Drug repurposing for COVID-19 from FDA approved and experiment stage drugs by *in silico* methods with SARS CoV-2 spike protein

Sharanya CS., Arun KG., Abhithaj J., Sabu A., Haridas M*

*Inter University Centre for Bioscience and Department of Biotechnology & Microbiology, Kannur University, Dr. Janaki Ammal Campus, Palayad, Thalassery, Kannur, Kerala-670661, India.

*Corresponding author: Haridas M, Inter University Centre for Bioscience, Kannur University, Dr. Janaki Ammal Campus, Palayad, Thalassery, Kannur, Kerala-670661, India, Tel; 9446252450, E-mail; mharidasm@rediffmail.com

Abstract

Novel corona virus emerged from the Wuhan province of China is believed to be originated from an unknown animal from seafood market in China. This novel corona virus belongs to the genera beta corona virus and their phylogeny is close to SARS CoV and MERS CoV. The general structural feature of this virus includes structural proteins like envelop, matrix, nucleocapsid, spike and non-structural protein include RNA dependant RNA polymerase. In the present study the focus is on the spike glycoprotein which allows the attachment of virus to the human body. During the time of infection the receptor binding domain in the spike protein bound to cell surface receptor (ACE-2) leads to facilitate the fusion of viral and host cell membrane. Hence, by inhibiting the receptor binding domain in spike protein, its binding towards ACE-2 may be blocked. Thus, the viral fusion with cell surface receptor could be prevented. In this study e-pharmacophore based virtual screening of DrugBank database is carried out to identify candidate drugs for repurposing. The dug molecules were screened based on the pharmacophore generated and filtered through the 6000 drug molecule to obtain better 2000 of them. This filtered drug molecules further screened via structure based approach, involving molecular docking at different precisions. From the large database seven drug lead molecules were selected as hits and their binding energy with the spike protein were calculated. Cladribine, Clofarabine, Fludarabine from approved category and 7-methyl-guanosine-5'-triphosphate-5'-guanosine, Adenosine-2'-5'-Diphosphate, 8-Bromo-Adenosine-5'-Monophosphate, Alpha-Methylene Adenosine Monophosphate in the experimental category were found to be potent inhibitors of SARS CoV-2 spike protein to repurpose as drugs for COVID-19.

Keywords: SARS CoV-2; Spike protein; DrugBank; e-Pharmacophore; Virtual Screening Method

1. Introduction

COVID19 pandemic, a severe respiratory disease caused by novel corona virus SARS CoV-2 (Severe Acute Respiratory syndrome Coronavirus 2), originated from Wuhan, China rises serious challenge to humanity [Benvenuto et al., 2020; Guo et al., 2020]. According to WHO, on 13th May 2020, 4.1 million peoples were infected and 0.25 million were died by this infection, about 203 countries are reported COVID19 throughout the world [WHO]. COVID19 Infected people shows severe inflammation on the lungs which results in the alveolar damage leads to the acute respiratory syndrome and requires special treatment for recovery [Xiong et al., 2020]. Elderly people and peoples with cardiovascular disease, diabetes, chronic respiratory disease, and cancer are at high risk category to develop serious clinical symptoms [Favalli et al., 2020; South et al., 2020; Xiong et al., 2020; Zheng et al., 2020; Zhou et al., 2020]. COVID19 spreading may primarily due to the discharge of saliva or discharge from nose when an infected person cough or sneezes. The acute severe respiratory syndrome may be produced due to the over production of cytokines which produces uncontrolled immune responses that may leads to the multiple organ failure [Mehta et al., 2020]. COVID19 infection shows that the disease was associated with pneumonia and hepatic damages [Xu et al., 2020; Ai et al., 2020].

Corona viruses is a single stranded RNA virus enclosed in a glycoprotein membrane which infect both humans, animals, and certain animals like bats are acting like a reservoirs for them [Bhoopathi et al., 2020; Liu et al., 2020; Velavan et al., 2020]. They are grouped into four types namely α, β, γ, and Δ. The general features of SARS CoV-2 viral structure encompass a nucleo capsid protein within the membrane, consisting of three viral proteins, spike protein, a membrane spanning glycoprotein and a completely covered hydrophobic envelop protein. Furthermore, the genome structure of SARS CoV made of two ORF which are linked through ribosomal frame shift encoding two polyproteins, pp1a (~450 kDa) and

pp1ab (~750kDa). These overlapping replicases mediate the proteolytic cleavage of polypeptide to produce functional proteins by proteolysis [Bosch et al., 2020; Xia et al., 2020; Yuan et al., 2004]. Similarly SARS-CoV and SARS-CoV2 uses these spike proteins to enter into the host cells through the interaction with host cell adhesion protein named as angiotensin converting enzyme 2 (ACE 2) [Shang et al., 2020; Yan et al., 2020;]. Invading process requires a structural modification by trimming of S proteins which is mediated by TMRSS2II, which is serine protease released from the host cell [Hoffman et al., 2020]. The viral genome also poses non-structural proteins responsible for the virulence, which includes, RNA polymerase (RdRP), Corona virus main protease (3CLPro), and Papain like Protease (PLPro) [Sarma et al., 2020; Sanders et al., 2020; Robson et al., 2020; Jin et al., 2020; Elfiky et al., 2020]. After entry into the host cells, the Single Stranded RNA was released which is subsequently translated by using host translation machinery to produce viral polyproteins [Xia et al., 2020; Robson et al., 2020]. Further activation of viral proteins were done by cleaving mechanisms of viral main protease 3CLPro and PLPro. PLPro acts as a ubiquitinase that can deubiquitin certain host proteins like Inteferon factor 3 and NFκB which results immune suppression [Liu et al., 2020; Mothay et al., 2020; Pant et al., 2020].

