Medical Research Archives

European Society of Medicine

OPEN ACCESS

Published: June 30, 2024

Citation: Cobar O and Cobar S, 2024. Advances In SARS-COV-2 RdRp Inhibitors: A 2023-2024 Literature Review, Medical Research Archives, [online] 12(6). https://doi.org/10.18103/mra.v12i6.5389

DOI
https://doi.org/10.18103/mra.v12i6.5389

ISSN: 2375-1924

REVIEW ARTICLE

Advances In SARS-COV-2 RdRp Inhibitors: A 2023-2024 Literature Review

Cobar Oscar[1,2], Cobar Stella[3]

1. Pharmacogenomics and Nutrigenomics Research Laboratory, School of Chemical Sciences and Pharmacy, University of San Carlos, Guatemala.
2. Biomedical Sciences, Ph.D. Program, School of Medical Sciences, University of San Carlos, Guatemala.
3. School of Chemical Sciences and Pharmacy, University of San Carlos, Guatemala.

***Corresponding author:** ocobar@gmail.com

ABSTRACT

Background: One of the major problems in drug design is to enhance the drug's potency against genetic variants, for which adding a suitable pharmacophore to a newly designed molecule is preferred.

RNA-dependent RNA polymerase (RdRp) is the SARS-CoV-2 enzyme responsible for genome replication and gene transcription into the human cell.

Cryogenic Electron Microscopy resolved the first structure of the RdRp complex of SARS-CoV-2 in April 2020, followed by two other studies that reported similar structures that same year.

The RdRp complex is built up from several nonstructural proteins included nsp12, nsp7, and nsp8.

The protein nsp12 represents the core component and the catalytic subunit of RdRp, while nsp7 and nsp8 are accessory factors that increase the binding and processivity of the RdRp template.

The nsp12 subunit contains an N-terminal nidovirus RdRp-associated nucleotidyltransferase (NiRAN) domain, an interface domain and a C-terminal RdRp domain.

Subunits nsp7 and nsp8 bind to the thumb, and an additional copy of nsp8 binds to the fingers domain.

During replication, the active site of RdRp is responsible for incorporating free nucleotides into the daughter RNA strand of the replication complex.

RdRp inhibitors, once metabolized, compete with the viral ATP molecules for incorporation into the nascent RNA strand.

Once the RdRp drug replaces ATP in the new strand, the RNA synthesis process is terminated, thereby preventing further replication of the virus from occurring.

In several studies reviewed in this manuscript, Molecular Docking simulations was employed to screen inhibitors that showed binding interaction with the conserved residues of RdRp.

Aim: The purpose of the Review is to present a literature review from January 1, 2023, to April 30, 2024, on the advances in SARS-CoV-2 RdRp inhibitors as a therapeutic approach against the virus, emphasizing on the structure of the enzime, the non-structural proteins that comprises, in particular nsp12, nsp 8 and nsp 7, the mechanisms that underlie the antiviral activity of RdRp inhibitory substances, the structure of the nucleoside analogs that have demonstrated RdRp inhibition in structural biology and computational research studies, and examine the current understanding of the molecular mechanisms underlying the action of these nucleoside analogs.

Materials and Methods: Original scientific articles published in Medline, Pubmed, Science Direct, Web of Science, Scopus, EBSCO and BioMed Central databases, official health organizations (World Health Organization, U.S. Centers for Disease Control and Prevention, European Centre for Disease Prevention and Control) electronic publications, and specialized media in the subject, were electronically searched to accomplish the aim of the study. Articles published in any language were included from January 1, 2023, to April 30, 2024, using a variety of keywords in combination. The studies relevant to our review were analyzed and compared.

Results and Discussion:
Inhibition of RdRp´s has been an integral approach for managing various viral infections such as dengue, influenza, Hepatitis C (HCV), Bovine Viral Diarrhea Virus (BVDV), among others. Inhibition of the SARS-CoV-2 RdRp is currently rigorously explored for the treatment of COVID-19. Consequently, the importance of RdRp in developing anti-viral agents against this viral disease, has been discussed by the scientific community in the last four years. The structure activity relationship profile and binding conformations of the reported inhibitors are essential features to elucidate some hypothesis for the designing of further SARS-CoV-2 RdRp inhibitors.
The search on scientific literature on these inhibitors, the analyses of the interaction characteristics, together with the examination of the inhibitors chemical structure, it would guide the rational design of antiviral medications and research into viral transcriptional mechanisms.
Conclusions: Several RdRp inhibitors have shown promising results for their use in treating the SARS-CoV-2 virus.
While work must still be conducted to fully understand the mechanisms responsible for reducing the antiviral activity of SARS-CoV-2, their potential in healing infected individuals is extremely valuable.
The development of SARS-CoV-2 RdRp inhibitors, to relieve the severity of an infection for a SARS-CoV-2 variants that could emerge in the near future, it is an essential task for the scientific community.
The analyses of inhibitors chemical structure-RdRp, besides the analyses of the inhibitors-RdRp interactions, it would guide the rational design of antiviral medications and research into SARS-CoV-2 transcriptional mechanisms.
This review summarizes recent progress in studies of RdRp inhibitors, 87 compounds was tested, focusing on the chemical structure of the inhibitors and the interactions between these inhibitors and the enzyme complex.
Keywords: RNA dependent RNA Polymerase, RdRp, non-structural proteins, nsp 7, nsp 8, nsp 12, NIRAN, Molecular Dynamic Simulations, Cryo Electron Microcop

Introduction

In 2020, Patrick Cramer and colleagues from Department of Molecular Biology, Max Planck Institute for Biophysical Chemistry, Göttingen, Germany, publish in Nature "Structure of replicating SARS-CoV-2 polymerase"[1].

They present a cryo-electron microscopy structure of the SARS-CoV-2 RdRp that comprises the viral proteins non-structural protein 12 (nsp12), nsp8 and nsp7, and more than two turns of RNA template–product duplex (**Figure**)[2].

The RdRp of SARS-CoV-2 is composed of a catalytic subunit known as nsp12 as well as two accessory subunits, nsp8 and nsp7[3].

The nsp12 subunit contains an N-terminal nidovirus RdRp-associated nucleotidyltransferase (NiRAN) domain, an interface domain and a C-terminal RdRp domain.

The RdRp domain resembles a right hand, comprising the fingers, palm and thumb subdomains that are found in all single-subunit polymerases. Subunits nsp7 and nsp8 bind to the thumb, and an additional copy of nsp8 binds to the fingers domain4.

The active-site cleft of nsp12 binds to the first turn of RNA and mediates RdRp activity with conserved residues.

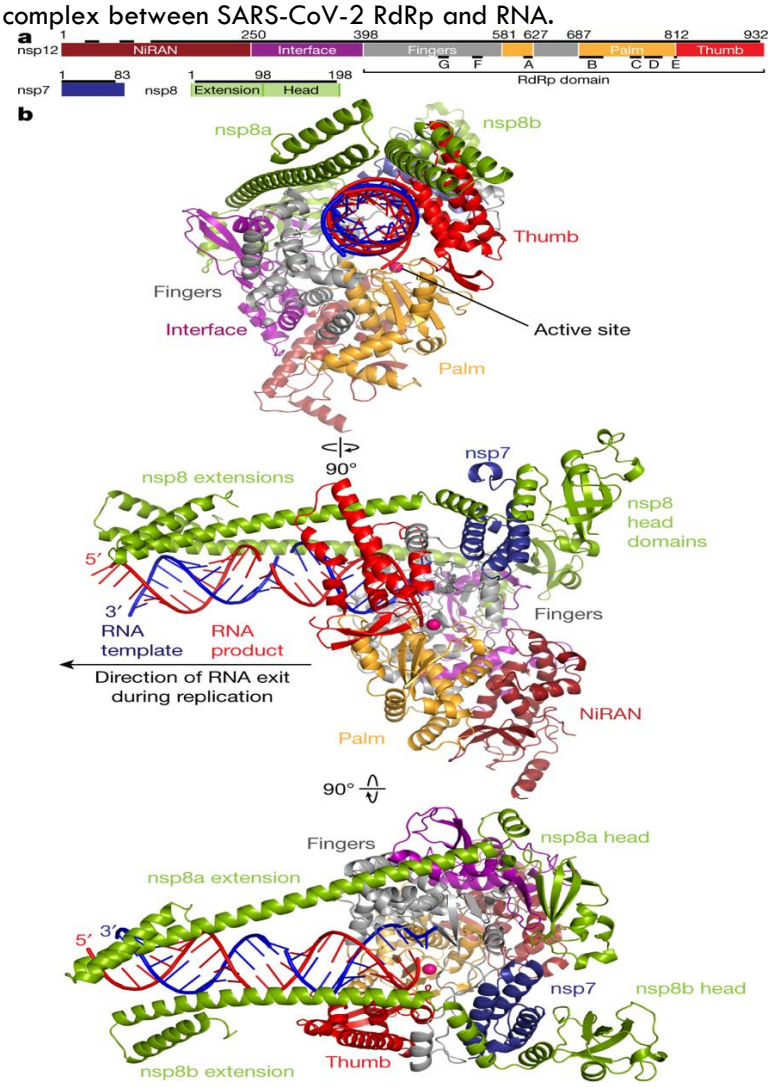
Two copies of nsp8 bind to opposite sides of the cleft and position the second turn of RNA.

Long helical extensions in nsp8 protrude along exiting RNA, forming positively charged 'sliding poles'.

These sliding poles can account for the known processivity of RdRp that is required for replicating the long genome of coronaviruses.

The study results enable a detailed analysis of the inhibitory mechanisms that underlie the antiviral activity of substances such as remdesivir, a drug for the treatment of COVID-19.

Figure. Structure of complex between SARS-CoV-2 RdRp and RNA.



***a**, Domain structure of nsp12, nsp8, and nsp7 subunits of RdRp. In nsp12, the conserved sequence motifs A–G are depicted. Regions included in the structure are indicated with black bars.*
***b**, Three views of the structure, related by 90° rotations (top, back view; middle, side view; bottom, top view). Colour code for nsp12 (NiRAN, interface, fingers, palm and thumb), nsp8, nsp7, RNA template (blue) and RNA product (red) used throughout. The magenta sphere depicts a modelled metal ion in the active site.*

Amar Jeet Yadav and colleagues from Laboratory for Computational Biology & Biomolecular Design, School of Biochemical Engineering, Indian Institute of Technology (BHU), Varanasi 221005, Uttar Pradesh, India, study the molecular features of nsp7 dimerization using leveraging computational protein design (CPD), machine learning (ML), AlphaFold v2.0-based structural analysis, and several related computational approaches[5].

They report that notably, RdRp forms dimers *via* non-structural protein (nsp) subunits, particularly nsp7, crucial for efficient viral RNA copying.

Similar to the main protease (M^{pro}) of SARS-CoV-2, there is a possibility that the nsp7 might also undergo mutational selection events to generate more stable and adaptable versions of nsp7 dimer during virus evolution.

Mahmoud E. S. Soliman, from Molecular Bio-Computation and Drug Design Laboratory, School of Health Sciences, University of KwaZulu-Natal, South Africa and colleagues fron other institutions[6], mapped out and fully characterized the variations between the two RdRp binding sites, BS1 and BS2, for significant differences in their amino acid architecture, size, volume, and hydrophobicity.

The interesting architecture of the catalytic chamber of RNA-dependent RNA polymerase (RdRp) with its two unique binding sites, BS1 and BS2, prompted the researchers to investigate the potential use of multiple inhibitors as combination therapy against COVID-19.

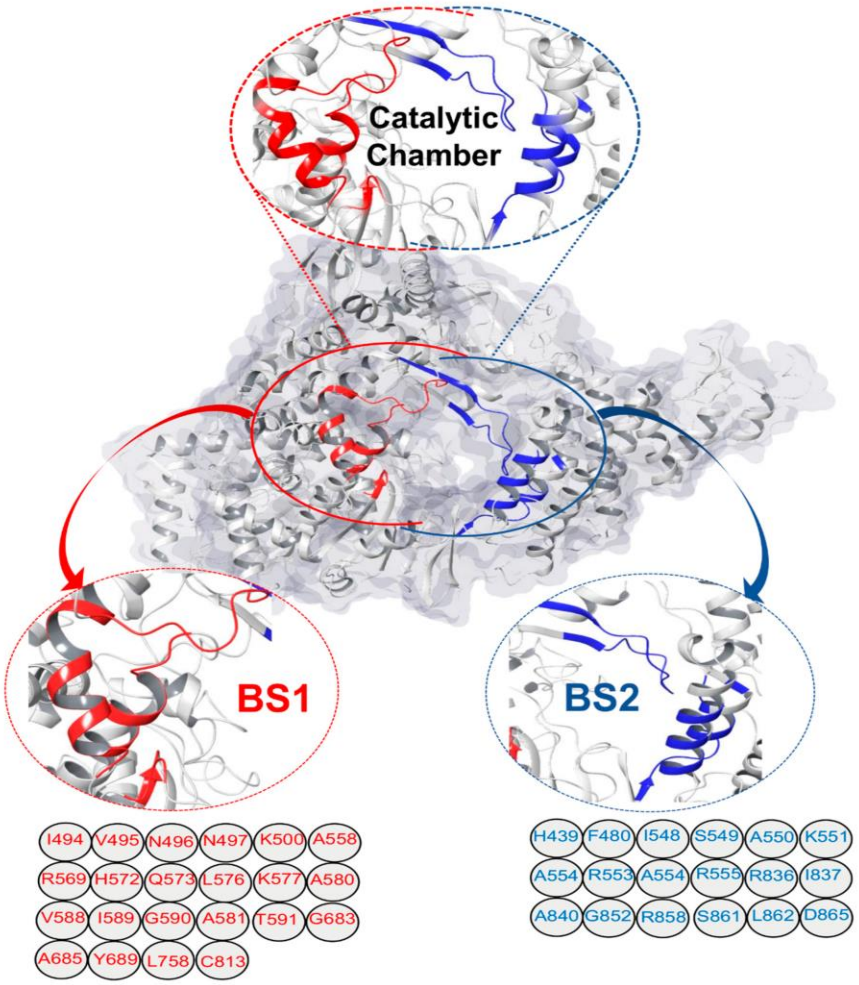
The maped out was followed by investigating the preferential binding of eight antiviral agents to each of the two binding sites, BS1 and BS2, to

understand the fundamental factors that govern the preferential binding of each drug to each binding site.

The results showed that, in general, hydrophobic drugs, such as remdesivir and sofosbuvir, bind better to both binding sites than relatively less hydrophobic drugs, such as alovudine, molnupiravir, zidovudine, favilavir, and ribavirin.

The **Figure** shows a Surface view of RdRp (PDB ID: 7D4F)[7], with the "catalytic chamber" housing two distinct binding sites/cavities, estimated with the Maestro Schrödinger software.

Figure. Surface view of RdRp (PDB ID: 7D4F), with the "catalytic chamber"



However, suramin, which is a highly hydrophobic drug, unexpectedly showed overall weaker binding affinities in both binding sites when compared to other drugs.

This unexpected observation may be attributed to its high binding solvation energy, which disfavors overall binding of suramin in both binding sites.

