*Disciplinarum Scientia*. Série: Naturais e Tecnológicas, Santa Maria, v. 23, n. 2, p. 57-83, 2022.
Recebido em: 20.04.2022. Aprovado em: 13.06.2022.
ISSN 2176-462X
DOI: 10.37779/nt.v23i2.4200

# IN-SILICO STUDY OF ANTIVIRALS AND NON-ANTIVIRALS FOR THE TREATMENT OF SARS-COV-2[1]

## *ESTUDO IN-SILICO DE ANTIVIRAIS E NÃO ANTIVIRAIS PARA O TRATAMENTO DE SARS-COV-2*

**André Flores dos Santos[2,*], Mirkos Ortiz Martins[3], Giane Engel Montagner[2], Júlia Vaz Schultz[4], Patrícia Gomes[3], Ivana Zanella da Silva[3] e Solange Binotto Fagan[3]**

**ABSTRACT**

In March 2020, it was declared a coronavirus (COVID-19) pandemic disease state by the World Health Organization, which represents a public health concern. Several antiviral and non-antiviral drugs are currently being tested against the COVID-19 disease, some of which have already been approved by health regulatory bodies in some countries. In this study, the objective was to identify the best drugs that are being used to fight the virus through computer simulations in addition to predicting toxicity and antiviral activity. Thus, molecular docking of the compounds arbidol, baricitinib, camostat mesylate, favipiravir, heparin, hydroxychloroquine, molnupiravir, paxlovid, and remdesivir was performed to discover the best points of interaction in the cellular organelles involved in the process of cellular infection by SARS-CoV- 2 and the prediction of toxicology and antiviral activity of the compounds was performed by online computer simulation programs: pkCSM, ProTox-II, GUSAR, ROSC-Pred, and AntiVir-Pred. The best results highlight the drugs heparin as a good protein S blocker, paxlovid as a protease inhibitor, arbidol as a viral entry blocker, baricitinib as an RNA replication inhibitor, and membrane fusion inhibitor. All compounds showed a low risk of toxicity, as most showed antiviral activity. Thus, all compounds, except favipiravir, demonstrated some type of interaction with target cell organelles, but more studies are needed, such as *in vitro* and *in vivo*, to obtain reliable results for treatment with small doses of the drug, since the prediction showed small rates of the toxicity, mainly hepatic.

**Keywords:** Coronavirus, Drugs, Efficacy, Infection, Pandemic, Toxicology.

***RESUMO***

*Em março de 2020, foi declarado estado de pandemia por coronavírus (COVID-19) pela Organização Mundial da Saúde, o que representa uma preocupação de saúde pública. Atualmente, vários medicamentos antivirais e não antivirais estão sendo testados contra a doença COVID-19, alguns dos quais já foram aprovados por órgãos reguladores de saúde em alguns países. Neste estudo, o objetivo foi identificar os melhores medicamentos que estão sendo utilizados para combater o vírus por meio de simulações computacionais, além de prever a toxicidade e a atividade antiviral. Assim, foi realizado docking molecular dos compostos arbidol, baricitinib, mesilato de camostato, favipiravir, heparina, hidroxicloroquina, molnupiravir, paxlovid e remdesivir para*

1 Study performed at Nanosciences Postgraduate Program - Franciscan University (UFN).

2 PhD Students of the Nanosciences Postgraduate Program - Franciscan University (UFN). E-mail: andre.santos@ufn.edu.br, giane.engel@ufn.edu.br

3 Contributors. Professors of the Nanosciences Postgraduate Program - Franciscan University (UFN). E-mail: mirkos@ufn.edu.br, patriciagomes@ufn.edu.br, ivanazanella@ufn.edu.br, sfagan@ufn.edu.br

4 Master's Students of the Nanosciences Postgraduate Program - Franciscan University (UFN). E-mail: julia.schultz@ufn.edu.br

*Corresponding author: Universidade Franciscana, Rua dos Andradas, 1614, Centro, Santa Maria - Rio Grande do Sul, 97010-030, Brazil. Email address: andre.santos@ufn.edu.br

*descobrir os melhores pontos de interação nas organelas celulares envolvidas no processo de infecção celular por SARS-CoV-2 e a predição da toxicologia e atividade antiviral dos compostos foi realizada por meio de programas de simulação computacional online: pkCSM, ProTox-II, GUSAR, ROSC-Pred e AntiVir-Pred. Os melhores resultados destacam os fármacos heparina como bom bloqueador da proteína S, paxlovid como inibidor de protease, arbidol como bloqueador de entrada viral, baricitinibe como inibidor de replicação de RNA e como inibidor de fusão de membrana. Todos os compostos apresentaram baixo risco de toxicidade, pois a maioria apresentou atividade antiviral. Assim, todos os compostos, exceto o favipiravir, demonstraram algum tipo de interação com organelas da célula-alvo, mas são necessários mais estudos, como in vitro e in vivo, para obter resultados confiáveis para o tratamento com pequenas doses do fármaco, pois a previsão mostrou pequenas taxas de toxicidade, principalmente hepática.*

***Palavras-chave:*** *Coronavirus, Fármacos, Eficácia, Infecção, Pandemia, Toxicologia.*

## INTRODUCTION

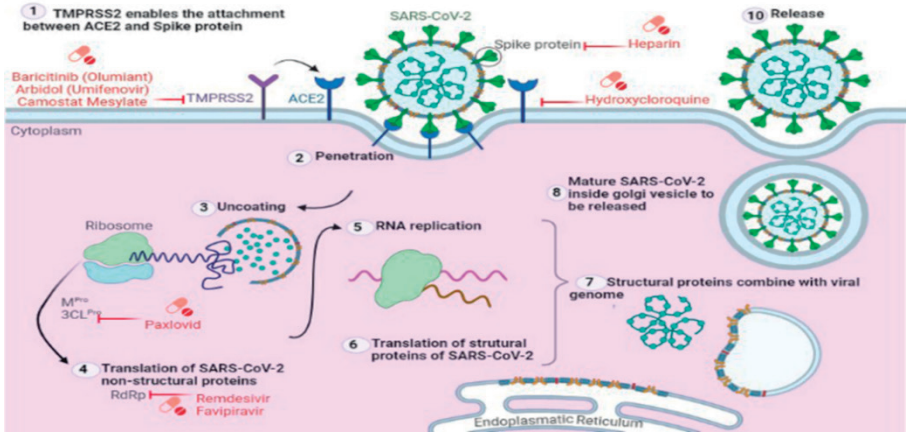
The COVID-19 pandemic caused by SARS-CoV-2 has currently recorded 6.204.423 (as of April 2022) deaths worldwide (HOPKINS, 2022). A part of patients affected by the disease requires hospitalization, largely the elderly and people with pre-existing conditions, for example, obesity, heart disease, diabetes, etc. Vaccines have been approved and are decreasing hospital admissions and deaths (HAAS *et al*., 2021; SANTOS *et al*., 2021) however, vaccination coverage remains insufficient. Some antiviral therapies that reduce the risk of progression of COVID-19 are being tested and so far few drugs have been approved (paxlovid, molnupiravir, and remdesivir) and are restricted only in some countries (FDA, 2020a, 2021a, 2021b).

Antiviral treatments for COVID-19 disease are currently based on experiences of similar viruses, for example, Severe Acute Respiratory Syndrome Coronavirus (SARS-CoV), Acquired Immunodeficiency Syndrome (HIV), Middle East Respiratory Syndrome (MERS-CoV), and influenza (H1N1). In this article, molecular docking tests were performed with some of the best-known antivirals that showed better responses to COVID-19 according to the literature and the Food and Drug Administration Database (FDA, 2022). Each antiviral exploits a way of blocking the virus and can be classified as endosomal acidification inhibitors, membrane fusion inhibitors, protein and virus entry blockers, virus replication blockers, and protease inhibitors (WANG; LI; LIU, 2020).

The SARS-CoV-2 virus infection process takes place in several stages. First, virus entry into host cells occurs through endocytosis, forming lysosomes to release viral RNA controlled by protease and respective pH. Some acidification inhibitors such as chloroquine and hydroxychloroquine and membrane fusion inhibitors block the virus before infection, for example, arbidol (umifenovir), baricitinib, camostat mesylate may be an alternative for the clinical treatment of COVID-19. In the next step of viral infection, RNA completes the transcription and translation of virus proteins and RNA replication takes place in the cytoplasm. Blocking these processes can be carried out by nucleoside analogs that resemble the natural ligands that build the chain, for example, remdesivir, protease inhibitors,

and replication like paxlovid, favipiravir, molnupiravir, arbidol. In the last step, viral protein structures combine with RNA to generate new coronavirus particles, being excreted into the extracellular environment through the Golgi complex to infect new cells (FREDIANSYAH *et al.*, 2021; WANG; LI; LIU, 2020). The SARS-CoV-2 infection process is described in Figure 1.

**Figure 1**. Steps in the process of infection by the SARS-CoV-2 virus in human cells.




Source: Author Construction (Biorender.com). TMPRSS2: Transmembrane Serine Protease 2, ACE2: Angiotensin-converting Enzyme, 3CLPro: 3C-like Cysteine Protease, RdRP: RNA-dependent RNA polymerase.

The combination of antivirals in the treatment of COVID-19 has been described in the literature (HUSSAIN *et al.*, 2021), and in April 2022, more than 6.434 clinical studies for the treatment of SARS-CoV-2 were registered on clinical trial registration platforms (CLINICALTRIALS, 2022). In this context, the study aimed to test targets (cellular organelles) that block the virus through specific antiviral drugs, for example, arbidol, baricitinib, camostat mesylate, favipiravir, heparin, hydroxychloroquine, molnupiravir, paxlovid, and remdesivir, in addition, to predict the toxicity and antiviral activity of the compounds.

