Identification of SARS-CoV-2 Cell Entry Inhibitors by Drug Repurposing using *in silico* Structure-based Virtual Screening Approach

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The rapidly spreading, highly contagious and pathogenic SARS-coronavirus 2 (SARS-CoV-2) associated Coronavirus Disease 2019 (COVID-19) has been declared as a pandemic by the World Health Organization (WHO). The novel 2019 SARS-CoV-2 enters the host cell by binding of the viral surface spike glycoprotein (S-protein) to angiotensin converting enzyme 2 (ACE2). The virus specific molecular interaction with the host cell represents a promising therapeutic target for identifying SARS-CoV-2 antiviral drugs. The repurposing of drugs can provide a rapid and potential cure towards exponentially expanding COVID-19. Thereto, high-throughput virtual screening approach was used to investigate FDA approved LOPAC library drugs against both the S-protein and ACE2 host cell receptor. Primary screening identified a few promising drugs for both the targets, which were further analyzed in details by their binding energy, binding modes through molecular docking, dynamics and simulations. Evidently, Eptifibatide acetate, TNP, GNF5, GR 127935 hydrochloride hydrate and RS504393 were found binding to virus binding motifs of ACE2 receptor. Additionally, KT185, KT203 GSK1838705A, BMS195614, and RS504393 were identified to bind at the receptor binding site on the viral S-protein. These identified drug molecules may effectively assist in controlling the rapid spread of SARS-COV-2 by not only potentially inhibiting the virus at entry step but also as anti-inflammatory agents which could impart relief in lung injuries. Timely identification and determination of an effective drug to combat and tranquilize the COVID-19 global crisis is the utmost need of hour. Further, prompt in vivo testing to validate the anti-SARS-COV-2 inhibition by these drugs could save lives is justified.
INTRODUCTION

The world is facing a dire situation of global public health emergency due to a viral pandemic of severe febrile pneumonia like respiratory syndrome caused by novel coronavirus, provisionally named as 2019-nCoV and later SARS-CoV-2 causing COVID-19 disease. SARS-CoV-2, a member of the *Coronaviridae* family, is a type of positive-sense, single-stranded enveloped RNA viruses responsible for causing infections in avian, mammalian and marine species across the world (1). Clinical onset of infection in COVID-19 is characterized by symptoms as headache, dry cough, and fever; in severe cases multi-organ failure, and even deaths are reported (2). As of March 14th 2020, the outbreak has adversely affected more than 1,42,539 people globally, and about 5393 deaths have already been reported from Mainland China and rest of the 134 affected countries (3).

Infections caused by alpha-coronavirus are usually mild and asymptomatic, whereas beta-coronaviruses like severe acute respiratory syndrome coronavirus (SARS-CoV) and Middle East respiratory syndrome coronavirus (MERS-CoV), have caused serious epidemics (4). In the year 2002, SARS-CoV emerged as an epidemic in China and resulted in approximately 8000 reported cases (5). Recurrence in the form of MERS-CoV was later reported in Saudi Arabia, with a fatality rate of 35% (6)(7). NL63-CoV, HCoV-OC43, and HCoV-HKU1 are a few other coronaviruses responsible for causing infections in humans (8).

Re-emergence of coronaviruses, as SARS-CoV-2 in the end of year 2019, has put the world on high alert and has created an alarming situation demanding an urgent treatment to preclude the potential death of infected patients (9)(10). Despite extensive efforts worldwide by researchers, there is still no effective antiviral drugs or therapies available that could treat patients or prevent the virus transmission. Current prevention and treatment efforts are directed on quarantine and containment of infected patients to prevent human to human transmission (10)(11). However, reports are available on repurposing the antiviral drugs like remdesivir, lopinavir, ritonavir, chloroquine etc. present in the market, and neutralizing monoclonal antibody-based therapeutics are also being developed to combat COVID-19 (12)(13)(14)(15).