On the other hand, hydrophobic drugs displayed higher binding affinities towards BS1 due to its higher hydrophobic architecture when compared to BS2, while less hydrophobic drugs did not show a significant difference in binding affinities in both binding sites.

Analysis of binding energy contributions revealed that the most favorable components are the DEele, DEvdw, and DGgas, whereas DGsol was unfavorable.

The DEele and DGgas for hydrophobic drugs were enough to balance the unfavorable DGsol, leaving the DEvdw to be the most determining factor of the total binding energy.

The amino acid residues of binding site1 (BS1) and binding site2 (BS2) are also shown in the **Figure** above.

Additionally, the architecture of the binding sites BS1 and BS2 within the catalytic chamber and the preferential binding modes of eight antiviral drugs in each binding site were thoroughly analyzed in order to understand the underlying factors that govern preferential drug binding[8].

Tingting Feng and colleagues from Institute of Biology & Medical Sciences, Jiangsu Key Laboratory of Infection & Immunity, Soochow University, Jiangsu Province, China[9], analyze the minimal RNA synthesizing machinery established from cryo-electron microscopy structural data there has been development of high-throughput screening assays for directly screening inhibitors that target the SARS-CoV-2 RdRp.

Previous data from *in vitro* assays has shown that recombinant SARS-CoV nsp12 fused with a glutathione S-transferase tag displays weak and nonprocessive polymerase activity, contrary to the demand for synthesis of a large RNA genome *in vivo*, a phenomenon that might hinder development of antiviral drugs which target the RdRp[10,11].

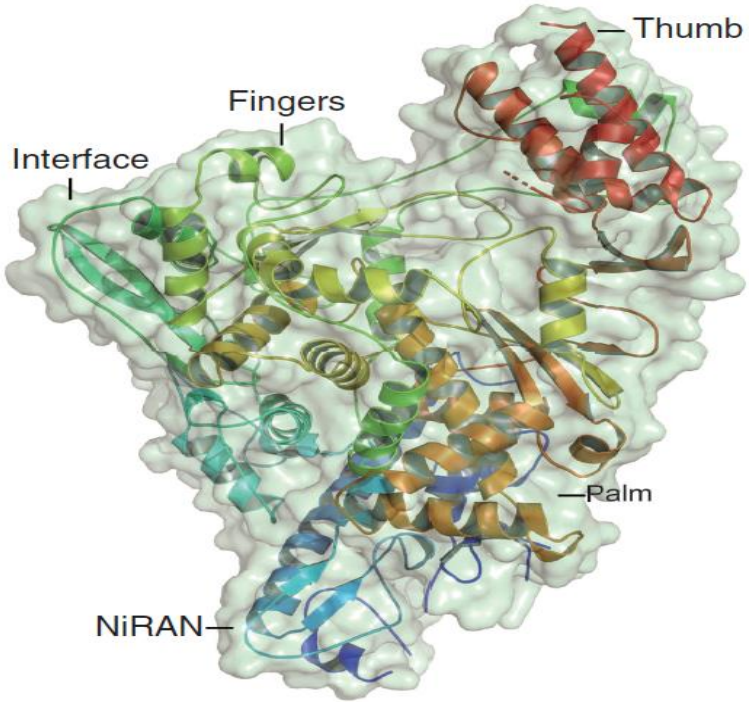
They present verified techniques that could be used to discover potential anti-RdRp agents or repurposing of approved drugs to target the SARS-CoV-2 RdRp and, highlight the characteristics and application value of cell-free or cell-based assays in drug discovery.

Collectively, summarized cell-free and cell-based assays for detecting the enzymatic activity of SARS-CoV-2 nsp12–nsp8–nsp7 complex and evaluating the inhibitory effect of prospective anti-RdRp compounds.

The structural and functional insights into coronavirus replication and transcription will contribute to the improvement and optimization of *in vitro* screening schemes which can be formatted into High Throughput Screening conveniently.

The Figure shows the structure of nsp12.

Figure. Structure of SARS-CoV-2 nsp12



NiRAN: Nucleotidyltransferase.

The nsp12 subunit consists of a nidovirus N-terminal RNA-dependent RNA polymerase (NiRAN) associated domain, an interface domain, and a C-terminal polymerase domain composed of the fingers, palm and thumb subdomains.
Protein data taken from reference 12.

Results and Discussion

Daniel Haders and colleagues from Model Medicines, University of California San Diego and DSG Pharma Consulting LLC, publishes "New Results, Discovery of RdRp Thumb-1 as a novel broad-spectrum antiviral family of targets and MDL-001 as a potent broad-spectrum inhibitor thereof - Part I: A Bioinformatics and Deep Learning Approach"[13].

The authors investigated the broad-spectrum potential of the allosteric Thumb-1 cryptic site of the RdRp, which to date has only been adequately studied in Hepatitis C Virus (HCV).

To explore this potential antiviral target, they used a suite of bioinformatics techniques, including homology modeling and multiple sequence alignments, to reveal the conserved landscape of the Thumb-1 site across +ssRNA viruses[14].

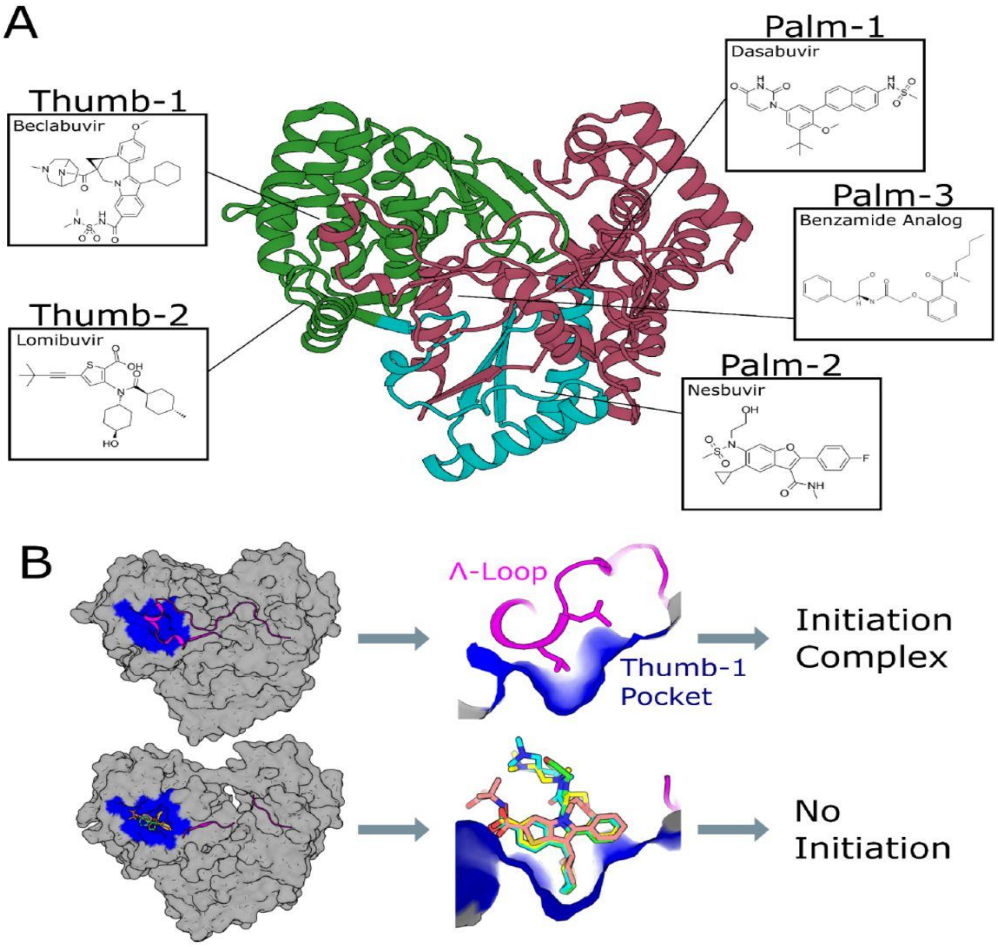
Then used ChemPrint, our Mol-GDL (Molecular-Geometric Deep Learning) machine learning model to predict drug inhibition of the Thumb-1 site in RdRp across +ssRNA viruses.

They identify MDL-001 as a promising broad-spectrum antiviral candidate with favorable properties that enable oral and once-a-day dosing.

Also shows how the cryptic nature of the Thumb-1 site masks itself to conventional virtual screening techniques, like docking, where activity prediction is heavily based on solving or predicting an accurate structure of the open pocket.

The **Figure 1** shows the HCV RdRp, known as NS5B, features a conserved hand-like structure with three domains (palm, fingers, and thumb), and harbors five identified allosteric sites (Thumb-1, Thumb-2, Palm-1, Palm-2, and Palm-3) for drug discovery efforts[15].

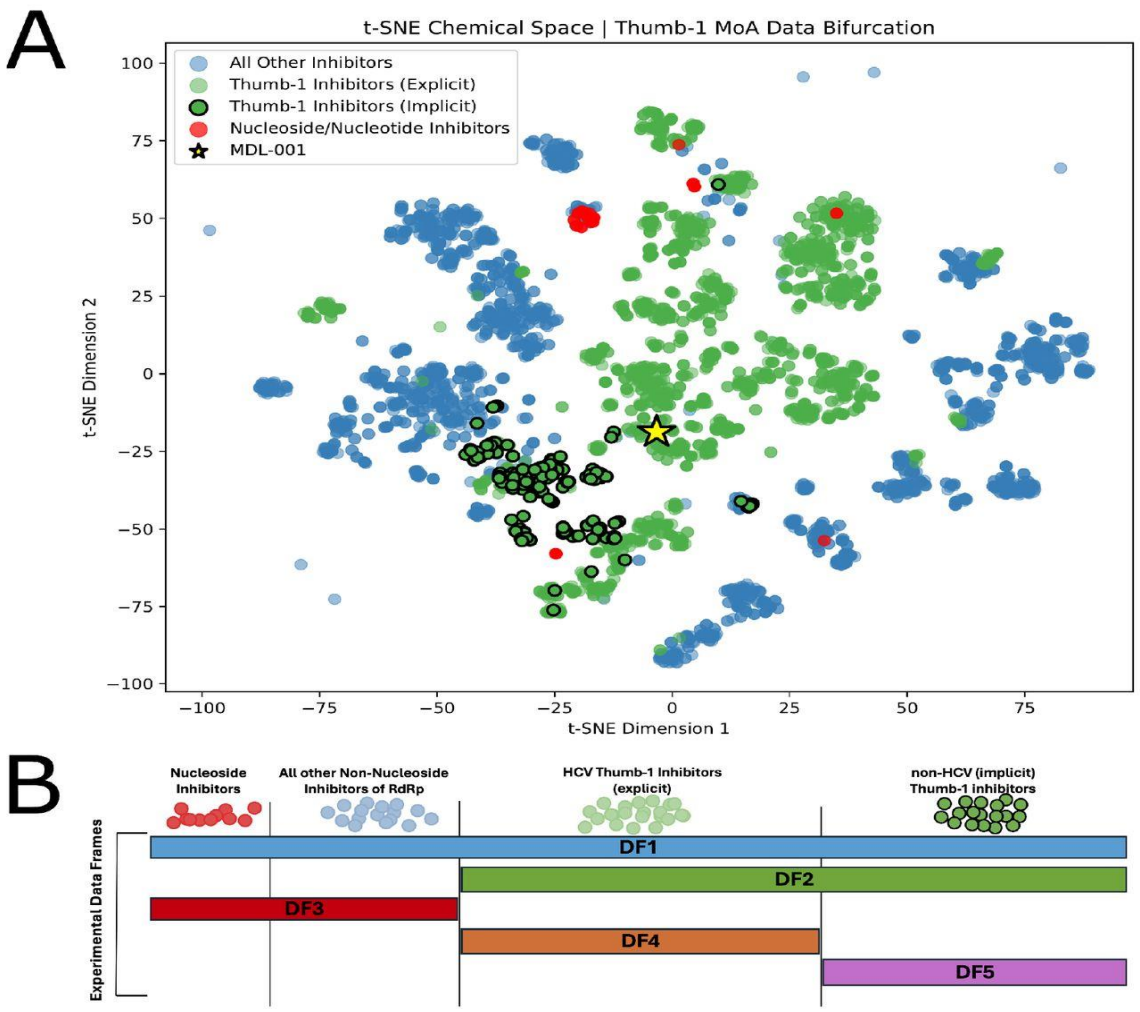
Figure 1. The Thumb-1 Site Mechanism of HCV RdRp



(A) *View of HCV RdRp with sites highlighted and one example of an inhibitor for each site (PDB ID: 1C2P).*
(B) *View of the Λ1-thumb interaction and mechanism of thumb-1 inhibition.*
Top Left: *HCV RdRp apoprotein structure with a gray surface representation acquired from 1C2P.*
Top Right: *A Thumb-1 pocket view of the apoprotein α-helix and its two endogenous leucine side chains occupying the site.*
Bottom Left: *Overlay of five Thumb-1 inhibitors with structures available from the PDB (accession codes 2BRK, 2BRL, 2DXS, 2XWY, 2WCX), based on superposition of the holoprotein secondary structure 2XWY, with a gray surface representation.*
Bottom Right: *A Thumb-1 pocket view of the holoprotein and its superimposed Thumb-1 inhibitors.*

As shown in Figure 2 the chemical space representation of inhibitors places MDL-001 on top of a cluster of explicit Thumb-1 drugs, cleanly separated from compounds belonging to other target site classes[16].

Figure 2. t-SNE Chemical Space and Thumb-a MoA Data Bifurcation



(A) *TSNE of allosteric compounds that are known to target RdRp, showing the different classifications of nucleoside, non-nucleoside, and thumb-1 inhibitors (explicit and implicit data). MDL-001 is depicted as a yellow star.*
(B) *Schematic of the compositions of Datasets: (**DF1**) All Data (**DF2**) Thumb-1 (Explicit and Implicit) (**DF3**) All non-Thumb-1 Inhibitors (**DF4**) Thumb-1 (Explicit) (**DF5**) Thumb-1 (Implicit).*
t-SNE: t-distributed stochastic neighbor embedding parametric approach.

This placement further supported MDL-001's potential as a Thumb-1 site inhibitor.

Additionally, the t-SNE projection showed that MDL-001 fit relatively close to the grouping of implicit Thumb-1 inhibitors that inhibit non-HCV viruses, shown as black circle outlines in the figure, suggesting that MDL-001 may have similar activity outside of HCV.

As a conclusion the authors states that this study demonstrates the utility of this approach in drug discovery for broad-spectrum antivirals that target the Thumb-1 site.

Through the discovery of MDL-001 and in proposing its Thumb-1 MoA, this study has unveiled the Thumb-1 site of RdRp as a novel biological target for broad-spectrum drug discovery.

This advancement was propelled by the integration of AI, notably through our GALILEO platform and its ChemPrint prediction model.

The discovery of MDL-001, with its favorable pharmacokinetics and ADMET properties and potential as an orally administered therapy, offers hope as an orally administered therapy that effectively treats a broad swath of ssRNA viral diseases[17].

