Rapid Communication

Insilico drug repurposing using FDA approved drugs against Membrane protein of SARS-CoV-2

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A R T I C L E   I N F O

A B S T R A C T

The novel coronavirus (SARS-CoV-2) outbreak has started taking away the millions of lives worldwide. Identification of known and approved drugs against novel coronavirus disease (COVID-19) seems to be an urgent need for the repurposing of the existing drugs. So, here we examined a safe strategy of using approved drugs of SuperDRUG2 database against modeled membrane protein (M-protein) of SARS-CoV-2 which is essential for virus assembly by using molecular docking-based virtual screening. A total of 3639 drugs from Super-DRUG2 database and additionally 14 potential drugs reported against COVID-19 proteins were selected. Molecular docking analyses revealed that nine drugs can bind the active site of M-protein with desirable molecular interactions. We therefore applied molecular dynamics simulations and binding free energy calculation using MM-PBSA to analyze the stability of the compounds. The complexes of M-protein with the selected drugs were simulated for 50 ns and ranked according to their binding free energies. The binding mode of the drugs with M-protein was analyzed and it was observed that Colchicine, Remdesivir, Bafilomycin A1 from COVID-19 suggested drugs and Temozolomide from SuperDRUG2 database displayed desirable molecular interactions and higher binding affinity towards M-protein. Interestingly, Colchicine was found as the top most binder among tested drugs against M-protein. We therefore additionally identified four Colchicine derivatives which can bind efficiently with M-protein and have better pharmacokinetic properties. We recommend that these drugs can be tested further through in vitro studies against SARS-CoV-2 M-protein.

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Introduction

The coronavirus disease (COVID-19) is an infectious disease caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).[1,2] The disease was first reported in December 2019 at Wuhan (China) and currently spread all around the world.[3] As of 1st November 2020, approximately 46 million cases and 1.2 million deaths have been reported globally according to World Health Organization (WHO) (https://www.who.int/). The most common symptoms associated with disease reported are fever, cough, fatigue, breathing difficulties and pneumonia.[4,5] The SARS-CoV-2 was identified as the member of the family *Coronaviridae* which is subdivided into two subfamilies and have four genera: α, β, γ and Δ coronaviruses. The SARS-CoV-2 belongs to β-coronavirus together with SARS-CoV and

MERS-CoV viruses.[6] The virus have largest single stranded RNA genome of size 27–34 kb.[7,8] The current therapeutic options under investigation for treating COVID-19 were antiviral, convalescent plasma and hyper immune immunoglobulin.[9,10] There is still insufficient clinical data to recommend the use of these agents for the treatment. The urgent need of developing potential diagnostic, therapeutic, and preventive strategies till the vaccination arrives is drug repurposing. The main viral therapeutic proteins identified are spike protein (S-protein), 3C-like protease (3CL^{pro}), papain like protease (PL^{pro}), RNA-dependent RNA polymerase (RdRp), nucleocapsid protein (N-protein), transmembrane protease serine-2, envelope protein (E-protein) and the membrane (M) protein.[11] The spike protein is responsible for the interaction of virus to host cell receptor angiotensin-converting enzyme 2 (ACE2) and after the entry of the virus into the host cell, viral polyproteins are processed by 3CL^{pro} and PL^{pro} resulting in the release of nonstructural proteins (NSPs). The NSPs further forms a replication-transcriptase complex which is then assembled by RdRp and helicase leading to the production of mRNA.

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Simultaneously, sub genomic proteins translate structural and other accessory proteins S, M, N and E protein.[12−14] As stated earlier, S-protein helps in the entry of the virus inside the host cell while E-protein is an integral membrane protein responsible for envelop formation and assembly of the virus.[15] The M-protein is present in greater amounts in coronaviruses and conserved among β-coronaviruses.[15] The M-protein from SARS-CoV-2 shares over 98% sequence identity with Bat and Pangolin.[16] M-protein plays an important role in maintaining the shape of the virus envelop and also interacts with E-protein to form virions.[12] Furthermore, M-protein also appears to affect the immune responses by inhibiting nuclear factor kappa B (NF-κB) therefore resulting in the proliferation of the virus.[17] Additionally, M-protein inhibits the interaction of 3-phosphoinositide-dependant protein kinase 1 (PDK1) and protein kinase B (PKB) which resulted in release of caspases which eventually causes cell death.[18] This literature survey revealed that M-protein can be a potential target for limiting and targeting the formation of virions and preventing inflammation in host cells.[11,19] Recent studies have repurposed numerous drug-like candidates against well-known targets of SARS-CoV-2 viz. S-protein, 3CL^{pro}, PL^{pro}, RdRp and helicase.[9,20] Few of the drugs such as Umifenovir, Lopinavir, Ritonavir, Hydroxychloroquine, Remdesivir and Favipiravir are under clinical trials against COVID-19.[21] Only Remdesivir, a RdRp inhibitor has been found most effective till date and therefore approved by FDA for emergency use.[22−24] Researchers around the world are still testing and searching for effective solutions against COVID-19. Apart from the well-known protein targets (3CL^{pro}, PL^{pro}, RdRp), researchers are now targeting other structural (E, N and M-protein) and accessory proteins of the virus.[25−27] Among these proteins the M-protein whose role could be vital for viral entry, replication, assembly and maintenance of the virus envelop along with N, E and S proteins can be a potential targeting strategy for COVID-19 further seeks our attention.[11,12,19] Unfortunately, the 3 dimensional (3D) crystal structure of M-protein is not reported till date in protein data bank (PDB). Therefore, a homology modelled structure of SARS-CoV-2 M-protein was obtained from DeepMind algorithm AlphaFold system's project for COVID-19 structures prediction (https://deepmind.com/). The structure was validated using standard validation methods. Thereafter, the molecular docking-based virtual screening of 3639 drugs from SuperDRUG2 database along with 14 previously reported COVID-19 drugs was performed and the selected drugs were subjected to molecular dynamics simulations.[21,28−30] Additionally, the selected drugs were scrutinized for their binding affinity with M-protein by rigorous binding free energy calculations using MM-PBSA.

