Current Chemistry Letters 13 (2024) 199–206

Contents lists available at GrowingScience

Current Chemistry Letters

homepage: www.GrowingScience.com

In silico investigations on the repurposing of antivirals for Covid-19 and pharmacophore modelling

Vinod P Raphael[a*], K.S. Shaju[a], T.K. Bindu[a] and A. Sini[a]

[a]*Department of Chemistry, Government Engineering College Thrissur-680009, Affiliated to APJ Abdul Kalam Technological University, Thiruvananthapuram, Kerala, India*

C H R O N I C L E

Article history:
Received December 25, 2022
Received in revised form
June 3, 2023
Accepted July 7, 2023
Available online
July 7, 2023

Keywords:
Remdesivir
Docking
Pharmacophore
Binding Energy
ADME

A B S T R A C T

The pandemic COVID-19 has been spreading around the globe from December 2019 onwards and is considered the most infectious disease of this century. To date, there is no effective drug against SARS-CoV-2 discovered by pharmaceutical scientists, and the research is going rigorously all over the world. In this work, we examined the interaction of the already existing antivirals (Lopinavir, Atazanavir, and Remdesivir) with the structural proteins of SARS-CoV-2 using computational methods. Pharmacophore modeling of these drugs was conducted using molecular databases to determine the lead compounds from molecular databases. Pharmagist Webserver and Zinc Molecular Database were used to find out the pharmacophore and lead compounds, respectively. The drug-likeness properties of the compounds were evaluated by the SwissADME webserver. *In silico* studies showed that the binding affinities of the drugs followed the order Remdesivir > Atazanavir > Lopinavir. Docking and pharmacological studies revealed the potency and drug-likeness of the synthetic molecules.

1. Introduction

Severe acute respiratory syndrome COVID-19 is the most dreadful pandemic of the 21st century. It originated in China in the last quarter of 2019 and spread all over the world within a few months.[1] So far, there have been approximately 6.8 million reported fatalities and countless individuals who have contracted the coronavirus.[2] Doctors and immunologists believe that the spread of viral disease can be controlled by vaccination.[3,4] Many countries have started the vaccination drive, which is in its final stages. The treatment of COVID-19 patients may be ineffective due to a lack of effective pharmaceuticals. There is no effective drug against SARS-CoV-2 discovered by the pharmaceutical scientists, and the research is going rigorously all over the world. Repurposing of the medicines is tried by many doctors to alleviate the adverse effects of COVID-19 in patients. Some studies showed that anti-HIV drugs such as Lopinavir, Ritonavir, and Remdesivir are effective to some extent in controlling the impact of COVID-19.[5-7]

The structural or non-structural proteins of the COVID-19 virus may act as effective targets for drug molecules. The main protease of SARS-CoV-2 is an important non-structural protein.[8] Membrane protein, spike protein, envelope protein, and nucleocapsid protein are the structural proteins of coronaviruses. In our previous work, we had reported the interaction of some antiviral molecules with the main protease of the COVID-19 virus, which was investigated computationally.[9-10] In this work, we present the interactions of the already existing antivirals with the structural proteins of SARS-CoV-2 using *in silico* methods. Drugs such as Lopinavir, Atazanavir, and Remdesivir were taken and evaluated for their binding affinity with the structural proteins of coronaviruses using molecular docking studies. Molecules that are analogues to the antivirals

* Corresponding author.
E-mail address vinodpraphael@geetcr.ac.in (V. P Raphael)

doi: 10.5267/j.ccl.2023.7.001

were obtained by the pharmacophore modeling of the antivirals. Docking studies of the molecules were also carried out to check their effectiveness on the structural proteins of coronaviruses.

2. Results and Discussion

2.1 Docking studies of antivirals

Molecular docking studies established the binding interactions and the binding efficacy of various antivirals on the structural proteins of the COVID-19 virus. **Table 1** shows the binding energy values of protein-ligand complexes. The number of hydrogen bonds and the type of interaction between the receptor proteins and the antivirals are given in the subsequent paragraphs.