The highly polar residues within the active site often necessitate the use of highly polar or charged compounds, especially when designing nucleoside analog inhibitors, posing significant challenges in optimizing drug-likeness and membrane permeability for clinical efficacy.

Tiantian Zu and Lu Zhang from State Key Laboratory of Structural Chemistry, Fujian Institute

of Research on the Structure of Matter, Chinese Academy of Sciences, China, summarizes previous experimental findings and mechanistic investigations of nucleoside analogs inhibiting SARS-CoV-2 RdRp [18].

The review summarizes previous experimental findings and mechanistic investigations of nucleoside analogs inhibiting SARS-CoV-2 RdRp. It would guide the rational design of antiviral medications and research into viral transcriptional mechanisms.

The review could guide the rational design of antiviral medications and research into viral transcriptional mechanisms.

The authors state RdRp complex is the minimum replicase required to catalyze the replication of the RNA genome.

Considering the constant mutations of SARS-CoV-2 virus, the highly conserved and functionally essential enzyme RdRp has become a promising target for the development of antiviral drugs[19].

Nucleoside analogs structurally similar to natural nucleosides are promising inhibitors of viral replication and transcription.

The review begins by discussing the nucleoside analogs that have demonstrated inhibition in the experiments.

Second, examine the current understanding of the molecular mechanisms underlying the action of nucleoside analogs on the SARS-CoV-2 RdRp.

Then, recent findings in structural biology and computational research are presented through the classification of inhibitory mechanisms.
Nucleoside analogs structurally similar to natural nucleosides are promising inhibitors of viral replication and transcription.

They state the nucleoside analog's prodrug is typically metabolized into its 5'-triphosphate form in the cells.

It can therefore compete with the natural substrate nucleoside triphosphate (NTP) for incorporation into the primer strand during the nucleotide addition cycle[20-21].

Such incorporation would result in the presence of nucleoside analogs at the primer strand or later at the template strand during the second round of RNA synthesis, allowing nucleoside analogs to exert inhibition via different mechanisms[22-24].

In addition, concerted efforts have been made to comprehend the molecular details of inhibition, which would facilitate the mechanism-based drug design.

These analogs are classified according to the positions of their chemical modifications.

The current understanding of the inhibitory mechanisms exerted by nucleoside analogs at the molecular level is then reviewed.

For each mechanism, the previous structural biology and computational simulation findings are introduced.

The Figure shows the structure of the SARS-CoV-2 RdRp complex and the active site[25].
These analogs are classified according to the positions of their chemical modifications.

The current understanding of the inhibitory mechanisms exerted by nucleoside analogs at the molecular level is then reviewed.

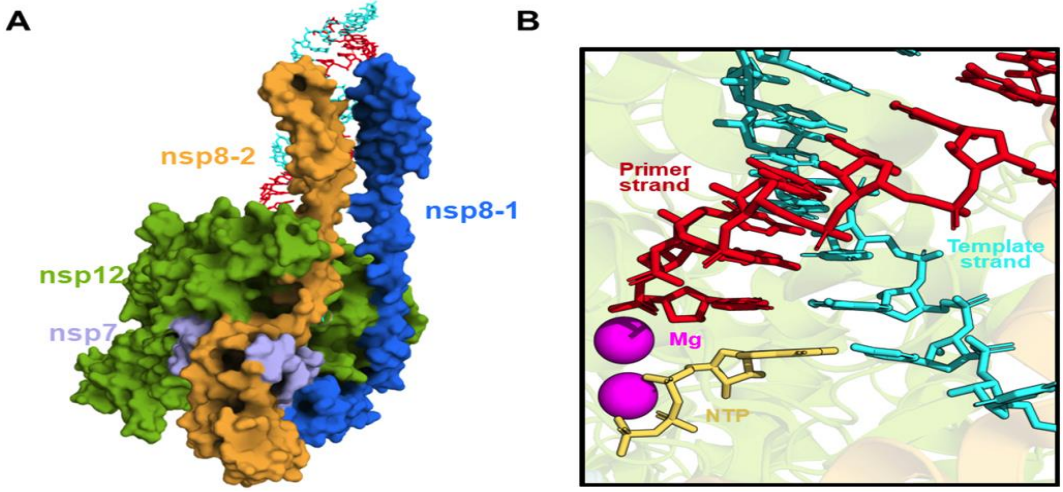
For each mechanism, the previous structural biology and computational simulation findings are introduced.

The study begins by introducing the nucleoside analogs that have experimentally demonstrated efficacy against SARS-CoV-2 by targeting the active site of RdRp.

In conclusion, the authors discuss the perspectives based on the subject current knowledge.

Overall, they have systematically reviewed the nucleoside analogs that show inhibition against SARS-CoV-2 RdRp and discussed the underlying molecular mechanisms.

Figure. The structure of the SARS-CoV-2 RdRp complex and the active site



(A) *The structure of nsp12 bound with nsp7 and nsp8 is shown in surface representation (PDB ID: 7UOB). Nsp12, nsp8–1, nsp8–2, and nsp7 are colored green, blue, orange, and light purple, respectively. The primer and template strand of the double-stranded RNA (dsRNA) are displayed with red and cyan sticks, respectively.*
(B) *The active site of RdRp complexed with NTP (shown in yellow). The primer strand and template strand are colored red and cyan, respectively. The two Mg2+ ions are shown in magenta spheres.*

Overall, the research has systematically reviewed the nucleoside analogs that show inhibition against SARS-CoV-2 RdRp and discussed the underlying molecular mechanisms.

This work is expected not only provide a comprehensive knowledge of nucleoside analogs inhibiting the SARS-CoV-2 RdRp but also be conducive to the further investigations of inhibitory mechanisms, orientating the mechanism-driven and structure-based drug design by optimizing the modifications on the nucleoside analogs.

In addition, concerted efforts have been made to comprehend the molecular details of inhibition, which would facilitate the mechanism-based drug design[26-28].

The authors hope this work will not only provide a comprehensive knowledge of nucleoside analogs inhibiting the SARS-CoV-2 RdRp but also be conducive to the further investigations of inhibitory mechanisms, orientating the mechanism-driven and structure-based drug design by optimizing the modifications on the nucleoside analogs.

Aparna S. Gana and James N. Baraniuk from College of Arts and Sciences, University of Virginia, and Division of Rheumatology, Immunology and Allergy, Department of Medicine, Georgetown University Medical Center, Washington DC respectively, use *in silico* methods to tested the hypothesis that structural biology approaches based on RdRp motifs that are conserved across evolution can define new drug binding locations and infer potential broad-spectrum inhibitors for SARS-CoV-2 and other ss(+)RNA viruses[29].

They identified sirolimus as a drug that would bind to a novel epitope extending from N-terminal domain to basic residues from motifs F, A and distal to D that form the NTP entry tunnel[30].

This novel drug target and platforms of limus, rifampin and digitalis drugs may generate new broad-spectrum antivirals directed at RdRp of these viruses.

A broad spectrum antiviral may be beneficial for cases and epidemics of ss(+)RNA viruses that are unknown, emerging or have no established therapies.

Such a drug could act as a “viral penicillin” directed at the structurally conserved motif, but with the caveat that over time the viruses would evolve to display some resistance.

Hypothesize that *in silico* structural biology approaches can discover novel drug binding sites for RNA-dependent-RNA-polymerases (RdRp) of positive sense single-strand RNA (ss(+)RNA) virus species.

RdRps have a structurally conserved active site with seven motifs (A to G), despite low sequence similarity.

The approach of searching for a widely conserved drug binding motif, led to identification of a novel inhibitory site that may have the potential to lead

to development of broad-spectrum antivirals that obstruct the NTP tunnel[31].

Future stages of drug development will proceed by *in vitro* drug RdRp binding and inhibition studies, mutagenesis of the binding region, drug bound structure analysis, cell and animal testing with active viral infections.

Sirolimus was identify as a drug that would bind to a novel epitope extending from N-terminal domain to basic residues from motifs F, A and distal to D that form the NTP entry tunnel.

This original finding (the sirolimus docking site was in the nucleotide triphosphate entry tunnel between motifs A and F but distinct from the active site in motif C), supports the hypothesis that structural biology approaches based on RdRp motifs that are conserved across evolution can define new drug binding locations and infer potential broad-spectrum inhibitors for SARS-CoV-2 and other ss(+)RNA viruses.

The authors conclude that this novel drug target and platforms of limus, rifampin and digitalis drugs, may generate new broad-spectrum antivirals directed at RdRp of these viruses.

A broad spectrum antiviral may be beneficial for cases and epidemics of ss(+)RNA viruses that are unknown, emerging or have no established therapies.

Such a drug could act as a "viral penicillin" directed at the structurally conserved motif, but with the caveat that over time the viruses would evolve to display some resistance.

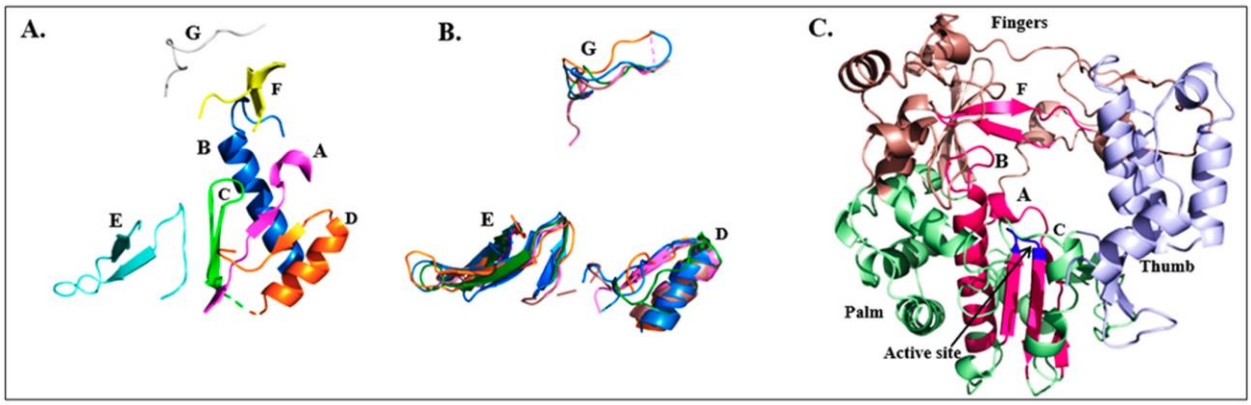
The **Figure** shows the "common core" for the active site of ss(+)RNA virus RdRps that is defined by structural motifs A to G[32].

Motifs A, B and C form the palm of the right-handed structure with motif F in the fingers.

Motifs D and E in the palm and G in the fingers are positioned in approximately the same three-dimensional space across species but cannot be exactly superimposed.

The thumb does not contribute to the active site. In general, the fingers interact with the major groove of the template RNA.

Figure. RdRp motifs



(A) *Conserved sequence motifs A to G were extracted from poliovirus (PDB ID: 2ijf) and superimposed. Motifs A, B, C and F defined by Peersen (Peersen, O.B. A Comprehensive Superposition of Viral Polymerase Structures. Viruses.* ***2019****. 11, 745) were highly conserved across ss(+)RNA viruses.*
(B) *Motifs D, E and G were not highly conserved when representative structures of each family were superpositioned: hepatitis C virus (gold; PDB ID: 1c2p), Those assign virus (blue; PDB ID: 4xhi), SARS-CoV-2 (green; PDB ID: 7bw4), enterovirus D68 (pink; PDB ID: 6l4r) and Norwalk virus (light blue; PDB ID: 2b43). The alpha helix of motif D was well aligned but the contiguous loop that follows deviated extensively. There was little structural conservation in motif G.*
(C) *Cartoon diagram of poliovirus RdRp (PDB ID: 2ijf) shows the palm (pale green), fingers (light brown) and thumb (light purple) domains, and motifs A, B, C and F that form the conserved motif FABC (magenta) in the manuscript.*
The active site (dark blue, black arrow) is at the tip of the motif C.
Figure created using PyMol 2.5.

Mahjabeen Saleem from School of Medical Lab Technology, Minhaj University Lahore, Pakistan, Mohd Ashraf Rather from Division of Fish Genetics and Biotechnology, Faculty of Fisheries, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir, India and colleagues from other academic institutions, publish "Identification of RdRp inhibitors against SARS-CoV-2 through E-pharmacophore-based virtual screening, molecular docking and MD simulations approaches[33].

The study aimed to employ E-pharmacophore-based virtual screening to reveal potent inhibitors of RdRp as potential leads to block the viral replication.

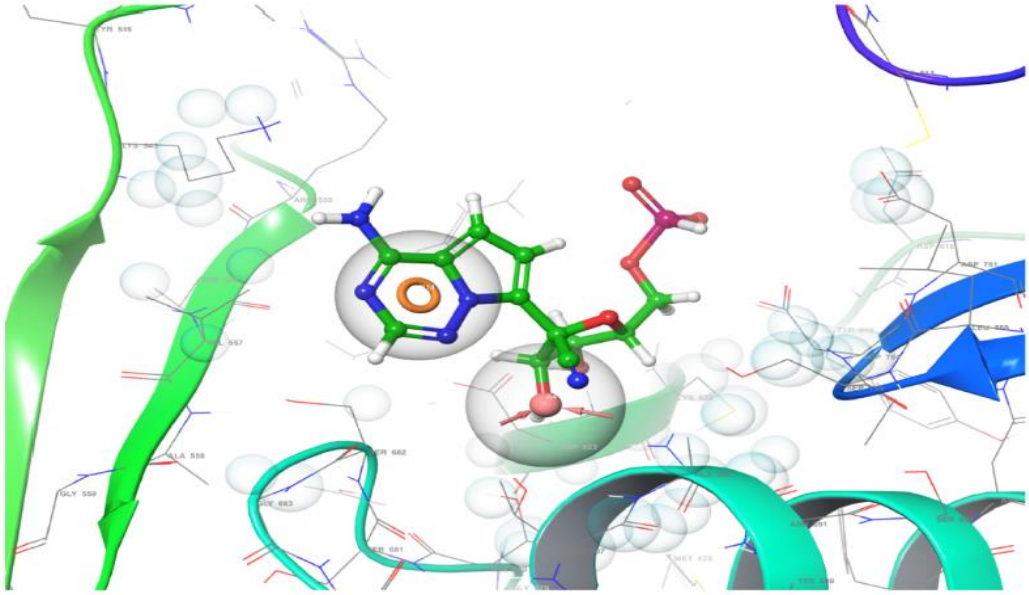
In the E-Pharmacophore model, they use remdesivir (constructed from PDB 7BV2) complexed with SARS-CoV-2 RdRp (see **Figure**)[12].

An energy-optimised pharmacophore model was generated to screen the Enamine REAL DataBase (RDB)[34].

Then, ADME/T profiles were determined to validate the pharmacokinetics and pharmacodynamics properties of the hit compounds.

Moreover, High Throughput Virtual Screening (HTVS) and molecular docking (SP & XP) were employed to screen the top hits from pharmacophore-based virtual screening and ADME/T screen.

Figure. Pharmacophore model of RdRP of SARS-CoV-2 with remdesivir (RTP) inhibitor[33].



The binding free energies of the top hits, were calculated by conducting MM-GBSA analysis followed by MD simulations to determine the stability of molecular interactions between top hits and RdRp protein[35,36].

