-H., Pei, Y-Y., Yuan, M-L., Zhang, Y-L., Dai, F-H., Liu, Y., Wang, Q-M., Zheng, J-J., Xu, L., Holmes, E. C. and Zhang, Y-Z. 2020. A new coronavirus associated with human respiratory disease in China. *Nature*. **579**, 265–269.
3. Anand, K., Ziebuhr, J., Wadhwani, P., Mesters, J. R. and Hilgenfeld, R. 2003. Coronavirus main proteinase (3CLpro) structure: basis for design of anti-SARS drugs. *Science*. **300**,1763-1767.
4. Hilgenfeld, R. 2014. From SARS to MERS: crystallographic studies on coronaviral proteases enable antiviral drug design. *FEBS J*. **281**, 4085–4096.

5. Zhang, L., Lin, D., Sun, X., Curth, U., Drosten, C., Sauerhering, L., Becker, S., Rox, K. and Hilgenfeld, R. 2020. Crystal structure of SARS-CoV-2 main protease provides a basis for design of improved alpha-ketoamide inhibitors. *Science*. **368,** 409-412.
6. Arab-Zozani, M., Hassanipour, S. and Ghoddoosi-Nejad, D. 2020. Favipiravir for treating patients with novel coronavirus (COVID-19): protocol for a systematic review and meta-analysis of randomised clinical trials. *BMJ Open*. **10**, e039730.
7. Goldhill, D. H., Te Velthuis, A. J., Fletcher, R. A., Langatb, P., Zambona, M., Lackenbya, A. and Barclayb, W. S. 2018. The mechanism of resistance to favipiravir in infuenza. *PNAS*. **115**, 11613–11618.
8. Zhu, W., Chen, C. Z., Gorshkov, K., Xu, M., Lo, D. C. and Zheng, W. 2020. RNA-Dependent RNA Polymerase as a Target for COVID-19 Drug Discovery. *SLAS Discov*. **25**, 1141-1151.
9. Elfiky A. A. 2021. SARS-CoV-2 RNA dependent RNA polymerase (RdRp) targeting: an *in silico* perspective. *J. Biomol. Struct. Dyn*. **39**, 3204-3212.
10. Han, X., Fan, Y., Wan, Y. L. and Shi, H. 2020. A diabetic patient with 2019-nCoV infection who recovered and was discharged from hospital. *J. Thorac. Imaging*, **35** W94-W95.
11. Haiping, Z., Konda, M. S., Yang. Y., Hossain, M. T., Li, J., Ren, X. and Wei, Y. 2020. Deep learning-based drug screening for novel Coronavirus 2019-nCov. *Interdiscip Sci*. **12**:368-376.
12. Jin Y-H, Cai, L., Cheng, Z-S., Cheng, H., Deng, T., Fan Y-P., Fang, C., Huang, D., Huang, L-Q., Huang, Q., Han, Y., Hu, B., Hu, F., Li, B-H., Li, Y-R., Liang, K., Lin, L-K., Luo, Li-S., Ma, J., Ma, L-L, Peng, Z-Y., Pan, Y-B., Pan, Z-Y., Ren, X-Q, Sun H-M, Wang Y, Wang Y-Y, Weng H, Wei C-J, Wu, D-F., Xia, J., Xiong, Y., Xu, H-B., Yao X-M., Yuan, Y-F., Ye, T-S., Zhang, X-C., Zhang, Y-W., Zhang, Y-G., Zhang, H-M., Zhao, Y., Zhao, M-J., Zi, H., Zeng, X-T., Wang, Y-Y. and Wang, X-H. 2020. A rapid advice guideline for the diagnosis and treatment of 2019 novel coronavirus (2019-nCoV) infected pneumonia (standard version). *Mil. Med. Res.* **7**, 4.
13. Sheahan, T. P., Sims, A. C., Leist, S. R., Schäfer, A., Won, J., Brown A. J., Montgomery, S. A., Hogg, A., Babusis, D., Clarke, M. O., Spahn, J. E., Bauer, L., Sellers, S., Porter, D., Feng, J. Y., Cihlar, T., Jordan, R., Denison, M. R. and Baric, R. S. 2020. Comparative therapeutic efficacy of remdesivir and combination lopinavir, ritonavir, and interferon beta against MERSCoV. *Nat. Commun*. **11**, 1-14.
14. 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. and Denison, M. R. 2018. Coronavirus susceptibility to the antiviral remdesivir (GS-5734) is mediated by the viral polymerase and the proofreading exoribonuclease. *mBio*. **9**, e00221-18.
15. Brown, A. J., Won, J. J., Graham, R. L., Dinnon IIIa K. H., Simsa, A. C., Fengb, J. Y., Cihlarb, T., Denisonc, M. R., Barica, R. S. and Sheahan, T. P. 2019. Broad spectrum antiviral remdesivir inhibits human endemic and zoonotic deltacoronaviruses with a highly divergent RNA dependent RNA polymerase. *Antivir. Res.* **169**, 104541.
16. Wang, M., Cao, R., Zhang, L., Yang, X., Liu, J., Xu, M., Shi, Z., Hu, Z., Zhong, W. and Xiao, G. 2020. Remdesivir and chloroquine effectively inhibit the recently emerged novel coronavirus (2019-nCoV) in vitro. *Cell Res.* **30**, 269-271.
17. Narkhede, R. R., Cheke, R. S., Ambhore, J. P. and Shinde, S. D. 2020. The molecular docking study of potential drug candidates showing anti-COVID-19 activity by exploring of therapeutic targets of SARS-CoV-2. *Eurasian J. Med. Oncol.* **4**, 185–195.
18. Dickerson, T. J., Beuscher, A. E., Rogers, C. J., Hixon, M. S., Yamamoto, N., Xu, Y., Olson, A. J. and Janda, K. D. 2005. Discovery of Acetylcholinesterase peripheral anionic site ligands through computational refinement of a directed library. *Biochemistry*, **44** 14845-14853.
19. Huang, H-J., Lee, C-C. and Chen, C. Y-C. 2014. *In silico* design of BACE1 inhibitor for Alzheimer disease by traditional Chinese medicine. *BioMed Res. Int.* 741703.
20. Hardy, J. and Selkoe, D. J. 2002. Medicine - the amyloid hypothesis of alzheimer's disease: progress and problems on the road to therapeutics. *Science*. **297**, 353-356.
21. Aguzzi, A. and O'Connor, T. 2010. Protein aggregation diseases: pathogenicity and therapeutic perspectives. *Nat. Rev. Drug Discov.* **9**, 237-248.
22. Hawkes, C. A., Ng, V. and MacLaurin, J. 2009. Small molecule inhibitors of alpha-beta aggregation and neurotoxicity. *Drug Dev. Res.* **70**, 111-124.
23. Cheng, F., Li, W., Zhou, Y., Shen, J., Wu, Z., Liu, G., Lee. P. W. and Tang, Y. 2012. admet-SAR: a comprehensive source and free tool for assessment of chemical ADMET properties. *J. Chem. Inf. Model*. **52**, 3099e3105.
24. Yang H, Lou C, Sun L, Li J, Cai Y, Wang Z, Li, W., Liu, G. and Tang, Y. 2019. admetSAR 2.0: web-service for prediction and optimization of chemical ADMET properties. *Bioinformatics*, **35**, 1067e1069.