Table 1. Binding energies (kcal/mol) of antiviral-protein complexes

Receptor\Drug	Lopinavir	Atazanavir	Remdesivir
6M3M	-10.35	-10.58	-11.11
6VXX	-8.34	-9.16	-8.82
7K3G	-9.54	-9.12	-10.25

2.1.1 Interaction with nucleoprotein

Lopinavir formed four conventional hydrogen bonds (Arg150-2 No.s, Ala157-2 No.s), three non-classical hydrogen bonds (Ala157, Ile158, and Ile75), three alkyl hydrophobic interactions (Ille158, Ala156, Ala157), and three pi-alkyl hydrophobic linkages (Arg69, Val159, and Ala156) with the 6M3M receptor. Lopinavir-6M3M complex displayed -10.35 kcal/mol binding energy.

Atazanavir exhibited a binding energy of -10.58 kcal/mol on the nucleocapsid protein and showed with three conventional hydrogen bonds (Arg150-2 No.s, Tyr110), two non-conventional hydrogen bonds (Pro152, Ala56), two alkyl hydrophobic interactions (Ille158-2 No.s), and three pi-alkyl interactions (Ala56, Ala156, Ala157).

Remdesivir-6M3M complex was stabilized by ten classical hydrogen bonds. Out of which five numbers were established with the amino acid residue Arg89 and the drug molecule, and the others were between Glu63, Thr92, Lys66, Asp129, Arg90, and Remdesivir. In addition to this, six non-classical hydrogen bonds (Glu63-3No.s., Gly130, Ala91, Arg90), two electrostatic interactions (Glu63, Lys66), two hydrophobic linkages (Pro118, Lys66), and four pi-alkyl type interactions (Lys62-2No.s., Ala91, Pro169) were found in the receptor-drug complex. Two repulsive interactions were also noted between the Remdesivir molecule and two aminoacid residues, Arg89 and Lys66. A very good score of -11.11 kcal/mol binding energy was shown by Remdesivir on the nucleocapsid protein of the COVID-19 virus.

2.1.2 Interaction with Spike protein

Antivirals Lopinavir and Remdesivir showed binding energies of -8.34 and -8.82 kcal/mol on the receptor binding domain (RBD) of spike protein respectively. The 6VXX-lopinavir complex displayed only one hydrogen bond (Gly339) and three non-classical hydrogen bonds (Gly339, Gly340, and Asn343). This complex also displayed three hydrophobic interconnections with the residues Leu335 (2 No.s) and Val367. Remdesivir is bound to the RBD of spike protein using four conventional hydrogen bonds (Phe338-2 No.s, Ser371, Asn343), five non-classical hydrogen bonds (Cys336, Gly339, Ser373-2 No.s, Ser371), one π-π hydrophobic bond (Trp436), and one alkyl hydrophobic bond (Val367).

According to the docking studies, the receptor binding domain (RBD) of the spike protein combined with the atazanavir to form the drug-protein complex with a binding energy of -9.16 kcal/mol. The complex didn't possess any conventional hydrogen bonds but consisted of one non-classical hydrogen bond with Glu 224. One π-π interaction (Trp104), three alkyl interactions (Leu126, Val227, and Leu229), and three pi-alkyl linkages (Hsd207-2 No.s., Val126) were also noticed in the 6VXX-atazanavir complex.

2.1.3 Interaction with Envelope protein

Atazanavir formed a stable complex with the envelope protein of the coronavirus using one conventional (Ala32) and one non-conventional hydrogen bond (Leu31) and showed a binding energy of -9.12 kcal/mol. In addition to these interactions, the complex displayed seven alkyl-hydrophobic linkages (Leu31, Ala32, Leu28, -3 No.s, Ala32, Ala-30) and three pi-alkyl type bonds (Ala36-2. Nos, Ala-32-2 No.s) with the amino acid residues of envelope protein.

The 7K3G-Lopinavir complex did not display any conventional or non-conventional hydrogen bonds. But the complex was stabilized by fourteen hydrophobic interactions, namely pi-hydrophobic interactions (Leu31, Ala32,-4 No.s, Ala36) and alkyl-hydrophobic interactions (Val25-2 No.s, Leu28-4 No.s, Ala32, Ala36) with the various chains of envelope protein.

Remdesivir antiviral formed one conventional hydrogen bond with Ala32. Amino acid residues Val25 (3 No.s), Leu21 (3 No.s), and Val24 (2-No.s) of different chains of envelope protein made alkyl hydrophobic interactions with Remdesivir. Five pi-alkyl bonds were also established between the drug and the envelope protein.

