Identification of entry inhibitors for SARS-CoV-2 using computational drug repurposing approach

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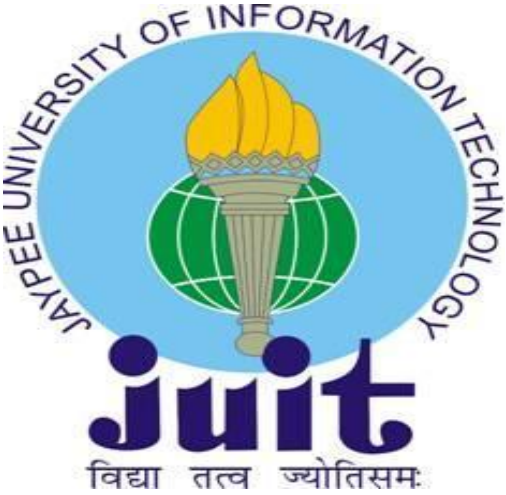

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Lastly, we would like to thank all our friends, colleagues and our parents for their blessing and moral support that always encourages us to overcome all the difficulties in our life with a positive attitude.

DECLARATION BY THE STUDENT

We hereby declare that the work reported in the B.Tech project report entitled "**Identification of entry inhibitors for SARS-CoV-2 using computational drug re-purposing approach**" submitted at **Jaypee University of Information Technology, Waknaghat, India**, is an authentic record of our work carried out under the supervision of **Dr. Raj Kumar**. We have not submitted this work elsewhere for any other degree or diploma.

(Signature of the Students)

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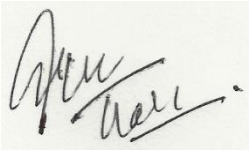
Date: 15/05/2021

CERTIFICATE

This is to certify that the project entitled "Identification of entry inhibitors for SARS-CoV-2 using computational drug re-purposing approach", submitted by Maharishi Kalla and Riya Dhiman is in its partial fulfillment for the award of degree of Bachelor of Technology in Bioinformatics to Jaypee University of Information Technology Waknaghat,Solan (H.P), India is an authentic record of candidate's own work carried out by them under my supervision.

This work has not been submitted partially or fully to any other university or institute in order to achieve any award or other degree.



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1.ABSTRACT

Coronavirus disease (COVID-19) is an infectious, growing and deadly disease caused by the Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) which is a new evolving viral pathogen causing respiratory disease and has hit the planet, affecting the society, economy, and health-care facilities. Recognizing the virus receptor recognition mechanism is necessary for dealing with the pandemic. SARS-CoV-2 has been characterized as a virus that controls the range of its infectivity, pathogenicity, and host cell interaction. In the present situation, drug repositioning is seen as a potential therapeutic option for COVID-19.There is high sequence similarity between the spike proteins of SARS-CoV and SARS-CoV-2, therefore ACE2 is also a identified as a functional target for SARS-CoV-2. SARS-CoV-2 mainly damages lungs after binding to its receptor ACE2.

Our study focuses on the screening of potential drugs against our target protein i.e., SARS-CoV-2 spike protein forming a complex with ACE2 (PDB ID: 6VW1). We performed docking based virtual screening against a library of pre-approved drugs. We had planned out to screen the potential drugs within the pre approved drugs and compare it with the reference inhibitor.The reference inhibitor taken was remdesivir that is responsible for the termination of SARS-CoV-2 replication.It was docked against the target protein resulting in a binding affinity of -6.4kcal/mol which was used as a reference for comparison against the DRUGBANK database pre-approved drug library of 800 drugs.Multi-docking of these drugs was performed against the target protein.Finally 7 potential drugs (ledipasivir, digitoxin, midostaurin, triptorelin, regorafebnib, ertapenem, hesperidin) were screened based upon their binding affinities and molecular interactions,with ledipasivir showing the best binding affinity of -10.3kcal/mol.Therefore,the results shows that these 7 potential drugs could prove effective against this fatal coronavirus disease.

2. INTRODUCTION

COVID-19 is a contagious,progressive,fatal respiratory disease that majorly affects respiratory tract and lungs.Corona virus has the ability to bind to angiotensin converting enzyme 2 (ACE2) receptors present in endothelial cells,lungs, heart, brain, kidneys, intestine therefore directly damaging these organs.[1]The word "coronavirus" comes from the crown-like appearance noticed for such viruses at the microscopic level (Figure 1).[1] Coronaviruses (CoVs) is a wide group of positive-sense, single-stranded,enveloped RNA viruses. The genome of the RNA virus has a very large size and it infects by attaching viral particles to the host cell targets.[2]

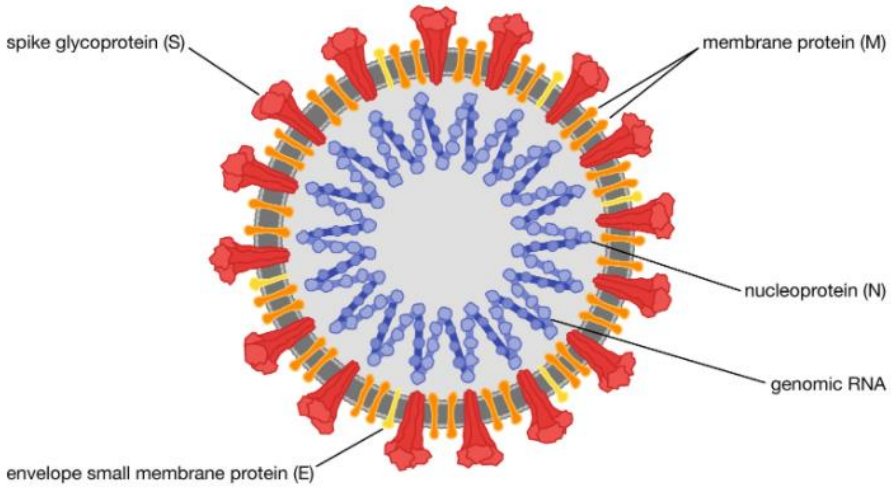

Figure1. Diagrammatic representation of SARS-CoV-2

SARS-CoV-2 S enters cells through ACE2, and the receptor-binding domains of SARS-CoV-2 and SARS-CoV-2 bind to human ACE2 with much greater, indicating that SARS-CoV-2 spreads efficiently among humans.[2] Coronavirus spike (S) glycoproteins make cell entry and are the primary target of antibodies.[2] ACE2 is widely present in a wide range of cells found in a number of human organs. In human biology, ACE2 functions as a key counter-regulatory enzyme to ACE by degrading angiotensin II, the core component in the renin-angiotensin-aldosterone system (RAAS) and ACE2's main target.[3]

Since the SARS-CoV-2 RBD is majorly involved in virus attaching to host cells, targeting the RBD may be a wise option for the production of COVID-19 therapeutic agents.[4] There are some resemblance between SARS-CoV-2 and SARS-CoV.[1] Further to the analysis of the SARS-CoV-2 genomic studies, a number of molecular simulations have been found to be particularly effective to discover a viable candidate to defeat the novel coronavirus SARS-CoV-2.

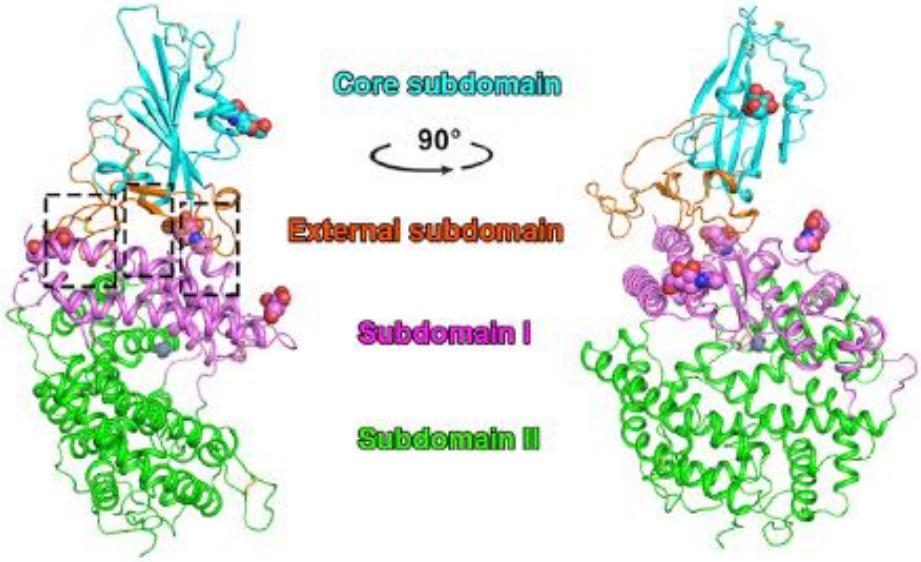

Figure 2. Cartoon representation of SARS-CoV-2 and ACE2 complex.

