ACCSCIENCE PUBLISHING

INNOSC Theranostics and Pharmacological Sciences

ORIGINAL RESEARCH ARTICLE

Evaluating the SARS-CoV-2 spike glycoprotein as a molecular target for therapeutic development

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Abstract

The SARS-CoV-2 virus gains entry into host cells by binding its spike glycoprotein (S-glycoprotein) to the angiotensin 2 receptor. This viral protein contains several conserved regions, such as the receptor binding domain region, making it an ideal target for treating COVID-19. Notably, the majority of existing vaccines elicit antigenic reaction by targeting this protein epitope. This study evaluated the binding affinities of 44 different drugs against the SARS-CoV-2 S-glycoprotein, considering their toxicity profiles and previous clinical studies at different testing stages. Our results revealed that maraviroc and estradiol benzoate exhibited high affinities (−7.7 and −7.6 kcal mol^{-1}, respectively), while other ligands, such as indinavir and ritonavir, showed affinity at lower levels. Among the drugs with high affinity, toxicity levels ranged from harmful if swallowed (300 mg/kg < LD50 < 2000 mg/kg) to non-toxic (LD50 > 5000 mg/kg), with only three having undergone clinical testing, yielding promising or controversial results. Furthermore, emtricitabine and docosanol, previously explored as COVID-19 treatments, exhibited the lowest affinities (−4.7 and −3.9 kcal mol^{-1}, respectively), with associated harmful effects if swallowed. These results provide essential information about drug interaction against the SARS-CoV-2 S-glycoprotein and potential treatment pathways for COVID-19.

Keywords: SARS-CoV-2; Coronavirus spike glycoprotein; Molecular docking simulation; Pharmacology; Cluster MCMC

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Citation: Adame-Velasco BH, Octavio-Aguilar P, Mendoza-Huizar LH, Aguilar-Castro LM. Evaluating the SARS-CoV-2 spike glycoprotein as a molecular target for therapeutic development. *INNOSC Theranostics and Pharmacological Sciences*. 2024;7(2):1651. doi: 10.36922/itps.1651

Received: August 22, 2023

Accepted: December 4, 2023

Published Online: March 26, 2024

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1. Introduction

On March 11, 2020, the World Health Organization declared COVID-19, caused by the SARS-CoV-2 virus, a pandemic due to its rapid spread. This virus belongs to the *Coronaviridae* family, *Coronavirinae* subfamily, and *Betacoronavirus* genus. Coronaviruses derived their name from the multiple spikes (S-glycoproteins) surrounding their spherical lipoprotein capsule, giving them a crown-like appearance, which is recognized by receptor proteins on host cells susceptible to infection.[1,2] The S-glycoprotein is responsible for the attachment of the virus to host cells through angiotensin-converting enzyme 2 (ACE2), followed by binding to the transmembrane serine protease 2 (TMPRSS2), which allows cell membrane fusion and viral entry.[3,4] On

entering the host cell, the virus releases its RNA genome, which undergoes transcription through protein cleavage and assembly of the replicase-transcriptase complex. Viral RNA replication occurs, and structural viral proteins are synthesized, assembled, and packaged within host cell membranes before virions release.[5,6]

Understanding the variability of the S-glycoprotein is necessary for developing new vaccines, guaranteeing specificity, broad reactivity, and persistent immunity through cross-reactive responses.[7] Any change in the receptor binding domain (RBD) region of the spike protein can impact its binding affinity, necessitating close monitoring of mutant strains. Molecular docking studies of the spike virus and the ACE2 receptor junction are often utilized to assess potential changes in binding affinity.[8] However, a comprehensive understanding of S-glycoprotein binding does not equate to knowledge of its pharmacological interactions.

The pandemic underscores the critical need for effective medicines to prevent and treat viral infections, driving the urgency for the development of new treatments. However, given that the drug development process is both lengthy and costly, *in silico* evaluation through molecular simulation docking is employed to expedite this process.

At present, therapeutic options for COVID-19 are limited, primarily comprising antiviral medications such as molnupiravir, nirmatrelvir + ritonavir (Paxlovid), and remdesivir. In addition, monoclonal antibody therapies, exemplified by bamlanivimab/etesevimab and casirivimab/imdevimab, along with anti-inflammatory medications such as dexamethasone and immunomodulatory agents such as baricitinib and tocilizumab, constitute available treatment options. These treatments have been made available following clearance from the Food and Drug Administration (FDA). The majority have undergone testing in cell cultures, with a few demonstrating promising results in clinical trials.[9-14] In this context, a drug repositioning study has been carried out using *in silico* tools targeting the delta spike protein/ACE2 interface, employing a virtually screened library of 4388 approved drugs. The results suggest that several antihistaminic drugs form stable complexes against the spike RBD (with binding affinities between −32.92 and −53.78 kcal mol^{-1}), while practically all currently used antiviral drugs are not included in Spike-Antihistamine interaction.[13]

The primary objective of this study is to propose and evaluate various antiviral drugs with potential therapeutic effects, selecting those most promising for treating COVID-19 through serial simulations of molecular docking and a Markovian approach.