Materials and methods

Modeling of M-protein

Homology modeled three-dimensional (3D) structure of M-protein was downloaded from DeepMind algorithm AlphaFold system (https://deepmind.com/). The AlphaFold system uses convolutional neural network for protein structure prediction.[31] In brief, this neural networks approach uses protein sequence to predict distances and angles between chemical bonds which connects amino acids. Predicted properties were then combined into a score and these scores were then used to screen proteins database to find structures that match the prediction.[31] Recently Pandey et al., 2020 published a paper using drug repurposing approach for COVID-19 using the homology model of a membrane protein Nsp6 from DeepMind algorithm AlphaFold system.[32] For present study, server uses SARS-CoV-2 M-protein sequence (UniProtKB id VME1_SARS2) as input for the membrane protein model generation. The generated model was energy minimized using *Discovery Studio* (DS) v18 (www.accelrys.com Accelrys Inc. San Diego, USA) to remove the steric clashes and

lowest energy minimized structure was selected. To confirm the reliability of the SARS-CoV-2 M-protein model structure was then aligned with SARS-CoV sequence (UniProtKB id Q19QW6_SARS). The model was further evaluated by PROCHECK and ERRAT analysis using SAVES server (https://saves.mbi.ucla.edu/). The PROCHECK analysis evaluates the stereochemical quality of the protein backbone residues, while the ERRAT analysis provides details about protein folding.[33,34] The validated model was then selected and prepared using the *Clean Protein* protocol in DS for molecular docking. During the preparation of the M-protein, the hydrogen atoms were added, bonding order was checked and terminal residues were adjusted. The binding site of M-protein for further process of molecular docking was identified by using CASTp 3.0 online server (http://cast.engr.uic.edu). CASTp 3.0 detects the global topological and protein dimensions to identify the active site and its volume.[35] The top ranked site with larger surface area was considered as the active site of M-protein.[36] The selected ligand binding site have amino acids Ile48, Leu51, Leu52, Trp55, Phe96, Met109, Trp110, Phe112, Asn113 and Pro114.

Ligand selection and preparation

The drug repurposing strategy was performed by using 3639 drugs from SuperDRUG2 database.[28,29] We additionally selected 13 drugs (Lopinavir, Ritonavir, Hydroxychloroquine, Chloroquine, Ivermectin, Azithromycin, Favipiravir, Colchicine, Remdesivir, Tamiflu, Nitazoxanide, Toremifine, Umifenovir) reported active against other therapeutic target proteins of COVID-19 and downloaded them from PubChem database (https://pubchem.ncbi.nlm.nih.gov/).[21,30] Interestingly, Gordon et al. identified that Bafilomycin A1 can bind with SARS-CoV-2 M-protein, we therefore also included this drug in our dataset for binding mode analysis.[37] The drugs were saved in a single coordinated SDF format file. For each drug molecule, different possible conformers were generated and energy minimization was performed by using universal force field of PyRx software (https://pyrx.sourceforge.io/).

Molecular docking

To carry out the molecular docking studies, virtual screening software PyRx was employed.[38,39] PyRx works based on empirical-based free energy scoring function and Lamarckian Genetic Algorithm. Molecular docking was performed in the grid box generated based on the binding site information provided by the CASTp 3.0 online server.[35] The binding site residues inside the grid box with X, Y and Z axis and dimensions were adjusted to 13.49 Å × 0.41 Å × 2.45 Å with an exhaustiveness of 8, and the calculations were conducted in such a manner that only lowest energy pose was obtained as an output.

Molecular dynamics simulations

Molecular dynamics simulation technique is widely used in drug discovery to study the behavior of protein-ligand complexes at atomic level.[40,41] In the present investigation, top ranked compounds retrieved from the molecular docking calculations were subjected to molecular dynamics simulations with M-protein. Topology parameters of the potential drug candidates were generated by using Swiss-Param server.[42] Thereafter, these complexes were simulated for 50 ns using GROningen MAchine for Chemical Simulations (GROMACS v5.1.5),[43] following the same protocol as described in our previous report.[44]

Binding free energy calculations

Protein-ligand complexes obtained via molecular dynamics simulations were further subjected to Molecular Mechanics-based

Poisson-Boltzmann Surface Area (MM-PBSA) analysis for the calculation of their binding free energies.[45] Based on the RMSD plots, last 10 ns simulation trajectories were selected and a total of 40 snapshots were taken at a regular interval. The g_mmpbsa tool for GROMACS was employed to calculate the different parameters of binding free energies with the methodologies described in previous reports.[46−49]

Results

The present study provides a comprehensive details on targeting the M-protein of novel coronavirus using homology modeling, molecular docking-based virtual screening, binding mode analyses using molecular dynamics simulations with SARS-CoV-2 M-protein and free energy calculations. The schematic representation of the workflow has been described (Fig. 1).