The drug repurposing is a new methodology which imparts the use of an approved or investigational drug for another disease, other than its original medical use [Khan et al., 2020]. This policy makes several advantages over developing a fully new drug entity for a given indication [Muralidharan et al., 2020]. As the marketed drugs already passed the clinical trials and safety measures, drug repurposing is a cheaper and faster strategy. The regulatory and phase III costs may remain more or less the same for a repurposed drug as for a new drug in the same indication, but there could still be substantial savings in preclinical and phase I and II costs [Pushpakom et al., 2018]. Currently there is no specific vaccine or

therapeutics for COVID19 is available. Present study investigates the in silico drug repurposing to identify potent candidates against SARS-COV 2 by targeting spike receptor.

2. Materials and Methods

Computational applications are performed for the screening of specific spike glycoprotein inhibitors. The studies are carried out in Maestro version 11.4 from Schrodinger *Inc* and the methods include E-pharmacophore hypothesis generation, virtual screening, molecular docking and MM-GBSA. The molecular interactions were monitored using PYMOL and Discovery Studio 2.5

2.1. Protein Preparation and receptor grid generation

The structure of SARS CoV-2 spike protein (6VSB) was downloaded from PDB. Downloaded protein was prepared using the protein preparation wizard in the Maestro software (Maestro, v11.4, Schrodinger, LLC, NewYork, NY). In the pre-processing step in protein preparation wizard, the heterogroup having bond order and formal charges were added, hydrogen atom added to all the atoms in the system, disulfide bond and zero order bonds to metals were created and water molecule within 5Å in the heterogroups was removed. After pre-processing the structure refinement step followed in which missing side chain atoms were incorporated [Sastry et al., 2013]. Then for each structure a brief relaxation was performed with Impact Refinement module (Impref) were all atom constrained minimization was carried out with the aid of forcefield OPLS_2005 to relieve steric clashes present in the original PDB structure [Jorgensen et al., 1996]. After reaching the RMSD cut off of at 0.30 Å, which results in the termination of minimization job. Receptor grid was generated around the receptor binding site residues includes TYR449, TYR453, ASN487, GLY496, THR500, GLY502, TYR505 and receptor grid generation was performed by using receptor grid generation panel in the GLIDE module of Maestro [Halgren et al., 2004].

2.2. Ligand Preparation

The ligands for screening was selected from DrugBank [www.drugbank.ca] which comprises FDA approved drugs, drugs that are under investigational process and experimental drugs. Onset to screening, the ligands was prepared using the LigPrep module with Epik to expand protonation and tautomeric states at neutral pH (7±1). The energy minimization of ligands also carried out with OPLS_2005 force field.

2.3. Pharmacophore generation

The drug designing pursuit leads to lead optimization, hit identification, patent boosting and core hopping together come under the Pharmacophore modelling study. Simply a Pharmacophore model contains spatial arrangement of chemical features that interact with a receptor. The input information for pharmacophore generation includes the 3D structures of proteins, ligands, ligand-protein complexes, active site residues etc.

Generation of hypothesis for e-pharmacophores (energy optimized pharmacophores)

The e-pharmacophore hypothesis was developed based on the receptor binding domain residues TYR449, TYR453, ASN487, GLY496, THR500, GLY502, TYR505 reported by Wrapp et al., 2020. The e-pharmacophore model was developed using the 'Develop pharmacophore using receptor cavity' option in the phase module. For this, the prepared protein-ligand complex was imported to the workspace and default pharmacophore features such as hydrogen bond accepter (A), hydrogen bond donor (D), aromatic ring (R) and hydrophobicity (H) were mapped. The receptor cavity was marked by its active site residues and based on these residues hypothesis was generated [Salam et al., 2009].

2.4. Virtual screening of ligands based on e-pharmacophore

Based on the hypothesis created in the e-pharmacophore drugs having expected molecular features for receptor binding domain of spike glycoprotein interaction, virtual screening was carried with drug bank database using the Phase module of Schrodinger Suite. The fitness scores were used to select the best hits. Fitness score related to the alignment of ligands conformer match towards the hypothesis based on RMSD site matching, volume and vector alignment and it ranges from 0-3 as instigated in the default database screening in Phase module.

2.5. Virtual screening based on structure

About 4000 candidate molecules screened out after e-pharmacophore based virtual screening, molecular docking was followed in order to identify the most promising drug candidate from this subset. The programme used for molecular docking is the GLIDE (Grid-based Ligand Docking with Energetics) module of Schrodinger suite. The receptor binding site (RBD) amino acids were selected and a grid box was generated around this cavity with a size of 15×15×15 Å. The results were expressed based on the binding affinity and ranked by Glide scores (g score). The initial screening was performed using the High Throughput Virtual Screening module (HTVS) module of glide and the top scoring compounds were subjected to standard precision (SP) docking. Finally, extra precision (XP) docking method was used to identify the best hits. The G score obtained via ligand-receptor interaction was calculated based on the following equation

$$\text{Glide score} = 0.065 \times \text{vdW} + 0.130 \times \text{Coul} + \text{Lipo} + \text{Hbond} + \text{Metal} + \text{BuryP} + \text{RotB} + \text{Site}$$

where vdW related to van der Waals energy, Coul signifies Coulomb energy, Lipo represents lipophilic term derived from hydrophobic grid potential, Hbond denote hydrogen-bonding term, Metal shows metal-binding term, BuryP indicates penalty for buried polar groups, RotB

assumes penalty for freezing rotatable bonds, and Site implies polar interactions in the active site.

3. Result and Discussion

Covid-19 the pandemic disease cause increased death and infections day by day. So the entire world is behind the drug and vaccine development programme to combat the disease. Structure based studies are going on and drug repurposing also makes the development process easy and time consuming. Currently using drug specifications are antiviral drugs, antibiotics and convalescent plasma therapy. Moreover a constant drug treatment should be available for these deadly viral attacks and we want to make the situation controllable. The SARS novel corona virus structure was explored in 2020 and the main component of this virus is a protease and spike glycoprotein.