Coronavirus infection in humans is driven mainly by interactions between envelope-anchored spike glycoprotein (S-protein) of CoV and the host cell receptor, angiotensin-converting enzyme 2 (ACE2) (16)(17). The S-protein is made up of two subunits, S1 as the receptor-binding domain (RBD) and S2 subunit is responsible for the fusion of viral membrane and the host cellular membrane (18). The overall sequence similarities between S-protein of SARS-CoV-2 and previous SARS-CoV are approximately 75% (19)(20). Furthermore, the residues of S-RBD of SARS-CoV-2 are highly conserved when compared to SARS-CoVs from bats, human, and civet. The affinity between S-RBD of SARS-CoV-2 and ACE2 is found to be approximately ten times higher when compared with SARS-CoV RBD (year 2003), implying that ACE2 is the specific receptor which is responsible for the binding of virus to the host cell membrane (7)(21). Evidently, the key residues of SARS-CoV RBD (residues Phe442, Leu472, Asn479, Asp480 and Thr487) are hypothesized to have undergone natural selection in SARS-CoV-2 and have been proposed to play a critical role in cross-species transmission of SARS-CoV. Based on previous studies, Lys 31 and Lys 353 located on ACE2 are considered to be
virus-binding hotspot residues liable for binding of S-protein (1)(22). In human ACE2 receptor, hotspot 31(Lys 31) is made up of salt bridge between Lys31 and Glu35, and hotspot 353 is made up of another salt bridge between Lys353 and Asp 38, surrounded by a hydrophobic environment (22). SARS-CoV-2 recognizes human ACE2 by its residue Gln493 and Leu 455, which are proposed to form favorable molecular interactions with hotspot 31, thereby enhancing viral binding to human ACE2. Additionally other key residues of S-protein provide more support for hotspot 31(SARS-CoV-2: Leu455, Phe486, Ser494; SARS-CoV: Phe442, Leu472 and Asp480). In SARS-CoV-2, residue 494 which is a serine also strengthens structural stability of hotspot 353(Lys-353) of ACE2 receptor (1).

Intriguingly, detailed molecular analysis and characterization of these interactions between ACE2 receptor and S-RBD of SARS-CoV-2 is essential to develop vaccines or therapeutic drugs for prevention and treatment of infections SARS-CoV-2. Structure-based virtual screening of small-molecules based on epitopes, polyprotein, S-RBD domain of SARS-CoV-2, or the virus specific enzyme ACE2 present on host cell (PDB ID: 2AJF). Repurposing them for coronavirus infections can be an alternative approach that could help to discover potential antiviral molecules relatively quickly. To this end, structure-based virtual screening approach was used for identifying inhibitor molecules targeting SARS-CoV-2 virus-host cell interaction, using the crystal structure of ACE2 complexed with S-RBD and the newly released whole genome sequence of 2019-nCoV (SARS-CoV-2) (23)(24). Given that ACE2 is the key receptor for S-RBD, the hotspot 31 and hotspot 353 residues were targeted in this study, to identify small molecules that could help in preventing SARS-CoV-2 infections. This framework was reiteratively applied to identify small molecules targeting both the virus binding hotspot 31 and hotspot 353 on ACE2 receptor, and the residues of S-RBD of SARS-CoV-2. Binding interactions of antiviral drugs identified in this study, were validated using in silico structure based molecular docking and simulation approach. This study has identified drug molecules, which can be directly tested for in vitro and in vivo studies, to combat a global threat of COVID-19.

MATERIALS AND METHODS

Hardware and software

All computational study work was done on macOS Mojave workstation with 8-core Intel Xeon E5 processor. Bioinformatics software, such as PyRx (25), Open Babel (26), AutoDock Vina (27), PyMol (28), GROMACS (29) and online resources like SWISS MODEL (30), HADDOCK (31), RCSB PDB (32), NCBI (33), ProCheck at RCSB validation server (34), ProSA-web (35), SAVES-Verify3D (36) server, etc. were used in this study.

3D homology model generation of S1-Subunit:

Homology modelling for S1-subunit of S-protein (residues 319-529) of SARS-CoV-2 was done using SWISS-MODEL. NCBI was used to obtain target sequence for SARS-CoV-2 based on whole genome sequence of SARS-CoV-2 (GenBank accession number: MN908947.3). Crystal
structure of SARS-COV-2 S-protein (PDB ID: 6VSB) was the best template hit obtained which has a sequence identity of ~99%. This was used as a template to build three-dimensional model of S-RBD protein of SARS-COV-2. Quality assessment of the predicted 3D homology model of S-RBD protein was done using PROCHECK, followed by validation using ProSA plot, SAVES server, and Verify 3D. The best-mapped model with the least number of residues in the disallowed region was selected and used for the virtual screening to identify drug molecules that binds S-RBD.

**Choice of ligand library**

For structure based repurposing of clinically approved drugs, LOPAC drug library (Library of Pharmacologically Active Compounds, Sigma-Aldrich, St. Louis, MO) of ~1280 molecules, was used for screening the FDA approved potent antiviral drugs. These drug molecules were docked into crystal structures of ACE2 and modelled S-RBD of SARS-COV-2.