Among the three antivirals studied, Remdesivir exhibited more efficiency on the structural proteins 6M3M and 7K3G of corona virus. The two dimensional interaction plots of antiviral-receptor complexes having binding energy whose magnitude is >10 kcal/mol are shown in **Fig. 1**. There have been some clinical reports regarding the efficacy of Remdesivir to combat coronavirus coronavirus-19.[11]

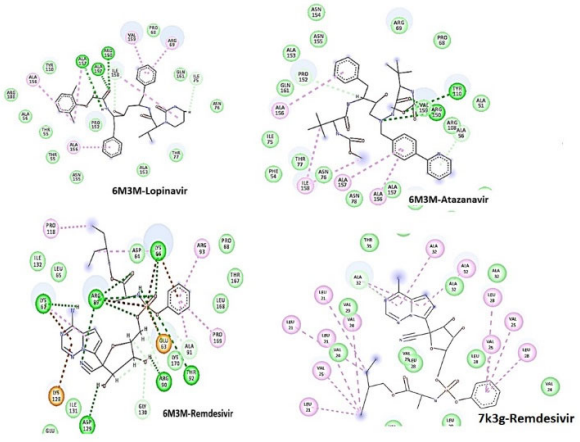
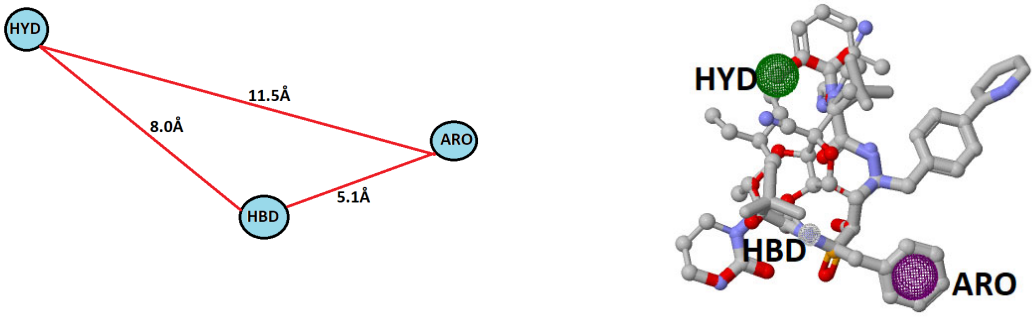


Fig. 1. 2D interaction plots of antiviral-protein complexes (B.E >10 kcal/mol)

2.2 Pharmacophore studies

To identify molecules with similar structural features to that of antivirals Lopinavir, Atazanavir, and Remdesivir, pharmacophore investigations were performed. The structures of these molecules were uploaded to the Pharmagist webserver and the resulting pharmacophore characteristics was analyzed using Pymol software. The derived pharmacophore consisted of three points; one aromatic ring system, one hydrogen bond donor, and one hydrophobic terminal. The pharmacophore structure obtained using the three antivirals is shown in **Fig. 2**. Using the pharmacophore model, four lead compounds (Zinc IDs 47163347, 12793669, 40476161, and 33138007) were selected from the Zinc database based on a low RMSD value and molecular mass <500. These compounds were then subjected to docking studies on various structural proteins of the COVID-19 virus. The molecular structures and binding affinities of the lead compounds with the structural proteins of the coronavirus are presented in **Fig. 3** and **Table 2**, respectively.



HBD: Hydrogen Bond Donor, HYD: Hydrophobic, ARO: Aromatic

Fig. 2. Pharmacophore derived from the drugs Lopinavir, Atazanavir and Remdesivir