2. Methods

An exhaustive search was conducted across scientific databases and publications such as PubMed, Google Scholar, FEBS Press, Springer Link, and Nature to identify literature pertaining to chemical compounds or drugs capable of interacting with the SARS-CoV-2 S-glycoprotein. The A chain of the S-glycoprotein (6VSB, EM Map EMD-21375)[15] was chosen using the UCSF Chimera Software v.1.16 developed by the University of California.[16] Using the Swiss-Model server provided by Biozentrum, University of Basel,[17] homology modeling of the protein structures was carried out to predict the missing structures in the protein and complete it. Subsequently, the Protox-II server provided by the Charité Universita Medizin Berlin[18] filtered out innocuous compounds by applying a score greater than four on the toxicity scale. Following this, the 3D molecular representation of the glycoprotein with 6VSB identification was downloaded in PDB format from the Protein Data Bank provided by the National Centre for Biotechnology Information,[19] and it was subjected to cleaning and preparation for further analysis.

To prepare and simulate ligand docking, we used the software PyRx v.0.8 developed by Sargis Dallakyan.[20] This software enabled us to obtain the binding free energy (ΔG) of the compounds and identify the most promising drugs. This modeling process involved 10 resampling steps to obtain the statistical parameters necessary for grouping medications with the highest affinity. Subsequently, a cluster analysis based on Euclidean distances was conducted using Ward's method. This grouping was achieved through 1000 bootstrap–MCMC, facilitated by the Past v.4.2 program developed by Hammer.[21]

3. Results

Forty-four drugs with therapeutic potential were identified within the consulted data sets. The binding affinity of these drugs ranges between −3.76 and −7.22 kcal mol^{-1}, as measured by their critical energy cost values (ΔG). The cytotoxicity predicted by the Protox-II assay ranges from 4 to 6 toxicity points (Table 1).

Overall, the clustering analysis revealed two groups of drugs, with a high-affinity subgroup consisting of estradiol benzoate, itraconazole, maraviroc, indinavir, vicriviroc, dasabuvir, dolutegravir, and telaprevir (Figure 1). Within this high-affinity subgroup, estradiol benzoate and indinavir exhibited the lowest toxicity values. Hence, based on this study, they may be considered as the drugs with the most significant therapeutic potential. On the other hand, docosanol and valaciclovir comprised the lowest affinity subgroup. It is worth noting that while some low-affinity antiviral drugs were identified, they have proven effective

Table 1. Binding affinity of drugs tested against the spike protein of SARS-COV-2

Molecule	Binding affinity (kcal mol^{-1})	Predicted toxicity level	Clinical trials
Estradiol benzoate*	−7.22±0.58	5	Tested, controversial results[23–26]
Itraconazole*	−7.14±0.42	4	Tested, potential effect[25,28]
Maraviroc*	−7.07±0.41	4	Tested, controversial results[31,32]
Indinavir*	−7.04±0.32	5	Untested
Vicriviroc*	−7.03±0.4	4	Untested
Dasabuvir*	−6.87±0.47	4	Untested
Dolutegravir*	−6.84±0.45	4	Untested
Telaprevir*	−6.67±0.43	4	Untested
Nelfinavir	−6.55±0.31	4	Untested
Remdesivir	−6.47±0.33	4	Tested, potential effect[32,38]
Ritonavir	−6.44±034	4	Tested, controversial results[32,38,39]
Delavirdine	−6.38±0.38	4	Untested
Lopinavir	−6.37±0.49	5	Tested, controversial results[38,39]
Ceftazidime	−6.22±0.29	6	Tested, partial results[40]
Sofosbuvir	−6.08±0.34	6	Tested, potential effect[41]
Nevirapine	−8.89±0.32	4	Untested
Rilpivirine	−5.89±0.56	4	Tested, potential effect[42]
Tenofovir alafenamide	−5.67±0.29	6	Tested, potential effect[43,44]
Efavirenz	−5.64±036	4	Untested
Nitazoxanide	−5.46±0.36	4	Tested, potential effect[45]
Viramidine	−5.35±0.3	5	Tested, adverse effects[38]
Zanamivir	−5.33±0.37	5	Untested
Valganciclovir	−5.31±0.4	5	Untested
Tenofovir disoproxil	−5.27±0.49	6	Tested, unsupported[44]
Peramivir	−5.27±0.3	4	Tested, unsupported[46]
Ribavirin	−5.25±0.34	5	Tested, adverse effects[38,46]
Zidovudine	−5.19±0.31	6	Tested, unsupported[47]
Methisazone	−5.17±0.35	5	Untested
Telbivudine	−5.11±0.37	6	Untested
Famciclovir favipiravir	−5.09±0.49	4	Tested, potential effect[48]
Edoxudine	−5.08±0.36	6	Untested
Stavudine	−5.04±0.43	4	Untested
Didanosine	−4.92±0.45	4	Tested, potential effect[49]
Cidofovir	−4.84±0.27	4	Tested, potential effect[47]
Arbidol	−4.81±0.48	4	Tested, potential effect[38]
Zalcitabine	−4.8±0.38	5	Untested
Emtricitabine	−4.65±0.38	4	Tested, potential effect[42]
Lamivudine	−4.65±0.37	4	Tested, potential effect[50]
Penciclovir	−4.62±0.42	4	Untested
Aciclovir	−4.51±0.43	5	Tested, potential effect[51]
Ganciclovir	−4.46±0.47	5	Tested, potential effect[52]
Moroxydine	−4.41±0.36	4	Untested
Valaciclovir	−4.33±0.44	5	Tested, potential effect[47]
Docosanol	−3.76±0.42	4	Untested