SARS-CoV-2-S is a membrane protein comprised of S1 regions forming the NTD and CTD, S2, an intracellular region and cytoplasmic domain. Coronavirus entrance into host cells is a main factor of disease pathogenecity and infection. Coronaviruses enter host cells by first binding to a target cell for receptor binding, first entering cell surface and gradually integrating virus and intracellular surfaces. [5]The SARS-CoV virus has a binding site domain (RBD) that identifies the angiotensin-converting enzyme 2 (ACE2) as its target (Figure 2). ACE2 mainly identifies the RBDs of S protein by polar contact. [6]

3. MATERIAL AND METHODS

The basic understanding and techniques of the *In silico* screening, docking, drug repurposing reviewed and learnt from the various sources were used.[7] Some other bioinformatics tools like PyMol,PLIP,CHIMERA were also used.

3.1 LITERATURE REVIEW

Virus identification of receptors is the primary step in viral infection of host genome. [8] The S1 subunit of coronavirus spike protein has two separate domains, the N-terminal domain (S1-NTD) and the C-terminal domain (S1-CTD), which together act as receptor-binding domains (RBDs). Coronavirus entry into host cells is guided by an enveloped spike protein, which first binds to a receptor on the host cell surface before fusing viral and host membranes (Figure 3). The most significant factor of coronavirus relative abundance and inter species infection is the binding association between coronavirus RBD and its receptor. [9]

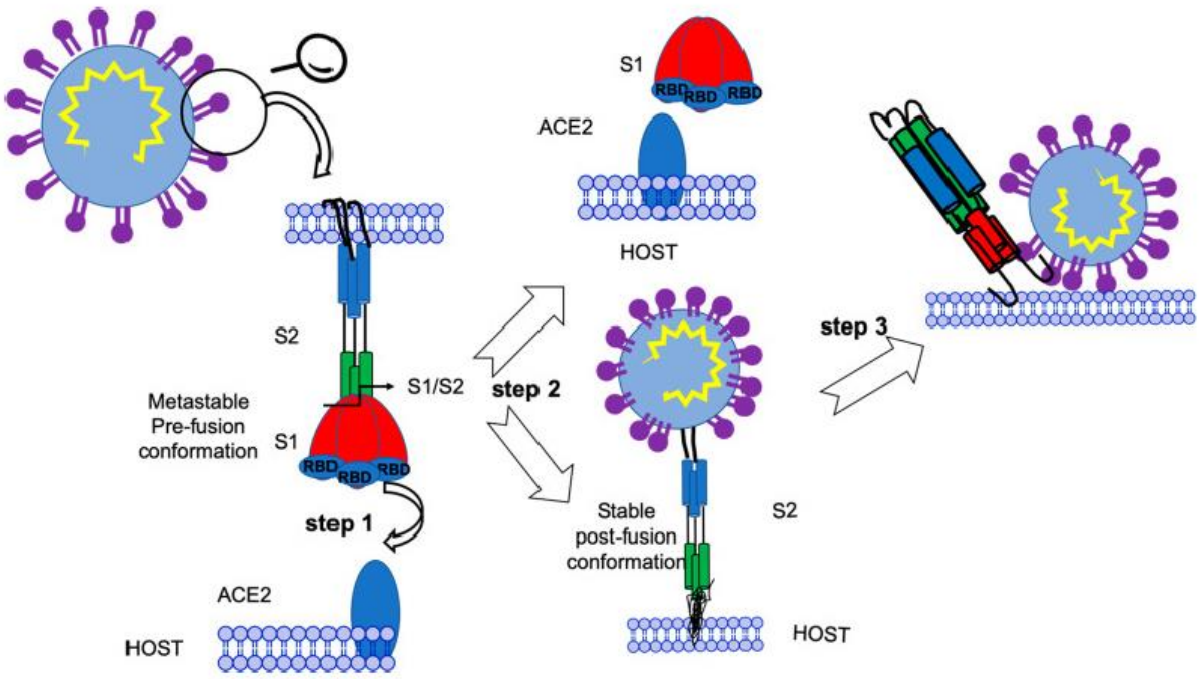

Figure 3. Receptor recognition mechanism of SARS-CoV-2.

3.2 STRUCTURE PREPARATION

The first step was selecting the Target/receptor molecule.PDB ID: 6VW1 was selected as the receptor molecule. 6VW1 may be a stable protein-ligand complex that inhibits the binding of spike glycoprotein to the cellular membrane's ACE-2 receptor.[5] The PDB structure was downloaded from the Protein Data Bank. The structure was chosen based on the RBD domain's structure consistency and completeness.[4] The resolution of 6VW1 is 2.68Å, the R-free value is 0.228, and there are no Ramachandran outliers. The structure was visualised using the tool UCSF Chimera as shown in Figure 4.

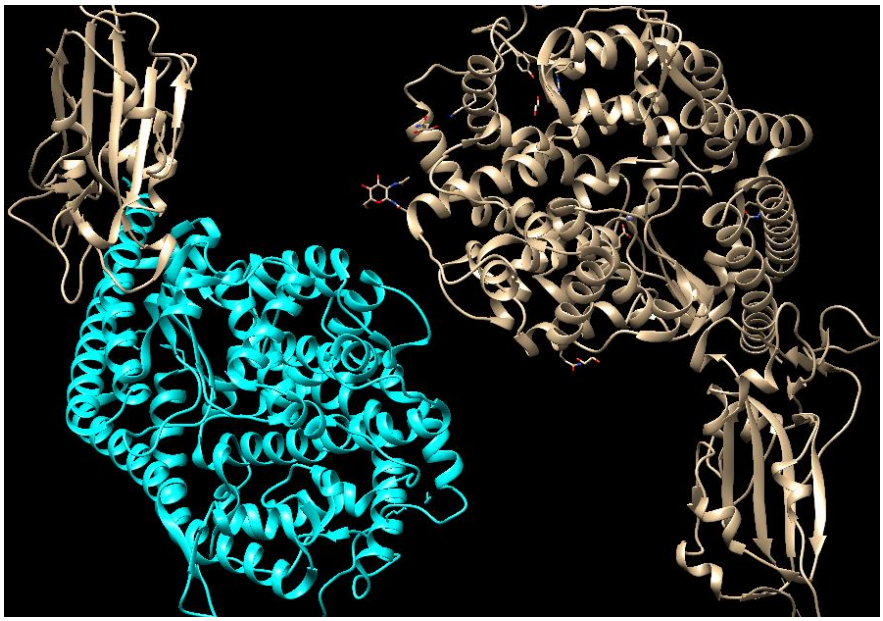

Figure 1. Visual representation of the PDB ID 6VW1 (SARS-CoV 2 and ACE2 complex)

The pdb consisted two chains of Human ACE2 and two chains of SARS-CoV-2 Spike RBD, in total four chains forming two SARS-CoV-2 Spike RBD and Human ACE2 complex. It also consisted other small molecules and ligands which were removed before docking steps.As mentioned in the literature review section the crystal structure was determined for the study of receptor binding mechanism of SARS-CoV-2 spike RBD and human ACE2 making it suitable target for the study.

3.3 SELECTION OF REFERENCE INHIBITOR

The Binding Database was used to search for inhibitor with literature review knowledge as well. The Binding database was used as it is free accessible database of measured binding affinities. It majorly focuses on interaction of proteins considered to be a drug target. The FASTA search option was used in the binding database where FASTA sequence of the Target protein was put in the search box. The search results presented best targets with various parameters such as EC_{50} and IC_{50}.

EC_{50} is the half maximal effective concentration it indicates how much of a drug is needed to active the 50% of maximum response. The more potent the drug smaller will be the EC_{50} will be. IC_{50} is the half maximal inhibitory concentration it is a measure of how much potent a substance in inhibiting a specific biochemical function, IC_{50} is a quantitative measure that indicates the amount needed of a particular inhibitory drug to inhibit.

Remdesivir in was the best result according to the database with EC_{50} value as IC_{50} value was provided as not available, the EC_{50} value however was the lowest from all the obtained results that came out to be 770, thus it was selected as the best result for the our target protein (Figure 5). After the literature review process remdesivir was taken into strong consideration, and with the result of Binding Database it was selected a reference inhibitor drug for the studies, thus the docking results of remdesivir and target protein will be considered as reference results and only the best and similar to results will be considered for final study.

