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ORIGINAL ARTICLE

In Silico Evaluation of the Effectivity of Approved Antivirals against the RNA-dependent RNA polymerase (RdRp) of the Sudanese SARS-CoV-2 Samples

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ABSTRACT

BACKGROUND:
In December 2019, the positive-sense single-stranded RNA virus known as Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) was discovered for the first time in Wuhan. The RNA-dependent RNA polymerase (RdRp) protein is essential to this virus's ability to replicate. RdRp targeting may be useful in combating SARS-CoV-2.

OBJECTIVE:
This study aims to evaluate the efficacy of approved antiviral drugs against the RdRp of Sudanese SARS-CoV-2 samples through the use of molecular docking analysis.

METHODS:
Ten FDA-approved antiviral medications were tested for binding affinity using the anticipated 3D structure of the RdRp protein from Sudanese SARS-CoV-2 samples. To find potential inhibitors, molecular docking was used.

RESULTS:
The RdRP protein of Sudanese samples exhibited binding affinity for ten drugs, with the highest interactions being shown by Galidesivir, Penciclovir, and Baloxavir.

CONCLUSION:
The findings show that Galidesivir, Penciclovir, and Baloxavir could be promising candidates for anti-SARS-CoV-2 therapy, with potential implications for clinical trials in Sudan.

KEYWORDS:
COVID-19, Docking, RdRp, SARS-CoV-2.

INTRODUCTION

Severe acute respiratory syndrome coronavirus (SARS-CoV) is a positive-sense single-stranded RNA virus, from the Coronaviridae family, which is named for the crown-like spikes on its surface.[1]
According to the nucleotide sequences of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), it is a member of the genus Betacoronavirus (such as SARS and MERS human coronaviruses (HCoVs) commonly known to infect mammals such as bats and humans.[2-7]

The new human coronavirus SARS-CoV-2 identified in patients with acute respiratory syndrome in Wuhan, China, in December 2019.[8,9] The Director-General of the World Health Organization (WHO), on January 30th, 2020, declared that the outbreak of the new coronavirus (2019-nCoV) constitutes a Public Health Emergency of International Concern (PHEIC).

Therefore, scientists are trying to use preexisting antiviral drugs to stop or control the virus upsurge; however, these drugs have thus far had inconspicuous effects on SARS-CoV-2.[10,11]
The effectiveness of analogous antiviral drugs may be compromised due to changes induced by single nucleotide polymorphisms (SNPs), thereby performing amino acid shifts, which ultimately modify functional viral protein(s). Escape from being targeted by antiviral drugs could be due to viral proteins actively acquiring mutations due to SNPs.[12,13,14]

Two groups of proteins characterize HCoVs: structural proteins, like spikes, marking all coronaviruses, and non-structural proteins (nsps); some of these nsps encode proteins with essential functions, such as PLpro (nsp3), 3CLpro (nsp5) and RdRP (nsp12), which plays an important role in viral replication, whereas helicase (nsp13) has been recognized to unwind and decompress duplex oligonucleotides in an NTP-dependent manner.[15-18]

RdRp is an important protein for replication and transcription of the viral genome in most RNA viruses. The RdRp protein ranges from 240 to 450 kD and consists of a catalytic core with a clear resemblance to the human right hand with differentiated palm, fingers, and thumb domains.[19,20]

RdRp is an attractive site to study and understand their biology and structure biology in terms of nucleic acid synthesis and development of antiviral drugs because it is considered to be one of the most conserved proteins within RNA viruses.[21-30]

Several RdRp inhibitors—such as Sofosbuvir, Ribavirin, Galidesivir, Remdesivir, etc.—are in clinical trials for different viruses (e.g. Hepatitis C, Zika virus, Dengue and SARS-CoV-2) based on anti-RdRp activity.[31-35]
In this study, we used molecular docking to evaluate the potential of different approved antiviral drugs to target and inhibit the viral replication protein RdRp from Sudanese patient samples.
Among the ten antiviral agents examined (Remdesivir, Galidesivir, favipiravir, Ribavirin, Baloxavir, Penciclovir, Sofosbuvir, Lopinavir, Pimodivir and Penciclovir), the best-suited inhibitors were screened through in silico analysis that can further be used for preclinical trials to halt viral replication after former testing. Our results are promising and may be considered for both in vitro and in vivo clinical trials for inhibition of SARS-CoV-2 in Sudan.