The first Cryo EM structure of Novel corona virus spike protein was revealed by Wrapp et al., 2020. The structure of spike glycoprotein (Figure 1) showed as a single polypeptide chain with 1300 amino acid in length which is a homo-trimer and cleaved by furin- like proteases of host cell into SI and S2 subunit. S1 subunit is the N-terminal domain which is responsible for the host cell attachment by bound to the cell membrane receptors through its receptor binding domain and S2 is the C-terminal domain [Walls et al., 2020; Wrapp et al., 2020; Ou et al., 2020]. The receptor binding domain in the S1 subunit of the corona virus is therefore responsible for the zoonotic transmission, recognition of host cell and invasion. The S2 subunit comprises two heptad region HR1 HR2 and a lipophilic fusion protein which make the coiled helix structure of the subunit. During the time of infection the receptor binding domain in the spike protein bound to cell surface receptor leads to conformational change in the both subunit (S1 and S2) and the fusion loop expose and fuse to membrane together with

the heptad region form a bundle fusion core and facilitate the fusion of viral and host cell membrane [Chen et al., 2020; Lan et al., 2020].

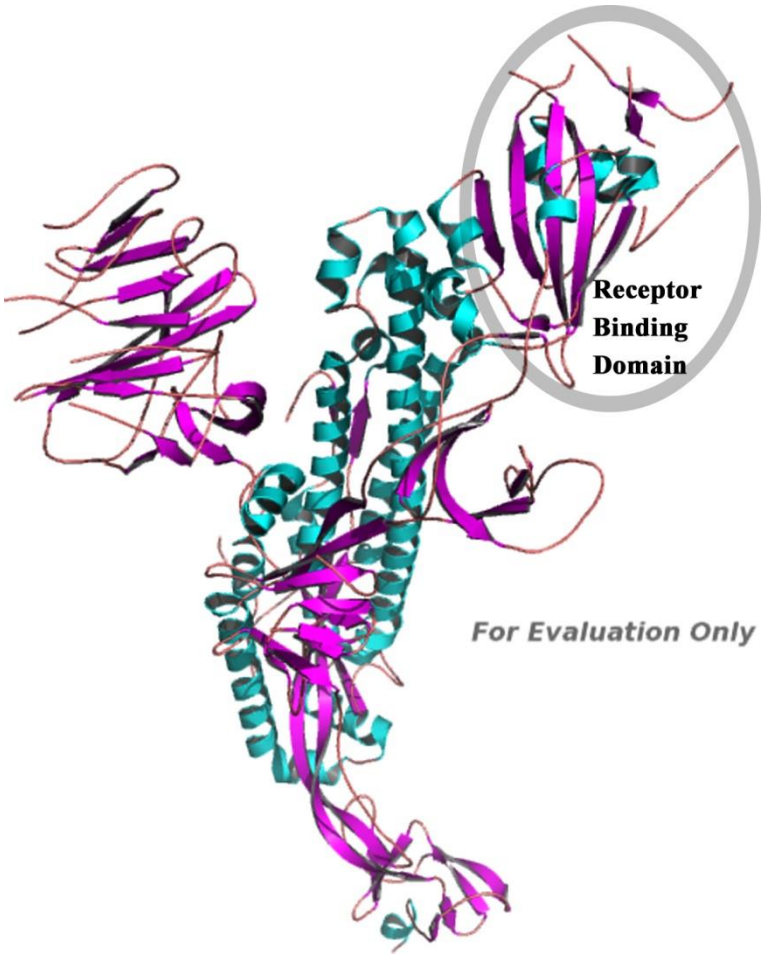


Figure 1: SARS CoV-2 Spike glycoprotein (PDB ID: 6VSB)

3.1. E-pharmacophore model and pharmacophore-based virtual screening

In the drug discovery process both structure based protein-ligand docking and ligand based pharmacophore modelling play significant roles. For the quick screening of large compound-database can be easily screened through ligand based virtual screening method like 3D pharmacophore modelling. The e-pharmacophores method achieves the advantages of both ligand- and structure-based approaches by generating energetically optimized, structure-based pharmacophores that can be used to screen millions of compounds rapidly. In our study we have developed an e-pharmacophore hypothesis based on the receptor cavity to screen

inhibitors of the spike protein of CoV-2 using its crystal structure. The receptor binding site amino acids were analysed and a seven-featured pharmacophore hypothesis, AAARRRR was generated. The generated e-pharmacophore model contains four aromatic rings (R) and three hydrogen bond acceptors. The acceptor A5 lies in the vicinity of GLY504, A8 lie in the vicinity of THR500, A9 lie in the vicinity of SER494, aromatic features R23, R25, R26, R27 found close to ARG403, ASP405, GLU406, ASN450 (Figure 2 & 3). The DrugBank library consists of 2 sets of compounds, one is approved FDA drugs, and the other is an experimental set. Based on the hypothesis, 1482 drug molecules from the approved drug category and 4562 molecules from experimental drugs category were selected to match the pharmacophore model that we have developed. During the screening, the phase module analyses the fitness of compounds with the query hypothesis and ranks the search results based on a fitness score Fitness score determines how strongly a ligand is fitted to the active site of the receptor molecule. Both sets were screened separately and 700 molecules from approved category and 1300 from experimental category were listed based on the fitness score obtained and these molecules were further undergone structure based screening based on molecular docking.