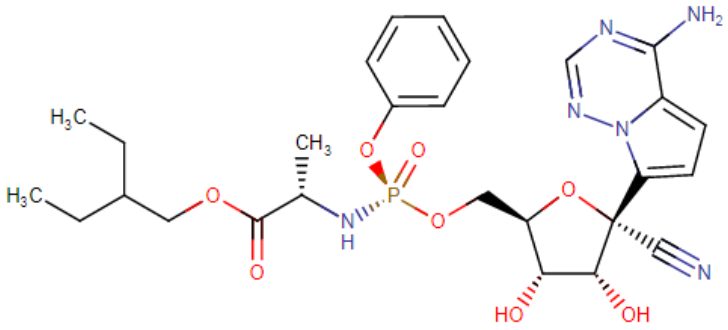

Figure 2. Chemical structure of remdesivir.

3.4 TARGET PROTEIN PREPARATION AND ACTIVE SITE IDENTIFICATION

After the selection of target protein structure 6VW1 it is essential to prepare this protein structure for docking purposes, with reference drug ligand and with pre-approved drugs respectively. 6VW1 initially consisted of two SARS-CoV-2 spike RBD and ACE 2 complex respectively thus four chains in total, as for the docking purposes we only need one complex thus 2 chains out of four chains are deleted manually through PyMol software. After the deletion the target protein pdb only have 1 complex consisting 2 chains this pdb was saved as “Targetprotein” using export molecule option from the PyMol software with pdb format.

Further protein cleaning procedure was performed, this was achieved by deletion of heteroatoams from the the “Targetprotein.pdb”, for deletion of the heteroatoms pdb file was opend in txt viewer which allows to view the file components in txt format that is every atom and heteroatoms and their respective position and element of the residue, from this txt viewer the all heteroatoms were selected and deleted manually they are represented as HETATM and their number. The modified file was again saved in pdb format with same name “Targetprotein.pdb”. After the deletion of heteroatoms all unnecessary residues and ligand molecules that were not needed for the docking purposes and would have hindered the docking calculations were removed. This completes initial phase of the protein cleansing next will be done using AutoDock Tool, after finding Active site for docking. The clean protein is shown in Figure 6.

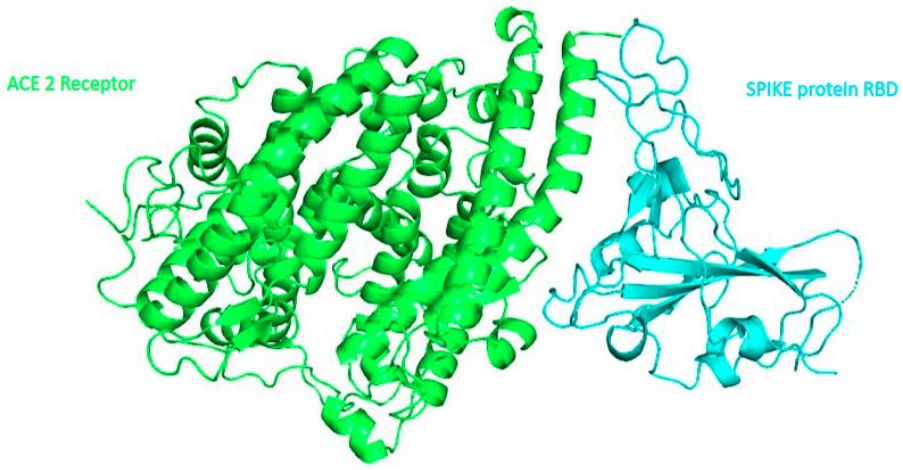

Figure 3. Prepared target protein structure.

For the docking purpose we needed the binding site where the inhibitor drugs will be docked for further analysis of their potential inhibiting properties. Thus for the identification of Binding site PyMol visualization software was used. After opening the PyMol window the Targetprotein.pdb was loaded → then under the setting option surface option option will appear, under that Cavities and Pockets Only criteria was chosen. → Then under the same surface option menu Cavity detection radius was set to 3 Solvent Radii (4.2 angstrom) → Cavity detection was also set to Solvent Radii (4.2 angstrong) → after this from the setting menu under Transparency option the transparency of surface was changed to 50 % . → After this "show surface" command was run in the PyMol shell.

This procedure represented the cavities and pocket present between the protein structure. (Figure 7) shows the outcome of the above procedure. The cavity between Human ACE2 and SARS-CoV-2 Spike RBD which is visible in the figure was selected as the docking site from the research analysis. Thus the residues around the cavity were selected as active binding site for the docking (Figure 7).

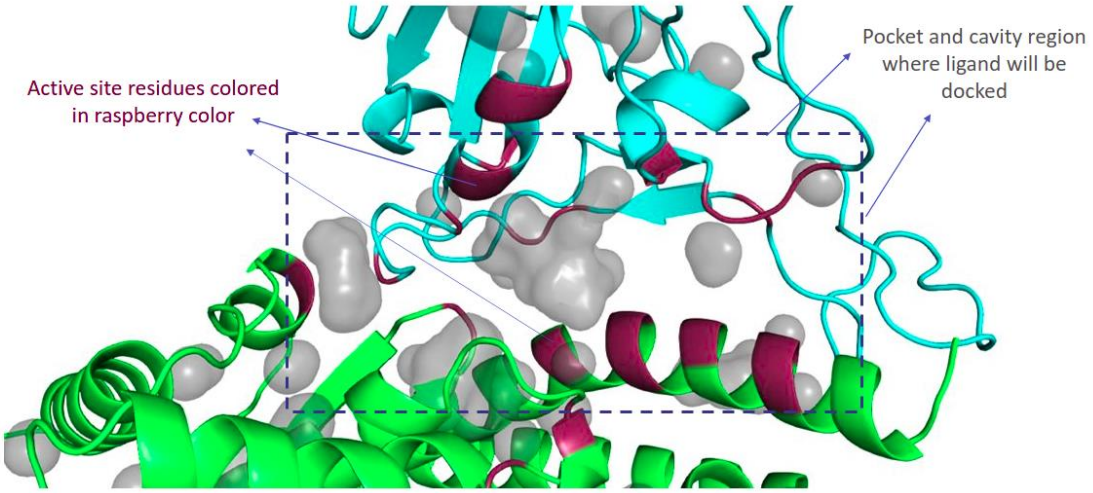

Figure 4. Visual representation of binding cavity of 6VW1.

The active site residues of 6VW1 includes: LYS25, THR26, ASN33, HIS34, GLU37, GLY326, LYS353, GLU37, PHE390, ARG393, LYS403, ASP405, ARG408, GLN409, VAL417, LEU455, PHE456, ARG457, PRO491, GLY496, TYR505 (Table 1).

The table of the active site residues of 6VW1 are also shown below:

Table 1. Table showing the active site residues forming the binding cavity.

ACE 2		Spike Protein	
Residue number	**Residue name**	**Residue number**	**Residue name**
25	Lysine(K)	403	Lysine(K)
26	Threonine(T)	405	Aspartate(D)
33	Asparagine(N)	408	Arginine(R)
34	Histidine(H)	409	Glutamine(Q)
37	Glutamic Acid(E)	417	Valine(V)
326	Glycine(G)	455	Leucine(L)
353	Lysine(K)	456	Phenylalanine(F)
37	Glutamic Acid(E)	457	Arginine(R)
353	Lysine(K)	491	Proline(P)
390	Phenylalanine(F)	496	Glycine(G)
393	Arginine(R)	505	Tyrosine(Y)

After finding the active site final preparation of our target protein for docking purposes was done using Autodock tool software, which included removal of water, addition of polar hydrogen, calculation of charges, converting the pdb file to pdbqt and generating grid, the grid was arranged in the manner as the selected active binding residues resides in the grid perfectly. This procedure was done through Autodock Tool. This grid files will contain the coordinates which will be read by AutodockVina as the site for docking of ligands in later steps.

Addition of charges is done as manually. Thus, it is necessary and sensible to add charges and missing atoms like hydrogen and in some cases non-hydrogen to protein before proceeding for docking experiment, irrespective of docking tool or software used.Kollman

charges represent the template values for each individual amnio acid that are derived from the corresponding electrostatic potential by using quantum mechanics. Gasteiger charges are determined on basis of electro negativity equilibrium, if the protein doesn't have any partial charge at hand.

The charges added were, Kollman charges and Gasteiger charges -9.229 and -24.0029 respectively. For the matter of coordinates grid file was prepared by placing the grid in position witch contained the desired cavity and pocket where drugs will be docked. Coordinates of receptor obtained from grid file are shown in Table 2 below.

Table 2. Table showing the grid coordinates of the receptor molecule.