**Structure based virtual screening against ACE2 receptor and S-RBD**

For this study, crystal structure of ACE2 receptor protein (PDB ID: 2AJF) and the spike protein S-RBD, which has been modelled using template of S-protein of SARS-COV-2 (PDB ID: 6VSB), was used. The three dimensional structures of drugs or small chemical molecules retrieved from LOPAC library were of SDF type. Open Babel software was used to convert all ligands into PDBQT type. AutoDock Vina (Version 4.2) and PyRx were used to screen FDA approved LOPAC library drug molecules against ACE2 protein of host cell. Additionally modelled structure of S-RBD of SARS-COV-2 was also used for in silico screening of therapeutic drugs from LOPAC compound library. Top hit drug molecules, targeting specific residues of ACE2 and S-RBD, were selected and further analyzed by AutoDock Vina for identifying specific interactions included in binding of molecules.

**Molecular docking**

Molecular docking studies of selected drugs into proteins were carried out using AutoDock Vina. Two different sets of docking studies were conducted- one set for modelled S-RBD of SARS-COV-2 and the other set for ACE2 protein of the host cell. For both studies, proteins were pre-processed by removal of all water and addition of kollman charges. H-bond optimization was done and Gasteiger charges were added to it. A receptor grid-box was generated by AutoGrid4 with spacing of 0.414 Å and grid box dimensions of 40 Å x 40 Å x 40 Å for ACE2 protein. Grid box for S-RBD was also set with spacing of 0.425 Å and dimensions of 60 Å x 60 Å x 60 Å. The program was run for a total number of 50 Genetic algorithm runs. Other parameters were set as default and the final result obtained was analyzed manually by PyMol and LigPlot.

**Molecular dynamics simulation**

Both ACE2 protein and S-RBD protein, and their respective screened drug molecules were subjected to molecular dynamics (MD) simulation studies to assess the flexibility and stability of protein-ligand interactions. For this purpose, GROMACS was used to carry out all simulation studies using GROMOS96 43a1 force field on a LINUX-based workstation. Ligand parameters
and topology files were generated using PRODRG server. Furthermore for solvation, ions and water molecules were added to neutralize whole cubic system. Using steepest descent method, energy minimization step was performed for 1000 steps followed by Isothermal-Isochoric (NVT) and Isobaric-Isothermal equilibration. Finally 50ns MD production run was performed with an integration time frame of 2fs. The conformations generated during the production step were used for calculating RMSD and RMSF values of protein-ligand complex (37).

RESULTS

Identification of ACE2 receptor binding drug molecules

To mediate entry inside host cell, the trimeric S-glycoprotein of coronavirus binds to the host cell surface receptor ACE2 via the viral S-RBD (38). ACE2 is a membrane glycoprotein containing a claw like N-terminal peptidase domain made up of α-helical lobes present on outer surface, responsible for interacting with bowl-shaped cavity on S-RBD (22). In the sequence of SARS-COV-2, the S-RBD residues directly interacting with ACE2 receptor, are similar to that of SARS-CoV, strongly signifying that ACE2 is playing a central role in SARS-COV-2 entry into host-cell (38)(39). Lys31 and Lys353 are reported to be the two main hotspot virus-binding residues located on ACE2 at the virus-receptor interface for NL63-CoV and SARS (1)(22). Recent published data suggests that hotspot 31 is made up of salt bridge between Lys31 and Glu35, and hotspot 353 comprises of a salt bridge between Lys353 and Asp38, both buried in hydrophobic environment (1)(8).

Therefore in this study, the virus binding hotspots on ACE2 receptor were targeted to identify small drug molecules from FDA approved LOPAC library, which is expected to block ACE2 receptor and virus interactions. Screening was done using computer based high-throughput protocol of PyRx and AutoDock Vina with a grid box centering on Lys31 and Lys353 hotspot residues (Figure 1A). The top hit ligand candidates were scored based on their binding energies for ACE2 protein. The primary screen resulted in identification of ~10 hits having potentially high binding energies for ACE2 (Table 1). Out of these, the best 5 molecules were selected on the basis of RMSD values, molecular interactions with interface residues and binding energies. Eptifibatide acetate, GNF-5, GR 127935 Hydrochloride hydrate, TNP and RS504393 were the top hit drugs obtained, which targeted ACE2 host-virus interface (Figure 1B-1F). To gain further insights into the interactions present at ligand-ACE2 interface, each of the selected drug molecule was docked into ACE2 protein using AutoDock Vina. Top scoring ligands based on their binding affinities and visual analysis of docked complexes for their capability of forming H-bond and other interactions with ACE2 virus-binding motifs are documented in Table 2.