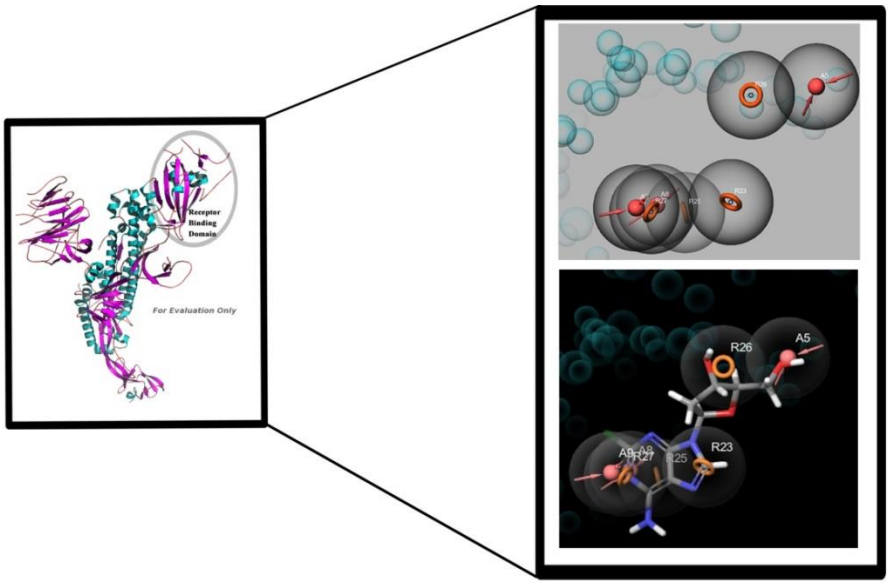


Figure 2: Pharmacophore model of RBD site of spike protein generated by e-pharmacophore method in Phase module

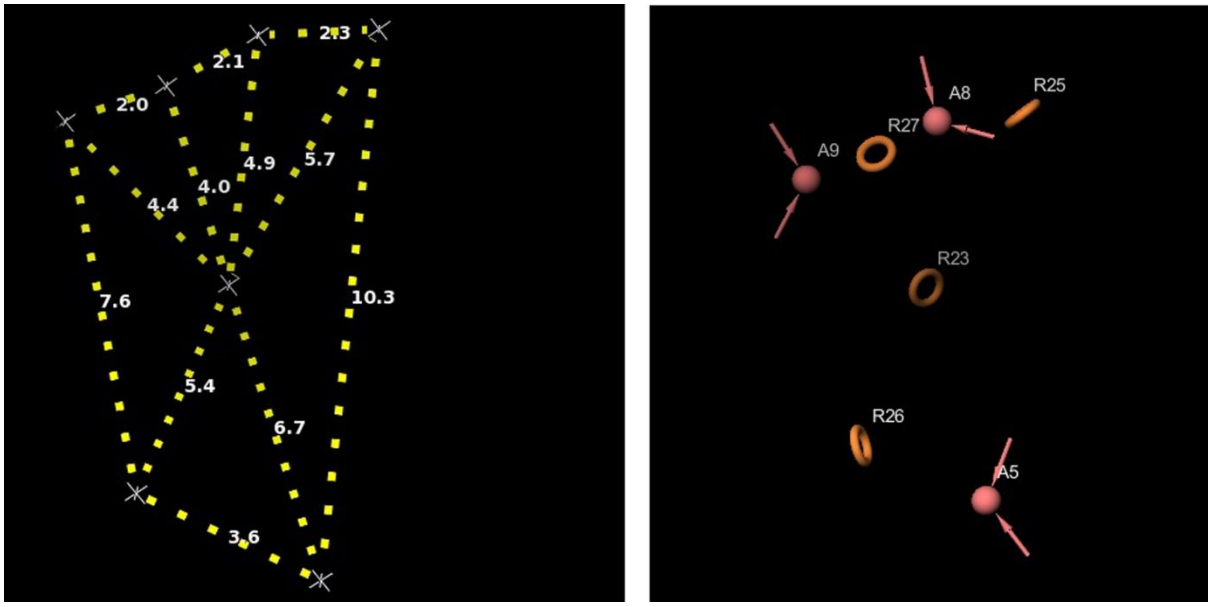


Figure 3: The distance between each pharmacophore groups and the generated pharmacophore AAARRRR (A5, A8, and A9 are hydrogen bond acceptors; R23, R25, R26 and R27 are aromatic rings)

Structure-based virtual screening and MM-GBSA

Since the ligand bounded structure of spike protein was not readily available, we opted to go for hypothesis generation and grid preparation based on the receptor binding site residues of spike protein. From the pharmacophore based virtual screening we reached to 2000 compounds from 6000 drug molecules based on the fitness score (Table 1). Then these drug molecules were urther undergone structure based screening in which initially a high throughput screening was carried out (HTVS). This initial screening was followed when there was a large number of compounds for confirm. HTVS, compared to standard precision conformational sampling, is much more restricted and cannot be used with score-in-place. About 297 compounds from 700 compounds in the approved drugs and 913 compounds from 1300 molecules in the experimental category were filtered out based on dock score from HTVS docking. This large number of compounds cannot be directly moved on to extra precision docking; hence standard precision was carried out. The scoring function in SP

includes lesser terms and its processing require less CPU time. This decreased further the number of ligand molecules. XP used explicit water technology and descriptors. The HTVS screening showed a docking score between -4 to -7 kcal/mol and SP docking having a docking score between -6 to -8 kcal/mol. Thus, from 297 molecules, SP docking filtered the approved category compounds to 26, and 913 molecule from experimental category to 90. These filtered drug molecules then further were undergone extra precision docking which gave a better correlation between scores and poses. Extra precision lowered the number of molecules from standard precision into less number of more strongly featured molecules for drug development process. The final screening and docking results in the generation of 7 molecules (Figure 4) and their binding affinity towards spike RBD showed in the Table 1.