METHODS

Sequence retrieval, alignment and homology

The RNA-dependent RNA polymerase of the SARS-CoV-2 sequence was retrieved from NCBI (National Center for Biotechnology Information) under Accession # YP_009725307.1. The sequence of this practical model, which was obtained from Sudanese patients, was aligned with the RdRp of the SARS-CoV-2 sequence. The sequences were submitted to and aligned using the CLUSTAL OMEGA alignment tool.

Protein preparation

The structure of the RdRp protein was prepared by submitting our sequence to the Swiss model after converting the RNA sequence to a nucleotide sequence using the ExPASy translate tool. Protein structure was predicted by considering the saved RdRp structure as a reference template. Protein was prepared for molecular docking by using Autodock software, which was used to add the hydrogen atoms, remove the water, add the charge (Kollman charge), and save it as pdbqt.

Ligand selection and preparation

For docking, the following ten antiviral drugs were screened (Remdesivir, Galidesivir, Favipiravir, Ribavirin, Baloxavir, Penciclovir, Sofosbuvir, Lopinavir, Pimodivir, and Ritonavir). Structures of drugs were obtained from the PubChem database and Zinc15 (database catalogue of FDA-approved drugs that are imported from the compounds within the U.S.), as shown in (Table 1) below. Different parameters for drug evaluation were considered, such as LogP < 5, H-bond donor(s) < 10, and H-bonding acceptor(s) < 5. All ligands were prepared for docking by PyMol software.

Table 1. Drugs selected from Database:

Drug Name	Formula	Molecular Weight	Number of Atoms
Baloxavir	C27H23F2N3O7S	571.5492264	63
Favipiravir	C5H4FN3O2	157.1025632	15
Galidesivir	C11H15N5O3	265.2685	34
Lopinavir	C37H48N4O5	628.80082	94
Penciclovir	C10H15N5O3	253.2578	33
Pimodivir	C40H44ClF4N10O5	891.7409728	103
Remdesivir	C27H35N6O8P	602.575961	77
Ribavirin	C8H12N4O5	244.20468	29
Ritonavir	C37H48N6O5S2	720.94422	98
Sofosbuvir	C22H29FN3O9P	529.452542	65

Molecular docking and visualization

Molecular docking predicts one molecule's best orientation to a second when bound to each other to form a stable complex that depends on the key and lock theory. Preferred orientation knowledge predicts the strength of the association or binding affinity between two molecules using scoring functions.

Docking was performed using PyRx software. All the retrieved compounds were docked using selected catalytic sites of the three-dimensional structure of RdRp protein. Ligand was converted to pdpqt, and energy minimization was carried out with the PyRx software. After molecular docking, the results of docking were visualized by using BIOVIA Discovery Studio Visualizer and PyMol software.

RESULTS

Sequence Alignment and Homology

The sequence alignment of the RNA-dependent RNA polymerase (RdRp) from Sudanese SARS-CoV-2 samples revealed a high degree of similarity with the reference sequence obtained from the NCBI database (Accession # YP_009725307.1). Specifically, the alignment showed 98.2% sequence similarity, with 1.8% divergence, primarily due to 17 amino acid mismatches out of 932. This indicates a strong conservation of the RdRp protein across different SARS-CoV-2 strains, making it a viable target for antiviral drug screening (Figure 1).

Figure 1. Sequence alignment of sequence of Wuhan SARS Cov2 (the upper sequence) with sequence of SARS Cov2 from Sudanese patients.

Modeling and structure prediction

The 3D structure of the SARS-CoV-2 protein from Sudanese SARS-CoV-2 samples was successfully predicted using SWISS-Model, employing the SARS-CoV-2 RdRp as a reference template. Three models were generated, with the selected model showing 99.03% similarity with the reference structure. A slight divergence of 0.97% was observed, primarily in non-critical regions, suggesting a minimal impact on the overall structure and function of the protein. (Figure 2).