The results revealed six compounds having binding free energies, calculated by the MM-GBSA method of, -57.498, -45.776, -46.248, -35.67, -25.15 and -24.90 kcal/mol respectively[37].

The MD simulation studies confirmed the stability of protein ligand complexes, hence, indicating as potent RdRp inhibitors and are promising candidate drugs to be further validated and translated into clinics in future.

E-pharmacophore and structure-based virtual screening followed by MD simulations have evolved as the most efficient computational approach to expedite the drug discovery process[38].

Comp-1 and -6 (**Table**) were identified as the high XP Gscoring thereby high binding free energy-containing hit exhibiting strong and stable binding affinity, validated by MD simulation, towards the RdRp active site.

A favourable pharmacokinetic profile and Lipinski properties evinced the promising potential of these compounds as an effective inhibitors of RNA-dependent RNA polymerase delineating its prospective utilization against SARS-CoV-2.

However, a rational assessment regarding the benefits and possible side effects of the proposed hit compound is mandatory to ascertain its reliability and efficacy against COVID-19.

Table. The IUPAC names and smiles of the top six hit compounds

Compound no.	Name	Smiles
Comp-1	(4-[({[(2*S*,3*R*)-1-Methyl-2-(1-methyl-1H-imidazol-2-yl)-6-oxo-3-piperidinyl]methyl}amino)methyl]benzamide)	NC(=O)c1ccc(cc1)C[NH2+]C[C@@H](CCC2=O)[C@H](N2C)c3n(*C*)cc[nH+]3
Comp-2	(N^2-(2-{1-[(1-Methyl-4-piperidinyl)acetyl]-3-piperidinyl}ethyl)glycinamide)	NC(=O)C[NH2+]CC[C@H]1CCCN(C1)C(=O)C[C@H]2CC[N@H+](C)CC2
Comp-3	(5-(3,4,5-trimethoxyphenyl)-1H-pyrazole-3-carbohydrazide)	NNC(=O)c1cc([nH]n1)-c2cc(*OC*)c(OC)c(c2)OC
Comp-4	(N-[(2,1,3-benzothiadiazol-4-yl)methyl]-5-[(morpholin-4-yl)methyl]pyridin-2-amine)	n1snc(c12)cccc2CNc(nc3)ccc3CN4CCOC(
Comp-5	(2-Chloro-3-isopropoxy-N-[(1-oxo-1,2,3,4-tetrahydro-4-isoquinolinyl)methyl]benzamide)	CC(*C*)Oc(ccc1)c(*Cl*)c1C(=O)NC[C@@H](CNC2=O)c(c23)cccc3
Comp-6	(N-[(5-Fluoro-1H-benzimidazol-4-yl)methyl]-2-(methoxymethyl)-6-methyl-4-pyrimidinamine)	COCC1=NC(=CC(=[NH+]1)C)NCC2=C3[NH]C=NC3=CC=C2F

Aqib Iqbal from Institute of Biotechnology and Genetic Engineering, The University of Agriculture, Pakistan, Ajmal Khan from Natural and Medical Sciences Research Center, University of Nizwa, Sultanate of Oman, Ahmed Al-Harrasi from Natural and Medical Sciences Research Center, University of Nizwa, Sultanate of Oman and colleagues from other academic institutions, published "Identifying non-nucleoside inhibitors of RNA-dependent RNA-polymerase of SARS-CoV-2 through per-residue energy decomposition-based pharmacophore modeling, molecular docking, and molecular dynamics simulation"[39].

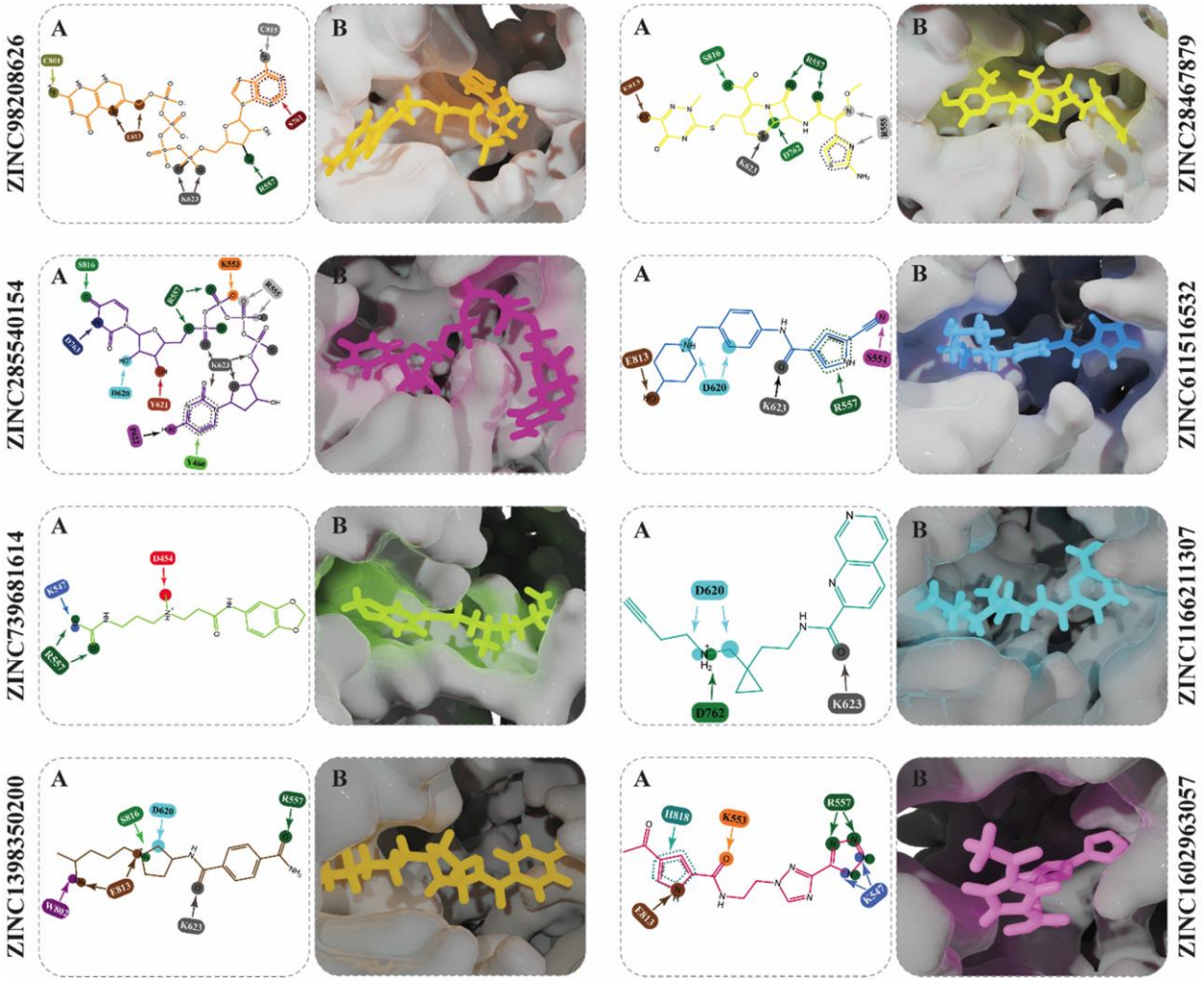
In the study, the researchers have computationally screened ~690 million compounds from the ZINC20 database and 11,698 small molecule inhibitors from DrugBank to find existing and novel non-nucleoside inhibitors for SARS-CoV-2 RdRp.

A combination of the structure-based pharmacophore modeling and hybrid virtual screening methods, including per-residue energy decomposition-based pharmacophore screening, molecular docking, pharmacokinetics, and toxicity evaluation were employed to retrieve novel as well as existing RdRp non-nucleoside inhibitors from large chemical databases[40].

Besides, molecular dynamics simulation and Molecular Mechanics/Generalized Born Surface Area (MM/GBSA) method were used to investigate the binding stability and calculate the binding free energy of RdRp-inhibitor complexes[41].

Based on docking scores and significant binding interactions with crucial residues (Lys553, Arg557, Lys623, Cys815, and Ser816) in the RNA binding site of RdRp, three existing drugs, ZINC285540154 (1), ZINC98208626 (2), ZINC28467879 (3), and five compounds from ZINC20 (ZINC739681614 (4), ZINC1166211307 (5), ZINC611516532 (6), ZINC1602963057 (7), and ZINC1398350200 (8) were selected, and the conformational stability of RdRp due to their binding was confirmed through molecular dynamics simulation (**Figure**).

Figure. Docked poses of selected inhibitors of RdRp.



*The binding interactions of ligands with protein are presented in 2D form **(A)** and 3D format **(B)**.*

Molecular dynamics simulation was employed to explore the structural and dynamic behavior of RdRp upon binding with these eight compounds.

The free energy calculations revealed these compounds possess strong binding affinities for RdRp, and exhibited drug-like features, good absorption, distribution, metabolism, and excretion profile and were found to be non-toxic.

Based on docking scores, protein-ligand interaction, and physiological and pharmacokinetics parameters, eight compounds (including five novel and three existing) were identified as promising inhibitors of RdRp.

MM/GBSA calculations revealed high binding affinity of (1), (3), (6), (8), (4), and (7), in range of -12.87 kcal/mol to -37.74 kcal/ mol, whereas (5) and (2) also showed good binding affinities for RdRp.

Based on the computational results, the authors propose that these compounds can hinder the replication of SARS- CoV-2 by specifically preventing RNA-primer strand attachment with the RdRp enzyme.

Hence, these compounds can act as potential non-nucleoside inhibitors of COVID-19 infection and warrant *in vitro* and *in vivo* testing to validate these findings.

The compounds identified in the study by multifold computational strategy can be validated *in vitro* as potential non-nucleoside inhibitors of SARS-CoV-2 RdRp and holds promise for the discovery of novel drugs against COVID-19 in future.

Aldo E. Elfiky from Biophysics Department, Faculty of Science, Cairo University, Egypt and colleagues from other academic institutions, published on December 27, 2023 “Novel sofosbuvir derivatives

against SARS-CoV-2 RNA-dependent RNA polymerase: an *in silico* perspective"[42].

The work combines molecular docking and dynamics simulation (MDS) to test 14 sofosbuvir-based modifications against SARS-CoV-2 RdRp.

Sofosbuvir, a uridine nucleotide analog that potently inhibits viral polymerase, has been found to help treat SARS-CoV-2 patients[43].

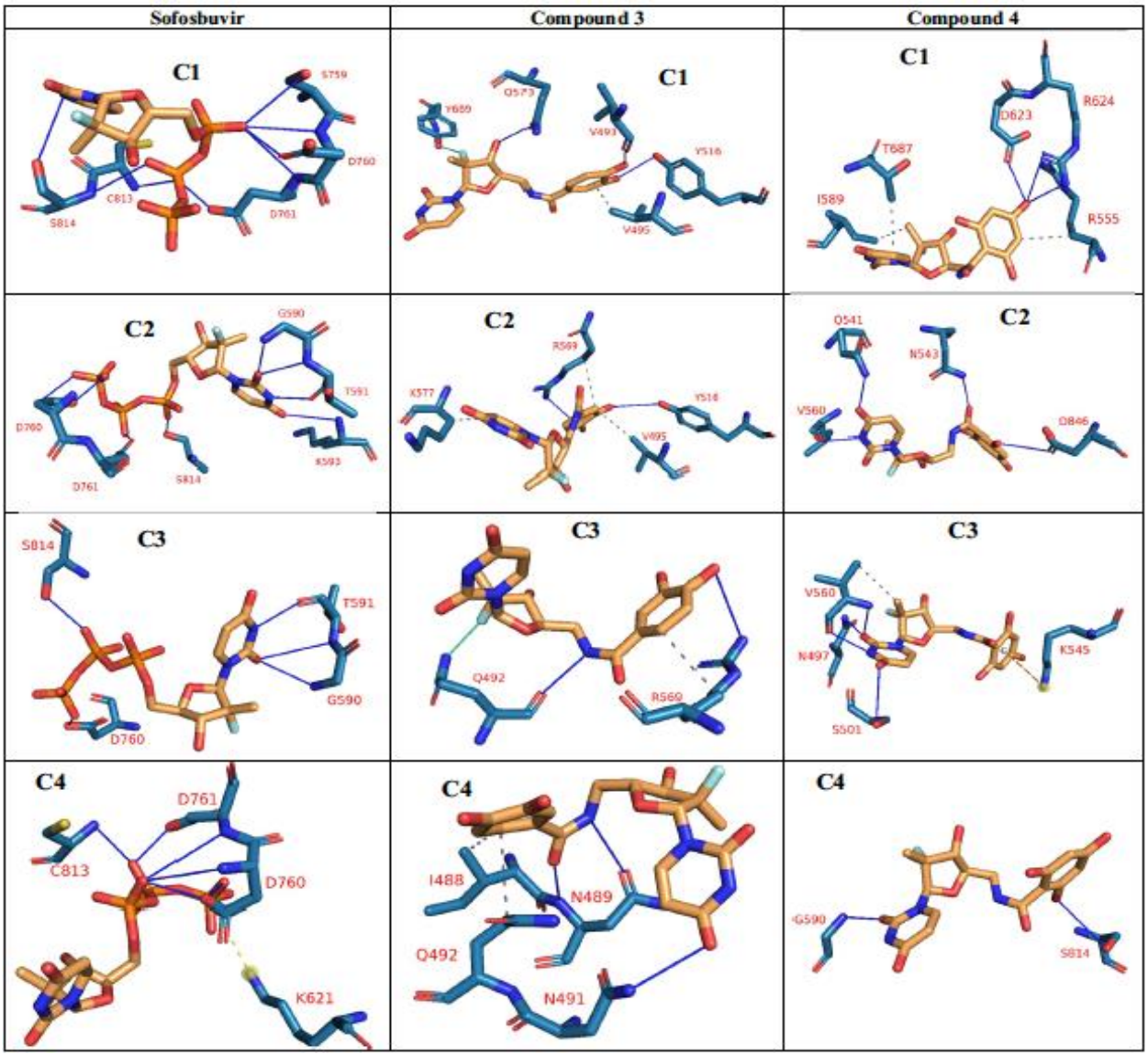
The study proposes compounds 3 and 4 as potential SARS-CoV-2 RdRp blockers, although this has yet to be proven experimentally.

The **Figure** shows the interactions detected using PILP webserver[44] and then depicted using PyMOL 2.0.4 software[45] on the representative frames obtained from the clustering of each trajectory after MDS.

The results reveal comparable (slightly better) average binding affinity of five modifications (compounds 3, 4, 11, 12, and 14) to the parent molecule, sofosbuvir.

Compounds 3 and 4 show the best average binding affinities against SARS-CoV-2 RdRp (-16.28 ± 5.69 and -16.25 ± 5.78 kcal/mol average binding energy compared to -16.20 ± 6.35 kcal/mol for sofosbuvir) calculated by Molecular Mechanics Generalized Born Surface Area (MM-GBSA) after MDS[37].

Figure. Interactions detected using PILP webserver and depicted using PyMOL 2.0.4.