Notes: *Drugs with high affinity, according to Ward's grouping. Predicted toxicity level: 4: Harmful if swallowed (300 mg/kg<LD50<2000 mg/kg); 5: May be harmful if swallowed (2000 mg/kg<LD50<5000 mg/kg); 6: Non-toxic (LD50>5000 mg/kg). Potential effects in co-infection treatment, pilot, phase I, II, III, or IV clinical trials. Controversial results are based on conclusions that contradict those of some publications.

against COVID-19 in auxiliary roles rather than as primary treatments. These drugs are often used in conjunction with antihistamines or in the treatment of comorbidities such as human immunodeficiency virus (HIV) and Epstein-Barr virus infections (Table 1).

4. Discussion

Estradiol benzoate, a female sex hormone of the estrogen group, exhibited the highest affinity among the drugs studied. Commonly prescribed for conditions such as menopause, female hypogonadism, vaginal atrophy, and osteoporosis in postmenopausal women, its therapeutic potential has garnered interest in the context of COVID-19. Recent studies have suggested a lower risk of severe COVID-19 complications in women, hypothesized to be linked to higher estradiol levels in women compared to men.[22] Estradiol benzoate inhibits the interaction between the spike protein and ACE2, thereby preventing intercellular fusion mediated by the SARS-CoV-2 protein.[23,24] However,

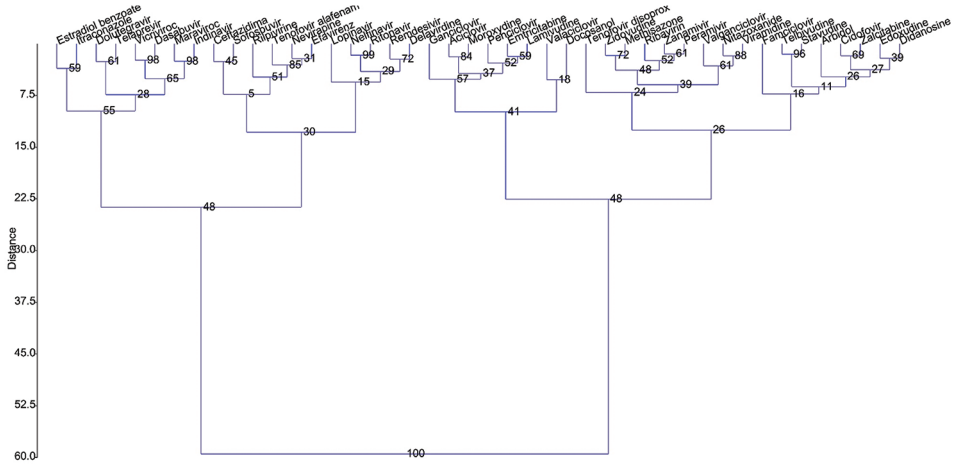


Figure 1. Clustering analysis based on Euclidean distances by Ward's method of resampling (1000 steps) binding free energy of the tested drugs.

in vitro studies have demonstrated an IC50 range between 1.02 and 1.75 μM for this drug, which is much higher than the physiological concentration of estrogen. Despite the disparity, these doses have proven effective against viral infection.[25,26] These studies propose that the drug binds to the spike protein or ACE2 in a non-competitive manner outside the binding region, potentially inducing conformational changes in the protein and disrupting the spike–ACE2 interaction.[25] Therefore, it is suggested that future *in vitro* tests delve into these mechanisms in greater detail.