Comparison of molecular interactions between ACE2 receptor and ligands

Molecular docking using AutoDock Vina, for the top 5 drug molecules of the LOPAC library obtained by screening were analyzed by PyMol and Ligplot. Eptifibatide acetate displayed highest binding energy (-10.4 kcal/mol), makes 3 H-bonds with ACE2 receptor (Figure 2A). Apart from these, hydrophobic interactions are also observed including hotspot residue Lys31 and other adjacent residues like Glu35, Asp38, and Gln42 clearly depicting its ability to bind
and block interactions of hotspot 31 residues (Figure 1B & 2A). Ligand GNF5 and GR 127935 hydrochloride hydrate (GR Hydrochloride) interacted with Lys 31 thorough H-bond (Figure 1C & 1D). GNF-5 possessed maximum numbers of hydrogen bonds involving Glu35, Asp38 and Gln76 along with hydrophobic interactions, displaying its affinity towards hotspot 31 (Figure 1C, 2B). Key hydrophobic interactions playing a significant role for all three ligands involve His34, Leu39, Lys68, Phe72 and Glu75 along with other residues (Figure 1B-1D and Figure 2A-2C). These interactions clearly demonstrate that Eptifibatide acetate, GNF-5 and GR hydrochloride are drug molecules that could potentially inhibit virus, binding to hotspot 31 (Table 2). Docked conformations of ligand TNP (-10.3 kcal/mol) and RS504393 demonstrates that these ligands are displaying affinities towards hotspot 353 (Figure 1E, 1F). TNP interacts with ACE2 with three H-bonds, one with Lys353 also. Other residues, which interact with this drug, were at a distance to Lys31. Hydrophobic interactions reported here for TNP and RS504393 involves His34, Glu35, Asp38, Gly354, Ala386 as shown in Table 2 (Figure 2D & 2E). Given the results from all set of dockings, our study provides evidence that these identified molecules interacting with hotspot 31 and hotspot 353 specifically, if repurposed would prove to be potential drugs for further studies.

**Structure of S-RBD of SARS-COV-2**

The key determinant of host specificity of coronavirus is the surface anchored S-protein responsible for recognizing host cell receptor ACE2 through its S1 subunit. The central residues of S1 (NL63-CoV: 481-615; SARS-CoV: 306-527 for) are reported to contain the receptor binding domain (RBD), responsible for high affinity binding to ACE2 receptor (22). Because of sequence similarities between RBD of SARS-COV-2 and SARS-CoV, it is hypothesized that SARS-COV-2 infects the host cell via ACE2 receptor through binding of its RBD region of the S-protein (7).

Drug molecules targeting the S1 subunit of S-protein has the potential to cure COVID-19 infections and to tackle the pandemic. Therefore in this study, S1 subunit of SARS-COV-2 was targeted by *in silico* approach to repurpose drug molecule that binds the S-protein and blocks its interaction with ACE2 receptor, rendering it incapable to infect host cell. Since the newly published structure of SARS-CoV-2 S-protein (PDB ID: 6VSB) lacks important loop residues of S-RBD domain proposed to be involved in receptor binding, therefore a homology model was generated utilizing it as a template (Figure 3A&3B). For this objective, a 3D model of SARS-COV-2 S-protein was predicted using SWISS MODEL (NCBI reference sequence: MN908947.3) and the pre-fusion structure of 2019-nCoVspike glycoprotein (PDB ID: 6VSB) was used as template (Figure 3). The 3D model obtained for S-RBD of SARS-COV-2 was validated using PROCHECK, ProSA and SAVES-Verify 3D server. Ramachandran Plot of the predicted model of S-RBD domain of spike protein by PROCHECK and SAVES-Verify 3D server suggests that 82.8% of the residues were in the core allowed region, 15.2% in allowed region, 1.4 % in generously allowed region, and only 0.7% residues in disallowed region not part of loop involved in ACE2 receptor binding (Figure 4). Overall, the modelled structure was good as more than 99% of the residues, after summing up, were in allowed region of Ramachandran plot (40).
Further validation of model was done using ProSA, where the protein folding energy obtained through it was in good agreement with the plot. The Z-score value obtained through it was -7.39 (Figure 4). Overall quality factor evaluated by VERIFY3D was ~85%. These results suggested that the modelled S-RBD of SARS-COV-2 was acceptable and could be further used for structure based virtual screening. This predicted model of S-RBD of SARS-COV-2 was used for protein-protein docking studies to identify its residues interacting with ACE2 receptor and to further screen small drug molecules which could block these interactions of S-RBD: ACE2 interface. The predicted homology model for S-protein was submitted in PMDB database.