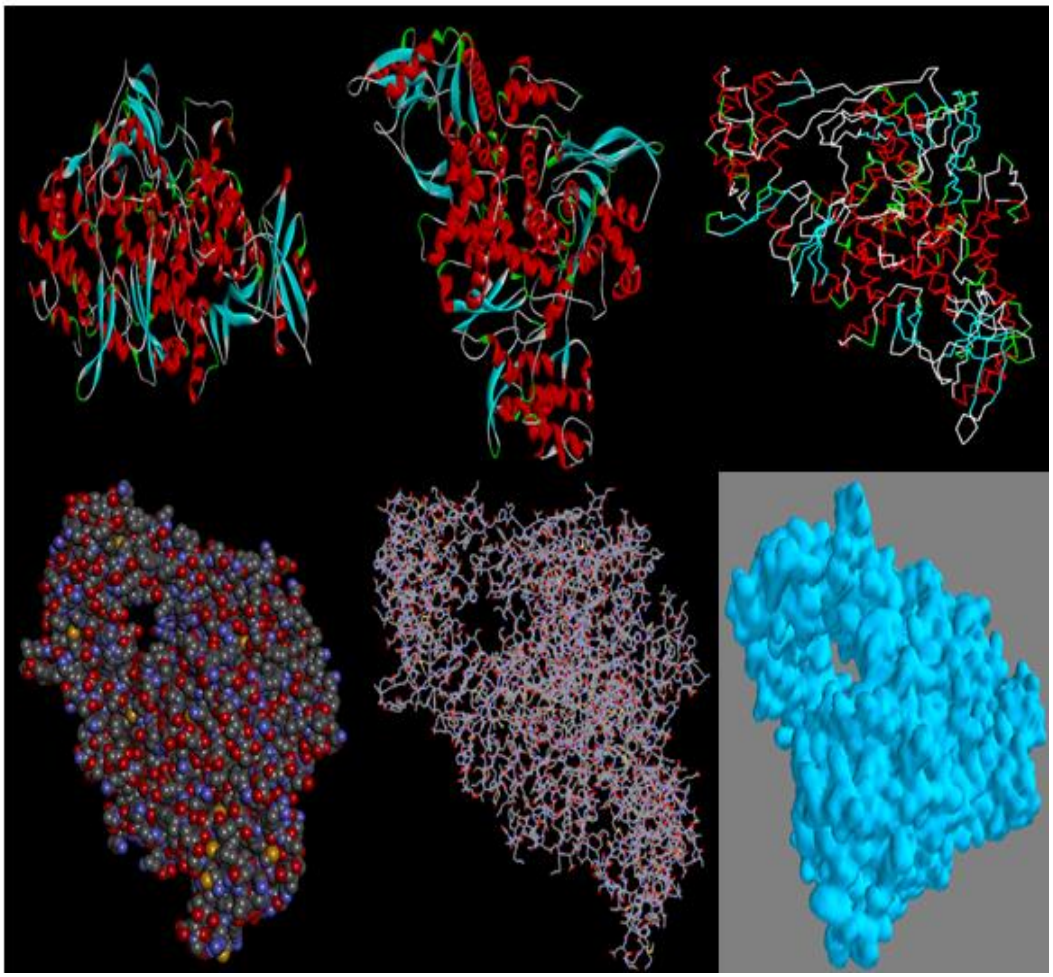

Figure 2. Structure of RdRp predicted from Sudanese samples sequence from several positions and in different forms.

Table 2. Binding Affinity of anti-RdRP drugs

No.	Drug name	Binding Affinity (kcal/mol)
1	Galidesivir	-6.3
2	Penciclovir	-6.3
3	Baloxavir	-6.1
4	Favipiravir	-5.9
5	Ribavirin	-5.6
6	Lopinavir	-5.6
7	Sofosbuvir	-5.3
8	Remdesivir	-5.1
9	Ritonavir	-4.2
10	Pimodivir	-1.8

Molecular docking and virtual screen

Molecular docking was performed on ten FDA-approved antiviral drugs against the predicted 3D structure of the Sudanese SARS-CoV-2 RdRp protein. The docking results indicated that all ten drugs could bind to the active site of the RdRp. Among them, Galidesivir, Penciclovir and Baloxavir exhibited the highest binding affinities, with scores of -6.3 kcal/mol, -6.3 kcal/mol and -6.1 kcal/mol, respectively (Table 2).

Visualization of the docking interactions further confirmed that these drugs formed stable complexes with the active site of the RdRp protein (Figures 3, 4, 5).