## MATERIAL AND METHODS

### ANTIVIRALS AND NON-ANTIVIRALS DRUGS CANDIDATES FOR THE TREATMENT OF SARS-COV-2 INFECTION

#### *Endosomal Acidification Inhibitors and Virus Entry Blockers*

##### *Chloroquine e Hydroxychloroquine*

Chloroquine and hydroxychloroquine are considered endosomal acidification inhibitors, they can enter cells and accumulate in organelles and increase the pH value to destroy their structure and functions, but misuse can cause serious side effects (SAVARINO *et al.*, 2003), e.g. retinopathy,

neuromyopathy, and cardiomyopathy (WANG; LI; LIU, 2020) however, the drug is indicated for the treatment of chronic rheumatic diseases that uses much smaller doses, so more studies are necessary for clinical application against COVID-19 with the safety of this drug. Chloroquine and hydroxychloroquine have also been shown to block virus entry, altering the structural configuration of cell receptors, for example by modifying ACE2 (Angiotensin Converting Enzyme) glycosylation which can prevent virus entry (FREDIANSYAH *et al.*, 2021).

## *Membrane Fusion inhibitors*

### *Arbidol*

Arbidol is an oral drug used in Russia and China mainly to treat some viruses, e.g. influenza A and B, hepatitis C, and can prevent the fusion of the virus with host cells and the replication of the virus (NOJOMI *et al.*, 2020). Blockade of virus fusion performed by arbidol is specifically in subunit 2 (S2) of the protein (S) trimer of SARS-CoV-2 and the Transmembrane Serine Protease 2 (TMPRSS2), being the region responsible for fusion in the host cell after protein (S) binding with the ACE2 cell receptor (SHUSTER *et al.*, 2021).

### *Baricitinib*

Baricitinib is indicated for use in conjunction with remdesivir for the treatment of COVID-19 in hospitalized adults and pediatric patients aged two years and older and requiring supplemental oxygen (FDA, 2020b, 2020c). Baricitinib is an inhibitor of Janus Kinase 1, 2, and can organize the signaling pathways that lead to hyperinflammation present in some diseases, such as COVID-19, it also can inhibit clathrin-mediated viral endocytosis through an interaction with the ACE2 (JORGENSEN *et al.*, 2020; STEBBING *et al.*, 2020).

### *Camostat Mesylate*

Camostat mesylate is an oral drug that has the potential to reduce the virus SARS-CoV-2 entry into the host cell through inhibition of the TMPRSS2 (BREINING *et al.*, 2021; HOFFMANN *et al.*, 2021).

## *Protease inhibitors*

### *Paxlovid*

The drug Paxlovid (nirmaltrervir) is an inhibitor of the 3CL-Pro protease (3C-like Cysteine Protease) to stop the virus from replicating and is used together with another drug called ritonavir that slows down the breakdown of paxlovid, helping it stay in the body for a longer period at higher concentrations (FDA, 2021c, 2021b), with potential antiviral activity (NCBI, 2022a).

## *Protein Blockers*

### *Heparin*

Heparin and heparan sulfate proteoglycans are negatively charged repeating chains and sulfated zones at the ends, making them attractive to bind with affinity to various types of ligands, e.g., cytokines, chemokines, enzymes, proteins, and bacterial and viral pathogens (DE PASQUALE *et al.*, 2021). The available heparan sulfate in the outer layer of the cells mediates the binding of the protein (S) with ACE2, so putting heparin in free circulation will cause the protein (S) to be blocked before it interacts with the outer layer of the host cells (CLAUSEN *et al.*, 2020a).

## *RNA Replication Inhibitors*

### *Remdesivir*

Remdesivir is an intravenous antiviral drug approved by the FDA and ANVISA (Agência Nacional de Vigilância Sanitária - Brazil) for use in adult and pediatric patients requiring hospitalization (FDA, 2020a, 2020d). Remdesivir is a monophosphoramidate prodrug, adenosine nucleotide analog, RNA-dependent RNA polymerase (RdRp) inhibitor with broad-spectrum antiviral activity against filovirus, paramyxovirus, pneumovirus, ebola virus, respiratory syncytial virus, coronavirus like SARS-CoV, MERS-CoV, SARS-CoV-2, reducing viral RNA replication in host cells preventing the severity of COVID-19 disease progression (MARTINEZ, 2020; NCBI, 2022b).

### *Favipiravir*

The drug favipiravir is a purine nucleoside analog (guanine), RdRP inhibitor, initially developed for the Influenza (H1N1) virus, has characteristics to act against RNA viruses, including Ebola and Coronavirus, especially SARS-COV-2. Clinical studies on this drug are being carried out, but there are many controversies about its effectiveness in mild and moderate cases of COVID-19 (BOSAEED *et al.*, 2021, 2022).

### *Molnupiravir*

Molnupiravir is an oral antiviral drug capable of reducing hospitalization for COVID-19 and death in mild cases on early treatment (SINGH *et al.*, 2021). This reduction also is verified in unvaccinated adults (JAYK BERNAL *et al.*, 2022). This drug was developed to treat influenza in the USA, and its way of action is related to RdRP inhibition, which can reduce the pathogenesis and replication of the virus (FDA, 2021d, 2021a).

## PREDICTION STUDY OF TOXICITY AND ANTIVIRAL ACTIVITY

A computer simulation study was performed to estimate the possible toxicological risks and antiviral activity of nine compounds: arbidol, baricitinib, camostat mesylate, favipiravir, heparin, hydroxychloroquine, molnupiravir, paxlovid, and remdesivir. For this, five online computer programs were used: pkCSM (PIRES; BLUNDELL; ASCHER, 2015), ProTox-II (BANERJEE *et al.*, 2018), GUSAR (Acute Rat Toxicity) (LAGUNIN *et al.*, 2011), ROSC-Pred (LAGUNIN *et al.*, 2018) and PASS Online (AntiVir-Pred) (POROIKOV *et al.*, 2019). Toxicity and antiviral activity predictions from online prediction software are based on molecular similarity, fragment propensities, most frequent characteristics, and machine learning based on predetermined real (in vitro and in vivo) models, thus predicting the toxic potential and activities of existing and virtual compounds (PIRES; BLUNDELL; ASCHER, 2015; BANERJEE *et al.*, 2018; POROIKOV *et al.*, 2019).

The SMILES (Simplified Molecular Input Line Entry System) were used as input for the different computer models. Isomeric SMILES strings were used in the case of stereoisomeric compounds. In contrast to the canonical SMILES strings, the isomeric SMILES strings allow for the differentiation between different stereoisomers. The SMILES strings were obtained from the website PubChem (https://pubchem.ncbi.nlm.nih.gov/).

The toxicity class was defined according to the Globally Harmonized System of Classification of Labelling of Chemicals (UNECE, 2019):

Class I: fatal if swallowed ($LD50 \leq 5$)

Class II: fatal if swallowed ($5 < LD50 \leq 50$)

Class III: toxic if swallowed ($50 < LD50 \leq 300$)

Class IV: harmful if swallowed ($300 < LD50 \leq 2000$)

Class V: may be harmful if swallowed ($2000 < LD50 \leq 5000$)

Class VI: non-toxic ($LD50 > 5000$),

where the LD50 values are given in [mg kg-1].

For the prediction of mutagenicity, the Ames test was used, using several strains of the bacterium Salmonella typhimurium (TA98, TA100, and TA1535). This test is capable of detecting mutations in the genetic material involved in the synthesis of the amino acid histidine (AMES; MCCANN; YAMASAKI, 1975). The result was considered positive when there was a reversal of the mutation in one or more bacteria, and negative when there was no reversion of the mutation in the bacteria. When there was a false positive, that is, there was no reversion and even though the program classified it as mutagenic, the result was not considered.

The prediction of carcinogenic potential in rodents (Rodent Carcinogenicity) was performed using data from the National Toxicology Program (NTP) and Food and Drug Administration (FDA), which analyzes the results of in vivo tests of carcinogenicity in rats and mice for two years. The presence

of toxicological risks, mutagenicity, carcinogenicity, and antiviral activity was indicated with the positive sign (+), and the absence with the negative sign (-).

## DOCKING MOLECULAR

Molecular docking, also known by the terms molecular docking, molecular coupling, molecular docking in the field of molecular modeling is a mechanism that predicts the preferential orientation of a macromolecule (usually a protein, peptides, or a stretch of DNA) called a target, to a second structure called a ligand, when linked together to form a stable complex. The prediction of the binding between the target and the ligand is performed using the three-dimensional location of the sites that can receive the volume of the ligand. The location is performed by algorithms that identify the space and the chances of biological compatibility.

The docking process at the molecular level can be described by the laws of quantum chemistry, where the temporal evolution of molecular systems is expressed in terms of the wave functions of atoms. In practical calculations, however, approximations are used, where the dynamics of the system are identified by atoms represented by point masses that move in molecular forces fields. Molecular forces are established by electrostatic and chemical bonding interactions between atoms (MARTINS *et al.*, 2021).

Molecular docking comprises a positioning relationship of a ligand to a target molecule and binding score based on some metrics, for example, score function and Root-mean-Square Deviation (RMSD), defined as a measure of mean distance in Angstrom between the atoms of the two ligands (Target and ligand). The RMSD is used to measure the quality of the molecular docking process, indicating a value smaller than 2 Angstroms, while the affinity value should be considered as negative as possible (TROTT; OLSON, 2009).

The software used to perform molecular docking was AutoDockFR (ADFR) (RAVINDRANATH *et al.*, 2015), which is one of the docking programs that are part of the AutoDock Suite. The Autodock energy function (HUEY *et al.*, 2007) is a weighted sum of terms representing van der Waals, hydrogen bond, electrostatic, and desolvation contributions, which are calculated between pairs of atoms. The ADFR score uses the energy function to independently score the interactions between the following three groups of atoms (L), Rigid Receptor atoms (RR), and Flexible Receptor atoms (RR) and Flexible Receptor atoms (FR).

The software used to define the affinity maps used by Autodock was AutoGridFR (ZHANG *et al.*, 2019), it supports the calculation of maps for various advanced covalent coupling techniques, hydrated, with flexible side chains and receptors. AutoGridFR uses a computational method to identify binding sites in macromolecules with a three-dimensional structure (receptor) called AutoSite (RAVINDRANATH; SANNER, 2016), where high-affinity binding sites are identified by clustering high-affinity points.

The initial steps of docking are composed of the preparation of the ligand and the receptor that was carried out using the AutoDockTools Software that is part of the AutoDock Suite. The software to generate the molecular docking images was BIOVIA Discovery Studio Visualizer.