M-protein modeling

Homology modeled structure of M-protein was downloaded from DeepMind algorithm AlphaFold system's project for COVID-19 structure prediction. The server uses deep neural network learning algorithm AlphaFold system (https://deepmind.com/). The server uses SASR-CoV-2 M-protein sequence (UniProtKB id VME1_SARS2) was used as input for the construction of 3D model. The obtained modeled structure was first aligned with SARS-CoV sequence (UniProtKB id Q19QW6_SARS). It was observed that the obtained model has 87.1% sequence identity and 93.1% sequence similarity with the SARS-CoV M-protein (Fig. 2a). This analysis is parallel with the reported sequence identity between both the viruses SARS-CoV and SARS-CoV-2.[16,50] The model was then submitted to SAVES server for the generation of Ramachandran plot to predict the distribution of residues.[51,52] The analysis revealed that 92.5% residues are in the allowed region (Fig. 2c). In addition, the model was also analyzed by ERRAT web server and the analysis revealed that the quality factor of our model was 92.04%. Generally, models with quality factor more that 50% is of acceptable quality.[50,53] The above detailed analysis of selected model using sequence alignment, PROCHECK and ERRAT revealed that the model is of reliable quality and can be used in further studies (Fig. 2b).

Molecular docking

The energy minimized drug database was used for docking-based virtual screening using PyRx software. PyRx works on empirical-based free energy scoring function and Lamarckian Genetic Algorithm. Molecular docking was performed in the grid box generated as stated above based on the binding site information provided by the CASTp 3.0 online server and the docking results were analyzed on the basis of binding energy scores. A total of 13 drugs from Super-DRUG2 database displayed acceptable binding energy score lower than −7.0 kcal/mol.[38,54,55] Additionally the drugs were also docked on two more possible sites predicted by CASTp 3.0 server. The results confirms that drugs have high binding affinity toward previously selected Top1binding site Table S2. The molecular interactions of selected drugs with were Top1 binding site were further analyzed and it was observed that five drugs displayed better molecular interactions with the active site residues of M-protein. The selected potential drugs formed hydrogen bonds with the active site residues Ala40, Asn41, Arg44, Asn113, and Glu115 of M-protein (Table S1). It has been observed that the drug Temozolomide from SuperDRUG2 database displayed highest binding affinity of −8.9 kcal/mol among selected drugs. The three drugs (Colchicine, Remdesivir and Bafilomycin A1) were also selected from the 14 COVID-19 reported drugs suggested on the basis of binding energy scores and molecular interactions (Table S1). The selected drug were additionally, docked with known drug targets. It was observed that drugs displayed comparable binding affinity towards SASR-CoV-2 M-protein complete details were provided in Table S3.
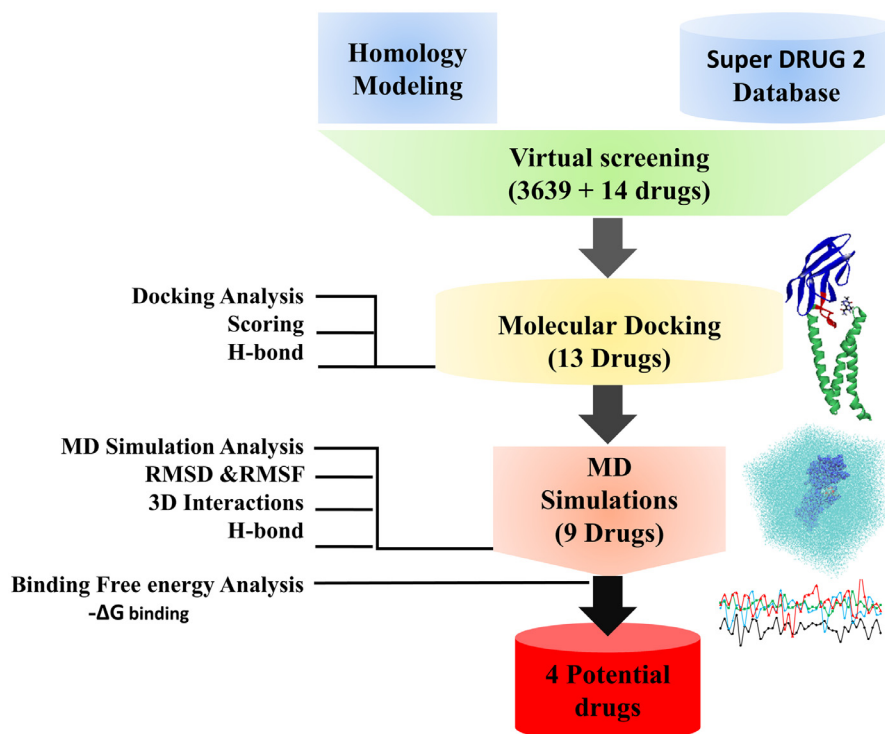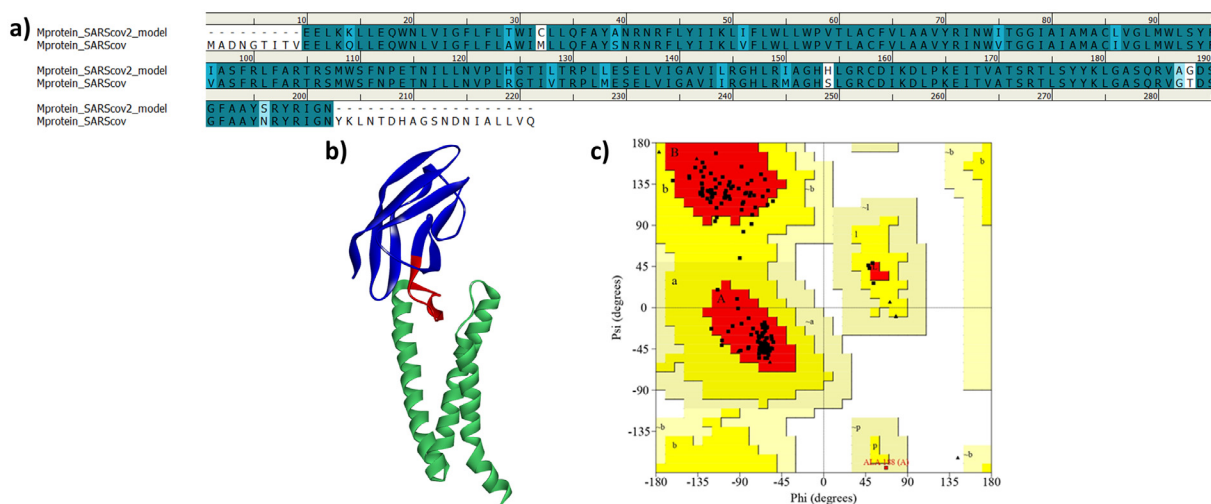