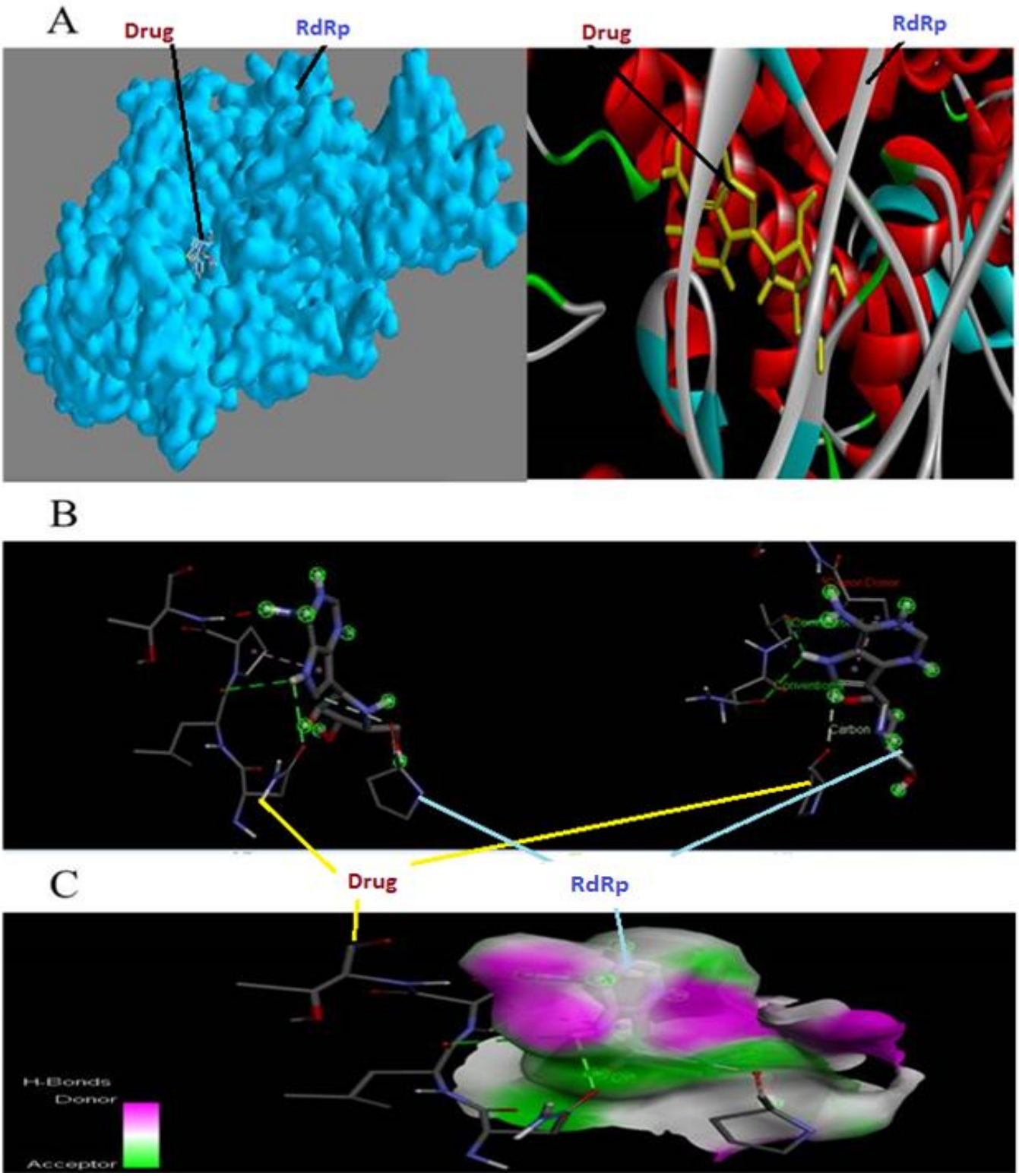

Figure 3. Interaction of Galidesivir with RdRp. **A:** Surface interaction. **B:** Interaction sites. **C:** Hydrogen bonds.