### *Ligant preparation*

The binder must be analyzed according to its structure to identify its possible torsion sites, which will allow its adaptation to different spatial conformations during the execution of the docking where it will make angular movements in its three-dimensional structure.

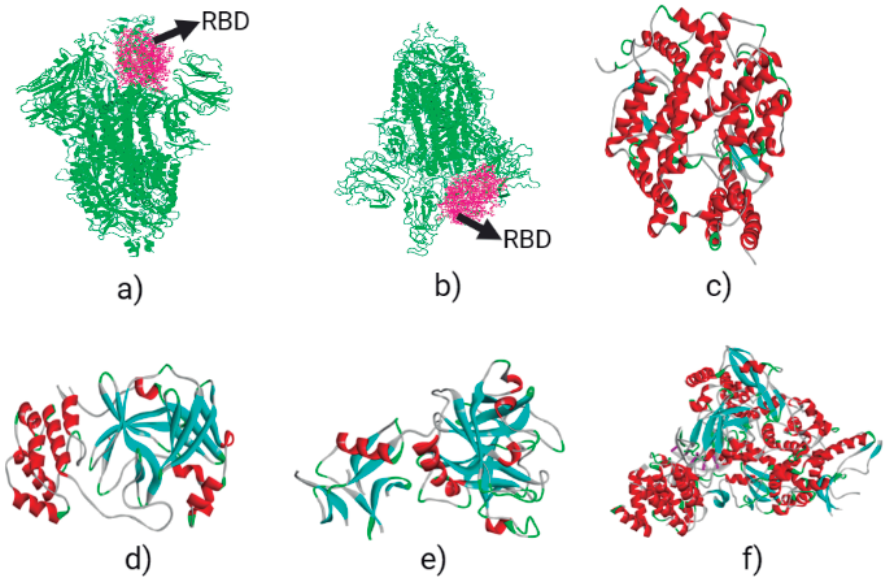
### *Target preparation*

The target molecule also called the receptor must be treated with the identification of charges, correction of unbound atoms for stabilization of the structure, and solvation of the medium with water molecules. According to (VAN DIJK; BONVIN, 2006) the solvation of the medium can improve the results for biomolecular complexes.

### *Structures used*

The structures used in this work are composed of different receptors and ligands for each type of specific drug target, as previously described. The targets used in this work are described in Figure 2.

**Figure 2 -** Structures used in the study.




a) Spike protein with RBD highlighted (6vyb), b) Omicron Spike protein with RBD highlighted (7qo7), c) ACE2 (7df4), d) Protease 3CLpro (7jst), e) TMPRSS2 (7meq), f) RdRP (6xqb).

For protein blockers, the receptor used was the Spike protein of SARS-CoV-2 (PDB ID: 6VYB) and Omicron B 1.1.529 (7QO7), and the target point for molecular docking was its Receptor-bind Domain (RBD). The Spike protein RBD is on S1 (Subunit 1) of its trimer, which runs from residue 330-527 (MANSBACH *et al*., 2021).

In the case of virus entry blockers and some membrane fusion inhibitors, the ACE2 receptor (PDB ID: 7DF4) SARS-CoV-2 S-ACE2 complex was used, which contains the Spike protein of SARS-CoV-2 and ACE2 together, then they were manually separated, using only the ACE2 complex for molecular docking. For other specific membrane fusion inhibitors, TMPRSS2 was used with the receptor (PDB ID: 7MEQ) manually removing additional complex ligands.

For protease inhibitors, the receptor (PDB ID: 7JST) protease 3CLpro was used (IKETANI *et al*., 2021).

The receptor for RdRP used was (PDB ID: 6XQB) SARS-CoV-2 RdRP/RNA complex.

## RESULTS

### PREDICTION STUDY OF TOXICITY AND ANTIVIRAL ACTIVITY

The results of the cytotoxicity, hepatotoxicity, immunotoxicity, toxicity class, mutagenicity, carcinogenicity, and antiviral activity prediction study are shown in Table 2.

**Table 2 -** Prediction of toxicity and antiviral activity of antivirals and non-antivirals against SARS-CoV-2.

| Compounds | Cytotoxicity | Hepatotoxity | Immunotoxicity | Toxicity Class |
|---|---|---|---|---|
| **Arbidol** | (-) [2] | (+) [1]<br>(-) [2] | (-) [2] | (IV) [2,3] |
| **Baricitinib** | (-) [2] | (+) [1]<br>(-) [2] | (-) [2] | (V) [2]<br>(IV) [3] |
| **Camostat** | (-) [2] | (-) [1,2] | (-) [2] | (V) [2,3] |
| **Favipiravir** | (-) [2] | (-) [1,2] | (-) [2] | (IV) [2,3] |
| **Heparin** | (-) [2] | (-) [1]<br>(+) [2] | (+) [2] | (V) [2]<br>(IV) [3] |
| **Hydroxychloroquine** | (-) [2] | (+) [1]<br>(-) [2] | (+) [2] | (IV) [2,3] |
| **Molnupiravir** | (-) [2] | (+) [1,2] | (-) [2] | (IV) [2]<br>(V) [3] |
| **Paxlovid** | (-) [2] | (+) [1]<br>(-) [2] | (-) [2] | (V) [2]<br>(IV) [3] |
| **Remdesivir** | (-) [2] | (+) [1]<br>(-) [2] | (-) [2] | (IV) [2,3] |

| Mutagenicity/ Carcinogenicity/ Antiviral Activity | | | |
|---|---|---|---|
| **Compounds** | **Mutagenicity** | **Carcinogenicity** | **Antiviral Activity** |
| **Arbidol** | (-) [1,2] | (-) [2,4] | (+) [5] |
| **Baricitinib** | (-) [1,2] | (+) [2]<br>(-) [4] | (+) [5] |
| **Camostat** | (-) [1,2] | (-) [2,4] | (+) [5] |

| | | | |
|---|---|---|---|
| **Favipiravir** | (-) [1,2] | (+) [2,4] | (+) [5] |
| **Heparin** | (-) [1,2] | (-) [2,4] | (-) [5] |
| **Hydroxychloroquine** | (+) [1,2] | (-) [2,4] | (+) [5] |
| **Molnupiravir** | (-) [1,2] | (-) [2]<br>(+) [4] | (+) [5] |
| **Paxlovid** | (-) [2] | (-) [2,4] | (+) [5] |
| **Remdesivir** | (-) [1,2] | (-) [2]<br>(+) [4] | (+) [5] |

[1]pkCSM; [2]ProTox-II; [3]GUSAR; [4]ROSC-Pred; [5]PASS-Online. (+) positive, (-) negative.

According to the results presented in Table 2, the nine compounds (heparin, baricitinib, camostat mesylate, favipiravir, hydroxychloroquine, molnupiravir, paxlovid, remdesivir, and arbidol) had a low probability of toxicity, being classified in class IV and V, according to the Protox-II and GUSAR platforms, did not show cytotoxicity (Protox-II) and there was no reversion of the mutation of *Salmonella typhimurium* bacteria, thus being non-mutagenic (pkCSM and Protox-II), except for hydroxychloroquine, which in both programs of prediction, proved to be mutagenic, but not considered carcinogenic. In addition, all compounds showed antiviral activity, except for heparin, according to the PASS Online (AntiVir-Pred) platform.

Regarding hepatotoxicity, the compounds were evaluated on two different platforms, pkCSM and Protox-II, presenting an ambiguity in the results, showing that only prediction analyzes are not enough to define whether the compound is toxic to the liver cells. However, camostat and favipiravir were shown not to be hepatotoxic in both platforms and molnupiravir was found to be hepatotoxic in both programs.

The compounds heparin and hydroxychloroquine showed immunotoxicity characteristics, but the other seven compounds, in the prediction, are not immunotoxic, according to the Protox-II program. Regarding the carcinogenicity of the compounds in rodents, heparin, camostat, hydroxychloroquine, paxlovid, and arbidol did not exhibit carcinogenic properties, however, baricitinib, molnupiravir, and remdesivir showed negative and positive results depending on the platform used, thus not being able to arrive at a definitive result of carcinogenicity without other tests. The compound favipiravir was the only one that proved to be carcinogenic in both platforms used, Protox-II and ROSC-Pred.

## DOCKING MOLECULAR

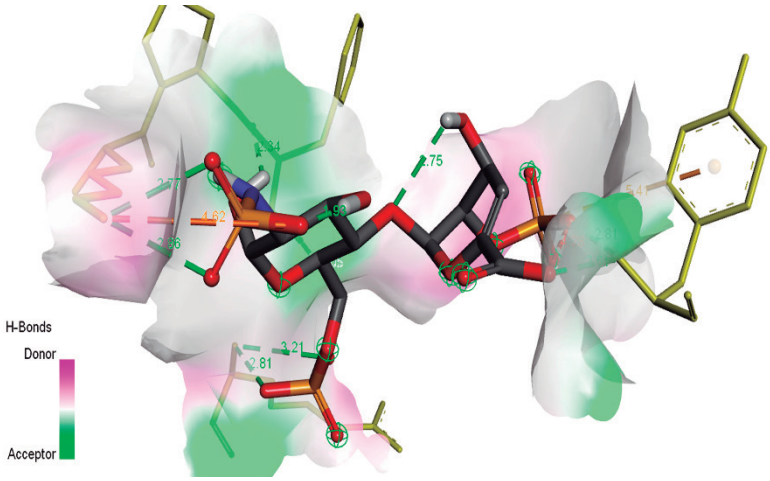
The results presented are separated by antiviral and non-antiviral drug targets, eg SARS-CoV-2 Spike protein blockers, 3CL protease inhibitors, and membrane fusion inhibitors. In the previous section, the drugs are described by type and target against COVID-19, but we decided to also test all drugs on the same target to see what their characteristics are.