H-bonds are shown in blue lines, while hydrophobic contacts are shown in dashed-grey lines.

Additionally, the molecular dynamics simulation revealed the effectiveness of compounds 3 and 4 as the best RdRp binders.

Compounds 3 and 4, form stable complexes with SARS-CoV-2 RdRp mainly through V415, V477, R489, and Q493 with binding energies of -16.28 and -16.25 respectively.

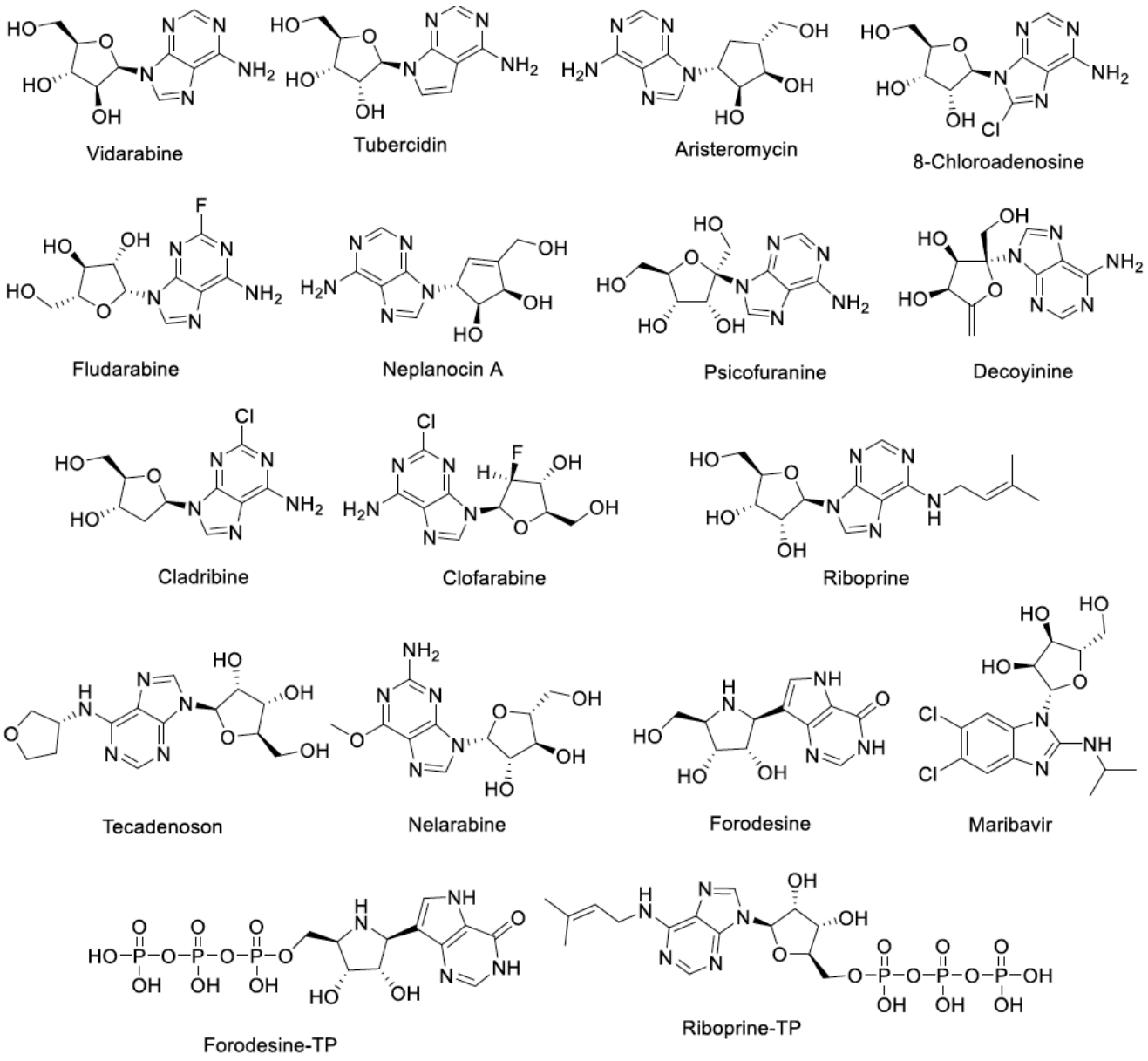
This study paves the way for the laboratory verification of for novel SARS-CoV-2 RdRp blockers.

Siva S. Panda, Adel S. Girgis from Department of Chemistry and Physics, Augusta University, USA, and Mohamed S. Bekheit from Department of Pesticide Chemistry, National Research Centre, Giza, Egypt, publishes the Review "Potential RNA-dependent RNA polymerase (RdRp) inhibitors as prospective drug candidates for SARS-CoV-2"[46].

This study highlights the most promising SARS-CoV-2 RdRp repurposed drugs in addition to natural and synthetic agents.

The **Figure** shows the structure of promising nucleosides as anti-RdRNAP, anti-ExoN, and anti-SARS-CoV-2 relative to Riboprine-TP and Forodesine-TP[47].

Figure. structure of promising nucleosides as anti-RdRNAP, anti-ExoN, and anti-SARS-CoV-2 relative to Riboprine-TP and Forodesine-TP.



Although many *in silico* predicted agents have been developed, the lack of *in vitro* and *in vivo* experimental data has hindered their application in drug discovery programs48,49.

The development of efficient anti-SARS-CoV-2 drugs over a short time is associated with many challenges, considerable obstacles, and unknown difficulties.

Among various modern approaches to adopt for developing potential drug candidates for SARS-CoV-2, rational drug design and drug repurposing strategies are key50,51.

Some drugs have been identified as anti-SARS-CoV-2 RdRp accessible for mild and weak infectious patients.

Numerous natural and synthetic molecules have also been investigated.

However, drug candidates with higher potency are still in demand.

In silico studies have been exploring the most in the search for potential drug candidates but without supporting *in vitro* and *in vivo* observations their accessibilities will be hindered for any drug discovery program[52-54].

The authors believe the compiled information will develop an interest in this field among the research community and provide them with a deeper understanding of the requirements and importance of chemical scaffolds for designing potential RdRp inhibitors for SARS-CoV-2.

Mingjia Yu and colleagues from Key Laboratory of Medical Molecule Science and Pharmaceutical Engineering, Ministry of Industry and Information Technology, School of Chemistry and Chemical Engineering, Beijing Institute of Technology, China, publishes "Structure-Based Drug Design of RdRp Inhibitors against SARS-CoV-2"[55].

In this article, the authors explore the inhibition of RdRp as a potential treatment for viral diseases, analysing the structural information of RdRp in virus proliferation and summarizing the reported inhibitor's pharmacophore features and structure–activity relationship profiles.

Although pharmacophore modelling and docking studies have successfully recognized remdesivir as an effective inhibitor of the RdRp enzyme, clinical trials are necessary to confirm these findings[56].

Apart from remdesivir, several nucleotides like ribavirin, EIDD-2801, favipiravir and galidesivir have shown efficacy in blocking SARS-CoV-2 replication.

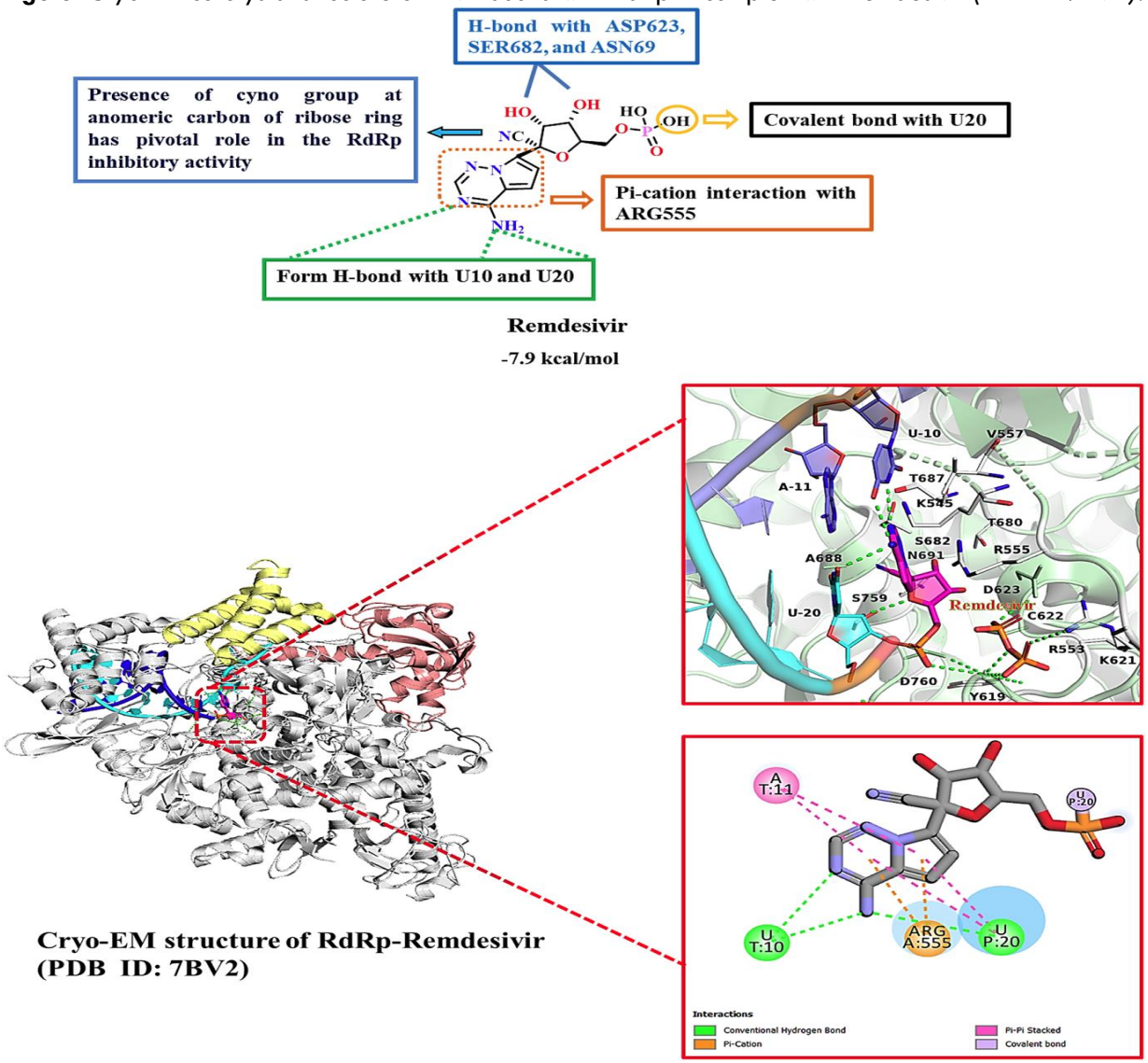
Additionally, some FDA-approved small molecules for clinical use have demonstrated effectiveness against SARS-CoV-2[57].

This review summarizes existing antiviral drugs and their structure–activity relationship and comparing or incorporating their characteristics is crucial to optimize or to design more effective drugs in the future.

The authors hope that the information provided by this review will aid in structure-based drug design and aid in the global fight against SARS-CoV-2 infection.

The **Figure** shows the Cryo-EM co-crystal structure of RNA bound with RdRp in complex with remdesivir (PDB ID: 7BV2).

Figure. Cryo-EM co-crystal structure of RNA bound with RdRp in complex with remdesivir (PDB ID: 7BV2).



The close-up view of RMP covalently bound with the catalytic site of RdRp primer (using PyMol software) and two-dimensional interaction analysis using BIOVIA Discovery Studio Visualizer v4.5.[58]

Eslam B. Elkaeed from Department of Pharmaceutical Sciences, College of Pharmacy, AlMaarefa University, Riyadh, Saudi Arabia, and colleagues from otrer academic institutions publishes "Computer-assisted drug discovery of potential natural inhibitors of the SARS-CoV-2 RNA-dependent RNA polymerase through a multi-phase in silico approach"[59].

The objective of the study was to identify the most effective natural inhibitors of SARS-CoV-2 RdRp among a set of 4,924 African natural products using a multi-phase *in silico* approach[60].

The study utilized remdesivir triphosphate (RTP), the co-crystallized ligand of RdRp, as a starting point to select compounds that have the most similar chemical structures among the examined set of compounds.

Molecular fingerprints and structure similarity studies were carried out in the first part of the study.

The second part included molecular docking against SARS-CoV-2 RdRp (PDB ID: 7BV2) and Molecular Dynamics (MD) simulations including the calculation of RMSD, RMSF, Rg, SASA, hydrogen bonding, and PLIP[61].

Moreover, the calculations of Molecular Mechanics with generalised Born and surface area solvation (MM-GBSA) Lennard-Jones and Columbic electrostatic interaction energies has been conducted[62].

Additionally, *in silico* ADMET and toxicity studies were performed to examine the drug likeness degrees of the selected compounds.

Molecular docking for the chosen 34 compounds preferred 10 candidates was performed, followingly, ADMET study excluded a compound.

The study depended on a multi-phase *in silico* approach to select the most structurally similar compounds to RTP, the co-crystallized ligand of the RdRp employing a molecular fingerprints and structure similarity studies.

Finally, MD simulation studies verified the correct binding of kaempferol 3-O-*β*-D-glucopyranoside against RdRp for 100 ns.

The study exposes eight compounds as the most effective natural inhibitors of SARS-CoV-2 RdRp (PDB ID: 7BV2).

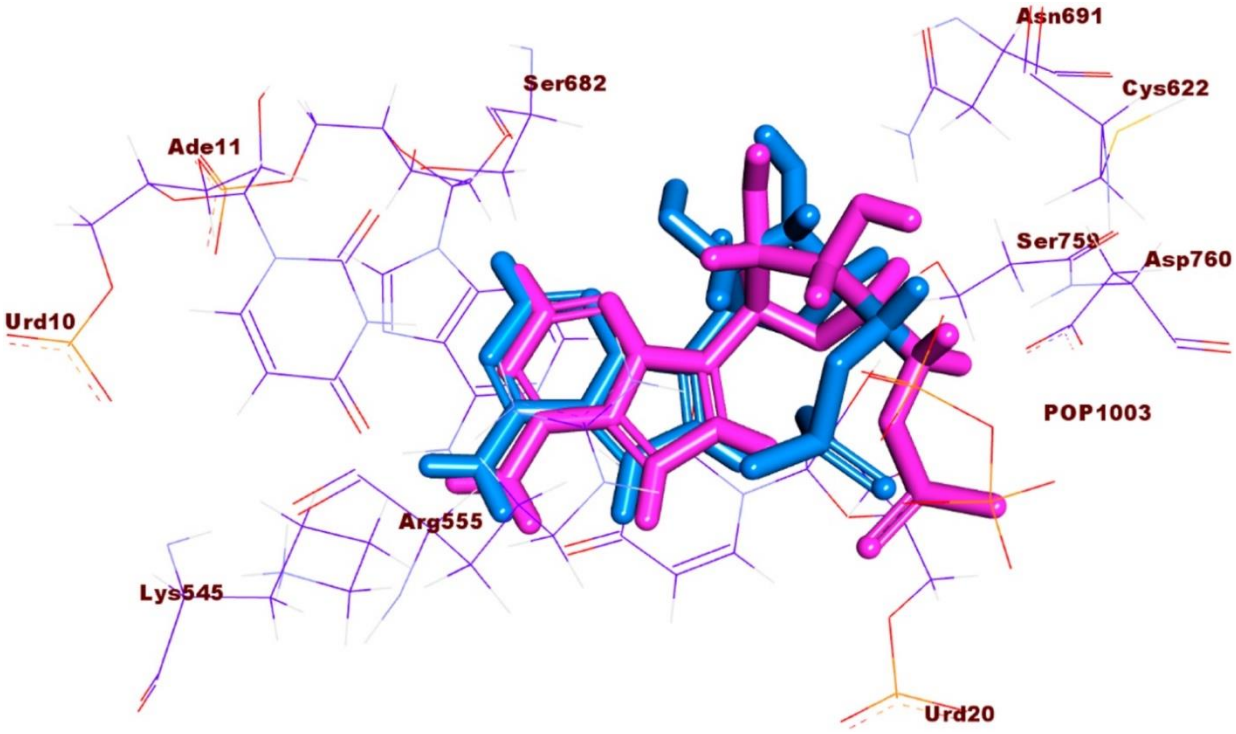
These compounds are kaempferol 3-galactoside, kaempferol 3-O-*β*-D-glucopyranoside, mangiferin methyl ether, luteolin 7-O-*β*-4C1-D-glucopyranoside, quercetin-3-glucopyranoside, 1-methoxy-3-indolylmethyl glucosinolate, naringenin, and asphodelin A 4'-O-*β*-D-glucopyranoside) were decided as the most potent inhibitors against SARS-CoV-2 RdRp.