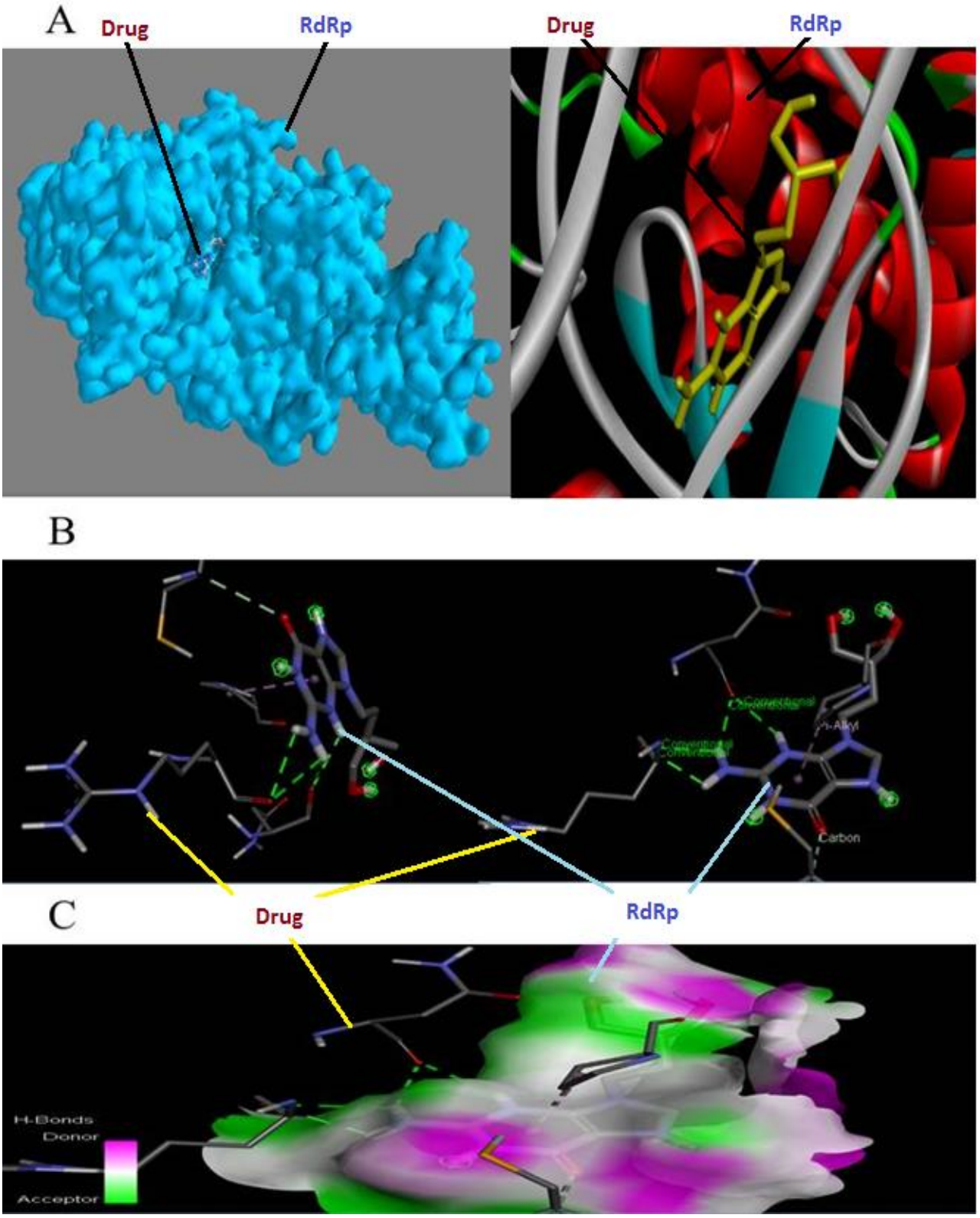

Figure 4: Interaction of Penciclovir with RdRp. **A:** Surface interaction. **B:** Interaction sites. **C:** Hydrogen bonds.