**Table 3** - The target of antiviral and non-antiviral drugs: Spike Protein (RBD) from SARS-CoV-2.

| Mode | Structure | Affinity (kcal/mol) SARS-CoV-2 | RMSD (Å -Angstrom) | Structure | Affinity (kcal/mol) SARS-CoV-2 Omicron B 1.1.529 | RMSD (Å -Angstrom) |
|---|---|---|---|---|---|---|
| Protein blocker | Heparin | -11.4 | 0.721 | Heparin | -7.6 | 0.719 |
| Protein blocker | Baricitinib | -6.0 | 1.862 | Camostat mesylate | -5.7 | 1.944 |
| Protein blocker | Paxlovid | -5.5 | 0.745 | Baricitinib | -5.7 | 1.898 |
| Protein blocker | Molnupiravir | -5.1 | 0.720 | Paxlovid | -5.7 | 0.766 |
| Protein blocker | Remdesivir | -4.4 | 0.242 | Arbidol | -5.3 | 0.935 |
| Protein blocker | Hydroxychloroquine | -4.3 | 1.916 | Remdesivir | -5.2 | 0.839 |
| Protein blocker | Camostat mesylate | -4.1 | 1.607 | Hydroxychloroquine | -5.2 | 1.842 |
| Protein blocker | Arbidol | -3.8 | 0.994 | Molnupiravir | -4.7 | 0.689 |
| Protein blocker | Favipiravir | -3.5 | 0.550 | Favipiravir | -3.5 | 0.531 |

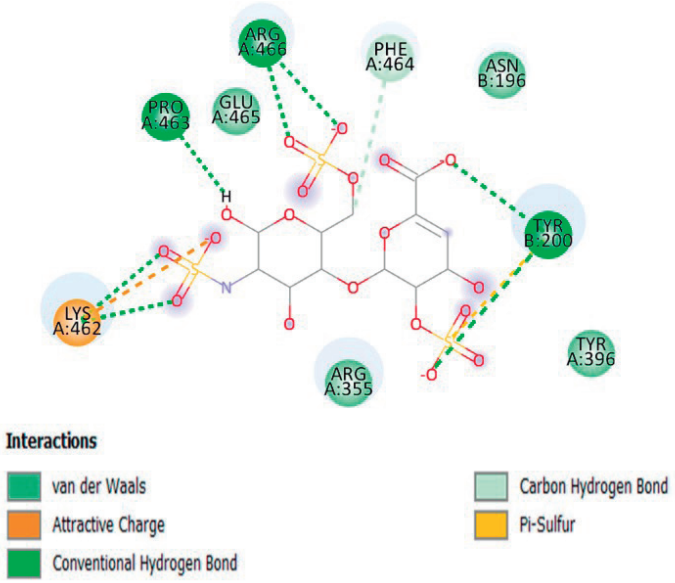
According to Table 3, the best result found for the RBD of the Spike protein of SARS-CoV-2 (unmodified) was the drug heparin, confirming the great blocking power of this drug in the Spike protein according to literature publications.

**Figure 3** - Interactions between Spike Protein of SARS-COV-2 RBD and Heparin.




The molecular docking image is shown in Figure 3, highlighting the hydrogen bonding regions.

**Figure 4** - 2D map of interactions between Spike Protein of SARS-COV-2 RBD and Heparin.

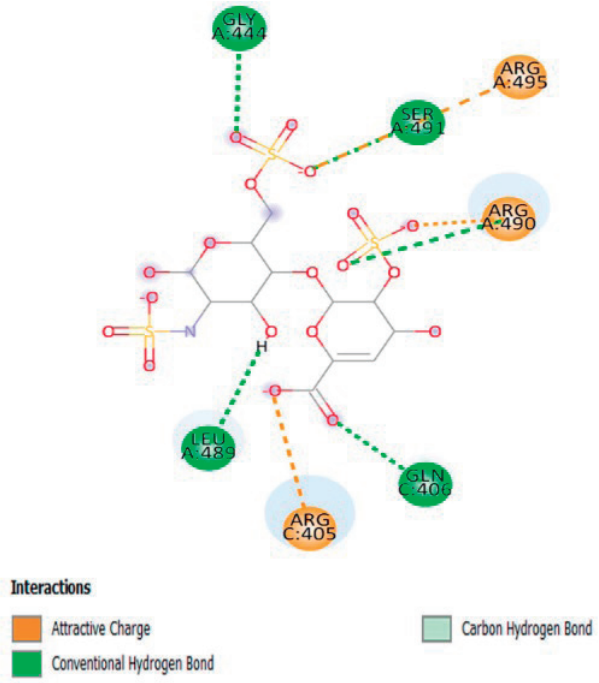



**Distances** (Å -Angstrom): Lysine-LYS462 (2.77, 2.86, 4.62), Arginine-ARG466 (2.81, 3.21), Proline-PRO463 (2.34), Phenylalanine-PHE464 (3.05), Tyrosine-TYR200 (2.61, 2.81).

The 2D interaction map described in Figure 4 and Figure 5 (Spike Protein Omicron) shows the residues that participate in the binding between protein (S) and heparin. According to Table 2 for the case of the Omicron variant of SARS-CoV-2, heparin did not show the same effectiveness and the binding energy and the number of bonds were reduced, which may be explained by the modifications that this variant presented in the Spike protein RBD (NI, 2021).

**Figure 5** - 2D map of interactions between Spike Protein of SARS-COV-2 Omicron B 1.1.529 RBD and Heparin.

**Distances** (Å -Angstrom): Glycine-GLY444 (2.98), Serine-SER491 (2.86), Arginine-ARG495 (5.39), Arginine-ARG490 (3.36), Glutamine-GLN406 (3.33), Arginine-ARG405 (4.03), Leucine-LEU489 (2.38).



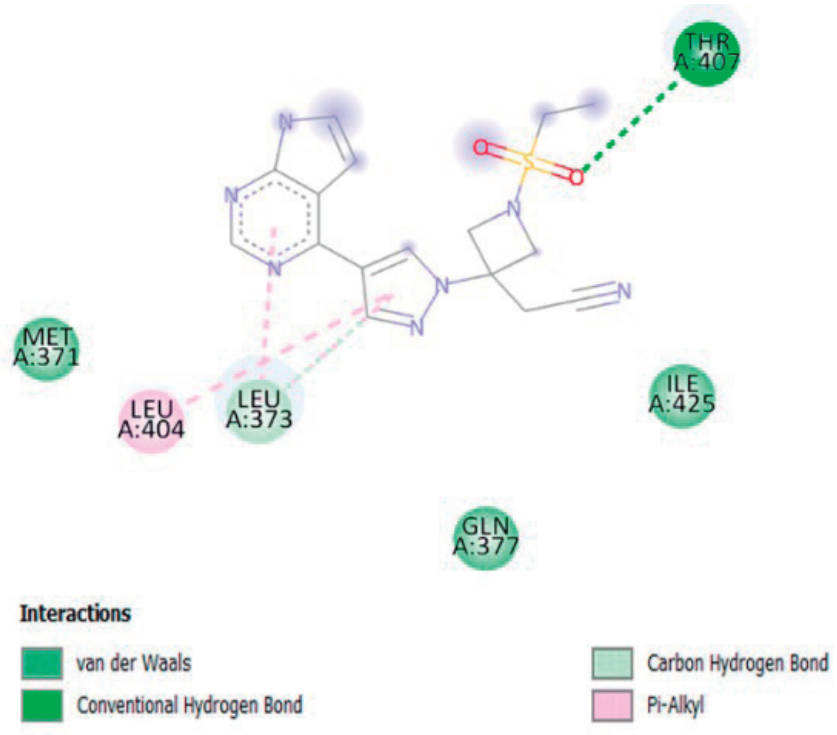
The 2D interaction map described in Figure 5 shows the residues that participate in the binding between Spike protein of Omicron B 1.1.529 and heparin.

**Table 4** - Target of antiviral and non-antiviral drugs: TMPRSS2.

| Structure | Mode | Affinity (kcal/mol) | RMSD (Å -Angstrom) |
|---|---|---|---|
| Baricitinib | Membrane fusion | -6.1 | 1.770 |
| Camostat mesylate | Membrane fusion | -5.4 | 0.894 |
| Heparin | Membrane fusion | -5.4 | 0.903 |
| Arbidol | Membrane fusion | -5.1 | 0.919 |
| Paxlovid | Membrane fusion | -4.9 | 0.739 |
| Molnupiravir | Membrane fusion | -4.4 | 1.105 |
| Hydroxychloroquine | Membrane fusion | -4.1 | 1.544 |
| Remdesivir | Membrane fusion | -3.9 | 0.306 |
| Favipiravir | Membrane fusion | -3.0 | 0.949 |

According to Table 4, the best result found for the TMPRSS2 receptor was the drug baricitinib, according to literature publications cited in the text, confirming the blocking power in the fusion of the virus with the host cells.

**Figure 6 -** 2D map of interactions between TMPRSS2 and Baricitinib.




**Distances** (Å -Angstrom): Theonine-THR407(2.95), Leucine-LEU404 (2.96), Leucine-LEU373 (5,33).

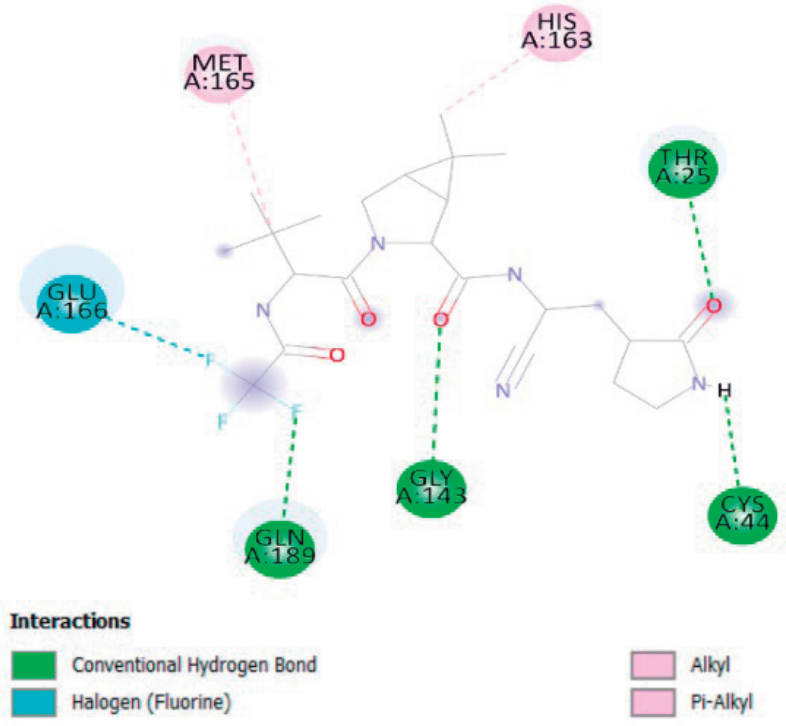
The 2D interaction map described in Figure 6 shows the residues that participate in the links between TMPRSS2 and the drug baricitinib.