Center X	**78.965**
Center Y	-6.534
Center Z	179.921

3.5 REFERENCE INHIBITOR DOCKING

Reference inhibitor drug remedesivir was docked using AutoDock Vina version 1.1.2, The drug was downloaded from Drugbank database in 3D with sdf format. OpenBabel was used to convert the sdf file into pdb file, AutoDock tool was used to prepare and convert the pdb ligand file to pdbqt file format.OpenBabel Minimize tool was used to minimize the enery of ligand molecule the selected force field for the procedure was MMFF94. Docking parameters were saved on configuration file containing the names of receptor and ligand with the coordinates of the grid. This configuration file lets directs AutoDock Vina for correct selection of molecules and coordinates for the working directory, Site specific docking was performed using AutoDock Vina. The docking grid size and dimension was noted from the protein preparation step that is size_x = 48, size_y = 72, size_x = 38 and centered at center_x = 78.965 center_y = -6.534, center_z = 179.921. The exhaustiveness was kept at 8 respectively.

The command used : vina --config conf.txt --log logSO.txt. This commands AutoDock Vina to run the docking procedure in terminal by reading conf.txt file and after completion of the procedure creating logSO file with the results of docking in it.

3.6 PRE APPROVED DRUGS LIGAND PREPARATION AND DOCKING

Pre-approved drugs were downloaded in compressed manner which contained 2D sdf files of all the FDA approved drugs in the DrugBank Database. Firstly the sdf files were converted to from 2D sdf file to 3D pdbqt file which was achieved by Open Babel version 3.1.0. Open Babel software uses combination of rules for generating a 3D file from 2D file.

The command used for the conversion of 2D sdf to 3D pdbqt conversion : obabel *.sdf –O *.pdbqt –gen3D

Energy Minimization : After the conversion process Energy minimization step was performed as a cleanup procedure after the 3D conversion, it was important step and it did not alter the stereochemistry of all the ligand files. This was done by using OpenBabel Minimize tool the selected force field for the procedure was MMFF94(Merck Molecular Force Field 94),with 1000 runs. MMFF94 performs well at optimizing geometries, bond lengths, angles, etc. and includes electrostatic and hydrogen-bonding effects.All the ligands name were listed in a text file using command: ls > *pdbqt.The txt file was named Ligand.txt

The command used for the procedure: obminimize –ff MMFF94 –n1000 *.pdbqt

Docking : Multiple site specific docking was performed using AutoDock Vina version 1.1.2 with receptor being the target protein and ligand being the drugs prepared prior to this step for docking.Docking parameters were same as used in reference docking i.e., saved in configuration file but only containing the name of receptor with the coordinates of the grid. This configuration file lets directs AutoDock Vina for correct selection of molecules and coordinates for the working directory, site specific docking was performed using AutoDock Vina. The docking grid size and dimension were same as that of the reference docking parameters.

A perl script file was used for commanding Autodock Vina to run docking procedure.After running the perl file in terminal it asked for the txt file containing all the ligand names in the working directory for Vina to take them as input for the docking procedure.After the docking procedure all the Output.pdbqt files and log.txt files were segregated to a specific folder.

4. RESULTS AND DISCUSSION

The complex SARS-CoV-2 spike protein and human ACE2 with the PDB ID: 6VW1 was selected as the drug target for the screening of potential compounds against SARS-CoV-2. The DrugBank database pre-approved drug library of 800 drugs was used for virtual screening studies. The selected drugs from the docking analysis were visually examined for the analytical interactions that help in destroying the interactions between the spike protein and ACE2 receptor leading to the inhibition of virus entry into human. The interactions for all the screened compounds with comparison to the reference inhibitor were visualised using PyMol and PLIP (Protein-Ligand Interaction Profiler) tools.

PyMol was used to visualize the interactions between drug ligands and target protein structure in 3D. While PLIP was used to tubulise the informative data regarding the bonds and interaction found in the docking results.

4.1. Reference Docking Analysis

The docking result for reference drug remdesivir was obtained after the docking process was completed. The result shown(Figure 8) represents the 7 pose perfectly binding within the selected cavity and pocket region of the target protein structure, the binding affinity for this pose was -6.4kcal/mol which was considered as reference binding affinity for the this research that means in the process of analysing pre-approved drugs docking results only those will be considered as viable for further study which will have binding affinity(kcal/mol) greater than that of reference drug i.e., -6.4kcal/mol also poses which will be docked perfectly inside the cavity and pocket region similar to that of reference drug inhibitor will be finally selected.(Figure 9)

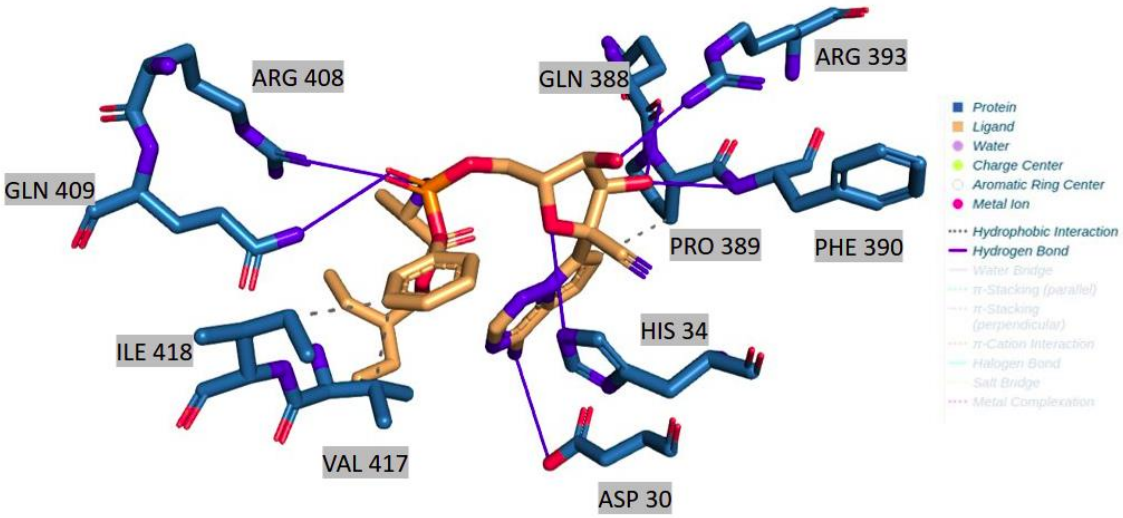

Figure 5. Three-dimensional representation of remdesivir with its molecular interactions.

Hydrophobic Interactions ····

Index	Residue	AA	Distance	Ligand Atom	Protein Atom
1	389A	PRO	3.79	16	3711
2	417E	VAL	3.75	45	6753
3	417E	VAL	3.23	31	6751
4	418E	ILE	3.66	31	6760

Hydrogen Bonds —

Index	Residue	AA	Distance H-A	Distance D-A	Donor Angle	Protein donor?	Side chain	Donor Atom	Acceptor Atom
1	30A	ASP	3.55	3.95	105.30	×	✓	17 [Npl]	164 [O.co2]
2	34A	HIS	3.38	3.91	114.59	✓	✓	211 [Npl]	1 [O3]
3	388A	GLN	2.84	3.55	130.28	×	×	20 [O3]	3698 [O2]
4	390A	PHE	3.31	3.73	106.60	✓	×	3714 [Nam]	20 [O3]
5	393A	ARG	2.80	3.24	106.40	✓	✓	3754 [Ng+]	46 [O3]
6	408E	ARG	3.17	4.04	144.41	✓	✓	6674 [Ng+]	33 [N3]
7	409E	GLN	2.78	3.41	120.51	✓	✓	6689 [Nam]	25 [O2]

Figure 9. Remdesivir and 6VW1 molecular interactions shown in tabulated form.

4.2. Pre-approved drugs docking analysis

Results of multiple site-specific docking of pre-approved drug ligands with target protein were obtained after the successful completion of docking procedure, result were analysed with reference docking result in comparative relation manner i.e., those who possessed greater binding affinity than that of selected reference pose binding affinity, were categorised as potential candidates for further analysis.

Further analysis of selected drugs on the parameter of binding affinity was done by studying the pose of those drugs, only those were considered as best results which perfectly docked under the given coordinates of grid which was based on the selected pocket and cavity region that was created previously in the study. Note that the grid coordinates for docking of reference drug and all the other pre-approved drugs was kept identical, as the docking of inhibitors at the selected cavity will bind with both the chains (ACE2 and spike protein RBD) of the protein structure.

We selected 7 potential drug compounds after the docking analysis of results from the DRUGBANK database approved drugs as shown in the Table 3 below.