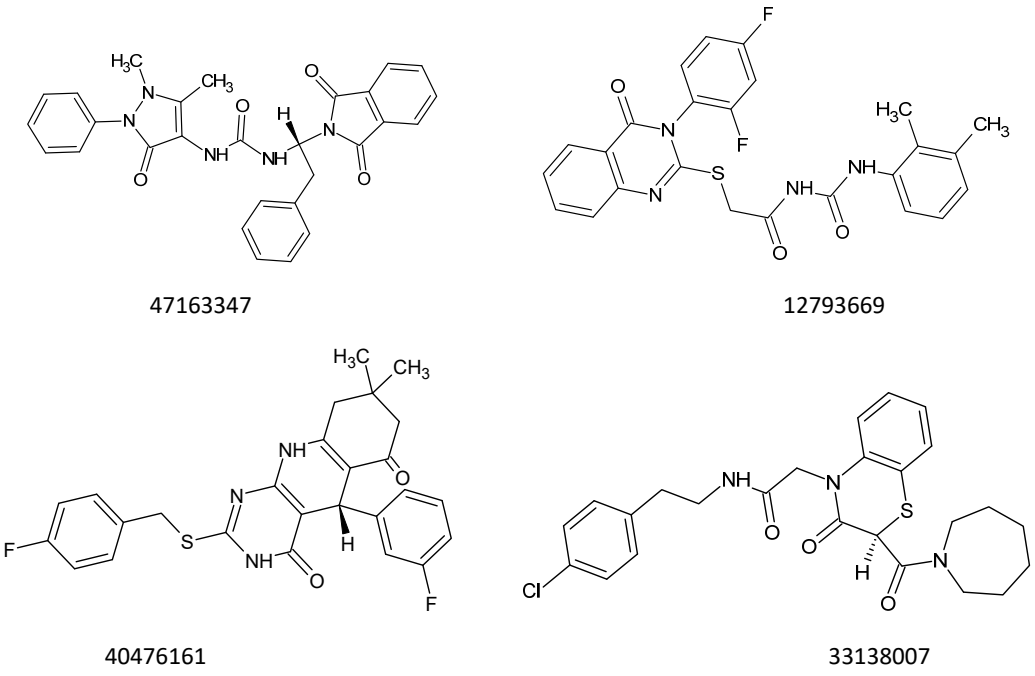


Fig. 3. Molecular structures of lead compounds from Zinc database

Table 2. Binding energy (kcal/mol) of lead molecules on structural proteins of SARS-CoV-2

	Ligand			
Receptor	47163347	2793669	40476161	33138007
6M3M	-9.18	-8.84	-8.47	-9.12
6VXX	-8.97	-7.98	-8.11	-8.22
7K3G	-9.12	-8.72	-8.74	-8.5

2.3 Pharmacokinetics and ADME Predictions

The lead compounds were computationally tested using the SwissADME webserver to evaluate their drug-likeness, physical properties, and pharmacokinetics. The bioavailability radar of the lead molecules was analyzed for Lipophilicity (LIPO): XLOGP3, Molecular weight (SIZE), Polarity (POLAR) TPSA, Solubility (INSOLU): logS, Flexibility (FLEX), and fraction of carbons in the sp3 hybridization (INSATU). The bioavailability radars predicted by SwissADME are shown in Figure 4, and all four molecules showed a bioavailability score of 0.55. The physical, pharmacokinetic, and drug-likeness properties of the lead molecules are presented in **Tables 3, 4**, and **5** respectively.

According to pharmacokinetic studies, the four lead molecules cannot penetrate the blood-brain barrier (BBB). All molecules obeyed the Lipinski rule, but three showed one violation in the MLOGP values. The four molecules followed the Veber and Egan rules for drug ability prediction but did not obey Ghose principles. The compound 12793669 violated the Muegge rule. By analyzing **Table 5**, it can be concluded that all the molecules possess good drug likeness. Gastrointestinal (GI) absorption of three molecules was high, but 12793669 showed poor absorption according to SwissADME analysis.

The bioavailability radar of the lead molecules (**Fig. 4**) showed that lipophilicity (LIPO): XLOGP3, molecular weight (SIZE), polarity (POLAR) TPSA, solubility (INSOLU): logS, and flexibility (FLEX): fitted within the standard range for 47163347 and 12793669. But the saturation (INSATU), i.e., a fraction of carbons in the sp3 hybridization, slightly deviated from the normal range. Lead compounds 40476161 and 33138007 exhibited a good range in bioavailability radar for all parameters such as XLOGP3, SIZE, TPSA, logS, FLEX, and INSATU. The molecule 12793669 displayed poor water solubility, which may be attributed to the high value of Total Polar Surface Area (118.39Å²). The other three showed moderate water solubility. The LogP values of all molecules were appreciable and fell within the standard range for drug molecules.

Molar refractivity (MR) is an important molecular descriptor that denotes the overall polarity of a molecule. In general, the greater the MR value, the greater the polarizability of a molecule. Molar volume and molar mass are the two contributing factors to MR. The drug-like molecule usually has an MR value between 40 and 130.[12] The four molecules obtained from the zinc database exhibited MR values between 130-143, suggesting that they show appreciable polarity.

