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Drug repurposing against SARS-CoV-2 using E-pharmacophore based virtual screening, molecular docking and molecular dynamics with main protease as the target

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ABSTRACT

Since its first report in December 2019 from China, the COVID-19 pandemic caused by the beta-coronavirus SARS-CoV-2 has spread at an alarming pace infecting about 5.59 million, and claiming the lives of more than 0.35 million individuals across the globe. The lack of a clinically approved vaccine or drug remains the biggest bottleneck in combating the pandemic. Drug repurposing can expedite the process of drug development by identifying known drugs which are effective against SARS-CoV-2. The SARS-CoV-2 main protease is a promising drug target due to its indispensable role in viral multiplication inside the host. In the present study an E-pharmacophore hypothesis was generated using a crystal structure of the viral protease in complex with an imidazole carbaximide inhibitor. Drugs available in the superDRUG2 database were used to identify candidate drugs for repurposing. The hits obtained from the pharmacophore based screening were further screened using a structure based approach involving molecular docking at different precisions. The binding energies of the most promising compounds were estimated using MM-GBSA. The stability of the interactions between the selected drugs and the target were further explored using molecular dynamics simulation at 100 ns. The results showed that the drugs Binifibrate and Bamifylline bind strongly to the enzyme active site and hence they can be repurposed against SARS-CoV-2. However, U.S Food and Drug Administration have withdrawn Binifibrate from the market as it was having some adverse health effects on patients.

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1. Introduction

COVID-19, a severe viral pneumonia, was first reported on December 31, 2019, from the city of Wuhan in the Hubei province of China by the Chinese Centre for Disease Control (CDC, China). The causative virus was shortly identified as a novel beta-coronavirus, dubbed SARS-CoV-2. The virus belongs to the order Nidovirales of the Coronaviridae family comprising of the alpha- and beta-coronaviruses. These are enveloped, positive-sense RNA viruses with comparatively large genomes among known RNA viruses (26.4–31.7 kb) (Zheng, 2020; Su et al., 2016). Six members of the family are previously known to infect humans including SARS-CoV and MERS-CoV, which are known to cause severe respiratory ailments in the host (Paules et al., 2020; Corman et al., 2018). SARS-CoV-2 is the latest addition to the group and has presented itself as a potent human respiratory pathogen due to a mutation in the Receptor Binding Domain (RBD) of its spike protein that enables high affinity binding to the ACE2 receptor in humans and a polybasic furin cleavage site at the junction of the S1 and S2 subunits of the spike protein (Andersen et al., 2020; Hasan et al., 2020). Since the first report on the virus, it has spread across continents inflicting a global health-care and economic emergency. In view of the global spread of the outbreak, the World Health Organization (WHO) declared it as a pandemic in January 2020. As of 28th May 2020, the number of infections reported was 5,596,550 and the number of deaths is 353,373. The numbers are increasing relentlessly despite concerted efforts to contain the spread of the virus using rigorous diagnostic testing, isolation of positive cases and tracing of contacts. The scenario is further made grim by the fact that there are no specific drugs or vaccines against the virus currently. The current treatments focus on symptom management and supportive therapy (Prajapat et al., 2020). Government agencies, pharmaceutical companies and research institutes across the globe have taken up the formidable challenge of inventing a specific, viable and validated therapeutic agent against SARS-CoV-2 as it is probably the only solution to the ongoing crisis.

Drug repurposing refers to the identification of novel applications/targets for an approved or investigational drug outside the premise of its medical indication (Ashburn & Thor, 2004). At present, the strategy would be a logical

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choice for developing a therapy for COVID-19 considering the substantial time-scales and attrition rates associated with new drug discovery and the trial-based validation of its safety and efficacy. The major advantage lies in the fact that a repurposed drug has been already evaluated for its safety by pre-clinical and clinical trials, which would save significant amounts of time and money (Pushpakom et al., 2019), a priority concern in SARS-CoV-2 drug discovery. Indeed, most of the drugs that are currently under investigation for efficacy against SARS-CoV-2 are repurposed known medicines. Drugs that are either under development or prescribed off-label against COVID-19 include ribavirin, interferon-α, mycophenolic acid, ritonavir, lopinavir, oseltamivir and remdesivir (Mitjà & Clotet, 2020; Baden & Rubin, 2020; Cao et al., 2020; Muralidharan et al., 2020; Hendaus, 2020; Arya & Dwivedi, 2020). Among these, hydroxychloroquine, an approved antimalarial drug and two known antivirals ritonavir and remdesivir have been reported to be effective against SARS-CoV-2 in vitro (Yan, 2020). However, more recent studies have reported harmful effects of chloroquine and hydroxychloroquine. Inhibition of autophagy by these drugs may induce tissue damage and worsen organ injury in COVID-19 patients (Edelstein et al., 2020). A number of computational studies that aim to repurpose drugs against COVID-19 have been reported. The drug targets selected in these studies include the host cell protease TMPRSS2 (Elmezayen et al., 2020), the spike (S) protein (Oliveira et al., 2020; Sinha et al., 2020), the envelope (E) protein ion channel (Gupta et al., 2020), a putative immune evasion molecule, 2′-O-ribosemethyltransferase (Khan et al., 2020), the nucleocapsid protein (Salma et al., 2020) and the RNA dependant RNA polymerase (RdRp) enzyme (Elfiky & Azzam, 2020; Elfiky, 2020).

