A PROJECT REPORT ON

IN-SILICO MOLECULAR DOCKING STUDIES FOR FDA APPROVED DRUGS AGAINST M-pro SARS-CoV-2

Submitted by

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JULY 2025

INTRODUCTION OF THE BIOTECH PARK

The biotech park has been set up acres of land provided by the Department of Science and Technology, Government of Uttar Pradesh. The thrust areas identified for the initial phase of the park are; Health care-Human and animal health care products including therapeutic aids, immunodiagnostics, biosensor, vaccines, gene therapy, monoclonal antibodies, nutraceuticals and cosmeceutical , Agriculture-Improvement in the quality and yield of crops, horticulture and forest tree species, biopesticides and biofertilizers, processed food and quality enhancers; Environment bioremediation, safe disposal of waste; Industrial Application-plants as bioreactors, enzymes, chemicals and polymers and energy-biofuels and renewable energy source.

Block-I: Bio-Business Block The Bio-Business Block houses the following:

1. Business Support Facilities
2. Bioinformatics center
3. Conference Hall
4. Cafeteria
5. Laboratory Space

Bioinformatics Centre

Bioinformatics Centre has been set up to establish a close network with various institutions and provide information to industries regarding technologies, facilities and expertise available with them in the area of biotechnology city. Bioinformatics center has been established as A state of-the-art hub for performing high-end computational. processing and houses a dedicated server with a heavy-duty processor to carry out elusive computational jobs. A computer facility with good quality processors has been

Block-II: Solvent Extraction Block: The solvent extraction block comprises:

1. Solvent extraction unit with solid-liquid solvent extraction and solvent recovery system for extraction
2. of photochemical/lead molecules from high-value medicinal plants.
3. S.S. high vacuum fractionating column unit.
4. Multipurpose reaction cum hydrolysis and solvent
5. recovery unit along with chromatography

Block-III: Biofertilizer, Tissue culture and Centre support Block:

1. Biofertilizer Unit-Ground Floor.
2. Plant Tissue culture -First floor.
3. Molecular and analytical laboratory- second floor

1. Biofertilizer unit:

This unit has facilities for perfecting the technology and production of bacterial fertilizers Phosphorous Solubilizing Bacteria (PSB), Azotobacterial (with a capacity of up to 500 tons/annum). The batch production has been started.

2. Tissue culture and Hardening facility

The tissue culture laboratory has a capacity of producing 10,000 to 100,000 plans per batch and can produce 2 million plants/ annum. The facility has a modern polycarbonate house for micropropagation and a hardening facility with net houses, and air-conditioned glass houses.

3. Molecular Biology and Analytical Facility

The facility is equipped with high-pressure liquid liquid chromatography (HPLC), Nano spectrophotometer polarimeter High pressure thin layer chromatography (HPTLC) and other support equipment. HPLC has a versatile functioning since it is equipped with UV/VIS, Fluorescence, PDA and Ion chromatography detectors. A wide range of metabolites or molecules can be quantitatively analyzed using HPLC and HPTLC. It also houses a common facility for storage.

Diagnostic Unit

This unit has been constructed in an area of about 60.f00 sq

Vermicomposting Unit

The Vermicomposting unit is the fastest and effective way of recycling of organic waste with the help of earthworms for the production of useful compost. To utilize the large quantity of agro-waste generated from distillation and solvent extraction units the Vermicomposting unit has been set up at the Park. The unit will serve as demonstration cum-training facility for the farmers. Facilities being operated under Public-Private Partnership mode Distillation & Vermicomposting unit, Bio fertilizers unit and Tissue culture and Hardening facilities are being run under Public-Private Partnership mode as given below: 1. Distillation and Vermicomposting Units M/s Next Gen - Bio fertilizers & Aroma Products, Lucknow 2. Bio fertilizers Unit M/s Next Gen - Bio fertilizers & Aroma Products, Lucknow 3. Tissue Culture and Hardening Facility M/s Sheel Biotech, Manesar, Haryana

Entrepreneurs in the Park:

1. Aqua Biochip Genomics (India) Pvt Ltd., Lucknow 2. Chandan Health Care Ltd., Lucknow 3. NISCO Agri Tech (Bangalore) Pvt. Ltd. 4. Life Care Innovations Pvt. Ltd., Gurgaon 5.PIRINCSPharmaceuticalsPvt.Ltd. 6. ABC Genomics (India) Pvt. Ltd. 7. Nextec Pvt. Ltd 8.Devaa biofuels, Indore 9. IQRA Biotech services, Lucknow

ACKNOWLEDGEMENT

I am very thankful and express my deepest gratitude to **Prof. J.P Pandey, CEO, Biotech Park**, Lucknow for providing me such a great opportunity of training at an institute with an esteemed status in the field of Biotechnology.

I would express deep gratefulness to **Dr. Deepika Yadav, Scientist, Bioinformatics Centre, Biotech Park** for her invaluable guidance and great support throughout the project. She has been extremely supportive and understanding throughout the project. Without her invaluable guidance and support this project would have not been possible.

I would say thanks to whole Bioinformatics team at Biotech Park for their help and cooperation. They all provided me a healthy working environment in Lab.

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ABSTRACT

A worldwide scientific reaction to the COVID-19 pandemic, which was brought on by SARS-CoV-2, is centred on finding efficient treatment medicines. Since the virus's primary protease (Mpro) is essential for breaking down viral polyproteins, it is a desirable target for medication. Using molecular docking experiments, this study investigates whether FDA-approved medications have the ability to inhibit Mpro. Twenty approved medications were chosen. Docking of the ligands against PDB structure 6LZE was done using PyRx. The binding energy and interaction profiles were evaluated by visualising and analysing the results using Discovery Studio. Nirmatrelvir, Remdesivir, Compoud 11a and Sofosbuvir are among the promising candidates that the study found to have a high binding affinity for the Mpro active site.