**Receptor binding residues on S-RBD of SARS-COV-2**

Crystal structure of S-protein of nCoV-2019 (PDB ID: 6VSB), published recently, lacks residues present in the S-RBD region of SARS-COV-2. Chimeric S-RBD of SARS-COV-2 (PDB ID: 6VW1) has been reported, but the structure comprises majorly of SARS-CoV residues and contains only S-RBM of SARS-COV-2. Therefore, S-protein of SARS-COV-2 was modelled and used to identify molecular interactions with ACE2 receptor using HADDOCK based protein-protein docking tool. Hotspot 31 and hotspot 353 were fed as central residues on the basis of which S-RBD residues of the predicted model were docked (Figure 5).

**Identification of SARS-COV-2 S-RBD binding drugs**

The residues present at the interface region of S-RBD: ACE2 were targeted and used for structure based screening and selection of drug molecules using PyRx. Top scoring 10 drugs were selected on the basis of binding energies as shown in table 3. With respect to interface residues, AutoDock Vina based docking calculations were performed for 5 top molecules selected on the basis of RMSD values, binding energies and for their ability to form hydrogen and hydrophobic bonds. KT185 and KT203 were the first hits obtained having binding energies of -8.9 and -8 kcal/mol respectively which were more than that of GSK1838705A (-7.9 kcal/mol) and BMS-195614 (-7.7 kcal/mol) (Figure 6B-6E). Interestingly drug RS504393 is identified for both ACE2 (-9.6 kcal/mol) and S-RBD (-7.7) (Figure 6F). A complete list of polar and hydrophobic interactions between the five ligands and S-RBD interface are shown in Table 4.

**Comparison of molecular interactions between S-RBD residues and ligands**

Two dimensional plot of the interaction networks of the ligands with S-RBD were prepared with the help of LigPlot, and the docking poses for each of these interactions are represented in Figure 6. The results obtained after docking calculations for all ligands suggests that the S-RBD residues of SARS-COV-2 interacting with the ligands are Leu455, Phe486, Asn487, Gln493, and Ser494. The residues Leu455, Phe486 and Gln493 of S-RBD have been reported to interact with hotspot 31, whereas residues Asn487 and Ser494 are described to interact with hotspot 353 (1)(38). Out of 1280 drug molecules, KT185 and KT203 displayed highest binding energies of -8.9 kcal/mol and -8 kcal/mol respectively and interact with S-RBD residues through two and one hydrogen bonds respectively (Figure 6B, 6C). In the docked conformations, KT185 and KT203 displayed maximum number of hydrophobic interactions with residues responsible for
recognizing both hotspot 31 and hotspot 353 (Figure 6B, 6C and 7A, 7B). GSK1838705A(GSK) interacts with Asn487, Pro491, Gln493 and Ser494 through hydrogen bonds but the hydrophobic interactions were less as compared to that of KT185 and KT203 (Figure 6D, 7C). BMS195614 (BMS) interacts mainly with residues responsible for recognizing hotspot 35 i.e., Asn487and Ser494 thereby would preferably block recognition and binding to S-RBD to hotspot 353 (Figure 6E, 7D). RS504393 was found to be a common ligand for ACE2 receptor and S-RBD, and shows polar interaction with Asn487 (Figure 6F, 7E) and few hydrophobic interactions were observed. It is observed that additional H-bonds are obtained in docked complexes of all ligands i.e., Lys417 and Leu492 which seems to contribute towards stability of docked drug complexes. Tyr489, Phe490 and Pro491 were additional important and common hydrophobic interactions observed for all ligands different from ACE2 interacting residues (Table 4). MD simulation analysis shows that S-RBD: ligand docked complexes of selected drugs were observed to be stable with RMSD values of less than 2Å.

**DISCUSSION**

Understanding virus-receptor recognition mechanism responsible for COVID-19 infection, pathogenesis and host range provides direction to develop antiviral therapy to combat and cure this global pandemic of 2020. There is no drug or antiviral treatment against SARS-COV-2, and development of new drug molecule will take years. Moreover, WHO has already declared COVID-19 infection as a global pandemic problem, therefore repurposing already characterized drugs would prove to be of great benefit as these can be quickly tested as anti-SARS-COV-2 drugs for experimental studies.