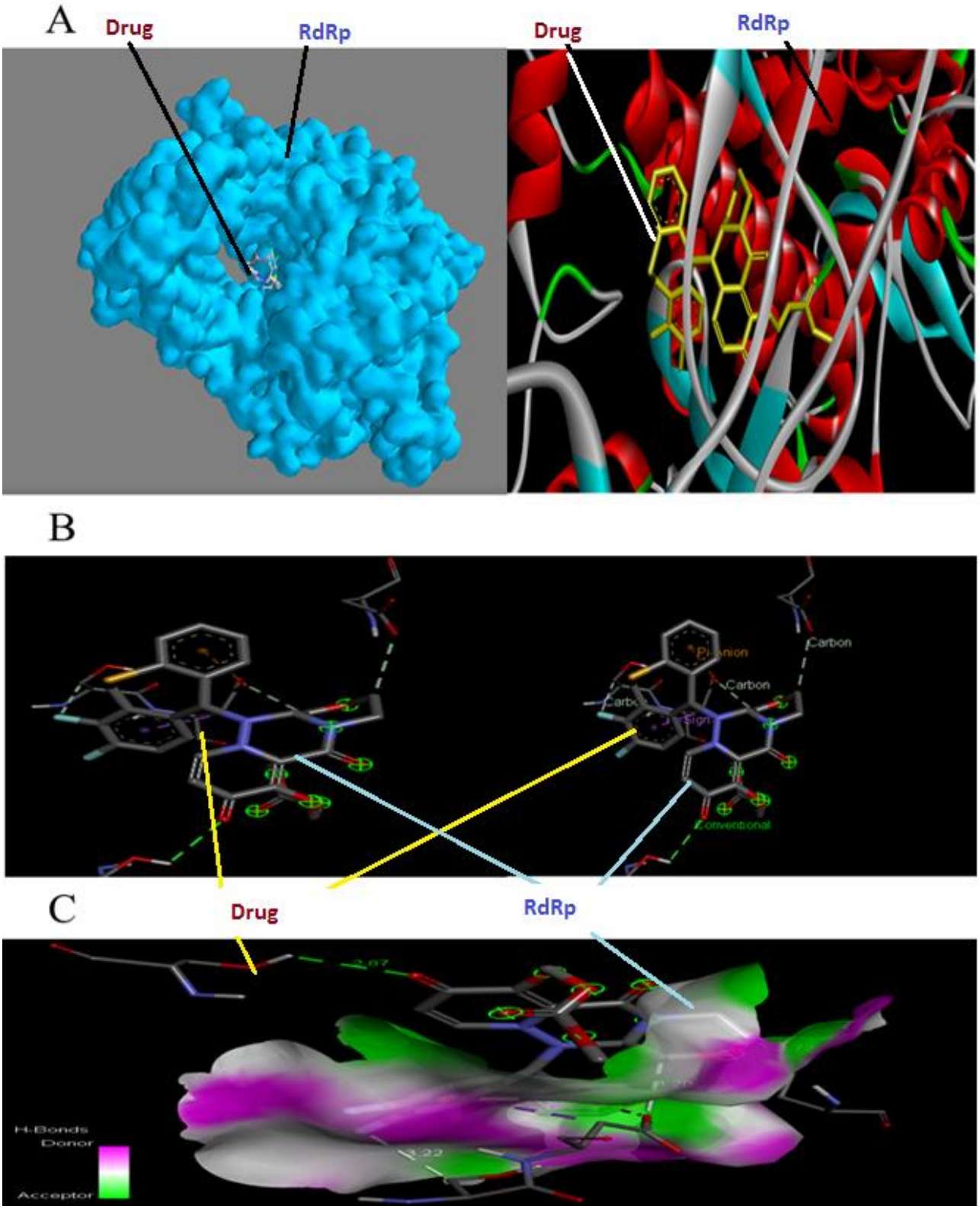

Figure 5: Interaction of Baloxavir with RdRp **A:** Surface interaction. **B:** Interaction sites. **C:** Hydrogen bonds.

DISCUSSION

Using cutting-edge computational techniques, this study thoroughly assessed the effectiveness of approved antiviral medications against the RNA-dependent RNA polymerase (RdRp) of Sudanese SARS-CoV-2 strains. According to our study, the antiviral drugs Galidesivir, Penciclovir, and Baloxavir had the highest binding affinities, indicating their strong capacity to stop viral reproduction.

The antiviral Favipiravir, on the other hand, showed a moderate level of binding efficacy, indicating that it might be used as a complementary treatment. However, the antiviral Pimodivir showed little interaction with RdRp, which raised serious questions about its clinical suitability for treating local variations. These findings highlight the necessity to modify treatment plans to take into consideration individual SARS-CoV-2 variations, especially in underserved areas like Sudan. This study not only clarifies the variable levels of effectiveness among currently available antiviral treatments, but it also offers crucial new knowledge about the molecular interactions between RdRp and these pharmaceuticals.