INTRODUCTION

What is SARS-CoV-2?

The positive-sense RNA virus that causes COVID-19, SARS-CoV-2, uses proteolytic enzymes to break down its polyproteins into useful pieces. The primary protease (Mpro), also known as 3CLpro, is a crucial component of viral replication and is a prime target for antiviral therapy because it does not have a near human counterpart.

The interest in repurposing FDA-approved medications to inhibit important viral proteins was sparked by the pandemic's urgency. In this regard, a quick and affordable way to assess drug-target interactions is through structure-based drug design with molecular docking. SARS-CoV-2 Mpro in complex with an investigational inhibitor (Compound 11a) is represented by PDB structure 6LZE, which offers a precise binding pocket for docking FDA-approved medications.

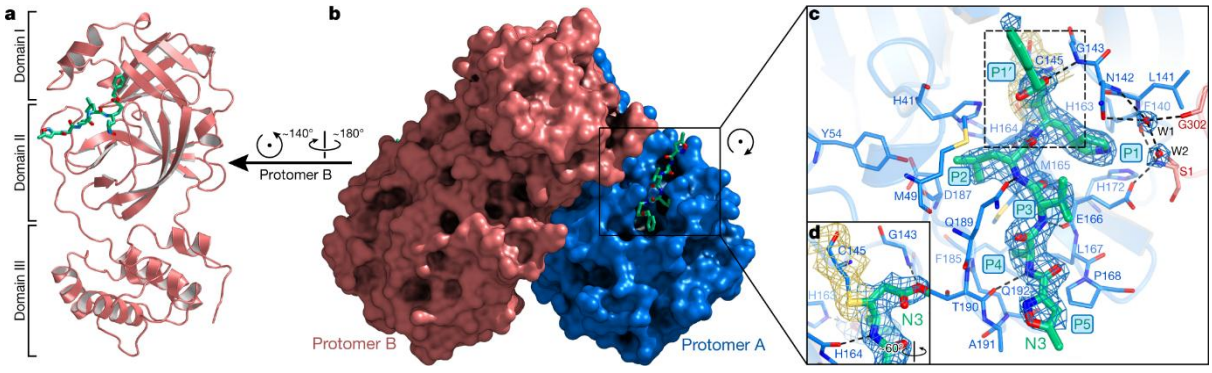


Fig.1. The crystal structure of SARS-CoV-2 Mpro in complex with N3

LITERATURE REVIEW

When SARS-CoV-2 first appeared in late 2019, structural biology studies to find potential treatment targets in the virus exploded. SARS-CoV-2 encodes a number of critical proteins required for transcription, replication, and host cell evasion, according to Wang et al. (2020). These consist of non-structural proteins such as RNA-dependent RNA polymerase (RdRp), papain-like protease (PLpro), and the major protease (Mpro or 3CLpro). Because of its conserved structure, lack of near human homologs, and crucial function in breaking down the viral polyprotein into functional components, the major protease (Mpro) has drawn the most attention of these.

According to the review, Mpro is crucial for viral replication because it cleaves the polyprotein at several different locations. Its catalytic mechanism centers on a catalytic dyad made up of Cys145 and His41, a characteristic that has made it a top target for inhibitor creation. The structure of Mpro coupled with several inhibitors, such as the well-known drug 11a (in PDB: 6LZE), has made it possible for researchers to thoroughly examine the active site of the enzyme.

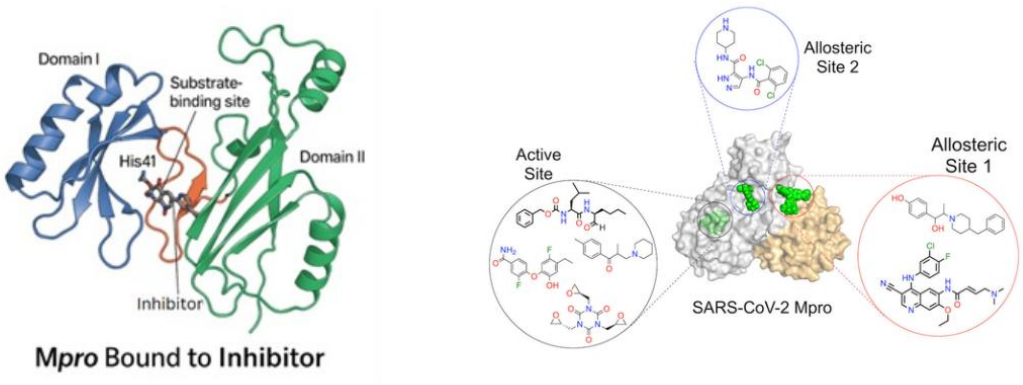


Fig.2. Mpro bound to Inhibitor

Additionally, Wang et al. examine how structure-based virtual screening and logical drug design have been made easier by the availability of high-resolution crystal structures, such as those in the Protein Data Bank (PDB). A variety of FDA-approved medications and investigational substances that exhibit inhibitory potential against SARS-CoV-2 targets are covered in the study, including in:

Another RdRp inhibitor that showed a modest interaction with Mpro was Remdesivir.

Nirmatrelvir: Paxlovid's active ingredient and a potent Mpro inhibitor.

Repurposed anticancer medication carmofur exhibits covalent interaction with Cys145.

Both PLpro and Mpro are covalently inhibited by Ebselen.