Viral S-protein present on the envelope of SARS-COV-2 is responsible for mediating interaction with ACE2 receptor present on host cells via its RBD unit. Since this interaction is essential for SARS-COV-2 infection, drugs targeting ACE2 receptor: S-protein interface sites could potentially inhibit virus entry into host cell and thus, provide quick solution to control SARS-COV-2 infections. Structure based drug repurposing using high-throughput virtual screening tools has been used to identify FDA approved drugs which could block interactions of SARS-COV-2 S-RBD: ACE2 receptor. The results of this study of modelling of S-RBD of SARS-COV-2, coupled with rapid screening of FDA approved LOPAC library drug molecules against both S-RBD and receptor ACE2, have identified potential drugs that are proposed to inhibit virus infection.

In concordance with results obtained after drug library screening, molecular docking studies were performed to gain insights into the binding mode and crucial molecular interactions of screened ligands with ACE2 protein of host cell and S-RBD protein of SARS-COV-2. With regards to ACE2 inhibitors, TNP and RS504393 interacted with hotspot 353 preferably and the remaining three, Eptifibatide acetate, GNF5and GR Hydrochloride hydrate interacted well with residues adjacent to hotspot31 through polar as well as hydrophobic bonds. Structure based rational drug design approach can be used to design a drug combining two separate ligands, that will possess ability to bind and block both hotspot 31 and hotspot 353 by interacting with all residues. KT185 and KT203 were predicted to be potential inhibitors against S-RBD of SARS-COV-2 in pursuit of their high binding energies and owing to their ability to
interact and block key RBD residues responsible for recognizing hotspot 31 and hotspot 353 of SARS-CoV-2 (Figure 6B, 6C and 7A, 7B). GSK and BMS were the other two ligands obtained, and BMS was observed to display a higher affinity towards S-RBD residue interacting with hotspot 353 (Figure 6D & 6E). Intriguingly RS504393 was screened to be common for both S-RBD and ACE2 interface residues, with a higher affinity towards ACE2 virus binding motif. RMSD values obtained after simulation studies suggested that each of the S-RBD and ligand complex was stable.

GNF5 identified in our study, is already a reported drug that blocks coronavirus S-protein induced fusion, prior to hemifusion, by inhibiting Abl kinase (41)(42). This drug also inhibits Dengue virus entry by its action on Abl kinase. Similarly TNP, identified against ACE2 is a selective inhibitor of Inositol hexakisphosphate kinase (IP6K) and Akt signaling, reported to be responsible for inhibiting MERS-CoV infection (43)(44). GR hydrochloride is an antagonist of 5-HT1B/1D serotonin receptor, and also plays a role in inhibiting entry of Ebola virus entry into host cell (45). Eptifibatide acetate protects lungs from inflammations caused by influenza virus (46). KT185 and KT203, inhibitors of S-RBD protein of SARS-CoV-2 are known to exert anti-inflammatory role on lungs (47). GSK is known to reduce inflammations posed by infections caused by influenza virus, whereas BMS, another inhibitor against S-RBD is proposed to inhibit Hepatitis B virus infection (48)(49). Drug RS504393, identified against both ACE2 and S-RBD, targets chemokine receptor, a mechanism by which SARS-CoV interferes with host immune system (50). Detailed role of screened compounds along with target sites are explained in Table 5. Therefore these molecules may target virus entry step as well as could act as anti-inflammatory drugs against damages caused by SARS-CoV-2.

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### Table 1: Top-10 ligands for ACE2 receptor obtained from LOPAC library of ~1280 molecules.

| Ligand                                | Binding Energies (kcal/mol) | 
|---------------------------------------|-----------------------------|
| Eptifibatide acetate                  | -10.4                       |
| TNP                                   | -10.3                       |
| GNF-5                                 | -9.6                        |
| GR 127935 hydrochloride hydrate       | -9.6                        |
| RS504393                              | -9.6                        |
| L732138                               | -9.4                        |
| Aurora A inhibitor                    | -9.4                        |
| Lometrexol Hydrate                    | -9.4                        |

### Table 2: Top hit ligand candidates for ACE2 receptor protein of host cells based on binding energies, polar and hydrophobic interactions.