Drug	**G Score Kcal/mol**	**Glide emodel kcal/mol**	**Fitness Score**	**Alignment Score**
7-methyl-guanosine-5'-triphosphate-5'-guanosine	-9.102	-58.068	1.055	0.832
Adenosine-2'-5'-Diphosphate	-8.569	-56.250	0.998	0.979
8-Bromo-Adenosine-5'-Monophosphate	-8.124	-54.548	0.954	1.026
Alpha-Methylene Adenosine Monophosphate	-7.606	-46.276	1.033	1.007
Cladribine	-7.865	-40.613	0.959	1.033
Fludarabine	-7.034	-32.543	0.998	0.927
Clofarabine	-7.200	-36.732	0.937	1.024

Table 1: The glide score, glide emodel, fitness score and alignment score analysis of top hits

The drug molecules which showed high glide score in the experimental and approved category includes, 7-methyl-guanosine-5'-triphosphate-5'-guanosine (-9.102 kcal/mol), Adenosine-2'-5'-Diphosphate (-8.569 kcal/mol), 8-Bromo-Adenosine-5'-Monophosphate (-8.124 kcal/mol), Alpha-Methylene Adenosine Monophosphate (-7.606 kcal/mol), Cladribine

(-7.865 kcal/mol), Clofarabine (-7.2 kcal/mol) and Fludarabine (-7.034 kcal/mol). These molecules are comes under the class of nucleosides and nucleotides (Figure 4, 5, 6 and 7; table 2) and the chemotherapeutic agents for leukaemia among the class of approved drugs. .

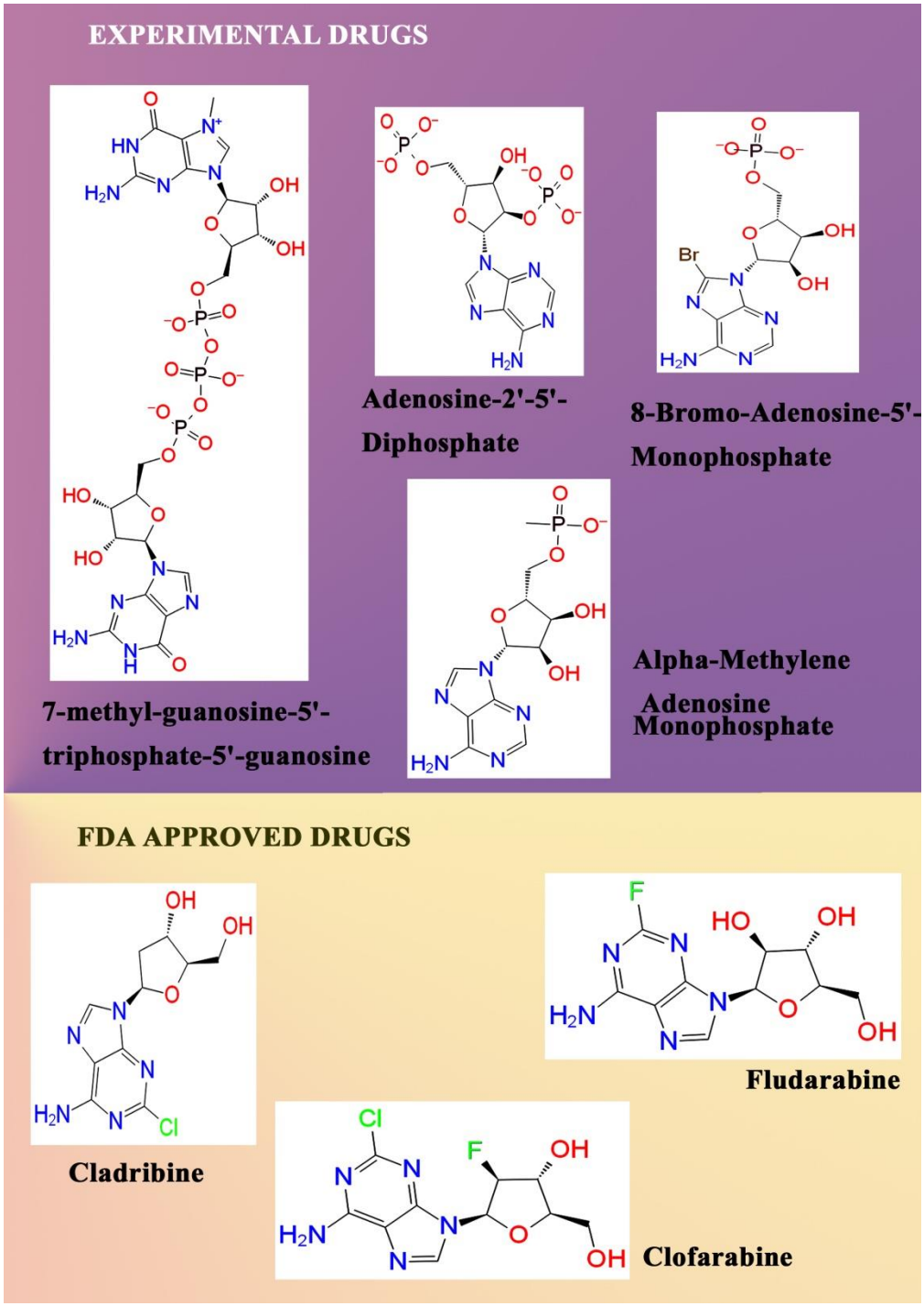


Figure 4: The 2D image of top hit molecules after structure based virtual screening