**Table 5 -** The target of antiviral and non-antiviral drugs: Protease 3CL.

| Structure | Mode | Affinity (kcal/mol) | RMSD (Å -Angstrom) |
|---|---|---|---|
| Paxlovid | Protease Inhibitor | -5.4 | 0.684 |
| Baricitinib | Protease Inhibitor | -5.1 | 1.157 |
| Camostat mesylate | Protease Inhibitor | -4.8 | 1.459 |
| Heparin | Protease Inhibitor | -4.5 | 0.715 |
| Arbidol | Protease Inhibitor | -4.1 | 0.996 |
| Molnupiravir | Protease Inhibitor | -3.7 | 0.691 |
| Hydroxychloroquine | Protease Inhibitor | -3.1 | 0.946 |
| Favipiravir | Protease Inhibitor | -3.0 | 0.533 |
| Remdesivir | Protease Inhibitor | -2.5 | 0.269 |

As shown in Table 5, the best result found for the 3CLpro protease receptor was the drug paxlovid, an oral antiviral drug approved by the FDA in December 2021 for the treatment of COVID-19, confirming the expected result.

**Figure 7 -** 2D map of interactions between protease 3CLpro and Paxlovid.




**Distances** (Å -Angstrom): GlutamicAcid-GLU166 (3.40), Glutamine-GLN189 (3.10), Glycine-GLU143 (3.23), Cysteine-CYS44 (2.42), Threonine-THR25 (3.39), Histidine-HIS163 (4.98), Methionine-MET165 (5.12).

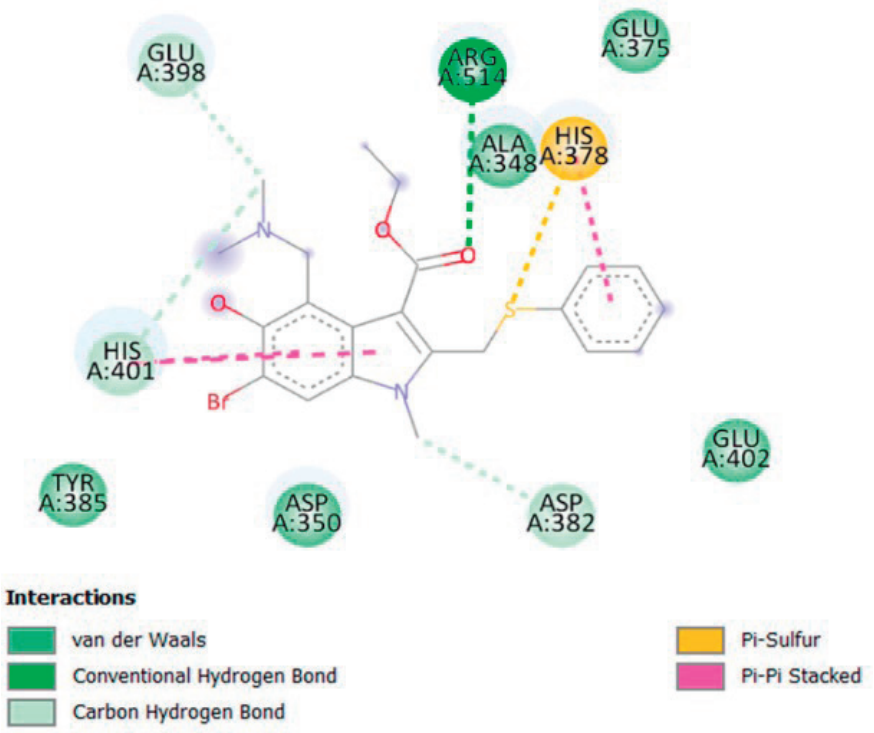
The 2D interaction map described in Figure 7 shows the residues that participate in the binding between the protease 3CLpro and the drug paxlovid.

**Table 6** - Target of antiviral and non-antiviral drugs: ACE2.

| Structure | Mode | Affinity (kcal/mol) | RMSD (Å -Angstrom) |
|---|---|---|---|
| Arbidol | Virus entry blocker | -5.9 | 0.929 |
| Baricitinib | Virus entry blocker | -5.7 | 1.780 |
| Camostat mesylate | Virus entry blocker | -5.0 | 1.157 |
| Hydroxychloroquine | Virus entry blocker | -4.9 | 1.521 |
| Paxlovid | Virus entry blocker | -4.8 | 0.726 |
| Molnupiravir | Virus entry blocker | -4.1 | 0.943 |
| Remdesivir | Virus entry blocker | -3.8 | 0.156 |
| Favipiravir | Virus entry blocker | -2.8 | 0.769 |
| Heparin | Virus entry blocker | -0.1 | 0.792 |

According to Table 6, the best result found for endosomal acidification inhibitors with the ACE2 receptor was the drug arbidol, demonstrating a better result than the hydroxychloroquine indicated for this function.

**Figure 8 -** 2D map of interactions between protease ACE2 and Arbidol.




**Distances** (Å -Angstrom): Arginine-ARG514 (2.92), GlutamicAcid-GLU398 (3.12), Histidine-HIS401 (3.37), AsparticAcid-ASP382 (3.57, 3.80), Histidine-HIS378 (3.99).

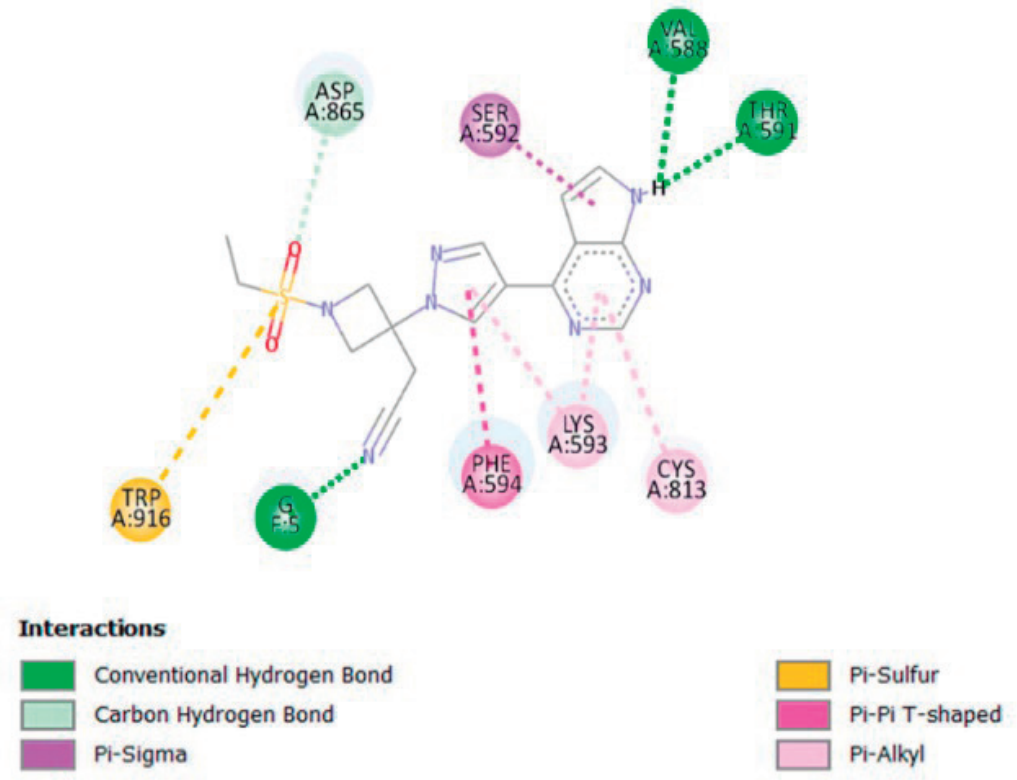
The 2D map of interactions is described in Figure 8 and shows the residues that participate in the binding between the ACE2 cell receptor and the drug arbidol.

**Table 7** - The target of antiviral and non-antiviral drugs: RdRP.

| Structure | Mode | Affinity (kcal/mol) | RMSD (Å -Angstrom) |
|---|---|---|---|
| Baricitinib | RNA Replication Inhibitors | - 6.6 | 1.781 |
| Camostat mesylate | RNA Replication Inhibitors | -5.3 | 1.165 |
| Hydroxychloroquine | RNA Replication Inhibitors | -5.3 | 1.853 |
| Paxlovid | RNA Replication Inhibitors | -5.2 | 1.023 |
| Heparin | RNA Replication Inhibitors | -5.2 | 0.733 |
| Molnupiravir | RNA Replication Inhibitors | -5.1 | 0.682 |
| Arbidol | RNA Replication Inhibitors | -5.0 | 0.986 |
| Remdesivir | RNA Replication Inhibitors | -4.7 | 0.302 |
| Favipiravir | RNA Replication Inhibitors | -3.9 | 0.518 |

According to Table 7, the best result found for the RdRP receptor was the drug baricitinib, showing a result well above that of the drugs remdesivir, favipiravir and molnupiravir indicated for this function, according to some articles cited in the text in the previous section.

**Figure 9 -** 2D map of interactions between RdRp and Baricitinib.




**Distances** (Å -Angstrom): AsparticAcid-ASP865 (2.76), Tryptophan-TRP916 (5.37), Phenylalanine-PHE594 (5.15), Lysine-LYS593 (5.26, 4.46), Cysteine-CYS813 (4.40), Threonine-THR591 (2.37), Valine-VAL588 (2.79), Serine-SER592 (2.85).