| Ligand                                | Binding Energy (kcal/mol) | Interactions                  |
|---------------------------------------|---------------------------|-------------------------------|
| Eptifibatide Acetate                  | -10.4                     |                               |
|                                       |                           | N10-OD2 (Asp38)               |                               |
|                                       |                           | N10-OE1 (Gln42)               |                               |
|                                       |                           | N11-OE2 (Glu75)               |                               |
| TNP                                   | -10.3                     |                               |
|                                       |                           | N3-O (His34)                  |                               |
|                                       |                           | N5-OE1 (Glu37)                |                               |
|                                       |                           | O1-N (Lys353)                 |                               |
| GNF-5                                 | -9.6                      |                               |
|                                       |                           | O2-NZ (Lys31)                 |                               |
|                                       |                           | O2-OE1 (Glu35)                |                               |
|                                       |                           | N4-OD2 (Asp38)                |                               |
| GR 127935 hydrochloride hydrate       | -9.6                      |                               |
|                                       |                           | N4-O (Lys31)                  |                               |
|                                       |                           | O1-NE2 (Gln42)                |                               |
| RS504393                              | -9.6                      |                               |
|                                       |                           | O1-NE2 (Gln42)                |                               |
|                                       |                           | O2-NZ (Lys68)                 |                               |
Table 3: Top-10 ligands predicted for S-RBD region of SARS-COV-2

| Ligand                          | Binding Energies (kcal/mol) |
|---------------------------------|-----------------------------|
| KT185                           | -8.9                        |
| KT203                           | -8.0                        |
| GSK1838705A                     | -7.9                        |
| BMS195614                       | -7.7                        |
| RS504393                        | -7.7                        |
| Calcimycin                      | -7.4                        |
| WIN62,577                       | -7.4                        |
| Dihydroergotamine Methanesulfate| -7.3                        |

Table 4: Top hit ligand candidates from LOPAC library for S-RBD of spike protein of SARS-COV-2.

| Ligand                | Binding Energy (kcal/mol) | Interactions                                                                 |
|-----------------------|---------------------------|------------------------------------------------------------------------------|
|                       |                           | H-Bonds | Bond length(Å) | Hydrophobic interactions |
| KT185                 | -8.9                      | O2-N(Asn487)       | 3.22               | Leu455, Lys458, Gly485, Phe486, Tyr489, Phe490, Pro491, Gln493, Ser494 |
|                       |                           | N4-O(Leu492)       | 2.64               |                                                                            |
| KT203                 | -8                        | O3-NZ(Lys417)      | 2.83               | Tyr453, Leu455, Glu484, Gly485, Phe486, Asn487, Tyr489, Phe490, Pro491, Gln493 |
| GSK1838705A           | -7.9                      | O3-N(Asn487)       | 2.99               | Leu452, Leu455, Lys458, Cys488, Tyr489, Phe490, Leu492                    |
|                       |                           | N3-O(Pro491)       | 2.9                |                                                                            |
|                       |                           | N4-OE1(Gln493)     | 2.88               |                                                                            |
|                       |                           | O1-N(Ser494)       | 2.74               |                                                                            |
| BMS195614             | -7.7                      | N2-O(Asn487)       | 2.94               | Leu455, Lys458, Cys488, Tyr489, Phe490, Pro491, Gln493                   |
|                       |                           | N1-O(Leu492)       | 2.72               |                                                                            |
|                       |                           | O3-OG(Ser494)      | 2.91               |                                                                            |
| RS504393              | -7.7                      | N2-O &O2-N(Asn487) | 2.75 & 2.94        | Leu452, Phe486, Cys488, Tyr489, Phe490, Gln493, Ser494                  |
|                       |                           | N3-O(Leu492)       | 2.61               |                                                                            |
Table 5: FDA approved LOPAC library drugs identified against SARS-CoV-2:ACE2 receptor interface with their reported functions and role on RNA viruses.