The approved category of drugs that showed a better inhibition rate for SARS CoV-2 are come under the class of purine nucleoside. The three drug molecules Cladribine, Clofarabine and Fludarabine are used for treatment of leukaemia. Cladribine mainly used to treat active hairy cell leukemia (leukemic reticuloendotheliosis) as defined by clinically significant anemia, neutropenia, thrombocytopenia, or disease-related symptoms [Warnke et al., 2010]. This drug molecule is further used as an alternative agent for the treatment of chronic lymphocytic leukemia (CLL), low-grade non-Hodgkin's lymphoma, and cutaneous T-cell lymphoma [Sigal et al., 2010]. Clofarabine is used in paediatrics to treat a type of leukaemia called relapsed or refractory acute lymphoblastic leukaemia (ALL) [Larson and Venugopal, 2010; Maranda et al., 2009; Sampat et al., 2009]. Its potential use in acute myeloid leukaemia (AML) and juvenile myelomonocytic leukaemia (JMML) has been investigated further [Pession et al., 2010; Saigal et al., 2010]. Fludarabine acts at DNA polymerase alpha, ribonucleotide reductase and DNA primase, resulting in the inhibition of DNA synthesis, and destroys the cancer cells [Tournilhac et al., 2004; Rai et al., 2000; Gonzalez et al., 1998]. The approved category drugs are coming under the class of purine ribonucleotides and their primary targets are only available. The primary target of 7-methyl-guanosine-5'-triphosphate-5'-guanosine is eukaryotic translation initiation factor 4E [www.drugbank.ca]. Likewise the primary target of Adenosine-2'-5'-Diphosphate is ribonuclease, 8-Bromo-Adenosine-5'-Monophosphate is cAMP-specific 3',5'-cyclic phosphodiesterase 4B and histidine triad nucleotide-binding protein 1, Alpha-methylene adenosine monophosphate primary target is ribose-phosphate pyrophosphokinase which involved in the biosynthesis of ribose 1,5-bisphosphate [www.drugbank.ca].

The drug interaction within the RBD site specifies these drug molecules as promising candidate as SARS CoV-2 inhibitor. The ligand interaction diagrams showed a potential H-bond with ASP405, ASN450, GLY496, and TYR505 (Table 2). The bond lengths between

each group were identified and found to be <3 Å. The binding energy of these molecules was studied through Prime MMGBSA and the DG binding energy was calculated. The Van der Waal's energy was also calculated within 4 Å; and the results are shown in the table 2.

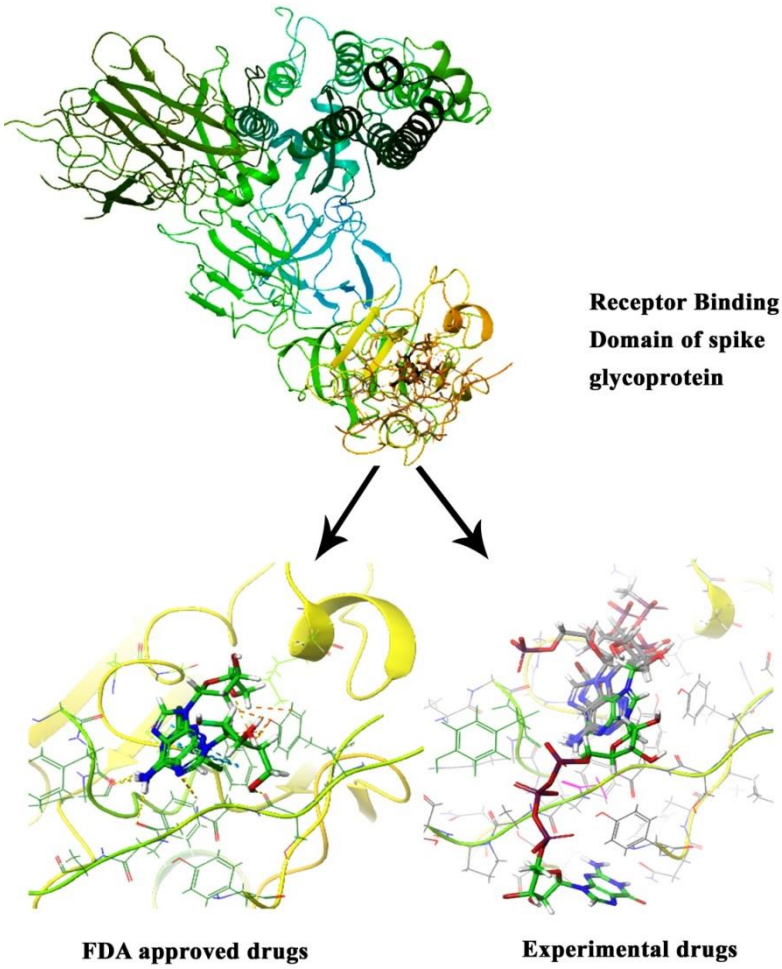


Figure 5: Interaction of top hits DrugBank molecules at the RBD site of spike protein