P-glycoprotein (Pgp) has a significant role in drug transportation and drug-drug interactions.[12,13] Drugs that inhibit Pgp may increase the bioavailability of the other molecules. According to pharmacokinetic studies, molecules 40476161 and 33138007 acted as Pgp substrates. Drug metabolism primarily occurs with the help of Cytochrome P450 enzymes. Similar to Pgp, some drugs may inhibit or agonize cytochrome P450 enzymes. This knowledge is important in the area of drug-drug interactions. Toxic effects and therapeutic failures can be minimized using this knowledge. The SwissADME predictions established the inhibitory nature of lead molecules on important cytochrome enzymes, which is shown in **Table 4**. It is worth noting that all of the molecules inhibited CYP2C19, CYP2C9, and CYP3A4. None of the molecules inhibit CYP1A2. CYP2D6 was inhibited only by the molecule 33138007.

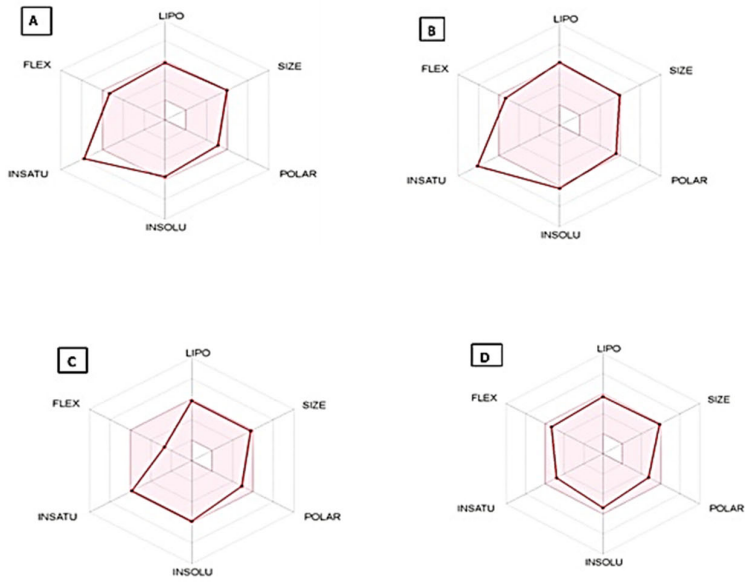


Fig. 4. Bioavailability radars for a) 47163347 b)12793669 c) 40476161 and d) 33138007

Table 3. Physical properties of the lead molecules predicted by SwissADME

Zinc Id of Molecule	M. wt (g/mol)	TPSA (Å²)	Consensus $LogP_{o/w}$	Water Solubility	Molar Refractivity
47163347	495.53	105.44	3.59	Moderately soluble	142.85
12793669	494.51	118.39	4.67	Poorly soluble	130.94
40476161	479.54	100.15	4.98	Moderately soluble	131.56
33138007	486.03	95.02	3.78	Moderately soluble	138.86

Table 4. Pharmacokinetics of the lead molecules predicted by SwissADME

Zinc Id of Molecule	GI absorption	BBB permeant	P-gp substrate	CYP1A2 inhibitor	CYP2C19 inhibitor	CYP2C9 inhibitor	CYP2D6 inhibitor	CYP3A4 inhibitor
47163347	High	No	No	No	Yes	Yes	No	Yes
12793669	Low	No	No	No	Yes	Yes	No	Yes
40476161	High	No	Yes	No	Yes	Yes	No	Yes
33138007	High	No	Yes	No	Yes	Yes	Yes	Yes

Table 5. Drug Likeness of the lead molecules predicted by SwissADME

Zinc Id of Molecule	Lipinski	Ghose	Veber	Egan	Muegge	Bioavailability Score
47163347	Yes (1)	No (2)	Yes (0)	Yes (0)	Yes (0)	0.55
12793669	Yes (1)	No (2)	Yes (0)	Yes (0)	No (1)	0.55
40476161	Yes (1)	No (2)	Yes (0)	Yes (0)	Yes (0)	0.55
33138007	Yes (0)	No (2)	Yes (0)	Yes (0)	Yes (0)	0.55

2.4 Docking studies of molecules from Zinc database

All the compounds showed fair binding affinity for the structural proteins (**Table 2**). The compound 47163347 showed a higher binding energy on all structural proteins than the other three compounds. Molecules 12793669 and 33138007 exhibited good binding scores on the nucleoprotein of the coronavirus. 40476161 displayed slightly more affinity towards envelope protein than other structural proteins. The molecular structures of the lead compounds are shown in **Fig. 3**.