The outputted results are a solid base to conduct further *in vitro* and *in vivo* studies on the preferred compounds to find a cure against COVID-19.

The results of the research provide valuable information for the development of natural product-based drugs against COVID-19.

However, the elected compounds should be further studied *in vitro* and *in vivo* to confirm their efficacy in treating COVID-19.

The Figure shows the Superimposition of the co-crystallized pose (purple) and the re-docking pose (blue) of Remdesivir triphosphate (RTP) indicate nearly the same binding mode.



By re-docking the co-crystalized ligands into their complexed enzyme, the measured RMSD between the docked and co-crystallized ligands was less than 1.20 Å, suggesting that the docking method was valid.

Discovery Studio 4.5 visualizer was used to visualize the obtained docking results[58].

Alejandro Morales-Bayuelo from GENOMA Group, School of Medicine, University of Sinú, Cartagena, Colombia and Jesús Sánchez-Márquez from Physical Chemistry Department, School of Sciences, University of Cadiz, Spain, publish "New findings on ligand series used as SARS-CoV-2 virus inhibitors within the frameworks of molecular docking, molecular quantum similarity and chemical reactivity indices, Version 3"[63].

In the updated article, the authors aim to evaluate a number of ligands used as SARS-CoV-2 virus inhibitors to determine the suitability of them for potential COVID-19 treatment.

They selected a series of ligands used as SARS-CoV-2 virus inhibitors such as: abacavir, acyclovir, amprenavir, ascorbic acid vitamin C, azithromycin, baloxavir, boceprevir, cholecalciferol vitamin D, cidofovir, edoxudine, emtricitabine, hydroxychloroquine and remdesivir (**Figure**)[64].

These ligands were analyzed using molecular docking, molecular quantum similarity, and chemical reactivity indices defined within a conceptual density functional theory framework.

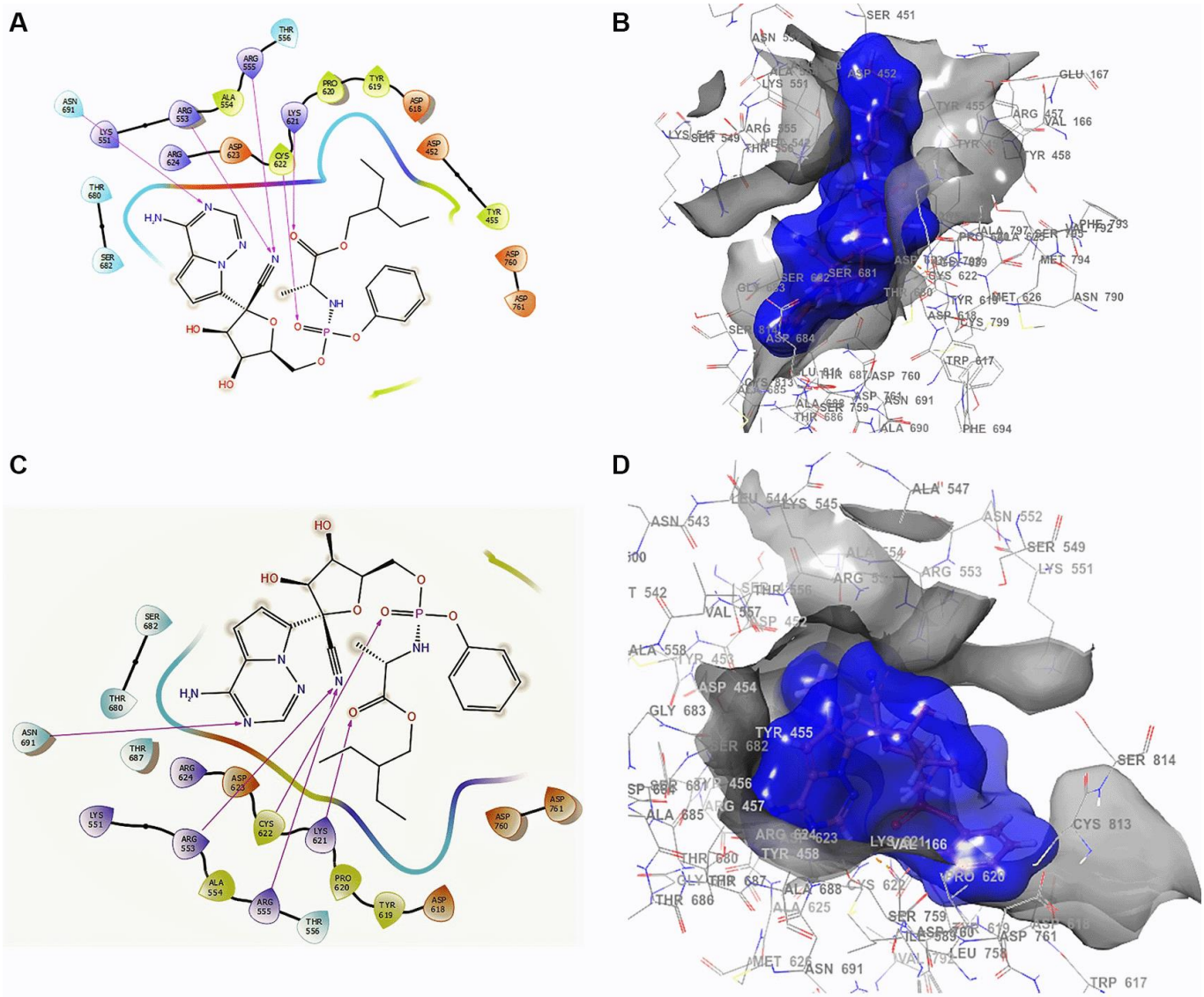
Global and local chemical reactivity indices were analyzed[65].

The analysis of molecular quantum similarity indices on inhibitors showed a high number of differences from a structural point of view.

However, they are quite similar in their electronic density, obtaining the highest values in the electronic similarity index.

Figure. Docking outcomes using remdesivir

A. Docking interactions using remdesivir with the crystal structure of SARS-CoV-2 RdRp (PDB accession number 6M71)[66].
B. Docking interactions using Receptor (gray) and ligand (blue) surfaces.
C. Docking interactions with the receptor site.
D. Surface of the binding pocket with the receptor site. SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; RdRp, RNA-dependent RNA polymerase; PDB, Protein Data Bank.



In the conformation of Figure A and B, the docking interaction for the structure of remdesivir with a higher RMSD shows an -H bond with ARG555, ARG553 with two lengths of 2.28 Å and a length of 2.35 Å, LYS621 with a length of 2.22 Å, CYS622 with a length of 2.42 Å, and ASN691 with a length of 2.38 Å.

As a conclusion, the authors pointed to that these studies allowed for the identification of the main stabilizing interactions using the crystal structure of SARS-CoV-2 RdRp.

The molecular quantum similarity and chemical reactivity descriptors provide novel insights into these ligands that can be used in the design of new COVID-19 treatments.

Radim Nencka and colleagues from Institute of Organic Chemistry and Biochemistry of the Czech Academy of Sciences, Prague, Czech Republic publishes "Novel analogues of a nonnucleoside SARS-CoV-2 RdRp inhibitor as potential antivirotics"[67].

In this study, the authors report three distinct modifications of HeE1-2Tyr: conversion of the core from a benzothiazole to a benzoxazole moiety and two different scaffold simplifications, respectively.

HeE1-2Tyr, a known inhibitor of flaviviral RdRp, has been discovered to also have antiviral potency against SARS-CoV-2.

HeE1-2Tyr (**1**) was originally identified by Tarantino *et al.* as a potent inhibitor of RdRp from all members of the genus *Orthoflavivirus* and was crystallized in complex with the RdRp from DENV-3[68].

They provide a novel synthetic approach and, in addition, evaluate the final molecules in an *in vitro* polymerase assay for biological activity.

Six of the novel compounds showed inhibitory activity in the fluorescence-based primer extension assay.

The two simplified molecules were the most promising inhibitors, with an IC50 value below 90µM, and the compounds 3a-c and 4a,b exerted stronger BEIs than 1 (**Figure 1**).

The authors focused on the modification of the central heterocyclic core and on the simplification and truncation of the relatively large molecule of HeE1-2Tyr (1).

Replacing the sulfur atom with a (bio)isosteric oxygen atom yielded two novel structural analogues, whilst our effort towards more simple molecules led to a series of pyridone derivatives.

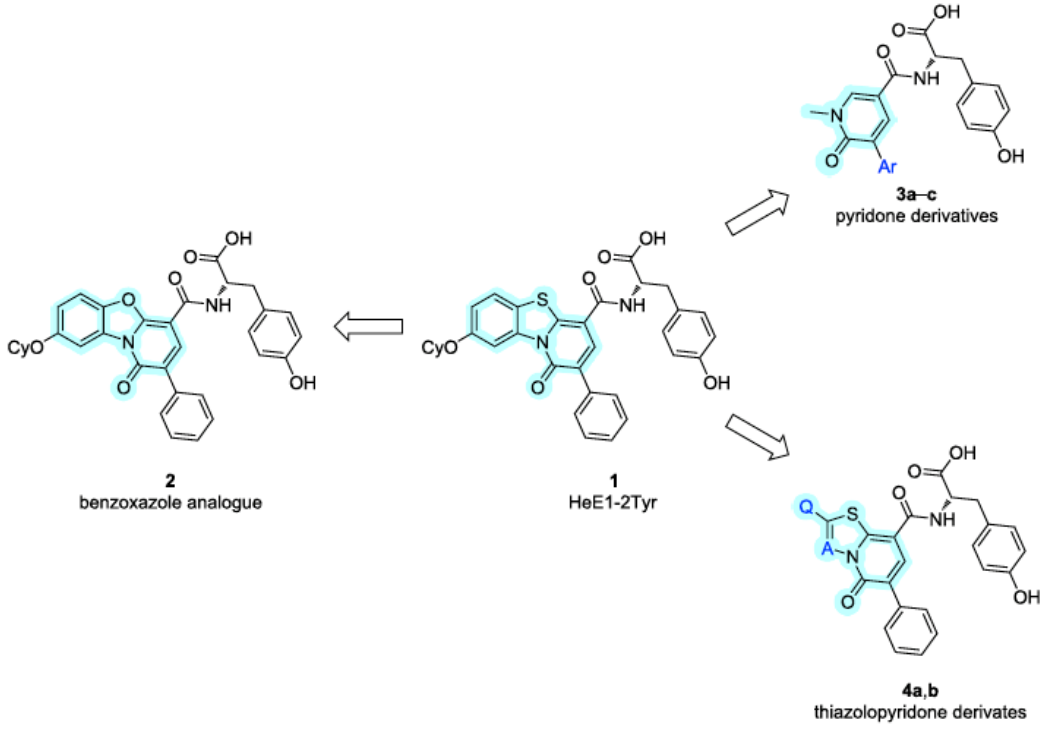
Out of these, **3a** had already been synthesized by a different approach[69].

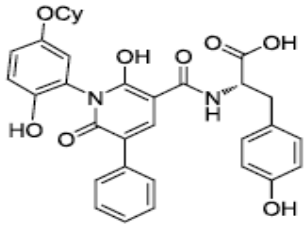
However, this study presents a novel and notably simpler synthetic route.

Furthermore, as part of the systematic truncation of the core, they synthesized thiazolopyridone and thiadiazolopyridone derivatives because molecules based on these cores have already shown promising antimicrobial activities[70,71].

They aimed to determine the inhibitory activity (IC50 values) of the final compounds 2, 16, 3a-c and 4a,b against SARS-CoV-2 RdRp.

Figure 1. Structure of HeE1-2Tyr (1) and of the derivatives synthesized in this work





16

In the Figure 2, the analysis of inhibitory activity against SARS-CoV-2 RdRp using primer extension assay is showed.

As a conclusion, in this study, novel analogues of the antiviral HeE1-2Tyr (**1**) were synthesized and evaluated with respect to the *in vitro* inhibitory activity towards SARS-CoV-2 RdRp.

To obtain the benzoxazole analogue, a new synthetic strategy avoiding base-mediated hydrolysis was successfully applied.