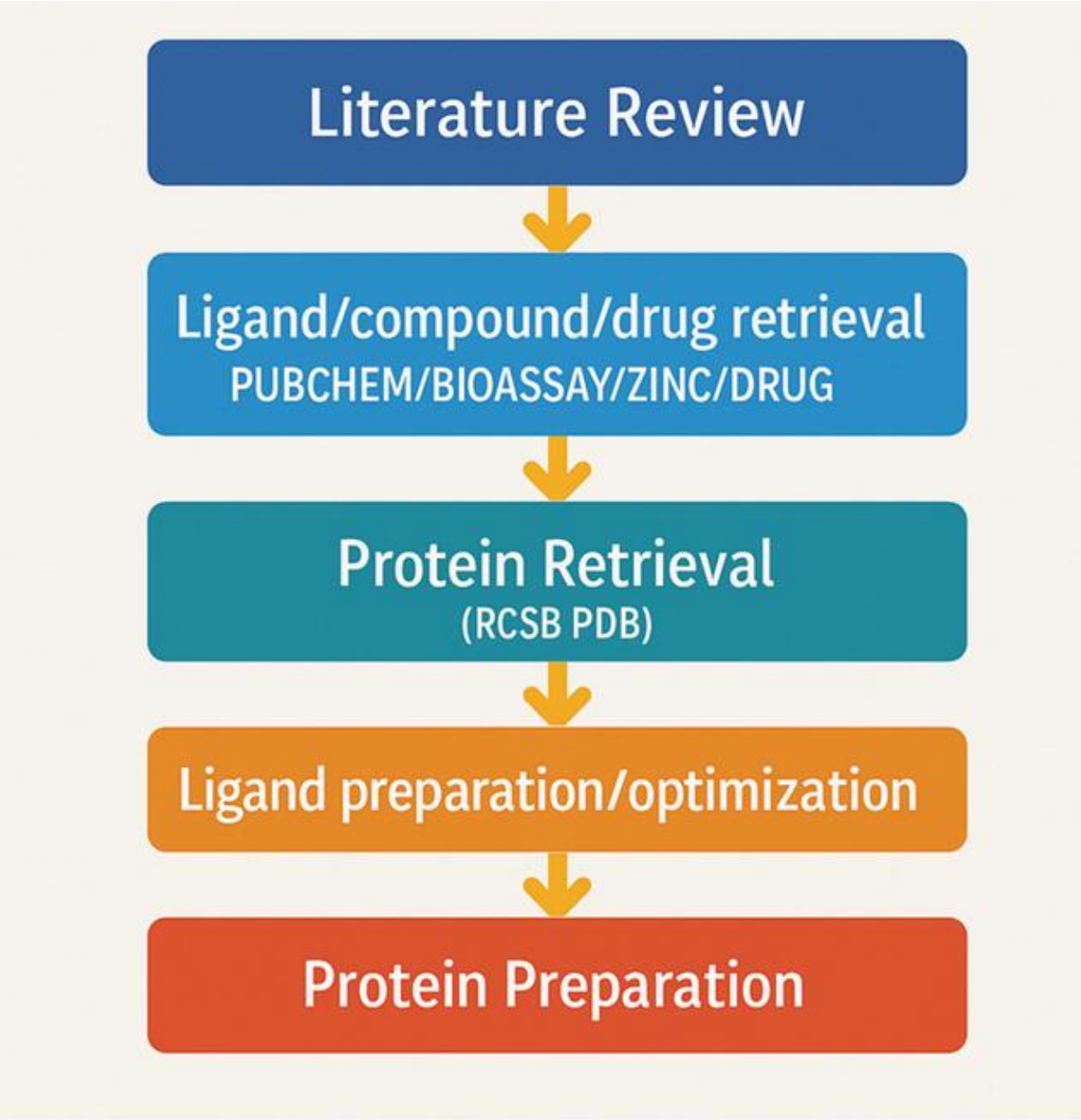
Disulfiram: A thiol-reactive substance that prevents viral cysteine proteases from working.

In vitro investigations have shown that boceprevir and GC-376, which were once protease inhibitors for other viruses, are efficient in inhibiting Mpro.

In silico docking is a quick and economical method for early-stage drug discovery, especially in pandemic situations where time is of the essence, according to the review. The binding affinities and interaction patterns of potential compounds can be predicted with the use of molecular docking in conjunction with programs such as PyRx, Discovery Studio etc. This facilitates the process of ranking compounds for validation in vitro.

The research addresses the structural plasticity of the Mpro active site and its implications for the creation of broad-spectrum antivirals, while also emphasizing prospective inhibitors. The current work, which uses molecular docking techniques to assess FDA-approved medications for possible interactions with the active site of Mpro (PDB: 6LZE), is based on these findings.

MATERIALS AND METHODS



SOFTWARE USED: PYRX

MOLECULAR DOCKING

A computer method called "**molecular docking**" is used to forecast the interactions between two molecules, usually a larger molecule (normally a protein) and a smaller molecule (ligand). It aids in comprehending molecular interactions and forecasts the orientation and binding affinity of ligands in the receptor's binding site.
The following are important phases in molecular docking:

- **STRUCTURE PREPARATION**: The ligand molecule is made by minimising its energy state and optimising its three-dimensional structure. In order to achieve a stable configuration, the receptor is prepared by eliminating the water molecules and co-factors and refining the receptor structure.

- **SELECTING A DOCKING METHOD**: Molecular docking simulations fall into one of the following categories:
  - Rigid Docking
  - Flexible Docking

- **SCORING FUNCTION**: After possible binding poses have been found, a scoring function is used to rank them. The degree of contact between the ligand and the receptor is estimated by the scoring function. This encompasses elements like:
  - Van der Waals forces
  - Electrostatic Interaction
  - Hydrogen Bond
  - Solvation Energy

  Assuming that the optimal binding mode is the one with the lowest energy, the scoring function assists in identifying the most likely binding modes.

- **ANALYSIS AND INTERPRETATION**:
  - **BINDING AFFINITY**: The docking scores can be used to determine the degree of contact between the ligand and the receptor. Stronger binding affinity is usually indicated by

lower energy ratings.
- **BINDING SITE**: The binding site key residues involved in ligand binding, such as hydrophobic areas, hydrogen-bonding residues, and ionic interactions, can be identified with the use of the docking result.