3. Conclusions

Four antiviral drugs were screened computationally on the structural proteins of SARS-CoV-2 to check the binding energies of protein-ligand complexes. Binding affinities of the drugs followed the order Remdesivir> Atazanavir> Lopinavir on the nucleocapsid protein. Remdesivir showed a higher maximum binding energy on the nucleocapsid protein than the other three drugs (-11.11 kcal/mol). It can be concluded that the efficacy of Remdesivir to alleviate the symptoms of COVID-19 may be due to the interaction between the drug and the nucleocapsid protein. Pharmacophore studies of three drugs Remdesivir, Atazanavir, and Lopinavir were performed to identify the probable lead compounds from the Zinc database. Molecular docking studies, pharmacokinetics, and ADME predictions of the derived compounds were done in this investigation. A molecule having the zinc id 47163347 showed very good affinity for the nucleoprotein and envelope protein.

5. Experimental

The X-ray crystallographic structures of the structural proteins of SARS-CoV-2 (**Fig. 5**) were obtained from the Protein Data Bank (PDB codes; 6M3M: nucleocapsid protein; 6VXX: spike protein; 7K3G: envelope protein).[14-18] The docking studies of the antivirals were done by the SwissDock webserver. After downloading the protein structure, it was prepared for docking studies by removing water molecules and co-crystallized ligands using Pymol software. PDB files of proteins and mol2 structures of the molecules were uploaded to the web server. The results of the protein-drug interaction were analyzed using UCSF Chimera and Biovia Discovery Studio software.

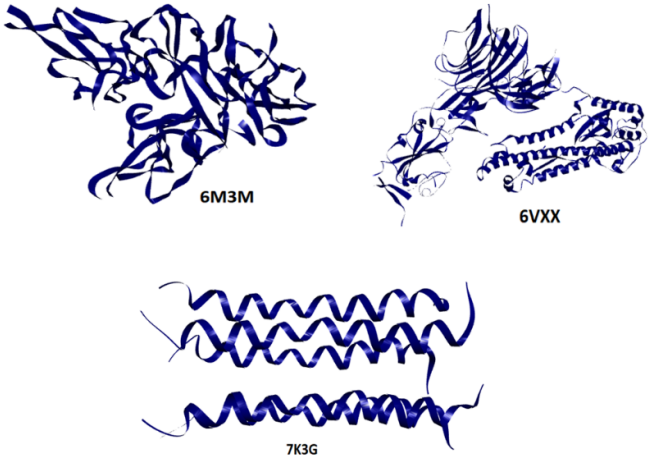


Fig. 5. Structures of nucleoprotein (6M3M), spike protein (6VXX) and envelope protein (73KG) of SARS-CoV-2

The pharmagist web server was used to derive pharmacophore features of antivirals.[19] The derived pharmacophore was uploaded to the Zinc database to choose lead compounds. The lead compounds were docked with the structural proteins

using Swissdock server and analyzed using UCSF Chimera and Discovery Studio software. The drug-likeness, pharmacokinetics, and physical properties of the compounds were evaluated by the SwissADME webserver.[20]

This work clarifies that chemical compounds have a lot of applications as reported before in different scientific papers.