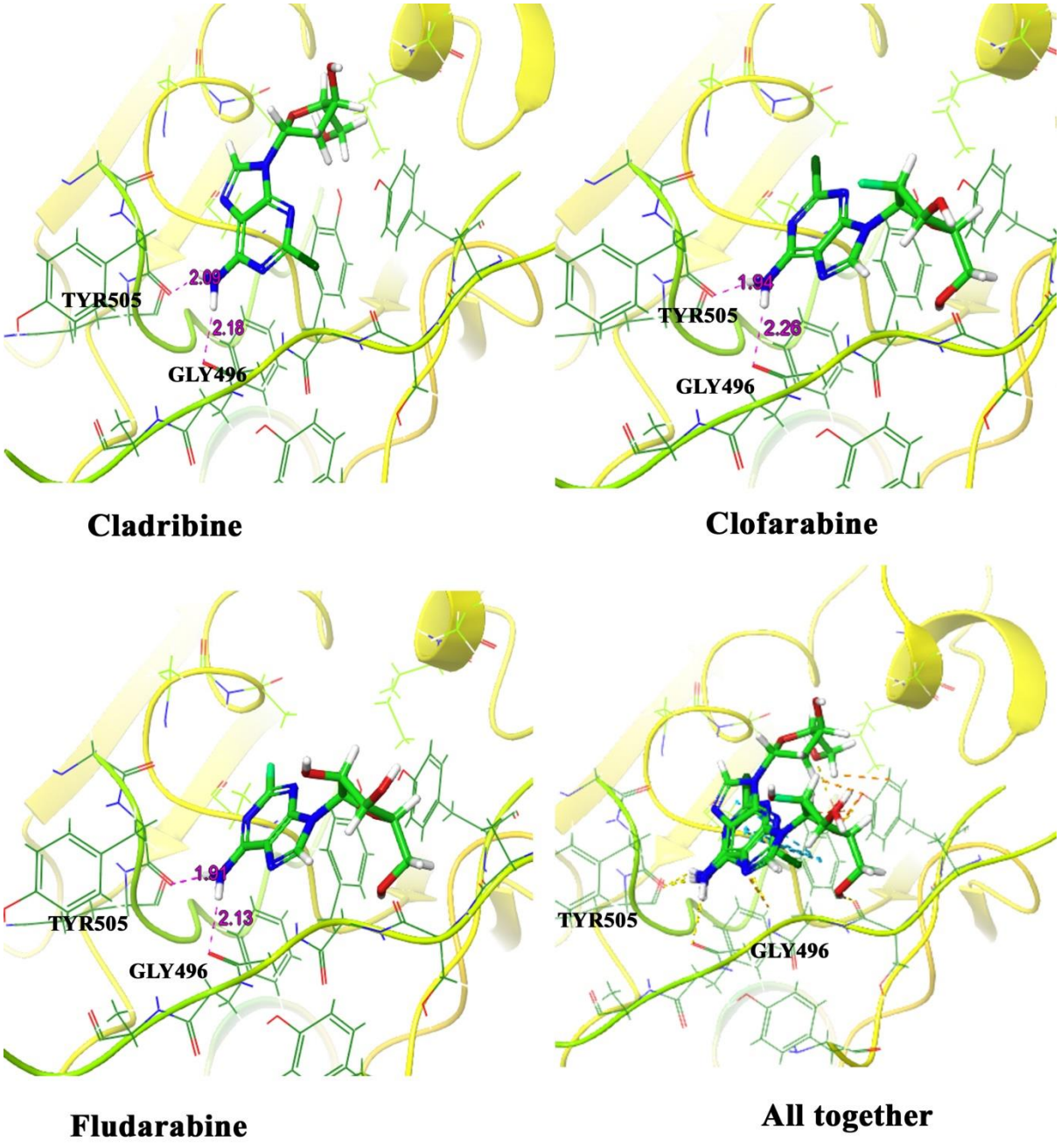
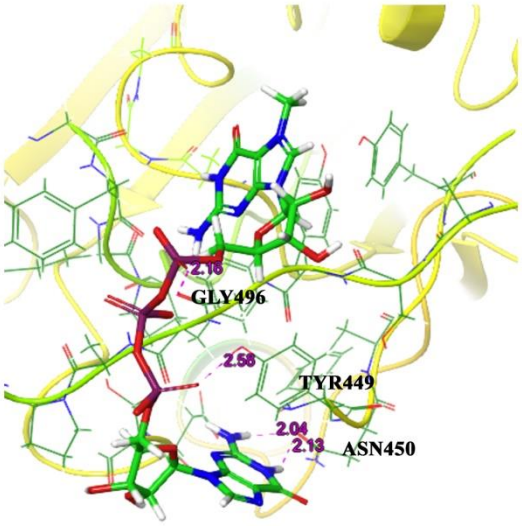
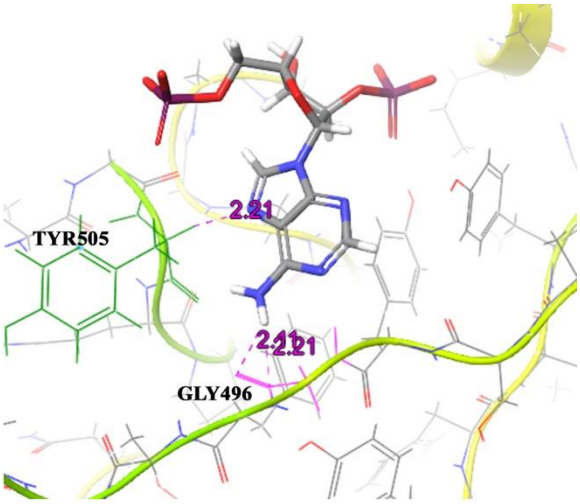


Figure 6: Interaction of the FDA approved drug molecules with RBD site of spike protein