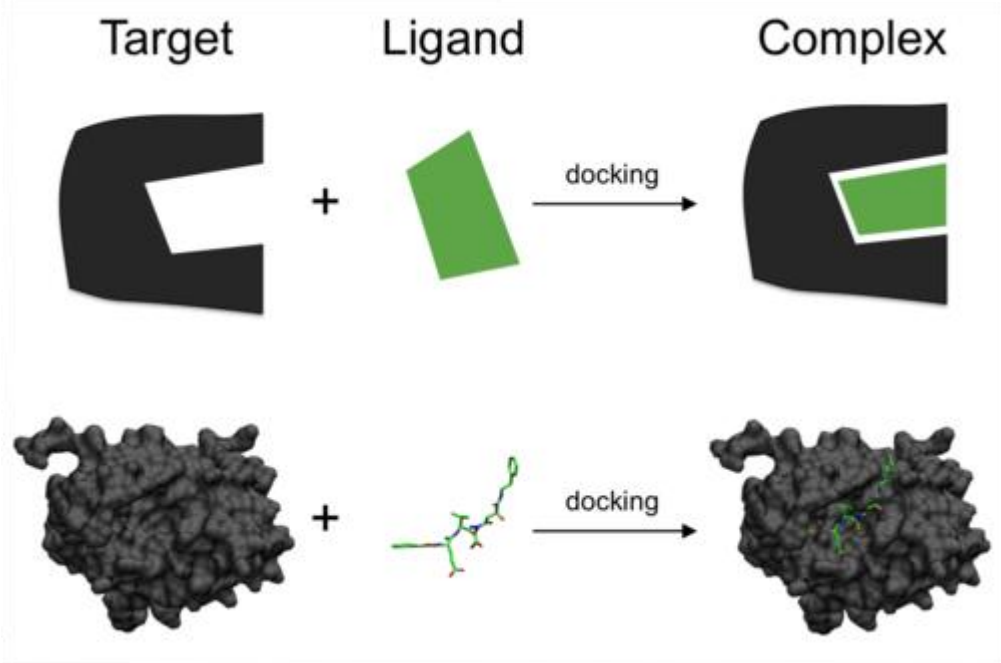


Fig.3. Overview of Molecular Docking

SOFTWARES USED IN MOLECULAR DOCKING

PyRx:

In drug discovery, it is a molecular docking tool and virtual screening program. It is intended to make it easier to identify possible medication candidates by mimicking how ligands, which are tiny molecules, interact with macromolecular targets, such proteins or enzymes. The basic engines for molecular docking in PyRx are Auto Dock Vina and Auto Dock.

Fig.4. PyRx

DISCOVERY STUDIO:

Developed by BIOVIA, Discovery Studio is a full suite of molecular modeling tools. It is a very effective tool for creating, altering, and displaying molecular structures.
Prior to simulations or experiments, the program helps to improve the geometry and stability of molecular structures by allowing for their optimization and refining.

Fig.5. Discovery Studio

LIST OF DRUGS/COMPOUNDS

1	Drug	PubChem ID
2	Suramin	5361
3	GRL- 0617	24941262
4	Sofosbuvir	45375808
5	Tideglusib	11313622
6	Remdesivir	121304016
7	alpha ketoamide 13 b	145996541
8	Narlaprevir	11857239
9	Boceprevir	10324367
10	Rupintrivir	6440352
11	Compound 11 a	73755219
12	Nirmatrelvir	1559039977
13	Ebselen	3194
14	Molnupiravir	145996610
15	Baricitinib	44205240
16	Ribavirin	37542
17	Carmofur	2577
18	Calpain inhibitor	72430
19	Favipiravir	492405
20	Disulfiram	3117
21	PX- 12	219104

DRUG WITH ITS BINDING AFFINITY FOR 6LZE

1	Drug	PubChem ID	Binding Affinity
2	Suramin	5361	-9.7
3	GRL- 0617	24941262	-8.4
4	Sofosbuvir	45375808	-7.7
5	Tideglusib	11313622	-7.9
6	Remdesivir	121304016	-7.5
7	alpha ketoamide 13 b	145996541	-7.8
8	Narlaprevir	11857239	-7.5
9	Boceprevir	10324367	-7.3
10	Rupintrivir	6440352	-7.2
11	Compound 11 a	73755219	-7.3
12	Nirmatrelvir	1559039977	-6.8
13	Ebselen	3194	-6.9
14	Molnupiravir	145996610	-6.5
15	Baricitinib	44205240	-6.3
16	Ribavirin	37542	-5.9
17	Carmofur	2577	-5.9
18	Calpain inhibitor	72430	-5.7
19	Favipiravir	492405	-5
20	Disulfiram	3117	-4
21	PX- 12	219104	-4

RESULTS

The molecular docking studies were performed using PyRx software using the secondary metabolites (ligands) and the target protein 3PTY. The 2D and 3D molecular interactions of the ligands and protein are shown below:

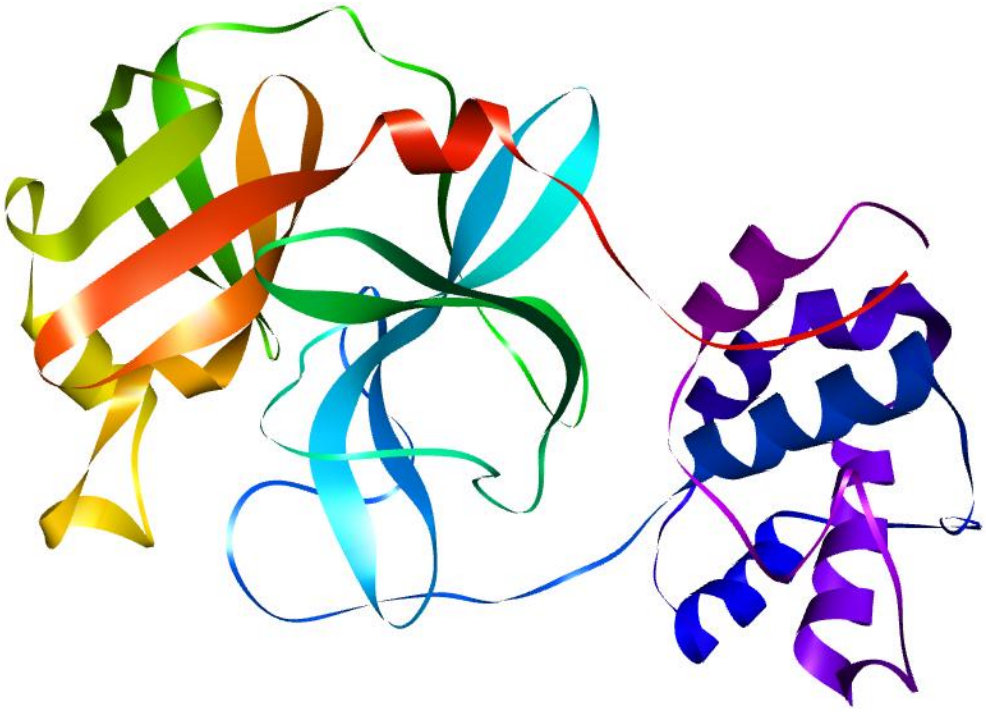


Fig.6. 3D Structure of Protein

SARS-CoV-2 PDB ID: **6LZE**

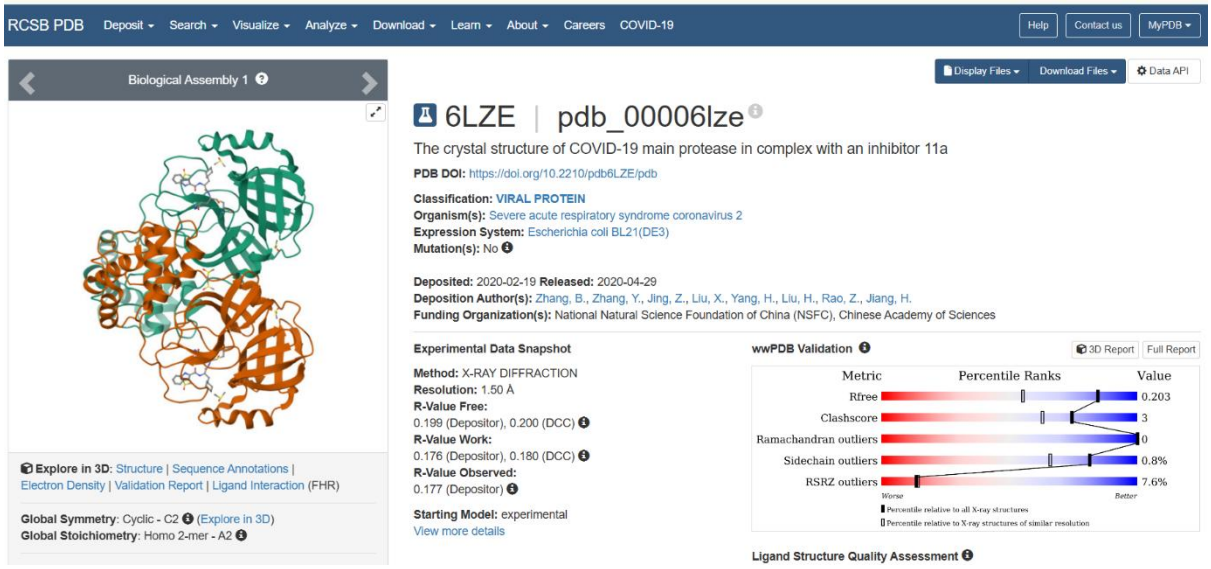
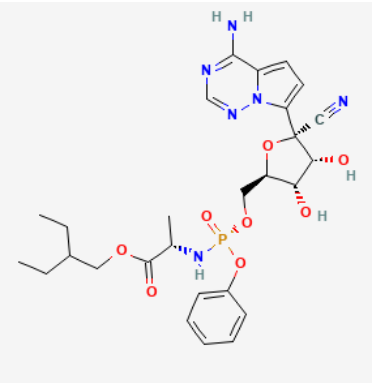
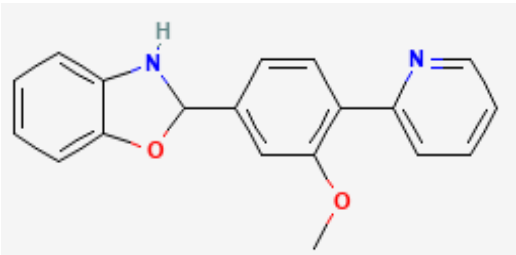


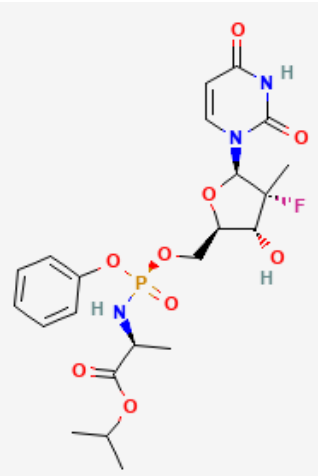
Fig.7.