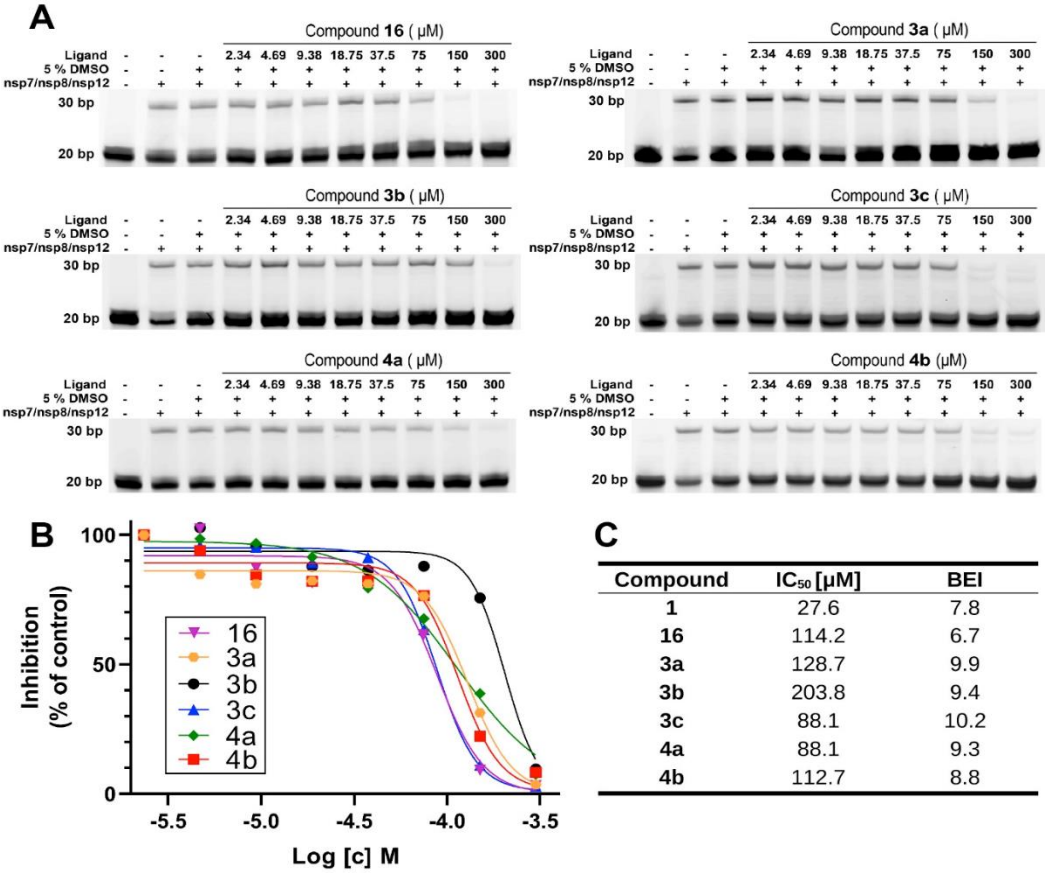
For the simplified structural derivatives, the applied routes were optimized for maximal efficacy of the synthetic work.

Regarding the inhibitory activity, six of the novel compounds showed inhibition in the fluorescence-based primer extension assay.

The two simplified molecules were the most promising inhibitors, with an IC_{50} value below 90 µM, and the compounds 3a-c and 4a,b exerted stronger BEIs than **1**.

The results provide important information about the structural requirements for the heterocyclic inhibitors based on HeE1-2Tyr (**1**), which can be used in the design of further generations of such antivirals.

Figure 2. Analysis of inhibitory activity against SARS-CoV-2 RdRp using primer extension assay



C

Compound	IC_{50} [µM]	BEI
1	27.6	7.8
16	114.2	6.7
3a	128.7	9.9
3b	203.8	9.4
3c	88.1	10.2
4a	88.1	9.3
4b	112.7	8.8

A) *Gel-based polymerase assay with a constant concentration of fluorescently labeled template/primer RNA (0.5 µM) and the polymerase complex (nsp7, nsp8 3 µM and nsp12 1 µM), along with increasing concentrations of compounds as indicated at the top. Reactions were initiated by adding 10 µM NTPs and run for 1 h at 30 °C. The reactions were stopped by adding stop buffer, and the products were separated on a 20% denaturing gel.*

B) *Graphical representation of the inhibitory activity of selected compounds evaluated from the gels obtained in the primer extension assay. The percentage of inhibition (against control) was plotted against the logarithm of the concentration of compounds. The results were fitted to sigmoidal dose–response curves.*

C) *The IC_{50} values were determined using the GraphPad algorithm (IC_{50} value of compound **1** was published by Dejmek et al.[72]), the BEI was calculated using the function pIC_{50} [mol/L]/MW [kDa].*

Matthias Götte and colleagues from Department of Medical Microbiology and Immunology, University of Alberta, Canada and Gilead Sciences, Inc., California, USA, publishes in *bioRxiv* on April 23, 2024, "Mechanism and spectrum of inhibition of a 4′-cyano modified nucleotide analog against diverse RNA polymerases of prototypic respiratory RNA viruses"[73].

The researches compare the mechanism and spectrum of inhibition of 1'-cyano modified C-adenosine with a newly discovered 4'-cyano modified C-adenosine against diverse RNA polymerases of prototypic respiratory RNA viruses.

In this study, among others, compared the biochemical properties of the active 5′-trisphosphate form of GS-7682, referred to as GS-646939, with GS-443902 against an array of RdRp enzymes representing multiple families of respiratory viruses[74].

Highly efficient rates of GS-646939 incorporation are seen with picornaviruses HRV-16 and EV-71 RdRp and, to a lesser extent, with pneumoviruses RSV and HMPV RdRp.

In contrast to GS-443902, inhibition with GS-646939 is based on immediate chain-termination at position "i".

Overall, the biochemical evaluation of the two nucleotide analogs against their targets provides mechanistic detail for the observed antiviral effects.

Subtle variations in the active site landscape seem to govern the specificity of inhibitor incorporation.

For HRV-16, EV-71, SARS-CoV-2, MERS-CoV, RSV, HMPV, PIV-5 and HPIV-3, both the 1'-cyano of GS-443902 and the 4'-cyano of GS-646939 are tolerated to varying degrees.

As previously described, the 1'-cyano of GS-443902 is particularly well positioned in the coronavirus active site, occupying a uniquely polar pocket defined by Thr-687, Asn-691 and Ser-759 in SARS-CoV-2, and similarly for MERS-CoV[75].

For GS-646939, the 4′-cyano appears to have a favorable interaction with Asn-296 of HRV-16 (**Figure A**).

The Motif C residue Gly-326 in HRV-16 allows for maximum flexibility in the 4' pocket, in contrast to the similar SARS-CoV-2 and MERS-CoV pocket, in which the corresponding residue is a serine (**Figure B**).

For RSV (**Figure C**) and HMPV, PIV-5 (**Figure D**) and HPIV-3, the 4'-cyano fits nicely in the pocket, but does't appear to convey any advantage.

In conclusion:
1'-cyano and 4'-cyano modified nucleotide analogs inhibit diverse RdRp enzymes of respiratory RNA viruses via different mechanisms.

Enzymes of the Coronaviridae (SARS-CoV-2 and MERS-CoV) and the Pneumoviridae (RSV and HMPV), are effectively targeted by the 1'-cyano modified C-adenosine GS-443902.

Selective incorporation and efficient template-dependent inhibition provide biochemical explanations for the observed antiviral activity.

Conclusions

Several RdRp inhibitors have shown promising results for their use in treating the SARS-CoV-2 virus.

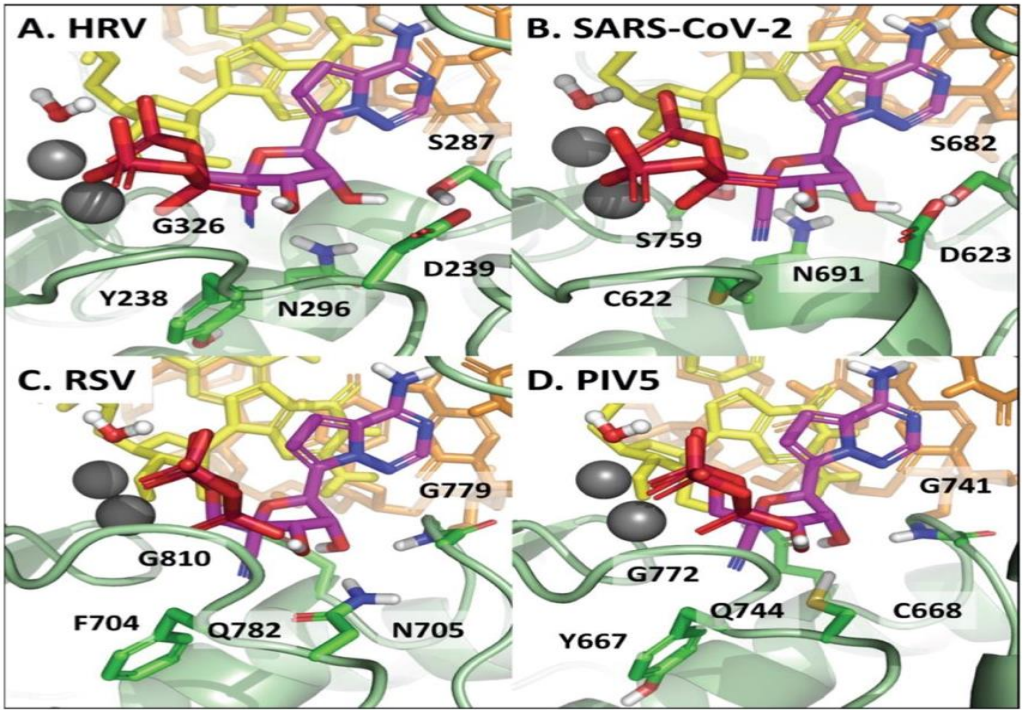
While work must still be conducted to fully understand the mechanisms responsible for reducing the antiviral activity of SARS-CoV-2, their potential in healing infected individuals is extremely valuable.

The development of SARS-CoV-2 RdRp inhibitors, to relieve the severity of an infection for a SARS-CoV-2 variants that could emerge in the near future, it is an essential task for the scientific community.

The analyses of inhibitors chemical structure-RdRp, besides the analyses of the inhibitors-RdRp interactions, it would guide the rational design of antiviral medications and research into SARS-CoV-2 transcriptional mechanisms.

This review summarizes recent progress in studies of RdRp inhibitors, 87 compounds was tested, focusing on the chemical structure of the inhibitors and the interactions between these inhibitors and the enzyme complex.

Figure. Models of GS-646939 in its pre-incorporated state for A, HRV, B, SARS-CoV-2, C, RSV, and D, PIV5.



Selectivity is largely driven by the nature of the 4' pocket and the specifics of how the 2'-OH is recognized by the polymerase. HRV and SARS-CoV-2 have a similar overall active site structure, differing primarily at Gly-326/Ser-759 and Tyr-238/Cys-622. While somewhat different from HRV and SARS-CoV-2, RSV and PIV5 also have a similar overall structure, differing primarily at Phe-704/Tyr-667 and Asn-705/Cys-668. In each case, the 4'-cyano of GS-646939 is at least tolerated. The interaction between the 4'-cyano and Asn-296 in HRV appears to be particularly ideal and is likely responsible for the inhibitor's increased affinity compared to ATP.

References

1. Hillen H, Kokic, G, Farnung L. *et al*. Structure of replicating SARS-CoV-2 polymerase. *Nature*. 2020;*584*:54-156. https://www.nature.com/articles/s41586-020-2368-8/
2. Gao Y, Yan L, Huang Y. *et al*. Structure of the RNA-dependent RNA polymerase from COVID-19 virus. *Science*. 2020;368:779–782. https://doi.org/10.1126/science.abb7498/
3. Ahn D, Choi J, Taylor D. *et al*. Biochemical characterization of a recombinant SARS coronavirus nsp12 RNA-dependent RNA polymerase capable of copying viral RNA templates. *Arch. Virol*. 2012;157:2095–2104. https://doi.org/10.1007/s00705-012-1404-x/
4. Kirchdoerfer R, Ward A. Structure of the SARS-CoV nsp12 polymerase bound to nsp7 and nsp8 co-factors. *Nat. Commun*. 2019;10:2342. https://doi.org/10.1038/s41467-019-10280-3/
5. Yadav A, Kumar Sh, Maurya Sh. *et al*. Interface design of SARS-CoV-2 symmetrical nsp7 dimer and machine learning-guided nsp7 sequence prediction reveals physicochemical properties and hotspots for nsp7 stability, adaptation, and therapeutic design. *Phys. Chem. Chem. Phys*. Advance Article. 2024;26:14046-14061. https://pubs.rsc.org/en/content/articlelanding/2024/cp/d4cp01014k/
6. Metwally K, Abo-Dya N, Issa M. *et al*. The Unusual Architecture of RNA-Dependent RNA Polymerase (RdRp)'s Catalytic Chamber Provides a Potential Strategy for Combination Therapy against COVID-19. *Molecules*. 2023;28:2806. https://doi.org/10.3390/molecules28062806/
7. Yin W, Luan X, Li Z. *et al*. Structural basis for inhibition of the SARS-CoV-2 RNA polymerase by suramin. *Nat. Struct. Mol. Biol*. 2021;28:319-325. https://doi.org/10.1038/s41594-021-00570-0/
8. Kloostra S. *Sitemap. Joomla! 3 SEO and Performance*; Apress: New York, NY, USA, 2015. https://www.amazon.com/Joomla-SEO-Performance-Simon-Kloostra/dp/1484211251/
9. Wu J, Chen Zh, Han X. *et al*. SARS-CoV-2 RNA-dependent RNA polymerase as a target for high-throughput drug screening. *Future Virol*. 2023;18(1):51-62. https://www.futuremedicine.com/doi/10.2217/fvl-2021-0335/
10. Cheng A, Zhang W, Xie Y. *et al*. Expression, purification, and characterization of SARS coronavirus RNA polymerase. Virology. 2005;335(2):165–176. https://pubmed.ncbi.nlm.nih.gov/15840516/
11. Velthuis A, Arnold J, Cameron C. *et al*. The RNA polymerase activity of SARS-coronavirus nsp12 is primer dependent. *Nucleic Acids Res*. 2010;38(1):203–214. https://pubmed.ncbi.nlm.nih.gov/19875418/
12. Yin W, Mao Ch, Luan X. *et al*. Structural basis for inhibition of the RNA-dependent RNA polymerase from SARS-CoV-2 by remdesivir. *Science*. 2020;368(6498):1499-1504. https://pubmed.ncbi.nlm.nih.gov/32358203/
13. Woods W, Umansky T, Ramesh N. *et al*. Discovery of RdRp Thumb-1 as a novel broad-spectrum antiviral family of targets and MDL-001 as a potent broad-spectrum inhibitor thereof - Part I: A Bioinformatics and Deep Learning Approach. *bioRxiv*. 2024;03.29:1-19. https://doi.org/10.1101/2024.03.29.587401/
14. Eltahla A, Luciani F, White P. *et al*. Inhibitors of the Hepatitis C Virus Polymerase; Mode of Action and Resistance. *Viruses*. 2015;***7***(10): 520624. https://pubmed.ncbi.nlm.nih.gov/26426038/
15. Lesburg C, Cable M, Ferrari, E. *et al*. Crystal structure of the RNA-dependent RNA polymerase from hepatitis C virus reveals a fully encircled active site. *Nat Struct Mol Biol*. 1999;6:937–943. https://pubmed.ncbi.nlm.nih.gov/26426038/
16. van der Maaten L, Hinton G. Visualizing Data using t-SNE. *Journal of Machine Learning Research*. 2008;9:2579-2605. https://jmlr.org/papers/volume9/vandermaaten08a/vandermaaten08a.pdf/
17. Nguyen L. Model medicines' oral anti-covid-19 drug candidate MDL-001 found to significantly reduce viral load in lungs; accepted into NIH's Antiviral Program for Pandemics. *Model Medicines*. 2023;12:20. https://www.modelmedicines.com/newsroom/mdl-001-viral-load-covid19/
18. Xu T, Zhang L. Current understanding of nucleoside analogs inhibiting the SARS-CoV-2 RNA-dependent RNA polymerase. *Comput Struct Biotechnol J*. 2023;21:4385-4394. https://doi.org/10.1016/j.csbj.2023.09.001/
19. Wu J, Chen Z, Han X. *et al*. SARS-CoV-2 RNA-dependent RNA polymerase as a target for high-throughput drug screening. *Future Virol*. 2023;18:1. https://doi.org/10.2217/fvl-2021-0335/
20. Gong P. Within and beyond the nucleotide addition cycle of viral RNA-dependent RNA polymerases. *Front Mol Biosci*. 2022;10:1. https://doi.org/10.3389/fmolb.2021.822218/