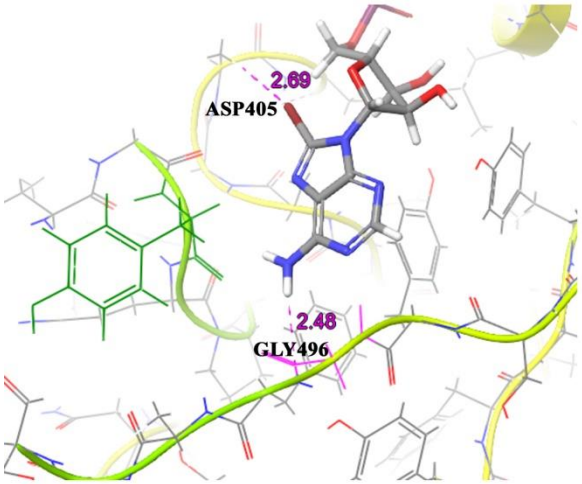


7-methyl-guanosine-

5'-triphosphate-5'-guanosine



Adenosine-2'-5'-Diphosphate



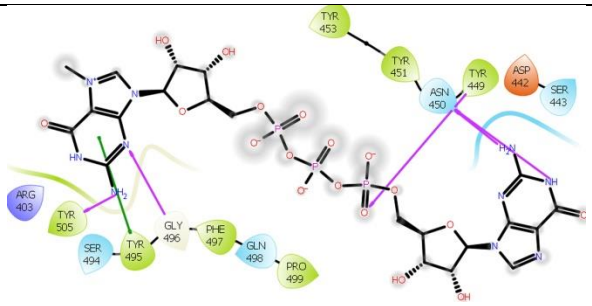
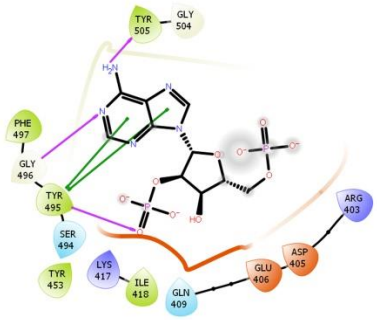
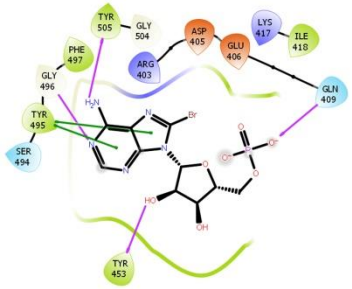
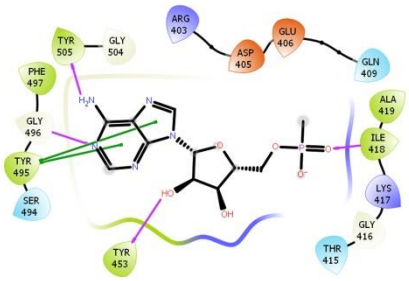
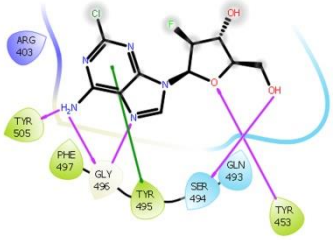
8-Bromo-Adenosine-5'-

Monophosphate

Alpha-Methylene Adenosine

Monophosphate

Figure 7: Interaction of the experimental drug molecules with RBD site of spike protein

Drug	Wander Vaal's contact (4Å)	Binding energy kcal/mol	Interaction Diagram	H-Bonds
7-methyl-guanosine-5'-triphosphate-5'-guanosine	68	-43.72		LYS417 ILE418 GLY496 TYR505
Adenosine-2'-5'-Diphosphate	57	-31.69		GLY496 TYR495 TYR505
8-Bromo-Adenosine-5'-Monophosphate	44	-39.49		GLN409 TYR453 GLY496 TYR505
Alpha-Methylene Adenosine Monophosphate	47	-46.63		TYR453 GLY496 TYR505
Cladribine (Litak; Movectro; Mylinax)	36	-45.90		GLY496 TYR495

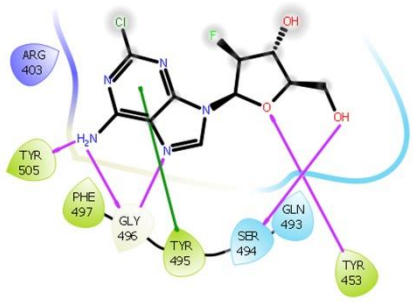
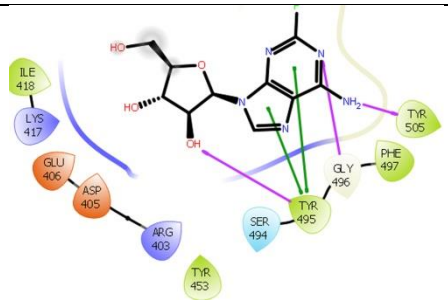
Clofarabine	38	-37.94		GLY496 SER494 TYR453 TYR505
Fludarabine	31	-35.61		GLY496 TYR505

Table 2: Summary of 2D interaction diagram with H-bonds, binding energy and Van der Waals contacts (4 Å)

Conclusion

Since the deadly virus going to increase its effect day by day scientist in all over the world are trying to knockdown the disease by discovering drugs and vaccines. As the latest report showed that about 4.1 million people were affected and 0.25 million lost their lives, and currently no specific drugs are available. At present only symptomatic and supportive treatments are available for this deadly disease. This is a highly mutation prone virus, hence specific drugs must be developed even if vaccine could be developed. In this context all methods of drug discovery must be employed. Hence, the present endeavour to identify inhibitors against SARS Cov-19 is important and it is found promising. It may be noted that the already available drugs for various other ailments could be repurposed and better ones may be considered for obtaining approval for treating Covid-19. In present study we have screened the already FDA approved drugs and experimental drugs from the DrugBank by e-pharmacophore based and structure based virtual screening method. From 6000 molecules

the screening could focus onto seven prominent molecules, the final top hits. Among this the FDA approved category of drugs are Cladribine (-7.865 kcal/mol), Clofarabine (-7.2 kcal/mol) and Fludarabine (-7.034 kcal/mol). They are top hits and are already in use for treating leukaemia. The remaining four molecules found experimentally fit to test as drugs for COVID-19 infection are 7-methyl-guanosine-5'-triphosphate-5'-guanosine (-9.102 kcal/mol), Adenosine-2'-5'-Diphosphate (-8.569 kcal/mol), 8-Bromo-Adenosine-5'-Monophosphate (-8.124 kcal/mol), and Alpha-Methylene Adenosine Monophosphate (-7.606 kcal/mol). The common feature behind all these drugs are, they all come under the class of purines nucleotides and nucleosides. These molecules can be of repurpose as inhibitors of SARS CoV-2. Further, they are fit to undergo in vitro and in vivo trials and can make good drug candidates, slashing time and resources. However, these drugs need be further evaluated using in-vitro studies to confirm their inhibitory activity before they can be adapted to a drug development pipeline.

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Disclosures

The authors indicate no potential conflicts of interest.

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