The 2D map of interactions described in Figure 9 shows the residues that participate in the binding between the RdRP and the drug Baricitinib.

## DISCUSSION

Computer simulation toxicology predictions are useful for organizing, modeling, simulating, visualizing, and predicting the toxicity of chemicals, thus minimizing the need for animal testing, reducing the cost and time of toxicity testing, improving toxicity prediction, and safety assessment of products (RAIES; BAJIC, 2016).

Drug-induced liver injury is a major safety concern for drug development and a significant cause of drug attrition. The compound is classified as hepatotoxic when there is at least one pathological or physiological event in the liver that is strongly associated with disrupted normal liver function (PIRES; BLUNDELL; ASCHER, 2015). In a study of drug reuse for use against COVID-19, the results of Hage-Melim *et al.* (HAGE-MELIM *et al.*, 2020) corroborate our findings, in which they demonstrated hydroxychloroquine and remdesivir have plausible hepatotoxicity, due to the quinoline group and organophosphorus di triester, respectively, present in their structures. Furthermore, Falcão *et al.* (FALCÃO *et al.*, 2020) in a case study on the use of hydroxychloroquine in patients with COVID-19, an increase in serum transaminases was reported, configuring a toxic effect on the liver of these patients using the medication. Other compounds in the present study showed hepatotoxicity, in different studies, such as heparin (BOSCO; KISH, 2019), baricitinib (RASCHI *et al.*, 2020), molnupiravir (ABU-MELHA *et al.*, 2022), and arbidol (HASANABADI, 2021) corroborating the results found and reinforcing the need for more tests, such as *in vitro* and *in vivo*. However, these drugs are already approved by the FDA for several uses, thus having an acceptable safety profile, so moderate use is indicated.

As far as mutagenicity is concerned, the Ames test uses several strains of the bacterium *Salmonella typhimurium* with mutations in the genes involved in the synthesis of histidine. These strains are auxotrophic mutants, that is, they require histidine for growth. Thus, the method tests the ability of a substance to reverse the mutation so that cells can grow in a histidine-free medium (AMES *et al.*, 1973). The mutagenesis test is considered a screening assay to predict the carcinogenic potential of substances that induce cancer by genotoxic mechanisms (PIRES; BLUNDELL; ASCHER, 2015). However, some substances induce cancer by non-genotoxic mechanisms, which involve mechanisms such as cytotoxicity with regeneration accompanied by an increase in DNA synthesis, immunosuppressants, and promoters of oncogenesis expression (AMES; GOLD, 1991) such as the compounds baricitinib, favipiravir, molnupiravir, and remdesivir that have a negative mutagenesis test and a positive carcinogenesis test on at least one prediction platform. However, baricitinib was considered non-carcinogenic in mice and rats, as shown in the study by Carfagna *et al.* (CARFAGNA *et al.*, 2018), corroborating the ROSC-Pred platform, in which the result was negative. As well as favipiravir and remdesivir, which through the ADMETSar web server were found to be non-carcinogenic (DAS *et al.*, 2021),

corroborating the negative results of the ROSC-Pred platform for remdesivir and contradicting the positive results of the Protox-II and ROSC-Pred for favipiravir.

Concerning the antiviral activity of compounds, AntiVir-Pred allows predicting whether a chemical compound can inhibit the activity of 66 proteins from 56 viruses at a concentration lower than or equal to 10,000 nM (POROIKOV *et al.*, 2019). Despite the prediction made in the study, which shows that heparin does not have viral activity, there is an increase in studies that indicate that heparin has this activity, as well as Gupta *et al.* (GUPTA *et al.*, 2021) who show that heparin has highly effective viral entry blocking properties, particularly for SARS-CoV-2, in addition to being a potential transmission blocker.

Considering the protein (S) RBD target that binds to host cells, the best result for protein blockers presented was that of the drug heparin demonstrating superiority over the other drugs, confirming its characteristics of a great blocker of the Spike protein of SARS-CoV -2 before fusion of the virus into cells (CLAUSEN *et al.*, 2020b; TAVASSOLY; SAFAVI; TAVASSOLY, 2020; YUE *et al.*, 2021).

Results for membrane fusion inhibitors using TMPRSS2 as a target were satisfactory for the three drugs studied, which are baricitinib, camostat mesylate, and arbidol including heparin which came in third with a better result than arbidol, but very upcoming. Spike protein cleavage and activation are mediated by TMPRSS2, an endothelial cell surface protein that mediates binding with ACE2 (LIMA; DE SOUSA; LIMA, 2020). Thus, TMPRSS2 and ACE2 inhibitors can also be considered an effective clinical therapy against COVID-19 (BOOPATHI; POMA; KOLANDAIVEL, 2020; WANG; LI; LIU, 2020).

For protease inhibitors using the 3CLpro protease target, the drug paxlovid approved against COVID-19 in December 2021 by the FDA (FDA, 2020c, 2021b) performed well, alongside baricitinib, demonstrating that we still have other drugs with the potential to be studied. However, it should be noted that paxlovid is used together with the drug ritonavir in some cases to slow down the circulation time of the drug in the human body (VANGEEL *et al.*, 2022), which can improve its performance.

For the viral entry inhibitors using ACE2 as a target, the best result was for arbidol, followed by baricitinib and camostat mesylate showing that these three drugs are very interesting for the treatment of COVID-19 and deserve to be highlighted in future studies.

For RNA replication inhibitors, the target used was RdRP and the best result found was for baricitinib followed by camostat mesylate, leaving these drugs highlighted again. The drugs molnupiravir, remdesivir, and favipiravir indicated for this function had similar values.

## CONCLUSIONS

According to the results presented, the drugs used in this study demonstrated great efficacy in their specific targets as described in the literature, in addition to having low rates of toxicity and

antiviral activities. However, more *in vitro,* and *in vivo* studies are needed to confirm the optimal conditions for use in humans and which will be most effective without causing side effects at adequate doses. Among the drugs used in this study, the only one that showed dubious results in molecular docking was favipiravir and it proved to be carcinogenic, however it did not show characteristics of cytotoxicity, hepatotoxicity, immunotoxicity, and mutagenicity, requiring further studies on it.

Viral infection has several stages from its binding and fusion in host cells, to its replication, several antiviral and non-antiviral drugs are being investigated in the world, and more satisfactory studies are needed to define which drug will be more effective. In future works, we intend to use nanostructures together with drugs to verify a possible improvement in the interaction between the targets and ligands used.

## Author's contributions

AFS, MOM, and GEM developed the conception and design of the study. JVS reviewed images and wrote the article under the supervision of IZS, SF, and PG. AFS, GEM, and PG reviewed the results. All authors reviewed and commented on the manuscript. All authors approved the final manuscript.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Acknowledgment

This work was carried out with the support of the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior-Brazil (CAPES)-Financing Code 001, TELEMEDICINA1690389P.

## REFERENCES

ABU-MELHA, Sraa; EDREES, Mastoura Mohamed; SAID, Musa A.; RIYADH, Sayed M.; AL-KAFF, Nadia S.; GOMHA, Sobhi M. Potential COVID-19 Drug Candidates Based on Diazinyl-Thiazol-Imine Moieties: Synthesis and Greener Pastures Biological Study. **Molecules**, *[S. l.]*, v. 27, n. 2, p. 488, 2022. DOI: 10.3390/molecules27020488. Available: https://bit.ly/3yaxm5c.

AMES, Bruce N.; DURSTON, William E.; YAMASAKI, Edith; LEE, Frank D. Carcinogens are Mutagens: A Simple Test System Combining Liver Homogenates for Activation and Bacteria for Detection. **Proceedings of the National Academy of Sciences**, *[S. l.]*, v. 70, n. 8, p. 2281-2285, 1973. DOI: 10.1073/pnas.70.8.2281. Available: https://bit.ly/3Adnpqn

AMES, Bruce N.; GOLD, Lios Swirsky. Endogenous mutagens and the causes of aging and cancer. **Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis**, *[S. l.]*, v. 250, n. 1-2, p. 3-16, 1991. DOI: 10.1016/0027-5107(91)90157-J. Available: https://bit.ly/3NtASgJ

AMES, Bruce N.; MCCANN, Joyce; YAMASAKI, Edith. Methods for detecting carcinogens and mutagens with the salmonella/mammalian-microsome mutagenicity test. **Mutation Research/ Environmental Mutagenesis and Related Subjects**, *[S. l.]*, v. 31, n. 6, p. 347-363, 1975. DOI: 10.1016/0165-1161(75)90046-1. Available: https://bit.ly/3NwMwYj

BANERJEE, Priyanka; ECKERT, Andreas O.; SCHREY, Anna K.; PREISSNER, Robert. ProTox-II: a webserver for the prediction of toxicity of chemicals. **Nucleic Acids Research**, *[S. l.]*, v. 46, n. W1, p. W257-W263, 2018. DOI: 10.1093/nar/gky318.

BOOPATHI, Subramanian; POMA, Adolfo B.; KOLANDAIVEL, Ponmalai. Novel 2019 coronavirus structure, mechanism of action, antiviral drug promises and rule out against its treatment. **Journal of Biomolecular Structure and Dynamics**, *[S. l.]*, p. 1-10, 2020. DOI: 10.1080/07391102.2020.1758788. Available: https://bit.ly/3OS5f1t

BOSAEED, Mohammad *et al.* Multicentre randomised double-blinded placebo-controlled trial of favipiravir in adults with mild COVID-19. **BMJ Open**, *[S. l.]*, v. 11, n. 4, p. e047495, 2021. DOI: 10.1136/bmjopen-2020-047495. Available: https://bit.ly/3Ai6fIq

BOSAEED, Mohammad *et al.* Efficacy of favipiravir in adults with mild COVID-19: a randomized, double-blind, multicenter, placebo-controlled trial clinical trial. **Clinical Microbiology and Infection**, *[S. l.]*, 2022. DOI: 10.1016/j.cmi.2021.12.026. Available: https://bit.ly/3ug9Oe6.