21. Malone B, Perry J, Olinares P. *et al*. Structural basis for substrate selection by the SARS-CoV-2 replicase. *Nature*. 2023;614:781-787. https://doi.org/10.1038/s41586-022-05664-3/
22. Johnson K, Dangerfield T. Mechanisms of inhibition of viral RNA replication by nucleotide analogs. *Enzymes*. 2021;49:39-62. https://doi.org/10.1016/bs.enz.2021.07.001/
23. Bravo J, Dangerfield T, Taylor D. *et al*. Remdesivir is a delayed translocation inhibitor of SARS-CoV-2 replication. *Mol Cell*. 2021;81:1548-1552. https://doi.org/10.1016/j.molcel.2021.01.035/
24. Gordon C, Lee H, Tchesnokov E. *et al*. Efficient incorporation and template-dependent polymerase inhibition are major determinants for the broad-spectrum antiviral activity of remdesivir. *J Biol Chem*. 2022;298:2. https://doi.org/10.1016/j.jbc.2021.101529/
25. Khan S, Attar F, Bloukh S. *et al*. A review on the interaction of nucleoside analogues with SARS-CoV-2 RNA dependent RNA polymerase. *Int J Biol Macromol*. 2021;181:605-611. https://doi.org/10.1016/j.ijbiomac.2021.03.112/
26. Biteau N, Amichai S, Azadi N. *et al*. Synthesis of 4'-substituted carbocyclic uracil derivatives and their monophosphate prodrugs as potential antiviral agents. *Viruses*. 2023;15:544. https://www.mdpi.com/1999-4915/15/2/544/
27. Schultz D, Johnson R, Ayyanathan K. *et al*. Pyrimidine inhibitors synergize with nucleoside analogues to block SARS-CoV-2. *Nature*. 2022;604:134-140. https://doi.org/10.1038/s41586-022-04482-x/
28. Zhao J, Guo S, Yi D. *et al*. A cell-based assay to discover inhibitors of SARS-CoV-2 RNA dependent RNA polymerase. *Antivir Res*. 2021;190:105078. https://doi.org/10.1016/j.antiviral.2021.105078/
29. Gana A, Baraniuk J. Identification of a New Drug Binding Site in the RNA-Dependent-RNA-Polymerase (RdRp) Domain. *BioMedInformatics*. 2023;3:885-907. https://doi.org/10.3390/biomedinformatics3040055/
30. Piplani S, Singh P, Winkler D. *et al*. Computationally repurposed drugs and natural products against RNA dependent RNA polymerase as potential COVID-19 therapies. *Mol. Biomed*. 2021;2:28. https://doi.org/10.1186/s43556-021-00050-3/
31. Malone B, Perry J, Olinares P. *et al*. Structural basis for substrate selection by the SARS-CoV-2 replicase. *Nature*. 2023;*614*:781-87. https://doi.org/10.1038/s41586-022-05664-3/
32. Peersen O. A Comprehensive Superposition of Viral Polymerase Structures. *Viruses*. 2019;11:*745*. https://doi.org/10.3390/v11080745/
33. Rehman H, Sajjad M, Akhtar M. *et al*. Identification of RdRp inhibitors against SARS-CoV-2 through E-pharmacophore-based virtual screening, molecular docking and MD simulations approaches. *International Journal of Biological Macromolecules*. 2023;237:124169. https://doi.org/10.1016/j.ijbiomac.2023.124169/
34. Shivanyuk A, Ryabukhin S, Bogolyubsky A. *et al*. Chimica Oggi-Chemistry Today. 2007;25:6. https://www.researchgate.net/publication/287001659/
35. Rather M, Dutta S, Guttula P. *et al*. Structural analysis, molecular docking and molecular dynamics simulations of G-protein-coupled receptor (kisspeptin) in fish, *J. Biomol. Struct. Dyn*. 2020;38:2422-2439. https://doi.org/10.1080/07391102.2019.1633407/
36. Ali J, Aldahham B, Rabeea M. *et al*. Synthesis, characterization and in-silico assessment of novel thiazolidinone derivatives for cyclin-dependent kinases-2 inhibitors. *Journal of Molecular Structure*. 2021;1223:129311. https://doi.org/10.1016/j.molstruc.2020.129311/
37. Genheden S, Ryde U. The MM/PBSA and MM/GBSA methods to estimate ligand-binding affinities. *Expert Opin Drug Discov*. 2015;10(5):449-61. https://doi.org/10.1517/17460441.2015.1032936/
38. Opo F, Rahman M, Ahammad F. *et al*. Structure based pharmacophore modeling, virtual screening, molecular docking and ADMET approaches for identification of natural anti-cancer agents targeting XIAP protein. *Sci Rep*. 2021;11:4049. https://doi.org/10.1038/s41598-021-83626-x/
39. Aziz S, Wagas M, Kumar T. *et al*. Identifying non-nucleoside inhibitors of RNA-dependent RNA-polymerase of SARS-CoV-2 through per-residue energy decomposition-based pharmacophore modeling, molecular docking, and molecular dynamics simulation. *Journal of Infection and Public Health*. 2023;16(4):501-519. https://doi.org/10.1016/j.jiph.2023.02.009/

40. Naqvi A, Mohammad T, Hasan G. *et al.* Advancements in docking and molecular dynamics simulations towards ligand-receptor Interactions and Structure-function Relationships. *Curr Top Med Chem.* 2019;18:1755-68. https://doi.org/10.2174/1568026618666181025114157/
41. Isaac T, Quadry O, Ooreoluwa T. *et al.* Molecular Mechanics with Generalized Born Surface Area (MMGBSA) Calculations and Docking Studies Unravel some Antimalarial Compounds Using Heme O Synthase as Therapeutic Target. *Chemistry Europe.* 2023;8:48. https://doi.org/10.1002/slct.202303686/
42. Alrehaily A, Elfiky A, Ibrahim I. *et al.* Novel sofosbuvir derivatives against SARS-CoV-2 RNA-dependent RNA polymerase: an *in silico* perspective. *Sci Rep.* 2023;13:23080. https://doi.org/10.1038/s41598-023-49712-y/
43. Roozbeh F, Saeedi M, Alizadeh-Navaei, R. *et al.* Sofosbuvir and daclatasvir for the treatment of COVID-19 outpatients: A double-blind, randomized controlled trial. *J. Antimicrob. Chemother.* 2021;76(3):753-757. https://doi.org/10.1093/jac/dkaa501/
44. https://plip-tool.biotec.Tu-dresden.de/plipweb/plip/index/
45. https://www.schrodinger.com/platform/products/pymol/
46. Bekheit M, Panda S, Girgis A. *et al.* Potential RNA-dependent RNA polymerase (RdRp) inhibitors as prospective drug candidates for SARS-CoV-2. *European Journal of Medicinal Chemistry.* 2023;252:115292. https://doi.org/10.1016/j.ejmech.2023.115292/
47. Newman D, Cragg G. Natural products as sources of new drugs from 1981 to 2014. *J. Nat. Prod.* 2016;79:629-661. https://doi:10.1021/acs.jnatprod.5b01055/
48. De Clercq E, Li G. Approved antiviral drugs over the past 50 years. *Clin. Microbiol. Rev.* 2016;29:695i747. https://doi.org/10.1128/cmr.00102-15/
49. Girgis A, D'Arcy P, Aboshouk D. *et al.* Synth/esis and bio-properties of 4-piperidone containing compounds as curcumin mimics. *RSC Adv.* 2022;12:31102-31123. http://doi:10.1039/d2ra05518j/
50. Nelson K, Dahlin J, Bisson J. The essential medicinal chemistry of curcumin. *J. Med. Chem.* 2017;60:1620-1637. http://doi:10.1021/acs.jmedchem.6b00975/
51. Chakravarti R, Singh R, Ghosh A. *et al.* A review on potential of natural products in the management of COVID-19. *RSC Adv.* 2021;11:16711-16735. http://doi:10.1039/d1ra00644d/
52. Thomas E, Stewart L, Darley B. A.M. *et al.* Plant-based natural products and extracts: potential source to develop new antiviral drug candidates. *Molecules.* 2021;26:6197. https://www.mdpi.com/1420-3049/26/20/6197/
53. Owen L, Laird K, Shivkumar M. Antiviral plant-derived natural products to combat RNA viruses: targets throughout the viral life cycle. *Lett. Appl. Microbiol.* 2021;75:476-499. http://doi:10.1111/lam.13637/
54. Khater S, Kumar P, Dasgupta N. *et al.* Combining SARS-CoV-2 proofreading exonuclease and RNA-dependent RNA polymerase inhibitors as a strategy to combat COVID-19: a high-throughput *in silico* screening. *Front. Microbiol.* 2021;12:647693. https://doi:10.3389/fmicb.2021.64769/
55. Shehzadi K, Saba A, Yu M. *et al.* Structure-Based Drug Design of RdRp Inhibitors against SARS-CoV-2. *Top Curr Chem (Z).* 2023;381:22. https://doi.org/10.1007/s41061-023-00432-x/
56. Gordon C, Tchesnokov P, Feng J. *et al.* The antiviral compound remdesivir potently inhibits RNA-dependent RNA polymerase from Middle East respiratory syndrome coronavirus. *J Biol Chem.* 2020;295(15):4773-4779. https://doi.org/10.1074/jbc.AC120.013056/
57. Ahmed M, Farag A, Boys I. *et al.* FDA approved drugs with antiviral activity against SARS-CoV-2: From structure-based repurposing to host-specific mechanisms. *Biomed Pharmacother.* 2023;162:114614. https://doi.org/10.1016/j.biopha.2023.114614/
58. https://mybiosoftware.com/biovia-discovery-studio-visualizer-4-5-molecular-visualization.html/
59. Elkaeed E, Alsfouk B, Metwaly A. *et al.* Computer-assisted drug discovery of potential natural inhibitors of the SARS-CoV-2 RNA-dependent RNA polymerase through a multi-phase in silico approach. *Antiviral Therapy.* 2023;28(5). https://journals.sagepub.com/doi/10.1177/13596535231199838/
60. Elkaeed E, Elkady H, Belal A. *et al.* Multi-Phase In Silico Discovery of Potential SARS-CoV-2 RNA-Dependent RNA Polymerase Inhibitors among 3009 Clinical and FDA-Approved Related Drugs. *Processes.* 2022;10(3):530. https://doi.org/10.3390/pr10030530/

61. Amin S, Banerjee S, Singh S. *et al*. First structure-activity relationship analysis of SARS-CoV-2 virus main protease (Mpro) inhibitors: an endeavor on COVID-19 drug discovery. *Mol Divers*. 2021;25(3):1827–1838. https://doi.org/10.1007/s11030-020-10166-3/
62. Sartori G, Nascimento A. Comparative Analysis of Electrostatic Models for Ligand Docking. *Front Mol Biosci*. 2019;6:52. https://doi.org/10.3389/fmolb.2019.00052/
63. *Morales-Bayuelo A, Sánchez-Márquez J*. New findings on ligand series used as SARS-CoV-2 virus inhibitors within the frameworks of molecular docking, molecular quantum similarity and chemical reactivity indices, Version 3. *F1000Research*. 2023;11:914. Last updated: 13 APR 2024. https://f1000research.com/articles/11-914/v3/
64. Morales-Bayuelo A: New insights in series of ligands used as SARS-CoV-2 virus inhibitors within molecular docking, molecular quantum similarity, and chemical reactivity indices frameworks. *Harvard Dataverse, V1*. [Dataset].2022. https://doi.org/10.12688/F1000RESEARCH.123550.1/
65. Morales-Bayuelo A, Matute R, Caballero J. New Insights from the CoMSIA Analysis within the Framework of Density Functional Theory. *J Mol Model*. 2015;21:156. https://bit.ly/3GLDQLs/
66. Friesner R, Banks J, Murphy R. *et al*. Glide: A New Approach for Rapid, Accurate Docking and Scor- ing. 1. Method and Assessment of Docking Accuracy. *J. Med. Chem*. 2004;47:1739-1749. https://doi.org/10.1021/jm0306430/
67. Tóth L, Krejčová, K, Dejmek M. *et al*. Novel analogues of a nonnucleoside SARS-CoV-2 RdRp inhibitor as potential antivirotics. *Beilstein J. Org. Chem*. 2024;20:1029-1036. https://doi.org/10.3762/bjoc.20.91/
68. Tarantino D, Cannalire R, Mastrangelo E. *et al*. Targeting flavivirus RNA dependent RNA polymerase through a pyridobenzothiazole inhibitor. *Antiviral Res*. 2016;134:226-235. https://doi.org/10.1016/j.antiviral.2016.09.007/
69. Cannalire R, Chan K, Burali K. *et al*. Pyridobenzothiazolones exert potent anti-dengue activity by hampering multiple functions of NS5 polymerase. *ACS Med. Chem. Lett*. 2020;11:773-782. https://doi.org/10.1021/acsmedchemlett.9b00619/
70. Wardakhan W, El-Sayed N. New Approaches for the Synthesis of 1,3,4-Thiadiazole and 1,2,4-Triazole Derivatives with Antimicrobial Activity. *Phosphorus, Sulfur Silicon Relat. Elem*. 2009;184:790-804. https://doi.org/10.1080/10426500802274534/
71. Targowska-Duda K, Maj M, Drączkowski P. *et al*. WaterMap-Guided Structure-Based Virtual Screening for Acetylcholinesterase Inhibitors. *ChemMedChem*. 2022;17:e202100721. http://doi.org/10.1002/cmdc.202100721/
72. Dejmek M, Konkoľová E, Eyer L. *et al*. Non-Nucleotide RNA-Dependent RNA Polymerase Inhibitor That Blocks SARS-CoV-2 Replication. *Viruses*. 2021;13:1585. https://doi.org/10.3390/v13081585/
73. Gordon C, Walker S, Tchesnokov E. *et al*. Mechanism and spectrum of inhibition of a 4′-cyano modified nucleotide analog against diverse RNA polymerases of prototypic respiratory RNA viruses". *bioRxiv*. 2024. https://doi.org/10.1101/2024.04.22.590607/
74. Siegel D, Hui, H, Pitts J. et al. Discovery and Synthesis of GS-7682, a Novel Prodrug of a 4'-CN-4-Aza-7,9-Dideazaadenosine C-Nucleoside with Broad-Spectrum Potency Against Pneumo- and Picornaviruses and Efficacy in RSV-Infected African Green Monkeys. bioRxiv, 2024. https://doi.org/10.1101/2024.04.17.589937/
75. Stevens L, Pruijssers, A, Lee, H. *et al*. Mutations in the SARS-CoV-2 RNA-dependent RNA polymerase confer resistance to remdesivir by distinct mechanisms. *Science translational medicine*. 2022;14(656):eabo0718. https://doi.org/10.1126/scitranslmed.abo0718/