Table3. Potential drugs selected after the docking analysis.

S.No	DRUGBANK ID	DRUG NAME	BINDING ENERGY(kcal/mol)
1	DB09027	Ledipasivir	-10.3
2	DB01396	Digitoxin	-9.2
3	DB06595	Midostaurin	-9.0
4	DB06825	Triptorelin	-9.0
5	DB08896	Regorafebnib	-8.6
6	DB00303	Ertapenem	-8.5
7	DB04703	Hesperidin	-8.4
8	**DB14761**	**Remdesivir**	**-6.4**

1.Ledipasivir

Ledipasvir is a pre- approved direct-acting antiviral agent which is used to treat specific hepatitis C virus (HCV) infections in combination with other antiviral agents.

The docking result shown (Figure 10) represents the second pose binding perfectly at the selected active site with high binding affinity of -10.4kcal/mol which was significantly greater than that of reference binding affinity (-6.4kcal/mol).Stabilized structure was found with ledipasvir making 7 hydrogen bonds and 7 hydrophobic interactions. The bonds and interaction were between drug and residues of both human ACE2 and SARS-CoV-2 spike RBD (Figure 11).

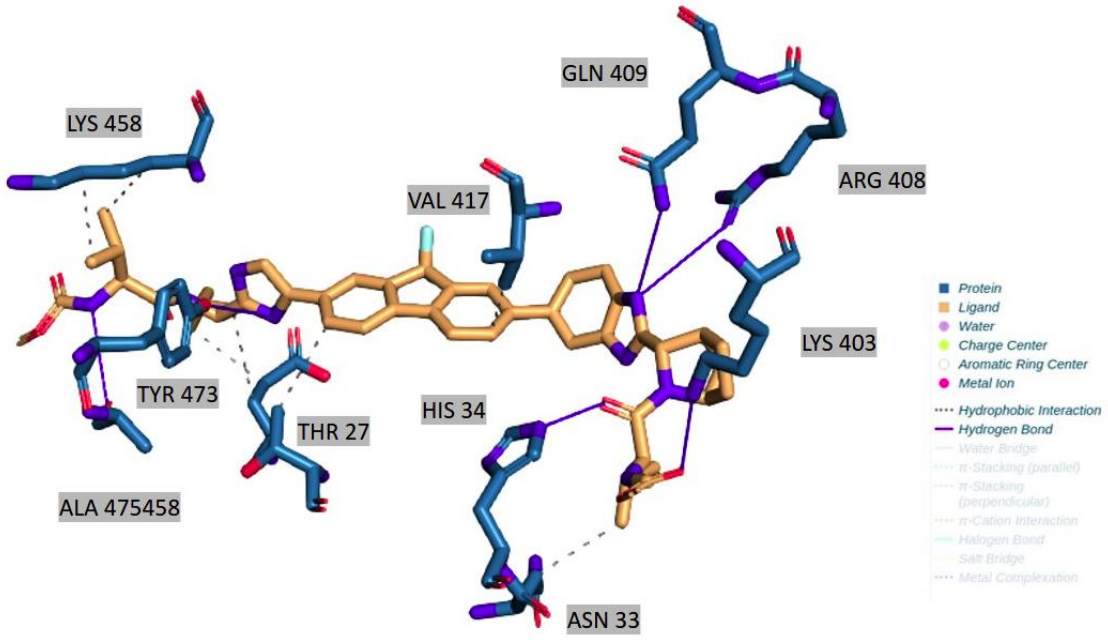

Figure 6. Three-dimensional representation of ledipasivir with its molecular interactions.

Hydrophobic Interactions ····

Index	Residue	AA	Distance	Ligand Atom	Protein Atom
1	23A	GLU	3.59	7823	44
2	23A	GLU	3.75	7819	43
3	27A	THR	3.84	7793	85
4	33A	ASN	3.63	7813	148
5	417E	VAL	3.42	7788	6706
6	458E	LYS	3.78	7830	7129
7	458E	LYS	3.64	7829	7131

Hydrogen Bonds —

Index	Residue	AA	Distance H-A	Distance D-A	Donor Angle	Protein donor?	Side chain	Donor Atom	Acceptor Atom
1	34A	HIS	2.35	3.04	123.73	✓	✓	164 [Npl]	7831 [O2]
2	403E	LYS	2.82	3.23	104.78	✓	✓	6582 [N3+]	7832 [O2]
3	408E	ARG	3.10	4.08	162.22	✓	✓	6627 [Ng+]	7775 [N3]
4	408E	ARG	3.78	4.08	100.07	×	✓	7775 [N3]	6627 [Ng+]
5	409E	GLN	3.47	4.01	114.86	✓	✓	6642 [Nam]	7775 [N3]
6	473E	TYR	3.34	3.96	124.94	✓	✓	7290 [O3]	7779 [N2]
7	475E	ALA	2.80	3.74	154.51	×	×	7781 [Nam]	7308 [O2]

Figure11. Ledipasivir and 6VW1 molecular interactions shown in tabulated form.

2. Digitoxin

Digitoxin is a pre-approved cardiac glycoside drug used for treatment and management of congestive arrhythmias, cardiac insufficiency and heart failure.

The docking result shown (Figure 12) represents the sixth pose binding perfectly at the selected active site with high binding affinity of -9.2kcal/mol which was significantly greater than that of reference binding affinity (-6.4kcal/mol).stabilized structure was found with digitoxin making 5 hydrogen bonds and 1 hydrophobic interactions. The bonds and interaction were between drug and residues of both Human ACE2 and SARS-CoV-2 Spike RBD. (Figure 13)

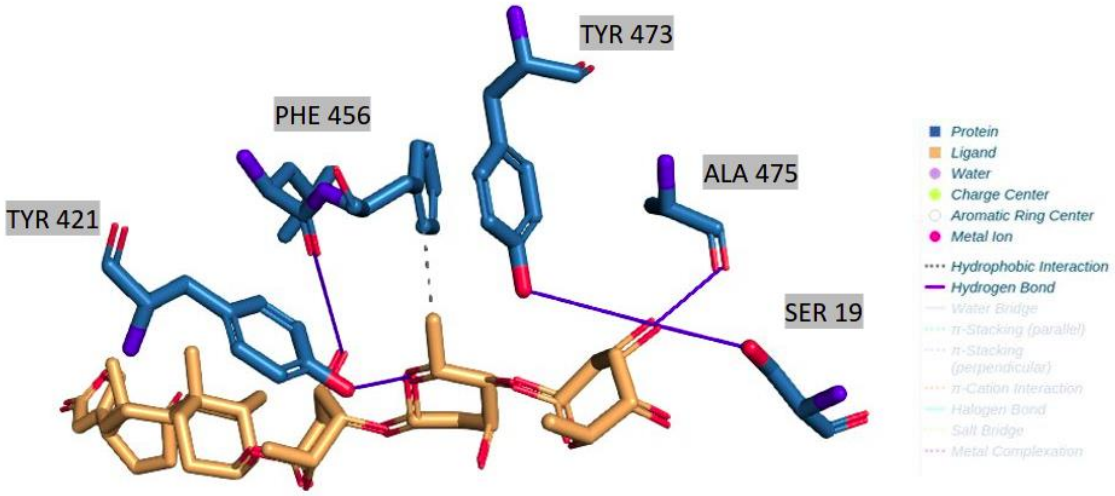

Figure 12. Three-dimensioanl representation of dgitoxin with its molecular interactions.

Hydrophobic Interactions ····

Index	Residue	AA	Distance	Ligand Atom	Protein Atom
1	456E	PHE	3.67	7806	7102

Hydrogen Bonds —

Index	Residue	AA	Distance H-A	Distance D-A	Donor Angle	Protein donor?	Side chain	Donor Atom	Acceptor Atom
1	19A	SER	2.21	2.81	120.17	✓	✓	6 [O3]	7827 [O3]
2	421E	TYR	2.29	3.23	171.27	✓	✓	6743 [O3]	7822 [O3]
3	455E	LEU	3.10	4.04	160.48	×	×	7816 [O3]	7090 [O2]
4	473E	TYR	3.41	3.92	116.62	✓	✓	7290 [O3]	7827 [O3]
5	475E	ALA	2.31	2.74	105.37	×	×	7827 [O3]	7308 [O2]

Figure13. Digitoxin and 6VW1 molecular interactions shown in tabulated form.

3. Midostaurin

Midostaurin is a pre-approved drug which is an antineoplastic agent used for treatment of high-risk acute myeloid leukemia .