BOSCO, Michael; KISH, Troy. Hepatotoxicity With Elevated Bilirubin Secondary to Prophylactic Doses of Unfractionated Heparin: A Case Report and Review of Heparin-Induced Hepatotoxicity. **Journal of Pharmacy Technology**, *[S. l.]*, v. 35, n. 1, p. 36-40, 2019. DOI: 10.1177/8755122518803363. Available: https://bit.ly/3AdoZbW

BREINING, Peter *et al.* Camostat mesylate against SARS-CoV-2 and COVID-19-Rationale, dosing and safety. **Basic & Clinical Pharmacology & Toxicology**, *[S. l.]*, v. 128, n. 2, p. 204-212, 2021. DOI: 10.1111/bcpt.13533. Available: https://bit.ly/3a2q3EM

CARFAGNA, Mark; CANNADY, Ellen; RYAN, Thomas; HERMAN, Jay; TRUEX, Lew; NARWANI, Kanchan; SULLIVAN, John. Carcinogenicity assessment of baricitinib in Tg.rasH2 mice and Sprague-Dawley (Crl:CD) rats. **Regulatory Toxicology and Pharmacology**, *[S. l.]*, v. 92, p. 458-471, 2018. DOI: 10.1016/j.yrtph.2017.11.020. Available: https://bit.ly/3Nz4Tf9

CLAUSEN, Thomas Mandel *et al.* SARS-CoV-2 Infection Depends on Cellular Heparan Sulfate and ACE2. **Cell**, *[S. l.]*, v. 183, n. 4, p. 1043- 1057.e15, 2020. a. DOI: 10.1016/j.cell.2020.09.033. Available: https://bit.ly/3AdnZV5

CLAUSEN, Thomas Mandel *et al.* SARS-CoV-2 Infection Depends on Cellular Heparan Sulfate and ACE2. **Cell**, *[S. l.]*, v. 183, n. 4, p. 1043-1057.e15, 2020. b. DOI: 10.1016/j.cell.2020.09.033. Available: https://bit.ly/3y3O4U8.

CLINICALTRIALS. **clinicaltrials.gov**. 2022. Available: https://bit.ly/3OYplaF

DAS, Sajan; MOHTASIM KHAN, Muhammad Shah; ISLAM BAKHTIAR, Md Shawkatul; SHAHRIAR, Mohammad. In silico Molecular Docking and ADMET Study of Some Potential Antiviral Drug Candidates as Potential Inhibitors of SARS-CoV-2 Protease Mpro (6Y2F). **Dhaka University Journal of Pharmaceutical Sciences**, *[S. l.]*, v. 20, n. 2, p. 177-183, 2021. DOI: 10.3329/dujps.v20i2.57168. Available: https://bit.ly/3y75ord.

DE PASQUALE, Valeria; QUICCIONE, Miriam Shasa; TAFURI, Simona; AVALLONE, Luigi; PAVONE, Luigi Michele. Heparan Sulfate Proteoglycans in Viral Infection and Treatment: A Special Focus on SARS-CoV-2. **International Journal of Molecular Sciences**, *[S. l.]*, v. 22, n. 12, p. 6574, 2021. DOI: 10.3390/ijms22126574. Available: https://bit.ly/3nrEHsi.

FALCÃO, Melissa Barreto; PAMPLONA DE GÓES CAVALCANTI, Luciano; FILGUEIRAS FILHO, Nivaldo Menezes; ANTUNES DE BRITO, Carlos Alexandre. Case Report: Hepatotoxicity Associated with the Use of Hydroxychloroquine in a Patient with COVID-19. **The American Journal of Tropical Medicine and Hygiene**, *[S. l.]*, v. 102, n. 6, p. 1214-1216, 2020. DOI: 10.4269/ajtmh.20-0276. Available: https://bit.ly/3y9gufp.

FDA. **Veklury Authorization**. 2020a. Available: https://bit.ly/3I2PPoU

FDA. **Baricitinib Authorization**. 2020b. Available: https://bit.ly/3NyLT0g

FDA. **Coronavirus (COVID-19) Update: FDA Authorizes Drug Combination for Treatment of COVID-19**. 2020c. Available: https://bit.ly/3I7iDMY

FDA. **Approves First Treatment for COVID-19-Veklury**. 2020d. Available: https://bit.ly/3ue8pVF

FDA. **Molnupiravir Authorization**. 2021a. Available: https://bit.ly/3yvGx1v

FDA. **Paxlovid Authorization**. 2021b. Available: https://bit.ly/3nMNG7P

FDA. **Coronavirus (COVID-19) Update: FDA Authorizes First Oral Antiviral for Treatment of COVID-19**. 2021c. Available: https://bit.ly/3I7iQzK

FDA. **Emergency use authorization (EUA) for Merck's molnupiravir for Covid-19**. 2021d. Available: https://bit.ly/3NzNxiq

FDA. **Drugs Database against Covid-19**. 2022. Available: https://bit.ly/3a8GJdJ

FREDIANSYAH, Andri; TIWARI, Ruchi; SHARUN, Khan; DHAMA, Kuldeep; HARAPAN, Harapan. Antivirals for COVID-19: A critical review. **Clinical Epidemiology and Global Health**, *[S. l.]*, v. 9, p. 90-98, 2021. DOI: 10.1016/j.cegh.2020.07.006. Available: https://bit.ly/3Ag2WRT

GUPTA, Yash; MACIOROWSKI, Dawid; ZAK, Samantha E.; KULKARNI, Chandrashekhar V.; HERBERT, Andrew S.; DURVASULA, Ravi; FAREED, Jawed; DYE, John M.; KEMPAIAH, Prakasha. Heparin: A simplistic repurposing to prevent SARS-CoV-2 transmission in light of its in-vitro nanomolar efficacy. **International Journal of Biological Macromolecules**, *[S. l.]*, v. 183, p. 203-212, 2021. DOI: 10.1016/j.ijbiomac.2021.04.148. Available: https://bit.ly/3AdB4xT

HAAS, Eric J. *et al.* Impact and effectiveness of mRNA BNT162b2 vaccine against SARS-CoV-2 infections and COVID-19 cases, hospitalisations, and deaths following a nationwide vaccination campaign in Israel: an observational study using national surveillance data. **The Lancet**, *[S. l.]*, v. 397, n. 10287, p. 1819-1829, 2021. DOI: 10.1016/S0140-6736(21)00947-8. Available: https://bit.ly/3Ns50cw.

HAGE-MELIM, Lorane Izabel da Silva *et al.* Virtual screening, ADME/Tox predictions and the drug repurposing concept for future use of old drugs against the COVID-19. **Life Sciences**, *[S. l.]*, v. 256, p. 117963, 2020. DOI: 10.1016/j.lfs.2020.117963. Available: https://bit.ly/3NsK4lL

HASANABADI, Parisa Saberi;Ramin Ataee. A Review on Current Side Effects of Used Drugs During Treatment of Patients With COVID- 19. **Pharmaceutical and Biomedical Research**, *[S. l.]*, v. 7, p. 257-266, 2021. Available: https://bit.ly/3OEAxJB

HOFFMANN, Markus *et al.* Camostat mesylate inhibits SARS-CoV-2 activation by TMPRSS2-related proteases and its metabolite GBPA exerts antiviral activity. **EBioMedicine**, *[S. l.]*, v. 65, p. 103255, 2021. DOI: 10.1016/j.ebiom.2021.103255. Available: https://bit.ly/3Afcg8A

HOPKINS, JONH. **Coronavirus Resource Center**. 2022. Available: https://coronavirus.jhu.edu/map.html.

HUEY, Ruth; MORRIS, Garrett M.; OLSON, Arthur J.; GOODSELL, David S. A semiempirical free energy force field with charge-based desolvation. **Journal of Computational Chemistry**, *[S. l.]*, v. 28, n. 6, p. 1145-1152, 2007. DOI: 10.1002/jcc.20634. Available: https://bit.ly/3bu5L7s

HUSSAIN, Nafisa; YOGANATHAN, Anusha; HEWAGE, Savini; ALOM, Samiha; HARKY, Amer. The effect of antivirals on COVID-19: a systematic review. **Expert Review of Anti-infective Therapy**, *[S. l.]*, v. 19, n. 4, p. 473-486, 2021. DOI: 10.1080/14787210.2021.1823832. Available: https://bit.ly/3OUHxBI

IKETANI, Sho *et al.* Lead compounds for the development of SARS-CoV-2 3CL protease inhibitors. **Nature Communications**, *[S. l.]*, v. 12, n. 1, p. 2016, 2021. DOI: 10.1038/s41467-021-22362-2. Available: https://go.nature.com/3OyczQk

JAYK BERNAL, Angélica *et al.* Molnupiravir for Oral Treatment of Covid-19 in Nonhospitalized Patients. **New England Journal of Medicine**, *[S. l.]*, v. 386, n. 6, p. 509-520, 2022. DOI: 10.1056/NEJMoa2116044. Available: https://bit.ly/3nqsZye

JORGENSEN, Sarah C. J.; TSE, Christopher L. Y.; BURRY, Lisa; DRESSER, Linda D. Baricitinib: A Review of Pharmacology, Safety, and Emerging Clinical Experience in COVID-19. **Pharmacotherapy: The Journal of Human Pharmacology and Drug Therapy**, *[S. l.]*, v. 40, n. 8, p. 843-856, 2020. DOI: 10.1002/phar.2438. Available: https://bit.ly/3ODebbq.