References

1. Centers for Disease Control and Prevention. Available from: https://www.cdc.gov/coronavirus/2019-ncov/symptoms-testing/symptoms.htm (accessed Apr 3, 2022).
2. COVID Live - Coronavirus Statistics - Worldometer. Available from: https://www.worldometers.info/coronavirus/?utm_campaign=homeAdvegas1 (accessed Apr 3, 2022).
3. Sapkal G. N., Yadav P. D., Ella R., Deshpande G. R., Sahay R. R., Gupta N., Neutralization Potential of COVAXIN Vaccinated Individuals Sera against Delta Variant (B.1.617.2) of SARS-CoV-2. bioRxiv 2021, 2021.07.09.452097, https://doi.org/10.1101/2021.07.09.452097.
4. Bharat Biotech. COVAXIN. Available from: https://www.bharatbiotech.com/covaxin.html.
5. Beigel J. H., Tomashek K. M., Dodd L. E., Mehta A. K., Zingman B. S., Kalil A. C. **(2020)** Remdesivir for the Treatment of Covid-19 — Final Report. N Engl J Med. 383 (19), 1813–1826, doi: 10.1056/NEJMoa2007764.
6. Sheahan T. P., Sims A. C., Leist S. R., Schäfer A., Won J., Brown A. J. **(2020)** Comparative Therapeutic Efficacy of Remdesivir and Combination Lopinavir, Ritonavir, and Interferon Beta against MERS-CoV. Nat Commun. 11 (1), 222, doi: 10.1038/s41467-019-13940-6.
7. Yanes T., Youngpairoj A. S., Dharajiya N., Gomez G., Rosario K., Benamu E., et al. **(2021)** Genetic Variants Associated with Severity of COVID-19 Disease: A Review of the Literature and Implications for Personalized Treatment. Pharmacotherapy. 41 (7), 721–735, doi: 10.1002/phar.2521.
8. Goyal B., Goyal D. **(2020)** Targeting the Dimerization of the Main Protease of Coronaviruses: A Potential Broad-Spectrum Therapeutic Strategy. ACS Comb Sci. 22 (6), 297–305, doi: 10.1021/acscombsci.0c00027.
9. Raphael V. P., Shanmughan S. K. **(2020)** Computational Evaluation of the Inhibition Efficacies of HIV Antivirals on SARS-CoV-2 (COVID-19) Protease and Identification of 3D Pharmacophore and Hit Compounds. Adv. Pharmacol. Pharm. Sci. 8818008, http://dx.doi.org/10.1155/2020/8818008.
10. Raphael V.P., Shanmughan S.K. **(2020)** In silico evaluation on the inhibition efficacy of five antivirals on SARS-CoV-2 protease (COVID-19). Indian J. Pharm. Educ. 54(4), 1031–1038. http://dx.doi.org/10.5530/ijper.54.4.197
11. Zhang L., Fang X., Liu X., Ou H. Zhang H., Wang J., et al. **(2020)** Discovery of sandwich type COVID-19 nucleocapsid protein DNA aptamers. Chem. Commun. 56(70), 10235–10238. http://dx.doi.org/10.1039/d0cc03993d
12. Smits V. A. J., Hernández-Carralero E., Paz-Cabrera M.C., Cabrera E., Hernández-Reyes Y., Hernández-Fernaud J. R. **(2021)** The Nucleocapsid protein triggers the main humoral immune response in COVID-19 patients. Biochem. Biophys. Res. Commun. 543, 45–49. http://dx.doi.org/10.1016/j.bbrc.2021.01.073
13. Elfiky A. A. **(2020)** Anti-HCV, nucleotide inhibitors, repurposing against COVID-19. Life Sci. 248, 117477.,
14. Vankadari N., Wilce J. A. **(2020)** Emerging WuHan (COVID-19) coronavirus: glycan shield and structure prediction of spike glycoprotein and its interaction with human CD26. Emerg. Microbes Infect. 9(1), 601–604. http://dx.doi.org/10.1080/22221751.2020.1739565
15. Walls A. C., Park Y. J., Tortorici M. A., Wall A., McGuire A. T., Veesler D. **(2020)** Structure, function, and antigenicity of the SARS-CoV-2 spike glycoprotein. Cell 181(2), 281–292.e6.
16. Schneidman-Duhovny D., Dror O., Inbar Y., Nussinov R., Wolfson H.J. **(2008)** PharmaGist: a webserver for ligand-based pharmacophore detection. Nucleic Acids Res. 36, W223–W228. http://dx.doi.org/10.1093/nar/gkn187
17. Muegge I., Heald S. L., Brittelli D. **(2001)** Simple selection criteria for drug-like chemical matter. J. Med. Chem. 44(12), 1841–1846.
18. Wang Y., Zhang D., Du G., **(2020)** Remdesivir in adults with severe COVID-19: a randomised, double-blind, placebo-controlled, multicentre trial. Lancet 395(10236), 1569–1578.
19. Fromm M. F. **(2004)** Importance of P-glycoprotein at blood-tissue barriers. Trends Pharmacol. Sci. 25(8), 423–429.
20. Wienkers L. C., Heath T.G. **(2005)** Predicting in vivo drug interactions from in vitro drug discovery data. Nat. Rev. Drug Discov. 4(10):825-33. doi: 10.1038/nrd1851.