The docking result shown (Figure 14) represents the ninth pose binding perfectly at the selected active site with high binding affinity of -9.0kcal/mol which was significantly greater than that of reference binding affinity (-6.4kcal/mol).Stabilize structure was found with midostaurin making 3 hydrogen bonds and 5 hydrophobic interactions. The bonds and interaction were between drug and residues of both human ACE2 and SARS-CoV-2 spike RBD.(Figure 15)

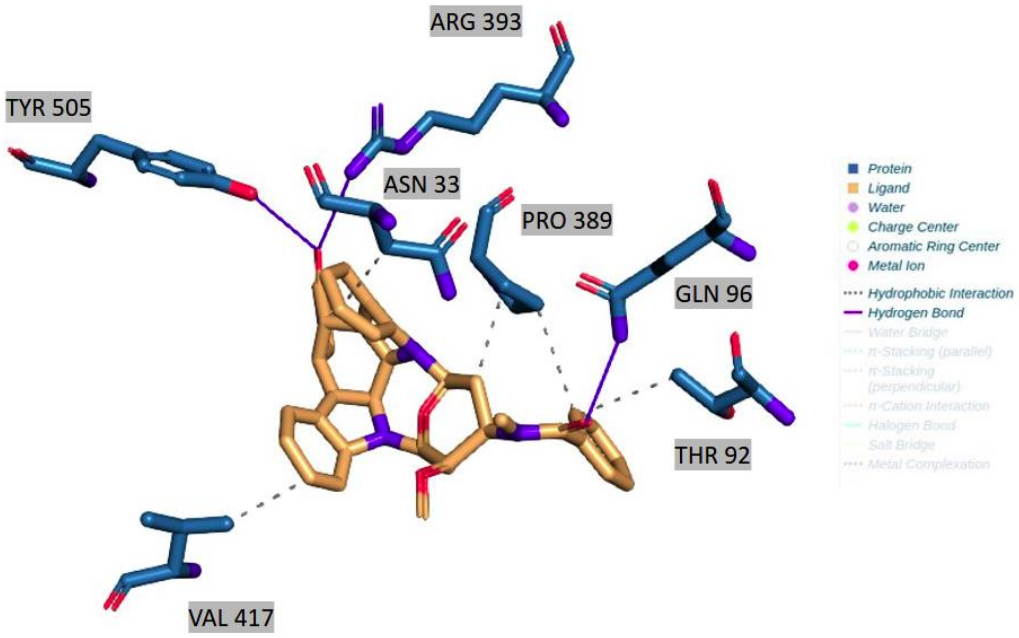

Figure 14. Three-dimensional representation of Midostaurin with its molecular interactions.

Hydrophobic Interactions ····

Index	Residue	AA	Distance	Ligand Atom	Protein Atom
1	33A	ASN	3.46	7789	148
2	92A	THR	3.68	7810	742
3	389A	PRO	3.86	7810	3665
4	389A	PRO	3.25	7797	3664
5	417E	VAL	3.36	7794	6706

Hydrogen Bonds —

Index	Residue	AA	Distance H-A	Distance D-A	Donor Angle	Protein donor?	Side chain	Donor Atom	Acceptor Atom
1	96A	GLN	2.22	2.95	127.18	✔	✔	783 [Nam]	7815 [O2]
2	393A	ARG	2.20	3.11	147.15	✔	✔	3707 [Ng+]	7814 [O2]
3	505E	TYR	2.42	2.99	118.35	✔	✔	7577 [O3]	7814 [O2]

Figure 15. Midostaurin and 6VW1 molecular interactions shown in tabulated form.

4. Triptorelin

Triptorelin is a GnRH agonist preferable for treatment of advanced prostate cancer by sedative means.

The docking result shown (Figure 16) represents the first pose binding perfectly at the selected active site with high binding affinity of -9.0kcal/mol which was significantly greater than that of reference binding affinity (-6.4kcal/mol).Stabilized structure was found with triptorelin making 10 hydrogen bonds and 13 hydrophobic interactions. The bonds and interaction were between drug and residues of both human ACE2 and SARS-CoV-2 Spike RBD.(Figure 17)

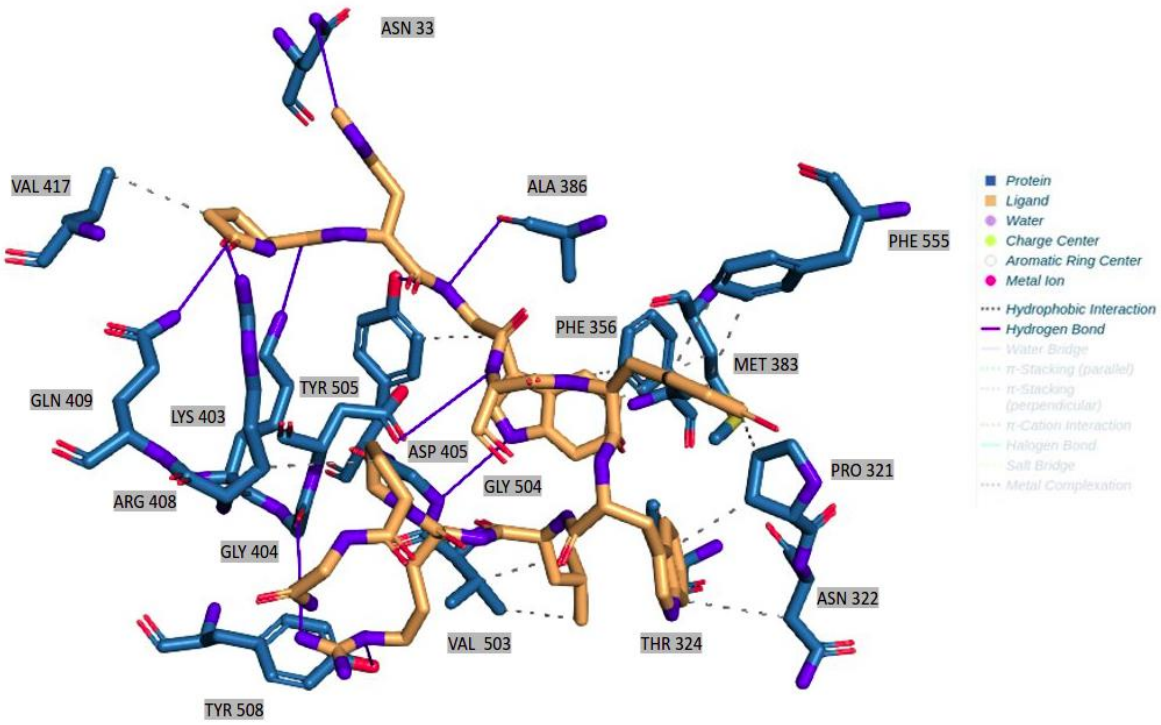

Figure 16. Three-dimensional representation of triptorelin with its molecular interactions.

Hydrophobic Interactions ····

Index	Residue	AA	Distance	Ligand Atom	Protein Atom
1	321A	PRO	3.94	7838	3016
2	321A	PRO	3.55	7816	3015
3	322A	ASN	3.92	7819	3022
4	324A	THR	3.48	7820	3043
5	356A	PHE	3.63	7863	3344
6	383A	MET	3.85	7867	3611
7	408E	ARG	3.91	7803	6622
8	417E	VAL	3.81	7830	6706
9	503E	VAL	3.79	7813	7559
10	503E	VAL	3.65	7814	7557
11	505E	TYR	3.70	7854	7574
12	555A	PHE	3.52	7834	5315
13	555A	PHE	3.60	7836	5314

Hydrogen Bonds —

Index	Residue	AA	Distance H-A	Distance D-A	Donor Angle	Protein donor?	Side chain	Donor Atom	Acceptor Atom
1	33A	ASN	3.43	3.90	110.24	✓	✓	150 [Nam]	7865 [Npl]
2	386A	ALA	2.67	3.21	113.50	×	×	7786 [Nam]	3639 [O2]
3	403E	LYS	2.56	3.11	113.50	✓	✓	6582 [N3+]	7849 [O2]
4	404E	GLY	3.04	4.05	178.17	×	×	7781 [Ng+]	6590 [O2]
5	405E	ASP	3.50	4.00	112.05	×	✓	7785 [Nam]	6598 [O3]
6	408E	ARG	2.18	3.08	146.31	✓	✓	6627 [Ng+]	7850 [O2]
7	409E	GLN	2.62	2.99	101.19	✓	✓	6642 [Nam]	7850 [O2]
8	504E	GLY	2.68	3.52	140.08	✓	×	7561 [Nam]	7864 [Npl]
9	505E	TYR	2.58	3.37	140.58	✓	✓	7577 [O3]	7786 [Nam]
10	508E	TYR	2.50	3.16	121.88	×	✓	7780 [Ng+]	7610 [O3]

Figure 17. Triptorelin and 6VW1 molecular interactions shown in tabulated form.