Fig. 1. Schematic representation of the workflow implemented in the current study.

Fig. 2. (a) Sequence alignment of the M-protein of SARS-CoV-2 with the SARS-CoV M-protein. Dark blue color and light blue color represents amino acid identity and similarity respectively, (b) 3D structure of the selected model with the transmembrane and C-terminal domain shown in blue and light green color respectively while the C-terminal conserved region is shown as red (c) Validation of the selected homology model of SARS-CoV-2 M-protein by Ramachandran plot. Plot displays 92.5% residues in the allowed region.

Molecular dynamics simulations

The selected drugs from molecular docking were subjected for molecular dynamics simulation for 50 ns run using GROMACS. The drugs were then analyzed for RMSD, potential energy, RMSF and formation of hydrogen bonds with M-protein. Furthermore, the binding free energies for selected drugs were calculated using MM-PBSA tool and drugs were ranked accordingly. Drugs which displayed acceptable binding were considered for detailed analyses (Table S1).

Stability analyses of simulated systems

To examine the structural fluctuations of the docked complexes, the RMSD value for the protein backbone and ligand was calculated for the time duration of 50 ns.[56,57] Except Remdesivir that showed stable RMSD from 5 ns till the end of the simulation run, the backbone RMSD of the Bafilomycin A1, Colchicine and Temozolomide initially increased between 10 and 25 ns and thereafter attained steadiness till the end of the simulation. (Fig. 3a). The mean RMSD value for M-protein backbone bound with Colchicine, Remdesivir, Temozolomide and Bafilomycin A1 was observed to be 0.29, 0.23,
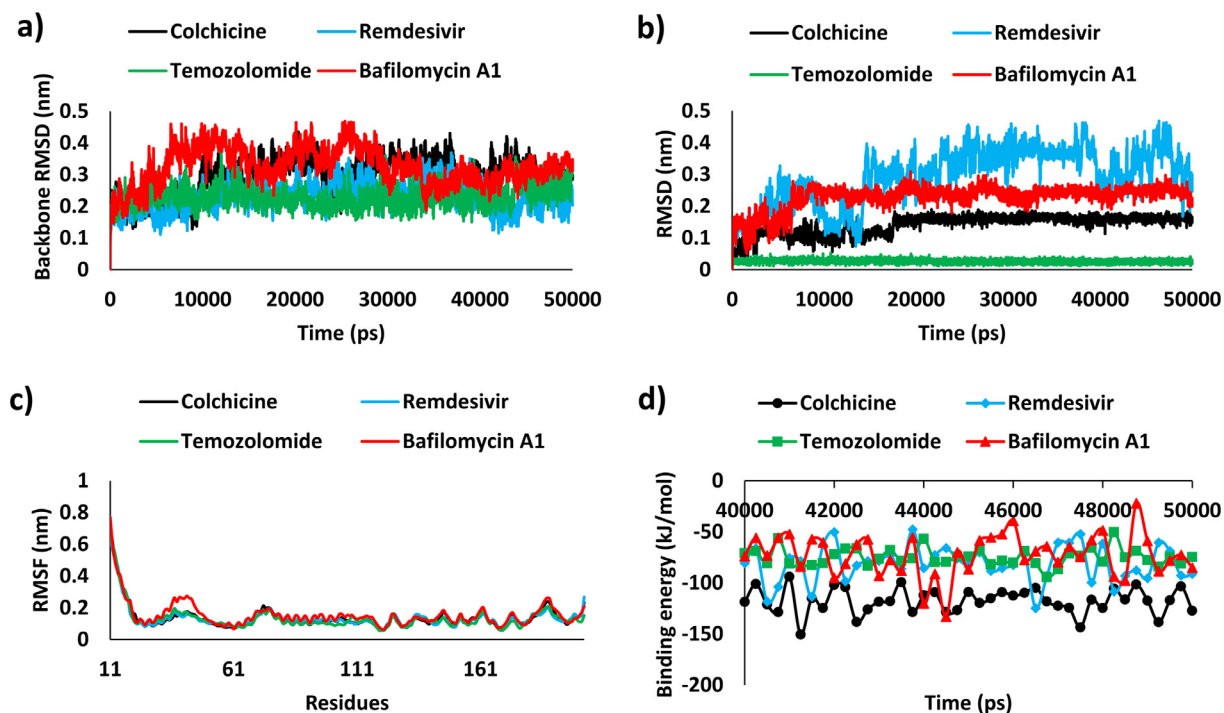
Fig. 3. Molecular dynamics simulation analysis. a) RMSD profile of the backbone atoms of M-protein, b) RMSD profile of bound drugs, c) RMSF analyses and d) MM-PBSA estimated binding free energy of selected potential drugs (Colchicine, Remdesivir, Temozolomide and Bafilomycin A1) from last 10 ns trajectories of simulation run.