LAGUNIN, Alexey; RUDIK, Anastasia; DRUZHILOVSKY, Dmitry; FILIMONOV, Dmitry; POROIKOV, Vladimir. ROSC-Pred: web-service for rodent organ-specific carcinogenicity prediction. **Bioinformatics**, *[S. l.]*, v. 34, n. 4, p. 710-712, 2018. DOI: 10.1093/bioinformatics/btx678. Available: https://bit.ly/3OybaZZ

LAGUNIN, Alexey; ZAKHAROV, Alexey; FILIMONOV, Dmitry; POROIKOV, Vladimir. QSAR Modelling of Rat Acute Toxicity on the Basis of PASS Prediction. **Molecular Informatics**, *[S. l.]*, v. 30, n. 2-3, p. 241-250, 2011. DOI: 10.1002/minf.201000151. Available: https://bit.ly/3OxBEdT

LIMA, Luana Nepomuceno Gondim Costa; DE SOUSA, Maisa Silva; LIMA, Karla Valéria Batista. As descobertas genômicas do SARS-CoV-2 e suas implicações na pandemia de COVID-19. **Journal of Health & Biological Sciences**, *[S. l.]*, v. 8, n. 1, p. 1, 2020. DOI: 10.12662/2317-3076jhbs. v8i1.3232.p1-9.2020. Available: https://bit.ly/3a2zCDE

MANSBACH, Rachael A.; CHAKRABORTY, Srirupa; NGUYEN, Kien; MONTEFIORI, David C.; KORBER, Bette; GNANAKARAN, S. The SARS-CoV-2 Spike variant D614G favors an open conformational state. **Science Advances**, *[S. l.]*, v. 7, n. 16, 2021. DOI: 10.1126/sciadv.abf3671. Available: https://bit.ly/3y6MnoX

MARTINEZ, Miguel Angel. Compounds with Therapeutic Potential against Novel Respiratory 2019 Coronavirus. **Antimicrobial Agents and Chemotherapy**, *[S. l.]*, v. 64, n. 5, 2020. DOI: 10.1128/ AAC.00399-20. Available: https://bit.ly/3QZHV3R

MARTINS, Mirkos Ortiz; SILVA, Ivana Zanella Da; FAGAN, Solange Binotto; SANTOS, André Flores Dos. Docking fundamentals for simulation in nanoscience. **Disciplinarum Scientia - Ciências Naturais e Tecnológicas**, *[S. l.]*, v. 22, n. 3, p. 67-76, 2021. DOI: 10.37779/nt.v22i3.4106. Available: https://bit.ly/3a7X7v9

NCBI. **National Center for Biotechnology Information**. 2022a. Available: https://bit.ly/3nu0aAX

NCBI. **National Center for Biotechnology Information**. 2022b. Available: https://bit.ly/3nzd8gA

NI, Dongchun. Structural analysis of the Spike of the Omicron SARS-COV-2 variant by cryo-EM and implications for immune evasion. **Biorxiv**, *[S. l.]*, p. 25, 2021. DOI: https://doi.org/10.1101/ 2021.12.27.474250. Available: https://bit.ly/3y4mnu9

NOJOMI, Marzieh; YASSIN, Zeynab; KEYVANI, Hossein; MAKIANI, Mahin Jamshidi; ROHAM, Maryam; LAALI, Azadeh; DEHGHAN, Nasir; NAVAEI, Mehrnaz; RANJBAR, Mitra. Effect of Arbidol (Umifenovir) on COVID-19: a randomized controlled trial. **BMC Infectious Diseases**, *[S. l.]*, v. 20, n. 1, p. 954, 2020. DOI: 10.1186/s12879-020-05698-w. Available: https://bit.ly/3R2nSBE

PIRES, Douglas E. V.; BLUNDELL, Tom L.; ASCHER, David B. pkCSM: Predicting Small-Molecule Pharmacokinetic and Toxicity Properties Using Graph-Based Signatures. **Journal of Medicinal Chemistry**, *[S. l.]*, v. 58, n. 9, p. 4066-4072, 2015. DOI: 10.1021/acs.jmedchem.5b00104. Available: https://bit.ly/3yvL6cg

POROIKOV, V. V. *et al.* Computer-aided prediction of biological activity spectra for organic compounds: the possibilities and limitations. **Russian Chemical Bulletin**, *[S. l.]*, v. 68, n. 12, p. 2143-2154, 2019. DOI: 10.1007/s11172-019-2683-0. Available: https://bit.ly/3btgzCP

RAIES, Arwa B.; BAJIC, Vladimir B. In silico toxicology: computational methods for the prediction of chemical toxicity. **Wiley Interdisciplinary Reviews: Computational Molecular Science**, *[S. l.]*, v. 6, n. 2, p. 147-172, 2016. DOI: 10.1002/wcms.1240. Available: https://bit.ly/3yuI4VO

RASCHI, Emanuel; CARACENI, Paolo; POLUZZI, Elisabetta; DE PONTI, Fabrizio. Baricitinib, JAK inhibitors and liver injury: a cause for concern in COVID-19? **Expert Opinion on Drug Safety**, *[S. l.]*, v. 19, n. 10, p. 1367-1369, 2020. DOI: 10.1080/14740338.2020.1812191. Available: https://bit.ly/3a7bwry

RAVINDRANATH, Pradeep Anand; FORLI, Stefano; GOODSELL, David S.; OLSON, Arthur J.; SANNER, Michel F. AutoDockFR: Advances in Protein-Ligand Docking with Explicitly Specified Binding Site Flexibility. **PLOS Computational Biology**, *[S. l.]*, v. 11, n. 12, p. e1004586, 2015. DOI: 10.1371/journal.pcbi.1004586. Available: https://bit.ly/3OOgfwS

RAVINDRANATH, Pradeep Anand; SANNER, Michel F. AutoSite: an automated approach for pseudo-ligands prediction-from ligand-binding sites identification to predicting key ligand atoms. **Bioinformatics**, *[S. l.]*, v. 32, n. 20, p. 3142-3149, 2016. DOI: 10.1093/bioinformatics/btw367. Available: https://bit.ly/3AcwWOB

SANTOS, André Flores Dos; FRAGA, Alessandra Soares Ayres; GOMES, Patrícia; FAGAN, Solange Binotto. Vaccines against COVID-19 using nanotechnology: a literature review. **Disciplinarum Scientia - Ciências Naturais e Tecnológicas**, *[S. l.]*, v. 22, n. 3, p. 99-112, 2021. DOI: 10.37779/nt.v22i3.4097. Available: https://bit.ly/3yz8Ts4.

SAVARINO, Adrea; BOELAERT, John R.; CASSONE, Antonio; MAJORI, Giancario; CAUDA, Roberto. Effects of chloroquine on viral infections: an old drug against today's diseases. **The Lancet Infectious Diseases**, *[S. l.]*, v. 3, n. 11, p. 722-727, 2003. DOI: 10.1016/S1473-3099(03)00806-5. Available: https://bit.ly/3ywPBTT

SHUSTER, Anton; PECHALRIEU, Dany; JACKSON, Cody B.; ABEGG, Daniel; CHOE, Hyeryun; ADIBEKIAN, Alexander. Clinical Antiviral Drug Arbidol Inhibits Infection by SARS-CoV-2 and Variants through Direct Binding to the Spike Protein. **ACS Chemical Biology**, *[S. l.]*, v. 16, n. 12, p. 2845-2851, 2021. DOI: 10.1021/acschembio.1c00756. Available: https://bit.ly/3NsHY5e

SINGH, Awadhesh Kumar; SINGH, Akriti; SINGH, Ritu; MISRA, Anoop. Molnupiravir in COVID-19: A systematic review of literature. **Diabetes & Metabolic Syndrome: Clinical Research & Reviews**, *[S. l.]*, v. 15, n. 6, p. 102329, 2021. DOI: 10.1016/j.dsx.2021.102329. Available: https://bit.ly/3bFxuSV

STEBBING, Justin; PHELAN, Anne; GRIFFIN, Ivan; TUCKER, Catherine; OECHSLE, Olly; SMITH, Dan; RICHARDSON, Peter. COVID-19: combining antiviral and anti-inflammatory treatments. **The Lancet Infectious Diseases**, *[S. l.]*, v. 20, n. 4, p. 400-402, 2020. DOI: 10.1016/S1473-3099(20)30132-8. Available: https://bit.ly/3uf1qvw

TAVASSOLY, Omid; SAFAVI, Farinaz; TAVASSOLY, Iman. Heparin-binding Peptides as Novel Therapies to Stop SARS-CoV-2 Cellular Entry and Infection. **Molecular Pharmacology**, *[S. l.]*, v. 98, n. 5, p. 612-619, 2020. DOI: 10.1124/molpharm.120.000098. Available: https://bit.ly/3I4xxUk

TROTT, Oleg; OLSON, Arthur J. AutoDock Vina: Improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. **Journal of Computational Chemistry**, *[S. l.]*, p. NA-NA, 2009. DOI: 10.1002/jcc.21334. Available: https://bit.ly/3ytKUub

UNECE. **Globally Harmonized System of Classification and Labelling of Chemicals (GHS)**. 2019.

VAN DIJK, Aalt D. J.; BONVIN, Alexandre M. J. J. Solvated docking: introducing water into the modelling of biomolecular complexes. **Bioinformatics**, *[S. l.]*, v. 22, n. 19, p. 2340-2347, 2006. DOI: 10.1093/bioinformatics/btl395.

VANGEEL, Laura *et al.* Remdesivir, Molnupiravir and Nirmatrelvir remain active against SARS-CoV-2 Omicron and other variants of concern. **Antiviral Research**, *[S. l.]*, v. 198, p. 105252, 2022. DOI: 10.1016/j.antiviral.2022.105252. Available: https://bit.ly/3uh2L4Z

WANG, Dongyuan; LI, Zigang; LIU, Yihui. An overview of the safety, clinical application and antiviral research of the COVID-19 therapeutics. **Journal of Infection and Public Health**, *[S. l.]*, v. 13, n. 10, p. 1405-1414, 2020. DOI: 10.1016/j.jiph.2020.07.004. Available: https://bit.ly/3NC7tRA

YUE, Jingwen *et al.* Heparan Sulfate Facilitates Spike Protein-Mediated SARS-CoV-2 Host Cell Invasion and Contributes to Increased Infection of SARS-CoV-2 G614 Mutant and in Lung Cancer. **Frontiers in Molecular Biosciences**, *[S. l.]*, v. 8, 2021. DOI: 10.3389/fmolb.2021.649575. Available: https://bit.ly/3NsL617

ZHANG, Yuqi; FORLI, Stefano; OMELCHENKO, Anna; SANNER, Michel F. AutoGridFR: / Improvements on AutoDock Affinity Maps and Associated Software Tools. **Journal of Computational Chemistry**, *[S. l.]*, v. 40, n. 32, p. 2882-2886, 2019. DOI: 10.1002/jcc.26054. Available: https://bit.ly/3a2VFKu.