| S. No | Identified Drugs | Target in SARS-CoV-2 | Reported function of drug | Inhibitory role on RNA viruses |
|-------|------------------|-----------------------|---------------------------|--------------------------------|
| 1     | RS504393         | SARS-CoV-2 receptor ACE2 and spike protein | Treatment of lung injury and bronchial wall thickening (51) | • Targets the chemokine receptor CCR2, responsible for intense up-regulation of chemokines, and represents a mechanism by which SARS-CoV interferes the host immune response (52)(50). |
| 2     | KT185            | SARS-CoV-2 spike protein | Anti-inflammatory | • Inhibitor of ABHD6 receptor.  
• Decreases macrophage activation and exerts anti-inflammatory effect on lungs (53)(47). |
| 3     | KT203            | SARS-CoV-2 spike protein | Cancer drug (54) | |
| 4     | GSK1838705A      | SARS-CoV-2 spike protein | Cancer drug (55) | • Inhibitor of Insulin like growth factor-1 receptor.  
• Regulates acute inflammatory lungs injury mediated by influenza virus infection (48). |
| 5     | BMS195614        | SARS-CoV-2 spike protein | Cancer drug (56) | |
| 6     | TNP              | SARS-CoV-2 receptor ACE2 | Tyrosine kinase inhibitor | • Inhibitor of IP6K and Akt signalling pathway.  
• Responsible for inhibiting MERS-CoV infection by targeting Akt signalling (43)(44). |
| 7     | GNF5             | SARS-CoV-2 receptor ACE2 | Kinase inhibitor | • Inhibits dengue virus entry and post entry step by targeting Abl kinase inhibitor (42).  
• Blocks coronavirus S-protein induced fusion prior to hemifusion by Abl kinase inhibition action (41). |
| 8     | GR127935 hydrochloride hydrate | SARS-CoV-2 receptor ACE2 | Control vasoconstriction | • Antagonist of 5-HT1B/1D serotonin receptor.  
• Serotonin antagonists are potent entry inhibitors of Ebola and Marburg virus (45). |
| 9     | Eptifibatide acetate | SARS-CoV-2 receptor ACE2 | Lungs injury and inflammation | • Inhibitor of glycoprotein IIb/IIIa receptor responsible for platelet aggregation.  
• Protects lungs from severe injury and inflammations induced by Influenza virus (46). |
FIGURE LEGENDS:

**Figure 1** | Molecular docking interactions and orientations of top-hit screened ligands from LOPAC library with ACE2 receptor of host cell (A) hotspot 31 and hotspot 353 residues of ACE2 receptor responsible for recognizing S-RBD of S-protein (B) Docking interactions of Eptifibatide acetate with ACE2 (C) Docking interactions of GNF-5 with ACE2  (D) Docking interactions of GR 127935 Hydrochloride Hydrate with ACE2 (E) Docking interactions of TNP with ACE2 (F) Docking interactions of RS504393 with ACE2. Blue ribbons corresponds to residues of ACE2 receptor and violet yellow stick model represents residues of Ligands. BE=Binding energy

**Figure 2** | Schematic representation of interactions made by screened drug molecules with ACE2 receptor upon analysis using Ligplot. (A) Eptifibatide Acetate (B) GNF5 (C) GR hydrochloride (D) TNP (E) RS5049393. Ligands are colored and represented in purple color, hydrogen bonds are displayed in green dotted lines, red stellations represents hydrophobic interactions and residues of proteins are shown in brown color. BE=Binding energy

**Figure 3** | Modelled structure of S-RBD protein of SARS-CoV-2 (A) Cartoon representation of modelled structure of S-RBD protein (B) Cartoon representation of predicted S-RBD homology model and template (PDB ID:6VSB). Predicted S-RBD and template are sky blue and green in color. Red circle represents the missing residues of template which were modelled for S-RBD protein of SARS-COV-2 using SWISS MODEL.

**Figure 4** | Validation of predicted S-RBD protein by ProCheck and ProSA server(A) ProCheck Ramachandran Plot where red, bright yellow and light yellow color represents favorably allowed area (99.4%) of structure residues of modelled spike protein of SARS-COV-2, and 0.7% residues in the disallowed area (lightest yellow) (B) Energy profile of modelled spike protein of SARS-COV-2 as calculated by ProSA. As concluded from these graphs, protein folding is in proper compliance with ProSA plot and Ramachandran plot with a Z-score of -7.39.

**Figure 5** | Identification of key interacting residues of S-RBD: ACE2 receptor interface by HADDOCK protein-protein docking approach.

**Figure 6** | Molecular docking interactions and orientations of top-hit screened ligands from LOPAC library with S-RBD of spike protein of SARS-COV-2 (A) residues of S-RBD responsible for interacting with ACE2 receptor. Molecular docking studies of S-RBD protein of SARS-CoV-2 with ligands, (B) KT185 (C) KT203 (D) GSK (E) BMS (F) RS504393. Blue ribbons corresponds to residues of S-RBD of spike protein of SARS-COV-2 and violet stick model represents residues of Ligands. BE=Binding energy

**Figure 7** | Schematic representation of interactions made by screened drug molecules with S-RBD of SARS-COV-2 upon analysis using Ligplot. (A) KT185 (B) KT203 (C) GSK (D) BMS (E) RS5049393. Ligands are colored and represented in purple color, hydrogen bonds are displayed in green dotted lines, red stellations represents hydrophobic interactions and residues of proteins are shown in brown color. BE=Binding energy