5. Regorafebnib

Regorafenib is a pre–approved kinase inhibitor drug used for treatment of patients with metastatic colorectal cancer, metastatic gastrointestinal stromal tumors and hepatocellular carcinoma.

The docking result shown (Figure 18) represents the sixth pose binding perfectly at the selected active site with high binding affinity of -8.6kcal/mol which was greater than that of reference binding affinity (-6.4kcal/mol). Stabilized structure was found with regorafebnib making 4

hydrogen bonds and 4 hydrophobic interactions and 1 halogen bond. The bonds and interaction were between drug and residues of both human ACE2 and SARS-CoV-2 spike RBD.(Figure 19)

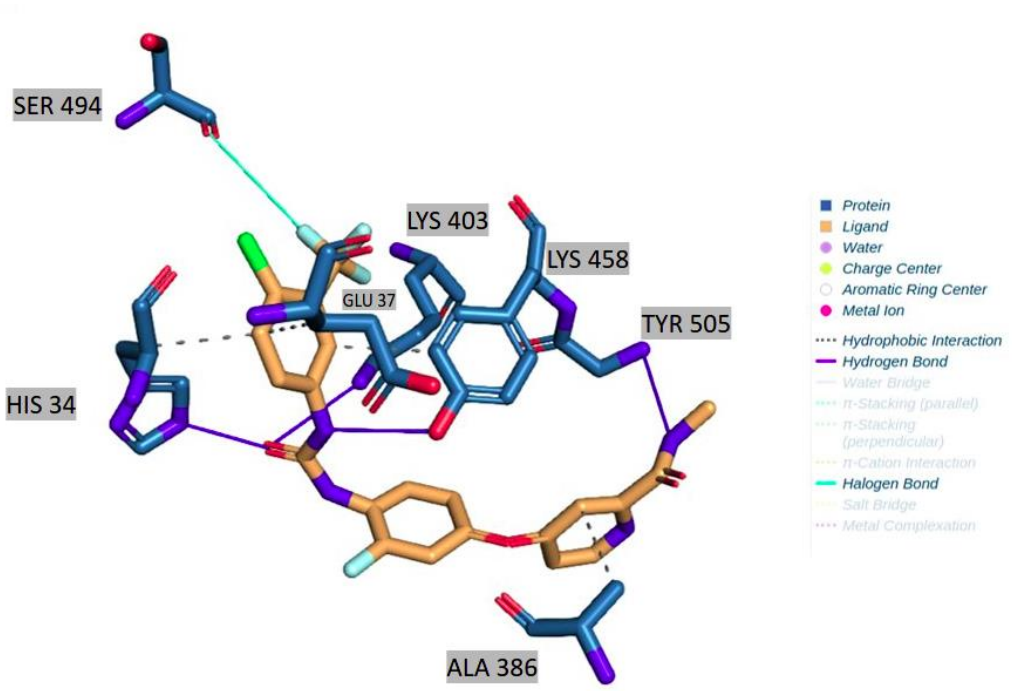

Figure 18. Three-dimensional representation of regorafebnib with its molecular interactions.

Hydrophobic Interactions ····

Index	Residue	AA	Distance	Ligand Atom	Protein Atom
1	34A	HIS	3.85	7786	159
2	37A	GLU	3.31	7786	188
3	386A	ALA	3.76	7796	3640
4	505E	TYR	3.14	7789	7575

Hydrogen Bonds —

Index	Residue	AA	Distance H-A	Distance D-A	Donor Angle	Protein donor?	Side chain	Donor Atom	Acceptor Atom
1	34A	HIS	1.98	2.68	122.78	✔	✔	164 [Npl]	7799 [O2]
2	403E	LYS	2.76	3.60	139.62	✔	✔	6582 [N3+]	7799 [O2]
3	504E	GLY	2.84	3.66	138.16	✔	×	7561 [Nam]	7777 [Nam]
4	505E	TYR	2.42	3.31	147.78	×	✔	7774 [Nam]	7577 [O3]

Halogen Bonds —

Index	Residue	AA	Distance	Donor Angle	Acceptor Angle	Donor Atom	Acceptor Atom
1	494E	SER	3.92	147.07	110.11	7806 [F]	7473 [O2]

Figure 19. Regorafebnib and 6VW1 molecular interactions shown in tabulated form.

6. Ertapenem

Ertapenem is a pre-approved carbapenem antibiotic drug used for the treatment of mild to severe bacterial infections caused by specific sensitive organisms.

The docking result shown (Figure 20) represents the second pose binding perfectly at the selected active site with high binding affinity of -8.5kcal/mol which was greater than that of reference binding affinity (-6.4kcal/mol).Stabilized structure was found with ertapenem making 6 hydrogen bonds and 3 hydrophobic interactions. In addition to these bonds 4 salt bridges were also found two with A chain and two with B chain.The bonds and interaction were between drug and residues of both human ACE2 and SARS-CoV-2 Spike RBD.(Figure 21)

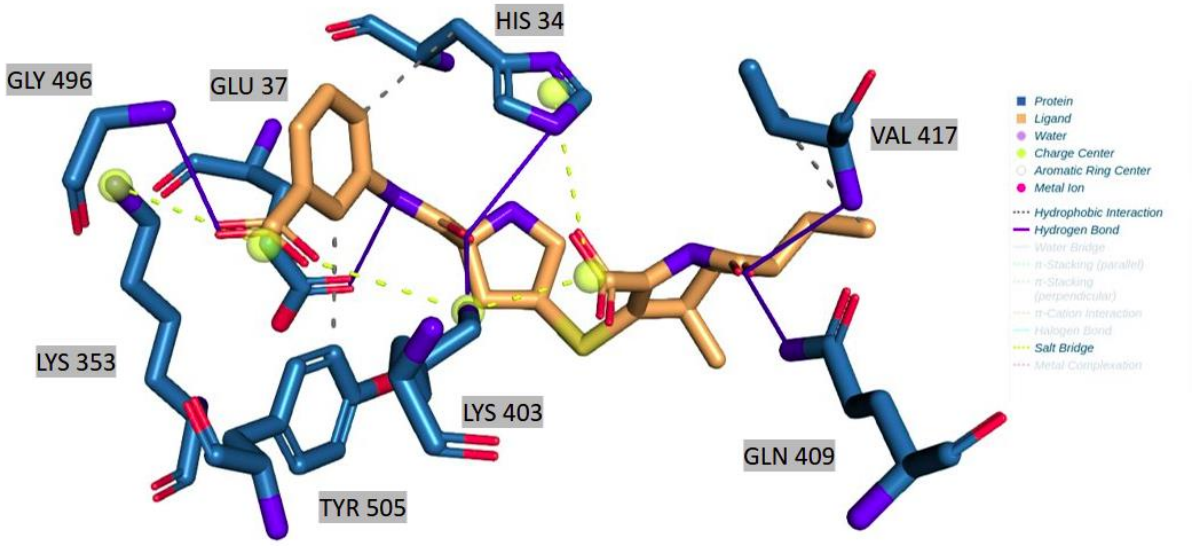

Figure 20. Three-dimensional representation of ertapenem with its molecular interactions.

Hydrophobic Interactions ····

Index	Residue	AA	Distance	Ligand Atom	Protein Atom
1	34A	HIS	3.89	7783	159
2	417E	VAL	3.79	7797	6706
3	505E	TYR	3.47	7787	7575

Hydrogen Bonds —

Index	Residue	AA	Distance H-A	Distance D-A	Donor Angle	Protein donor?	Side chain	Donor Atom	Acceptor Atom
1	34A	HIS	3.16	3.53	102.66	✓	✓	164 [Npl]	7799 [O2]
2	37A	GLU	2.67	3.25	116.76	×	✓	7775 [Nam]	191 [O.co2]
3	403E	LYS	2.47	2.86	101.96	✓	✓	6582 [N3+]	7799 [O2]
4	409E	GLN	2.34	3.21	142.25	✓	✓	6642 [Nam]	7802 [O2]
5	417E	VAL	2.26	3.26	168.58	✓	×	6700 [Nam]	7802 [O2]
6	496E	GLY	2.28	3.13	140.52	✓	×	7492 [Nam]	7800 [O.co2]

Salt Bridges ····

Index	Residue	AA	Distance	Protein positive?	Ligand Group	Ligand Atoms
1	34A	HIS	5.23	✓	Carboxylate	7804, 7805
2	353A	LYS	4.34	✓	Carboxylate	7800, 7801
3	403E	LYS	3.15	✓	Carboxylate	7804, 7805
4	403E	LYS	5.03	✓	Carboxylate	7800, 7801

Figure 21. Ertapenem and 6VW1 molecular interactions shown in tabulated form.