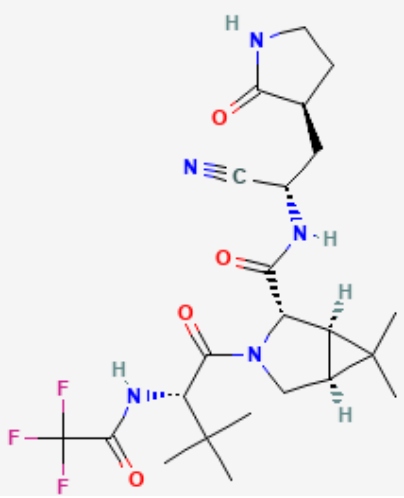
Remdesivir



Compound 11a



Sofosbuvir



Nirmatrelvir
(FDA Approved Drug)

Fig.8. 2D representation of Chemical Compounds

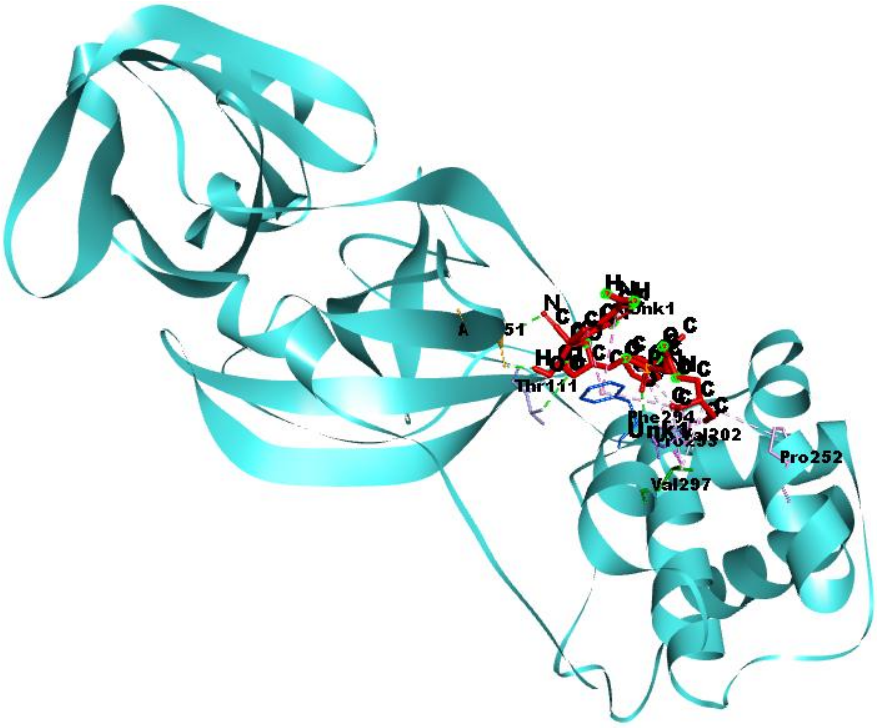


Fig.9. 3D Representation of molecular interaction Remdesivir against 6LZE

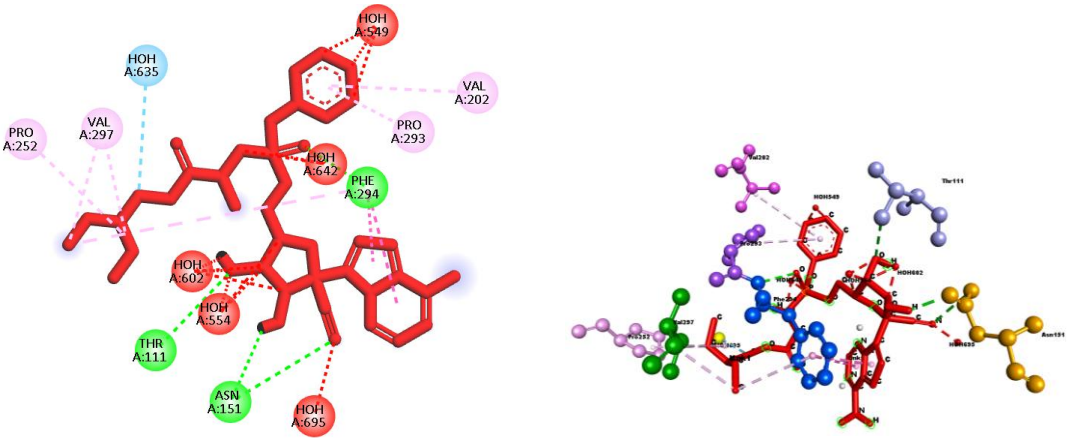


Fig.10. 2D Representation of 6LZE and Remdesivir interaction with its residue

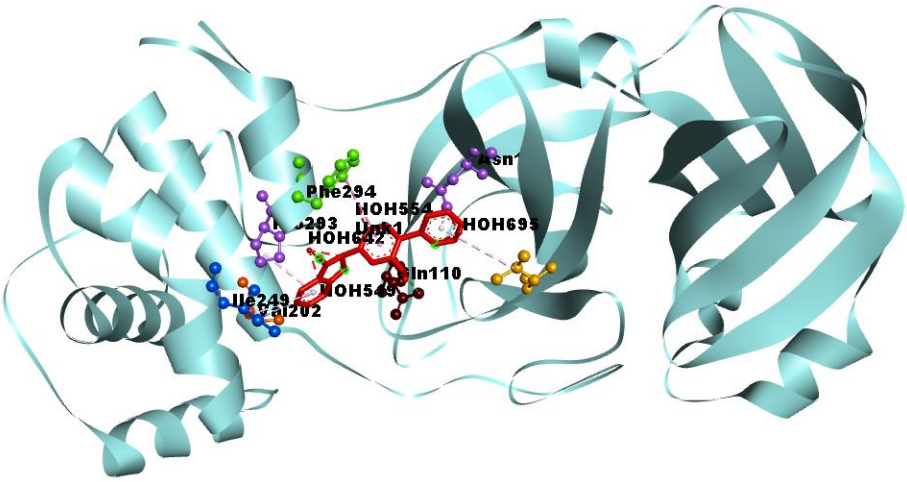


Fig.11. 3D Representation of molecular interaction between Compound 11a against 6LZE