Table 1
Molecular dynamics simulation analysis of selected drugs against SARS-CoV-2 M-protein.

Sr. No.	Drugs Name	Binding Free energy (kJ/mol)	RMSD backbone (nm)	RMSD ligand (nm)	RMSF backbone (nm)	Potential energy (kJ/mol)	No. of Hydrogen Bonds
1	Colchicine	−117.62	0.29	0.14	0.13	−1,056,643.76	0.75
2	Remdesivir	−81.17	0.23	0.29	0.13	−1,057,314.45	2.17
3	Temozolomide	−74.94	0.23	0.02	0.13	−1,057,813.98	2.48
4	Bafilomycin A1	−72.31	0.32	0.22	0.15	−1,056,947.96	1.29

0.23 and 0.32 nm, respectively. The RMSD pattern of M-protein bound drugs (Fig. 3b) revealed that Temozolomide showed stable RMSD from beginning till the end of the simulation run whereas Bafilomycin A1, Colchicine, Remdesivir complexed with M-protein reached equilibrium in the time duration of 15 ns. In drug discovery the RMSD values below 0.3 nm were generally considered as stable interestingly, our simulated systems were found within acceptable RMSD range.[44,58−60] The RMSF analysis showed minimal residual flexibility for all the M-protein and ligand complexes and were found stable throughout the simulation. The functionally important residues Ala40 and Asn41 displayed stable behavior and no fluctuations were observed towards the start of N-terminal and the end of C-terminal region of the SARS-CoV-2 M-protein. The mean RMSF value for M-protein with Colchicine, Remdesivir, Temozolomide and Bafilomycin A1 complexes was observed to be 0.13, 0.13, 0.13 and 0.15 nm, respectively (Fig. 3c).

Additionally, stability of the simulated complexes was also analyzed by potential energy plots.[61,62] The selected protein-ligand complexes attained equilibrium and were found to be stabilized throughout the period of simulation (Figure S2a). The binding stability of M-protein with Colchicine, Remdesivir, Temozolomide and Bafilomycin A1 was also estimated by hydrogen bonding (H-bond) analysis. H-bond analyses revealed that Temozolomide forms the highest number of H-bonds with M-protein followed by Remdesivir, Bafilomycin A1 and Colchicine (Figure S2b). The average values of RMSD, RMSF, potential energy and H-bond analyses has been displayed (Table 1). Additionally, all the above mentioned parameters were compared with SARS-CoV-RdRp-Remdesivir and were found satisfactory (Figure S3). The analysis revealed that the RMSD values of protein backbone atoms and ligand were found <0.3 nm which is very similar with our results with M-protein. RMSF analysis revealed the proteins showed residual fluctuations but the average values were found <0.3 nm. The stable potential energy and comparable number of hydrogen bonds were observed in both the protein.

Binding free energy calculations

The MM-PBSA method was used to estimate the binding affinity of ligands from the last 10 ns simulation trajectories (Table S1). Previous studies confirmed that binding free energy values lower than −30 kJ/mol can be considered for binding, however lower binding free energy values more favorable for interactions.[63,64] In present investigation, four drugs were able to display acceptable binding free energy values therefore other drugs were removed from further analysis. The predicted binding free energies (ΔG_{bind}) for selected drugs Colchicine, Remdesivir, Temozolomide and Bafilomycin A1 were −117.62,