7. Hesperidin

Hesperidin is a bioflavonoid compound found in a variety of food additives that is reproved to have many useful effects on blood vessel disorders and various other conditions related to it.

The docking result shown(Figure 22) represents the ninth pose binding perfectly at the selected active site with high binding affinity of -8.4kcal/mol which was greater than that of reference binding affinity (-6.4kcal/mol).Stabilized structure was found with hesperidin making 10 hydrogen bonds and 1 hydrophobic interactions. In addition to these bonds 1 salt bridges were also found two with A chain.The bonds and interaction were between drug and residues of both human ACE2 and SARS-CoV-2 spike RBD (Figure 23).

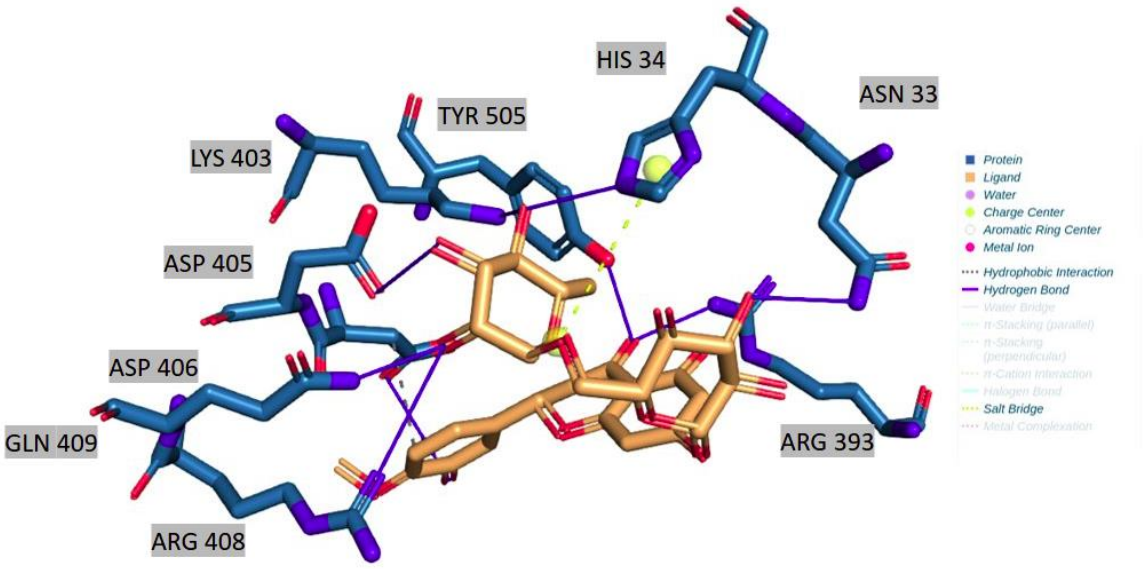

Figure 22. Three-dimensional representation of hesperidin with its molecular interactions.

Hydrophobic Interactions ····

Index	Residue	AA	Distance	Ligand Atom	Protein Atom
1	405E	ASP	4.00	7789	6596

Hydrogen Bonds —

Index	Residue	AA	Distance H-A	Distance D-A	Donor Angle	Protein donor?	Side chain	Donor Atom	Acceptor Atom
1	33A	ASN	2.35	3.36	171.14	✓	✓	150 [Nam]	7804 [O3]
2	34A	HIS	2.21	3.08	142.33	✓	✓	164 [Npl]	7815 [O3]
3	393A	ARG	2.02	2.89	141.82	✓	✓	3707 [Ng+]	7808 [O2]
4	403E	LYS	2.11	3.06	153.31	✓	✓	6582 [N3+]	7815 [O3]
5	405E	ASP	2.85	3.72	148.91	×	✓	7810 [O3]	6598 [O3]
6	406E	ASP	2.29	3.18	150.43	×	✓	7816 [O3]	6607 [O.co2]
7	408E	ARG	3.40	3.95	116.09	✓	✓	6627 [Ng+]	7814 [O3]
8	408E	ARG	3.37	3.95	120.19	×	✓	7814 [O3]	6627 [Ng+]
9	409E	GLN	2.25	2.81	112.79	✓	✓	6642 [Nam]	7814 [O3]
10	505E	TYR	2.16	2.75	118.92	✓	✓	7577 [O3]	7808 [O2]

Salt Bridges ····

Index	Residue	AA	Distance	Protein positive?	Ligand Group	Ligand Atoms
1	34A	HIS	5.23	✓	Carboxylate	7812, 7813

Figure 23. Hesperidin and 6VW1 molecular interactions shown in tabulated form.

5. CONCLUSION

COVID-19 is a pandemic that has not been managed to date. There are several other FDA-approved drugs available that are being used based on the COVID-19 trial, and this is known as the repurposing of drugs.These drugs screened could be the potential leads in fight against this fatal disease. Molecular docking can be considered to be an important method used to classify potential therapeutic agents for COVID-19 patients, and the screened agents should then be tested for efficacy in *in vitro* and *in vivo* trials. Therefore, by performing *in silico* screening of the pre-approved drugs from the drugbank library these 7 drugs(ledipasivir, digitoxin, midostaurin, triptorelin,regorafebnib,ertapenem,hesperidin) discussed above showed better results when compared to the reference inhibitor remdesivir,with ledipasivir showing the best results in terms of binding affinities and molecular interactions.This drug also being an anti-viral drug could be a better potential for further *in vitro* experiments effective as an inhibitor for the SARS-CoV-2 to prevent the host cell interaction.

6. REFERENCES

[1] Q. Wang *et al.*, "Structural and Functional Basis of SARS-CoV-2 Entry by Using Human ACE2," *Cell*, vol. 181, no. 4, pp. 894-904.e9, May 2020

[2] J. Shang *et al.*, "Structural basis of receptor recognition by SARS-CoV-2," *Nature*, vol. 581, no. 7807, pp. 221–224, May 2020

[3] A. R. Bourgonje *et al.*, "Angiotensin-converting enzyme 2 (ACE2), SARS-CoV -2 and the pathophysiology of coronavirus disease 2019 (COVID -19)," *J. Pathol.*, vol. 251, no. 3, pp. 228–248, Jul. 2020

[4] S. Unni, S. Aouti, S. Thiyagarajan, and B. Padmanabhan, "Identification of a repurposed drug as an inhibitor of Spike protein of human coronavirus SARS-CoV-2 by computational methods," *J Biosci*, vol. 45, no. 1, p. 130, Dec. 2020

[5] C. J. Alméciga-Díaz *et al.*, "Virtual Screening of Potential Inhibitors for SARS-CoV-2 Main Protease," LIFE SCIENCES, preprint, Apr. 2020

[6] T. U. Singh, S. Parida, M. C. Lingaraju, M. Kesavan, D. Kumar, and R. K. Singh, "Drug repurposing approach to fight COVID-19," *Pharmacol. Rep*, vol. 72, no. 6, pp. 1479–1508, Dec. 2020

[7] C. Gil *et al.*, "COVID-19: Drug Targets and Potential Treatments," *J. Med. Chem.*, vol. 63, no. 21, pp. 12359–12386, Nov. 2020

[8] L. Mousavizadeh and S. Ghasemi, "Genotype and phenotype of COVID-19: Their roles in pathogenesis," *Journal of Microbiology, Immunology and Infection*, vol. 54, no. 2, pp. 159–163, Apr. 2021

[9] K. Teralı, B. Baddal, and H. O. Gülcan, "Prioritizing potential ACE2 inhibitors in the COVID-19 pandemic: Insights from a molecular mechanics-assisted structure-based virtual screening experiment," *Journal of Molecular Graphics and Modelling*, vol. 100, p. 107697, Nov. 2020

7. APPENDIX

A perl script file was used for commanding Autodock Vina to run docking procedure.

The script of per file:

```
#!/usr/bin/perl

print"Ligand_file:\t";

$ligfile=<STDIN>;

chomp $ligfile;

open (FH,$ligfile)||die "Cannot open file\n";

@arr_file=<FH>;


for($i=0;$i<@arr_file;$i++)

{

print"@arr_file[$i]\n";

@name=split(/\./,@arr_file[$i]);

}

for($i=0;$i<@arr_file;$i++)

{

chomp @arr_file[$i];

print"@arr_file[$i]\n";

system("vina --config conf.txt --ligand @arr_file[$i] --log @arr_file[$i]_log.log");

}
```