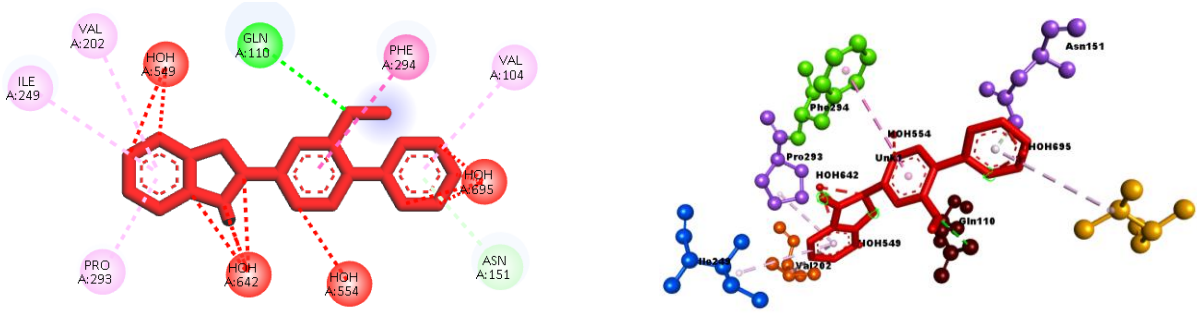


Fig.12. 2D representation of 6LZE target and Compound 11a interaction with its residue

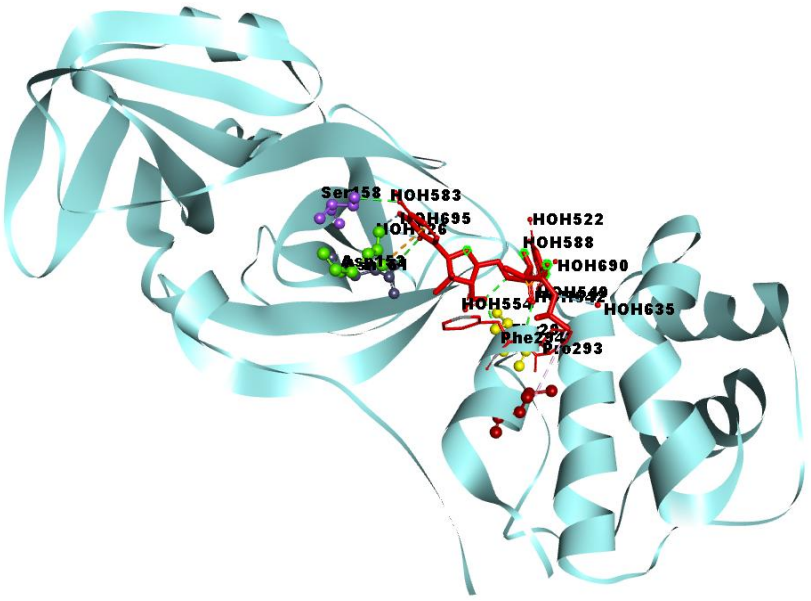


Fig. 13. 3D- representation of molecular interaction between Sofosbuvir against 6LZE

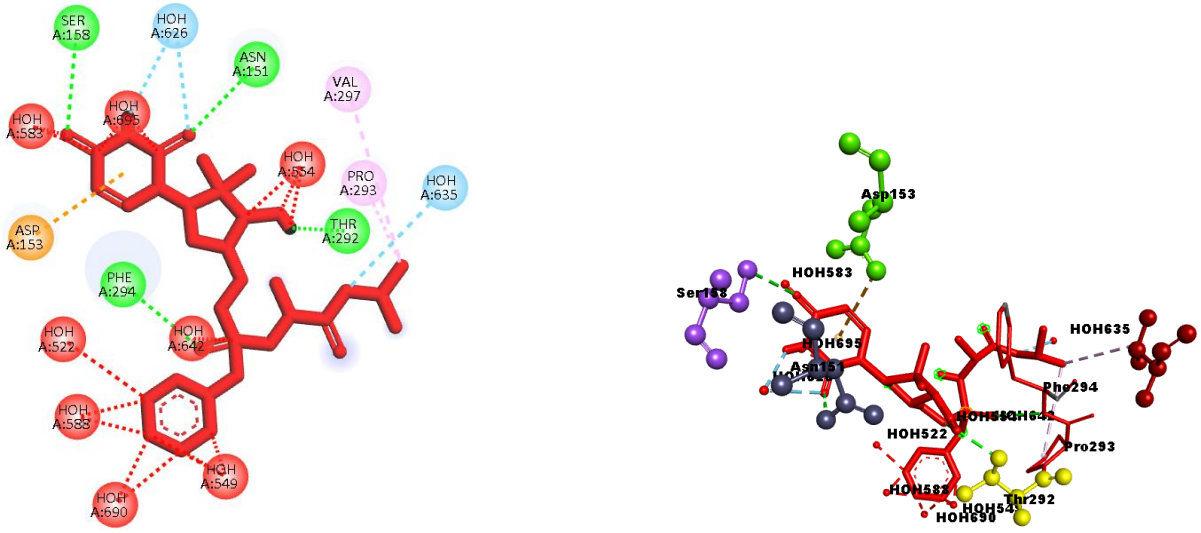


Fig.14. 2D Representation of 6LZE target and Sofosbuvir interaction with its residue