−81.17, −74.94 and −72.31 kJ/mol, respectively (Fig. 3d and Table 1). Our analyses confirmed that Colchicine was observed to show lowest ΔG_{bind} values among all drugs, therefore can bind more tightly to the M-protein this is followed by Remdesivir, Temozolomide and Bafilomycin A1. To further understand the binding in detail free energy values were decomposed in individual components (Table 2). Analysis revealed that the major favorable contributors for protein-ligand complexes were van der Waals interactions (ΔE_{vdW}) and solvent energy surface area SASA non-polar energy (ΔG_{np}). The greater electrostatic contribution (ΔE_{ele} = −69.88, −36.28, −47.62 kJ/mol) of Remdesivir, Temozolomide, Bafilomycin A1 in comparison to ΔE_{ele} = 18.88 kJ/mol of Colchicine was observed. The polar solvation energies (ΔG_{pol}) contributed positively to the total binding free energies and therefore opposes the complex formation.[65] The binding affinity of the drug Remedesivir was also analyzed with SARS-CoV-RdRp (Figure S3). As expected, Remdesivir displayed better binding affinity for its known target RdRp (−104 kJ/mol) but interestingly, showed considerable binding affinity toward M-protein (−81.17 kJ/mol) confirms the ability of the drug to target multi-targets.
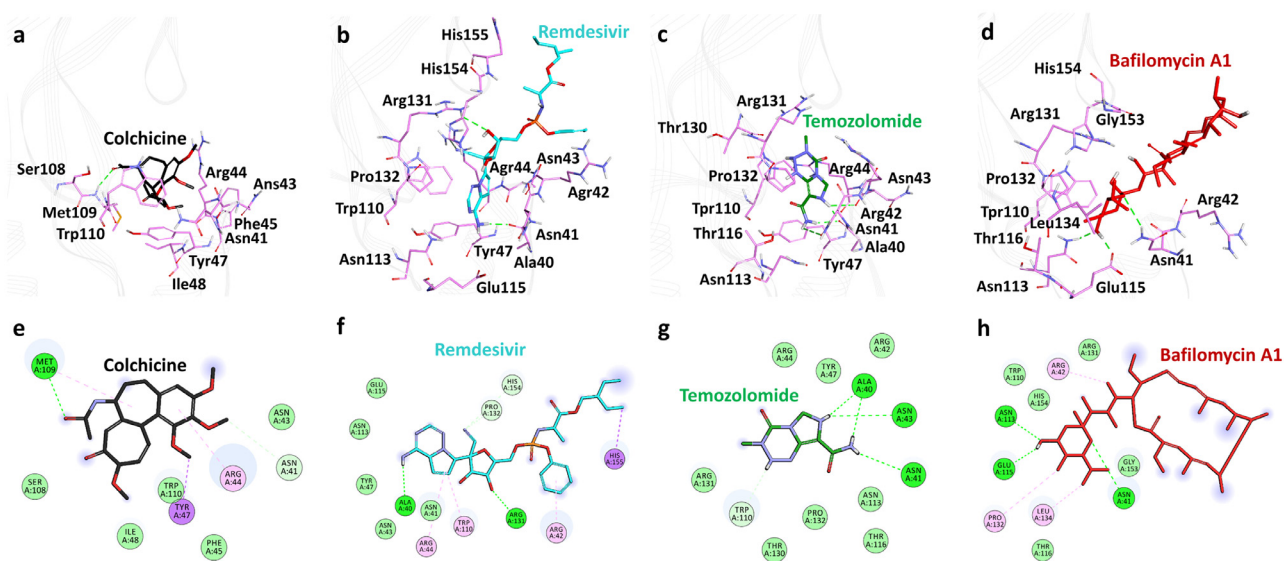
Molecular interaction analysis

The binding site of the M-protein was identified in between the conserved C-terminal and transmembrane domain (Fig. 2b). The average structure for all the selected complexes were calculated from the last 10 ns of stable MD trajectories and superimposed. All the selected drugs were observed to bind inside the defined binding site (Fig. 4). A deep insight on molecular interaction pattern revealed that Colchicine formed hydrogen bond interaction with the amino-acid Met109, Remdesivir showed hydrogen bonding with the amino-acids Ala40 and Arg131, Temozolomide displayed hydrogen bonding with amino-acids Ala40, Asn41 and Asn43 and Bafilomycin A1 showed hydrogen bonding with the amino-acid residues Asn41, Asn113 and Glu115 of SARS-CoV-2 M-protein (Fig. 5a, b, c and d). On further noticing the bonding distance between the mentioned drugs and their respective interacting amino-acids, it is clearly visible that the drugs have shown the bonding in less than 3.5 Å distance (Table 3).[44,61,66] The protein-drug molecular interactions were also stabilized by non-polar interactions within the active site residues of the protein. Colchicine was observed to form van der Waals interactions with active site residues of M-protein Asn41, Asn43, Ile48, Ser108, Trp110 and π-alkyl interactions with Arg44, Tyr47 whereas Remdesivir displayed van der Waals interactions with Asn41, Asn43, Tyr47, Asn113, Glu115, Pro132, His154 and π-alkyl interactions with residues Arg42, Arg44, Trp110, His155. Temozolomide formed van der Waals interactions with Arg42, Arg44, Tyr47, Trp110, Asn113,

Table 2
Binding free energies of simulated protein-ligand complexes through MM-PBSA method.