Itraconazole, a lipophilic compound drug with high bioavailability, is approved for the treatment of fungal infections. It inhibits viral entry by targeting the six-helix furoin core of the SARS-CoV-2 S-glycoprotein, as evidenced by cell culture tests.[27] Furthermore, investigations have explored its interaction with the heptad repeat 1 (HR1) region of the spike protein.[25] Our results place itraconazole as having the second highest affinity among the group of drugs with significant treatment potential. Despite its demonstrated *in vitro* activity, its clinical utility in hospitalized COVID-19 patients remains limited, having only been used in a pilot test. Consequently, further clinical trials are warranted.[25,28]

Maraviroc and vicriviroc[29] are antiretroviral drugs primarily used to treat CCR5-tropic HIV-1 infection. The pharmacological blockade of CCR5 has demonstrated promise in improving symptoms in severe COVID-19 patients and alleviating post-COVID-19 symptoms.[30] This effect is attributed to their mechanism of action, which involves blocking the entry of the virus into cells by binding to the CCR5 chemokine receptor, constituting a consistent and statistically significant inhibitory effect against SARS-CoV-2. This encouraging attribute makes these drugs promising candidates for COVID-19 treatment, as they not only prevent immune cell exhaustion but also redirect immunoreactive cells away from sites inflamed by COVID-19. Consequently, this effect reduces viral load and minimizes damage to organs such as the lungs.[31] However, it is crucial to note that these findings are subject to controversy, as other studies have failed to demonstrate any anti-SARS-CoV-2 activity of maraviroc.[32] Therefore, it is of the utmost importance to carry out additional studies and clinical tests to validate these findings.

Numerous antiviral drugs are currently under investigation for their efficacy against COVID-19, but only a selected few have demonstrated encouraging results in clinical and preclinical studies, even when there has been a significant reduction of viral load in cell cultures. Notable examples include remdesivir and ritonavir.[33] Our analysis grouped both of them with nelfinavir and delavirdine in a second cluster, exhibiting a binding affinity lower than -6.38 kcal mol^{-1}. However, clinical studies on remdesivir and ritonavir are lacking. These two drugs have been suggested solely as adjuvants due to their antiretroviral and protease inhibitory action, which could improve the efficacy of treatments involving molnupiravir, nirmatrelvir, or ensitrelvir[34-36] without directly interacting with the spike protein. Therefore, *in silico* studies do not substitute clinical treatments but rather offer potential treatment routes and drug interactions that could improve patient outcomes.

The effectiveness of a combination of an antiviral (−7 kcal mol^{-1}) and an antihistamine (−53.78 kcal mol^{-1})[14] in binding to the spike–ACE2 complex may potentially mislead our understanding of optimal treatment strategies, particularly when considering that antihistamines alone have shown promising outcomes. Antihistamines belonging to the histamine-1 receptor binding class are very effective

for treating COVID-19. Studies have demonstrated that the use of diphenhydramine, hydroxyzine, and azelastine correlates with a reduced incidence of SARS-CoV-2 positivity in individuals older than 61. While these drugs exhibit direct antiviral activity against SARS-CoV-2 *in vitro*, the specific mechanisms underlying their antiviral effects remain unclear. Hydroxyzine and potentially azelastine are known to bind to the ACE2 and sigma-1 receptors as unintended targets, which may explain their exceptional efficacy. Therefore, it is recommended that antivirals serve as adjuvants in combined treatments alongside these antihistamines.[37]

5. Conclusion

Within the realm of COVID-19 treatment, numerous other aspects, such as viral replication, enzymatic processes within infected cells, and molecular aspects of cellular response, serve as potential targets for therapeutic intervention. Molecular docking models, such as amixin, an antiviral approved by the FDA for treating MERS-CoV, offer promising avenues. While amixin has demonstrated partial success in treating COVID-19, its action does not rely on binding to the S-glycoprotein but on targeting the enzymatic complex involved in viral replication.[38]

Acknowledgments

Brandon H. Adame-Velasco acknowledges Wendolyne López Orozco for her useful comments and discussions during the preparation of this research.

Funding

None.

Conflict of interest

The authors declare no conflicts of interest.

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Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data

Data used in this work are available from the corresponding author on reasonable request.

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