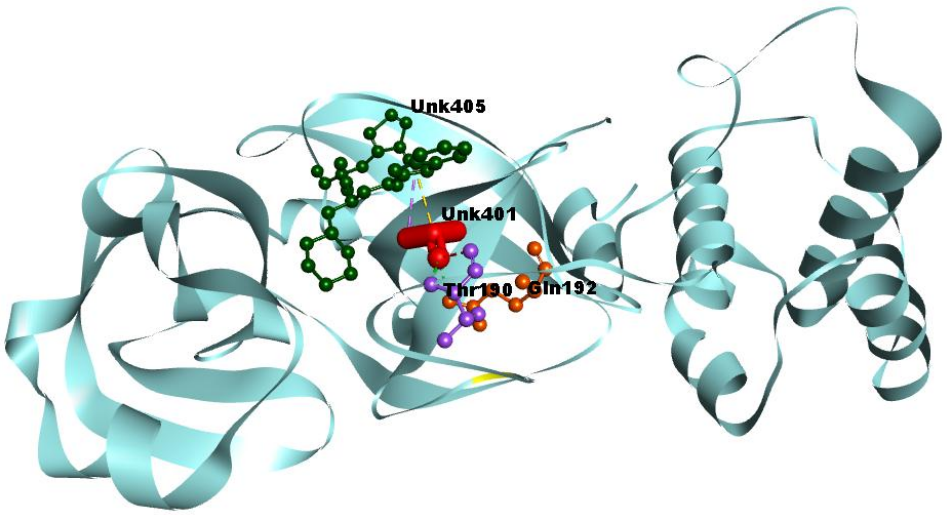


Fig.15. 3D Representation of molecular interaction between Nirmatrelvir against 6LZE target

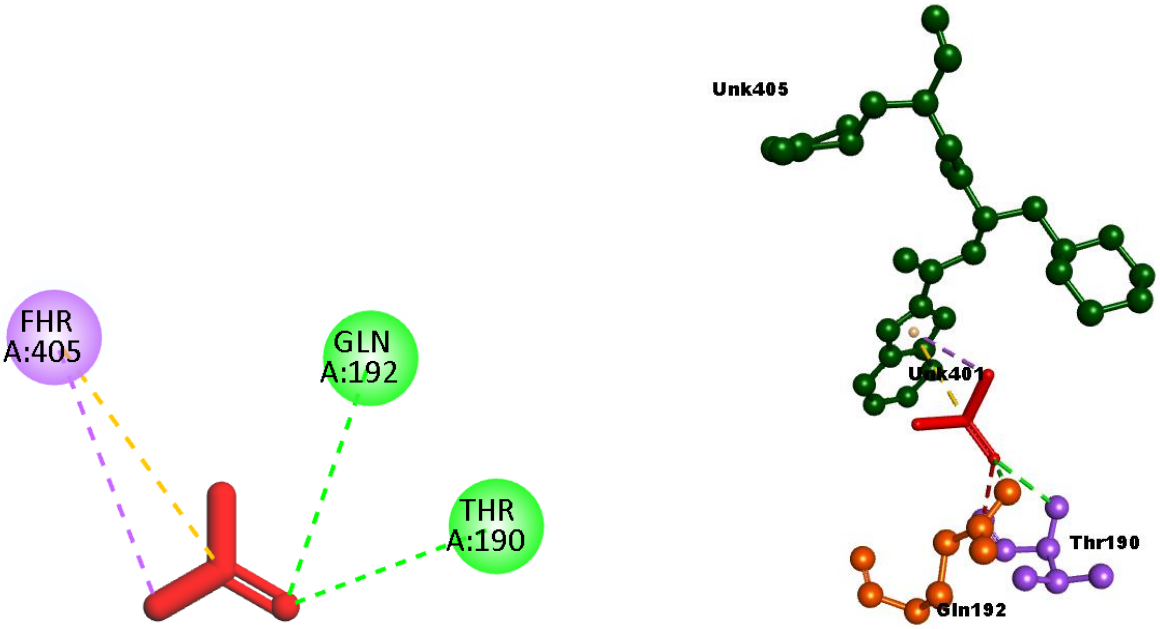


Fig.16. 2D representation of 6LZE target and Nirmatrelvir interaction with its residue

CONCLUSION

Several FDA-approved medications with a high binding affinity for the SARS-CoV-2 major protease (Mpro) were found by this in silico docking investigation. The work backs up the use of molecular docking and structural biology in medication repurposing. The results: **Remdesivir (-7.5kcal/mol), Compound 11a (-7.3kcal/mol), Sofosbuvir (-7.7kcal/mol) and Nirmatrelvir (-6.8kcal/mol)** offered a solid basis for additional experimental validation, considering the validated docking technique and the high-resolution structure of 6LZE.

Future research may involve in vitro antiviral testing, free energy calculations and molecular dynamics simulations. Newer SARS-CoV-2 or other viral target variants can be screened using the docking pipeline developed here.

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- RCSB Protein Data Bank. https://www.rcsb.org
- Mpro binding pocket with catalytic dyad (His41–Cys145). Refer to: Jin et al., 2020. *Nature*. https://www.nature.com/articles/s41586-020-2223-y
- Domain structure of SARS-CoV-2 Mpro (Figure 1). Refer to: Jin et al., 2020. *Nature*. https://www.nature.com/articles/s41586-020-2223-y/figures/1
- Ritonavir bound to Mpro (Docking visual). Reproduced or visualized using Discovery Studio. Reference image may be found via ResearchGate or recreated.
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- Hilgenfeld, R. (2020). SARS-CoV-2 replication enzyme overview. *Angewandte Chemie International Edition*, 59(46), 20837–20839. https://doi.org/10.1002/anie.202015961
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□ Inhibitor binding site at Mpro's S1/S2 pockets. *Chemical & Engineering News*, ACS. https://cen.acs.org/pharmaceuticals/drug-discovery/Crystal-structures-novel-coronavirus-protease/98/i12