Ligand	ΔE_{vdW} (kJ/mol)	ΔE_{ele} (kJ/mol)	ΔG_{pol} (kJ/mol)	ΔG_{np} (kJ/mol)	ΔG_{bind} (kJ/mol)
Colchicine	−148.40±9.55	18.88±8.41	27.39±9.63	−15.49±0.77	−117.62±12.20
Remdesivir	−177.51±14.43	−69.88±14.05	186.21±24.68	−19.98±2.00	−81.17±17.84
Temozolomide	−132.06± 8.25	−36.28±7.67	104.34±8.23	−10.94±0.44	−74.94±8.39
Bafilomycin A1	−155.44±20.09	−47.62±16.02	150.89±23.65	−20.13±2.01	−72.31±20.44
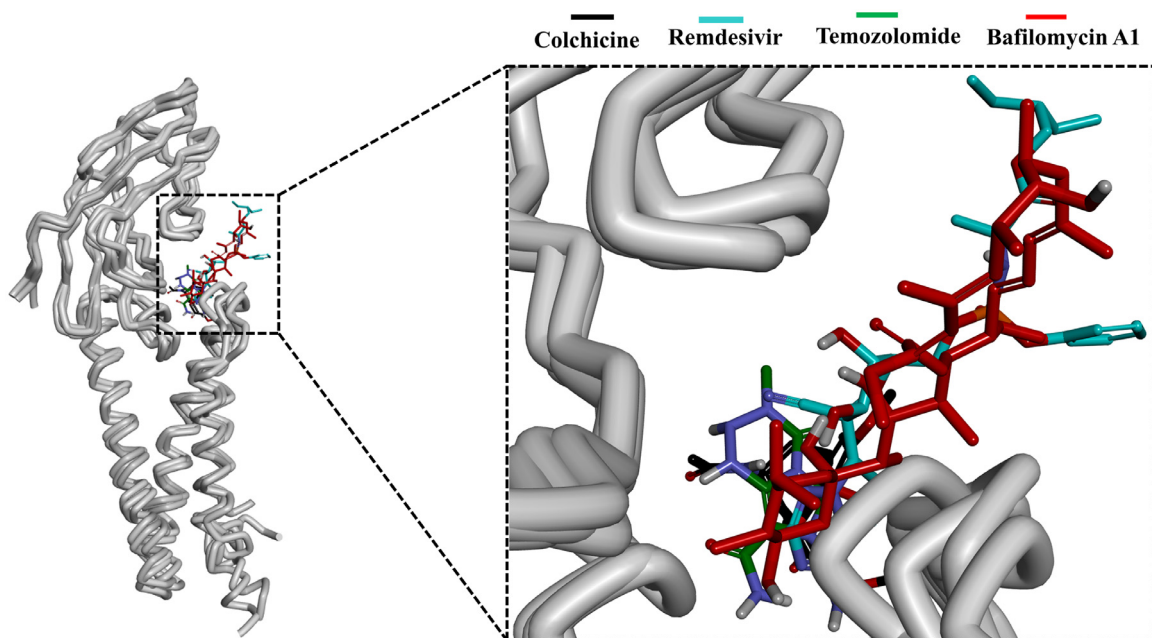
Fig. 4. Binding patterns of the potential drugs in the active site of SARS-CoV-2 M-protein. Superimposition of the selected drugs (left) and enlarged view (right). M-protein is shown as gray tube whereas Colchicine, Remdesivir, Temozolomide and Bafilomycin A1 are in black, cyan, green and red respectively.

Fig. 5. Molecular interaction analysis. Upper panel displaying 3D interaction patterns of the potential drugs in the active site of SARS-CoV-2 M-protein. Protein in background is shown as line ribbon (gray) whereas residues involved in interactions are shown as stick representation (light pink). Drugs are displayed as sticks with a) Colchicine (black) b) Remdesivir (cyan) c) Temozolomide (green) and d) Bafilomycin A1 (red). Hydrogen bonds were displayed in green dashed lines. The lower panel displaying 2D interactions of potential drugs in the active site SARS-CoV-2 M-protein. e) Colchicine f) Remdesivir g) Temozolomide and h) Bafilomycin A1. Residues shown in dark green color represent conventional hydrogen bonds, light green color represents van der Waals interactions and pink or purple color are alkyl interactions.

Thr116, Thr130, Arg131, Pro132 of M-protein and Bafilomycin A1 formed van der Waals interactions with Trp110, Thr116, Arg131, Gly153, His154 and π-alkyl interactions with Arg42, Pro132, Leu134 (Table 3). Recently, Bhowmik et al. identified through an *in silico* study that Caffeic acid and Ferulic acid can be effective against M-protein.[25] The identified compounds were reported to target Asn43, Tyr47 Lys51 through hydrogen bond and residues Leu46, Leu51, and Leu54 through hydrophobic interactions. It is noteworthy to mention here that our identified drugs target SARS-CoV-2 M-protein through hydrogen bonds and hydrophobic interactions hence providing enough support as future COVID-19 inhibitors (Table 3).

The repurposed drug Colchicine is an alkaloid-based drug derived from *Colchicum autumnale* and has been used against gout flare. The drug is currently under randomized clinical trials against COVID-19.[67] Colchicine is reported to target mitosis and microtubule assembly.[68] It is also reported to have antiviral effects against Dengue and Zika viruses and its derivatives were reported to reduce HIV virus load.[69,70] The drug Remdesivir which is a nucleotide analog is a well-known inhibitor of SARS-CoV-2 RdRp.[71−74] Interestingly, Remdesivir is the first drug got an emergency FDA approval against COVID-19.[75] Recently, *in silico* drug repurposing studies also identified that the drug can bind SARS-CoV-2 3CL^pro.[76,77] These observations conclude

Table 3
Molecular interactions of potential drugs with SARS-CoV-2 M-protein.