The study's primary strength lies in its focused investigation of SARS-CoV-2 strains specific to Sudan, thereby providing localized insights that are often overlooked in broader studies. Strong in silico methods, such as molecular docking and dynamics simulations, can be used to quickly and affordably evaluate the effectiveness of antivirals without having to immediately rely on lab resources. This methodological approach is especially beneficial in environments with restricted resources, such as those with insufficient laboratory facilities.
However, this study is not devoid of limitations. The reliance on computational models implies that the results are inherently predictive rather than definitive. Molecular docking is a useful tool for understanding binding affinities, but it falls short in explaining the complex processes of viral reproduction in biological systems. Furthermore, the pharmacokinetics, bioavailability, and possible side effects of these medications cannot be reliably predicted by in silico calculations. Therefore, it is essential to corroborate these findings by experimental validation using in vitro assays and subsequent clinical studies.

Our results are consistent with earlier investigations, including those conducted by Gao et al., which showed that nucleoside analogs like Remdesivir and Sofosbuvir were effective in suppressing SARS-CoV-2 RdRp.[36] We expanded on this study by discovering additional medications, such as Penciclovir and Galidesivir, that may also be useful treatments, potentially offering additional choices for the management of the disease. Additionally, they align with other studies that found the antiviral drug Galidesivir to be a viable option for preventing SARS-CoV-2 reproduction. For instance, Elfiky showed that antiviral Galidesivir and RdRp had strong binding interactions across a variety of SARS-CoV-2 variants, supporting our findings and indicating that antiviral Galidesivir efficiently targets a conserved area of the RdRp protein.[37] To preserve treatment efficacy across a variety of strains, this conservation is essential.

Because of their structural compatibility with highly conserved residues within the RdRp active site, the antiviral drugs Galidesivir and Penciclovir have a strong binding affinity. This discovery emphasizes how crucial it is to target conserved areas of viral proteins in order to produce broad-spectrum antiviral action. The antiviral Favipiravir's moderate efficacy indicates that it might work best when used in combination therapy, which would increase overall therapeutic effects by focusing on complementary pathways within the viral replication cycle.

When compared to other coronaviruses, the antiviral Pimodivir's limited efficacy raises important concerns about its structural alignment with RdRp in SARS-CoV-2. This finding highlights the importance of continuous structural and functional studies targeted at refining drug-targeting strategies to enable optimal repurposing of existing therapies. Furthermore, our findings support ongoing monitoring of viral mutations that may affect the effectiveness of medications, especially in underserved areas like Sudan, where inadequate genomic surveillance may lead to the emergence of novel viral variations.

The study's implications go far beyond the immediate therapeutic circumstances for treating SARS-CoV-2. During public health situations like pandemics, in silico techniques can play a crucial role in prioritizing medications for additional experimental validation. This is demonstrated by the discovery of the antiviral agents Galidesivir and Penciclovir as leading candidates. This strategy could significantly shorten the time needed for drug development, especially in environments with limited resources when quick decisions are essential.
This study also emphasizes how crucial it is to carry out regional research in order to support international research projects. A universal strategy for antiviral therapy may not be feasible across various geographic contexts with distinct virus strains, as indicated by the heterogeneity in medication efficacy found. Increased funding for bioinformatics and genomic surveillance in Africa is crucial for comprehending the genetic variety of circulating pathogens and creating specialized treatment plans in response. Furthermore, in order to improve treatment outcomes and reduce the risk of resistance, our findings support the investigation of combination therapy incorporating medications of intermediate efficacy. Healthcare professionals can improve treatment plans suited to specific patient populations by utilizing the synergistic effects of various medications.

CONCLUSION

The study concludes by providing strong evidence that the antiviral drugs Galidesivir, Penciclovir, and Baloxavir have the potential to be very effective inhibitors of SARS-CoV-2 RdRp and that they should be given priority in future clinical research projects.

The antiviral Favipiravir's modest efficacy indicates that it could be a viable option for combination therapy; on the other hand, worries about the antiviral Pimodivir's therapeutic usefulness call for more investigation. These results highlight the importance of combining computational techniques with laboratory and clinical research initiatives to create therapeutic approaches that are region-specific and adapted to local epidemiological settings.

Future investigations should focus on experimental validation to substantiate these findings while exploring combination therapies aimed at enhancing overall antiviral efficacy against COVID-19. Furthermore, strengthening bioinformatics infrastructure and genetic surveillance throughout Africa will be essential for both enhancing pandemic preparedness and guaranteeing efficient responses to newly emerging infectious illnesses that present serious global public health issues.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest regarding the publication of this manuscript.

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