Name	Hydrogen Bond Interactions				van der Waals Interactions	π -π /π-alkyl interactions
	Amino acid	Amino acid atom	Ligand atom	Distance (<3.5 Å)		
Colchicine	Met109	HN	O6	2.67	Asn41, Asn43, Ile48, Ser108, Trp110	Arg44, Tyr47
Remdesivir	Ala40	O	H20	2.08	Asn41, Asn43, Tyr47, Asn113, Glu115, Pro132, His154	Arg42, Arg44, Trp110, His155
	Arg131	HH21	O3	2.67		
Temozolamide	Ala40	O	HN	1.98	Arg42, Arg44, Tyr47, Trp110, Asn113, Thr116, Thr130, Arg131, Pro132	
	Asn41	OD1	H1	3.07		
	Asn43	O	HN	3.07		
Bafilomycin A1	Asn41	HD22	O1	3.01	Trp110, Thr116, Arg131, Gly153, His154	Arg42, Pro132, Leu134
	Asn113	HD22	O3	2.04		
	Glu115	OG1	H53	2.10		

Table 4
Pharmacokinetic properties analyses for Colchicine substructures.

Pharmacokinetic Properties	Compound name/PubChem ID				
	Colchicine	24,988,904	118,725,563	146,048,789	6,711,380
GI absorption	High	Low	Low	Low	Low
BBB permeant	No	No	No	No	No
P-gp substrate	Yes	Yes	Yes	Yes	Yes
CYP1A2 inhibitor	No	No	No	No	No
CYP2C19 inhibitor	No	No	No	No	No
CYP2D6 inhibitor	Yes	No	No	No	No
CYP3A4 inhibitor	Yes	Yes	Yes	Yes	No
Water solubility	Soluble	Moderately soluble	Moderately soluble	Moderately soluble	Soluble

that Remdesivir can bind both the proteins efficiently and hence found most effective against SARS-CoV-2 infections. The concept of multi-target drugs which can target multiple proteins simultaneously has been already in use and found effective and hence Remdesivir can be selected as a hit against M-protein.[78] The Bafilomycin A1 is reported to inhibit SARS-CoV-2 NSP6 and M-protein and is in pre-clinical studies.[37] The drug was also reported to inhibit SARS-CoV-2 in vitro studies and RdRp.[79] Here, our study revealed the binding mode of Bafilomycin A1 inside the active site of M-protein. In addition, Temozolomide from SuperDRUG2 database was found effective against SARS-CoV-2 M-protein as well. The drug is an anti-cancer agent approved by the FDA for use in the first-line treatment of glioblastoma. It is classified as an alkylating agent and is supposed to stop replication of cells.[80]

Substructure search for colchicine

According to binding free energy and binding mode analyses, out of the 14 COVID-19 reported drugs, Colchicine was found ranked on the top (Table 2). The drug is derived from *Colchicum autumnale* and has been approved by FDA for the treatment of gout flares and Familial Mediterranean fever.[81] Intriguingly, Colchicine is currently under randomized clinical trials against COVID-19.[67] It is hypothesized that Colchicine can interfere with SARS-CoV-2 spike protein function and therefore may block viral entry. The detailed mechanism of this is not fully reported yet. In the present study, we have observed that Colchicine binds M-protein with high affinity (−117.62 kJ/mol) with polar and non-polar interactions. Colchicine is reported to be a substrate of P-glycoprotein (P-gp) and CYP3A4 due to this drugs like Lopinavir and Ritonavir can increase the potential for Colchicine toxicity.[67] To avoid this toxicity effect we searched for similar compounds using PubChem database (https://pubchem.ncbi.nlm.nih.gov/) to obtained Colchicine-like substructures. A total of 683 compounds were retrieved and used for molecular docking with M-protein. The docking analysis revealed 10 compounds binding M-protein with better binding affinity than Colchicine and also formed more number

of hydrogen bonds than Colchicine (Table S4). These compounds were further subjected for calculation of their pharmacokinetic properties with online web tool SwissADME (http://www.swissadme.ch/index.php).[82] Four compounds were observed to display comparable pharmacokinetic properties with Colchicine (Table 4). Interestingly, the compound with PubChem ID 6,711,380 was found better among all selected derivatives in terms of pharmacokinetic properties predicted.

Conclusions

The membrane glycoprotein is conserved across the β-coronaviruses. The multiple sequence alignment shows a remarkable similarity over 96% among the SARS-CoV M variants. Membrane protein exists as a Homomultimer and interacts with envelope protein and nucleocapsid protein. In this study, four commercially available drugs Colchicine an alkaloid drug used in the management of gout, Remdesivir an investigational drug currently under trial against coronavirus, Temozolomide an oral alkylating agent used for the treatment of refractory anaplastic astrocytoma and Bafilomycin A1 an experimental toxic macrolide antibiotic is shown to be important and highly potent for the inhibition of SARS−CoV−2 M-protein. Moreover, Colchicine derivatives were also found effective against M-protein and displayed better pharmacokinetic properties. The lead drug candidates showed molecular interactions with binding pocket residues of the modelled M-protein of SARS−CoV−2. However further studies are required to authenticate the effectiveness of these purposed drugs.

Author contributions

K.A.P. and V.K. conceived the idea of the project. K.A. P. performed Molecular docking and contributed in writing and V.K. performed Molecular Dynamics simulation, binding free energy analyses and contributed in writing. K.A. P., V.K., K.W.L. and T.C.V., analyzed the results. All the authors have read and approved the manuscript for submission.

Declaration of Competing Interest

The authors report no conflicts of interest in this work.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.xphs.2021.03.004.

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