The aim of the present study is to identify clinically approved drugs that can be targeted to the main protease M^{pro}, which is also called $3CL^{pro}$. The enzyme has similar structural fold and cleavage site specificity as that of the picornavirus 3 C protease (Huang et al., 2004). The M^{pro} is an attractive and well characterized drug target in corona viruses owing to the pivotal role it plays in the propagation of the virus inside the host (Zhang et al., 2020; Boopathi et al., 2020). The non-structural proteins of the virus ($n = 16$) is encoded in the ORF1a/b of the RNA genome and gets transcribed and translated into two polyproteins (PP1a and PP1ab). Proteolytic cleavage of the PPs into its components is required to derive functionally active proteins. After its auto-cleavage from PP1a and PP1ab, M^{pro} cleaves PP1ab at about 11 sites. PP1ab contains the subunits of the replicase complex including the RNA dependant RNA polymerase (RdRP) and hence the cleavage becomes an essential requirement for viral replication (Zhang et al., 2020; Mousavizadeh & Ghasemi, 2020). Thus, the inhibition of M^{pro} would effectively stop viral spread by preventing its replication. Since, human proteases with the same cleavage specificity as the SARS-CoV-2 protease (Leu-Gln↓Ser,Ala,Gly) are not known, it is unlikely that an inhibitor would cross react with a human protease (Zhang et al., 2020). Very recently, Jin and co-workers reported the structure of the SARS-CoV-2 M^{pro} in complex with a potent inhibitor (Jin et al., 2020). The protein is 306 residues long and has a molecular weight 33.8 KDa. Being a pivotal drug target in SARS-CoV-2, various groups have reported *in silico* drug repurposing studies using different approaches against M^{pro} with a wide range of druggable molecules (Khan et al., 2020; Aanouz al., 2020; Pant et al., 2020; Joshi et al., 2020; Enmozhi et al., 2020; Choudhury, 2020; Kumar et al., 2020; Umesh et al., 2020; Das et al., 2020; Al-Khafaji et al., 2020; Gyebi et al., 2020).

In the present study in-order to identify clinically approved drugs that would bind to the catalytic site of M^{pro}, an E-pharmacophore model based virtual screening was performed on a chemical library of known drugs from the SuperDRUG2 database. The database contains more than 4600 active pharmaceuticals which are marketed or approved (Siramshetty et al., 2018). A subset of the drugs selected based on pharmacophore screening was further screened using molecular docking. The binding energies of the identified poses were calculated using MM-GBSA method. Molecular dynamics simulations were carried out on selected poses to understand the dynamic behaviour of the complexes and the stability of the protein-ligand binding.

2. Methods

All computational studies like E-pharmacophore hypothesis generation, virtual screening, molecular docking and MM-GBSA were carried out using Maestro version 11.4 (Schrödinger Release 2018-1) from Schrodinger Inc. Visualization of molecular interactions were done using PyMol.

2.1. Generation of E-pharmacophore model

The crystal structure of M^{pro} of SARS-CoV-2 bound to a non-covalent inhibitor X77 at a resolution of 2.1 Å was used to generate an energy-optimised pharmacophore hypothesis (E-pharmacophore). The structural coordinates of M^{pro}-X77 complex was downloaded from the PDB (ID: 6W63). The structure of the protein-ligand complex was pre-processed and water molecules within 5 Å distance from the ligand were removed. Missing hydrogens and loops were added and the structure was subjected to restrained energy minimization adopting OPLS3 force field (Harder et al., 2016). These steps were performed using the protein preparation wizard of the Schrodinger suite (Sastry et al., 2013). The E-pharmacophore model was developed using the 'Develop Pharmacophore from protein-ligand complex' option in the Phase module (Dixon et al., 2006). For this, the prepared protein-ligand complex was imported to the workspace and default pharmacophore features such as hydrogen bond acceptor (A), hydrogen bond donor (D), aromatic ring (R) and hydrophobicity (H) were mapped.

2.2. E-pharmacophore based virtual screening

E-pharmacophore based virtual screening was performed using the chemical structures of 4600 drugs (ligands) from the SuperDRUG2 database. Prior to the screening, the ligands were structurally optimised at near neutral pH (7 ± 1). All plausible tautomers and stereoisomers were generated and

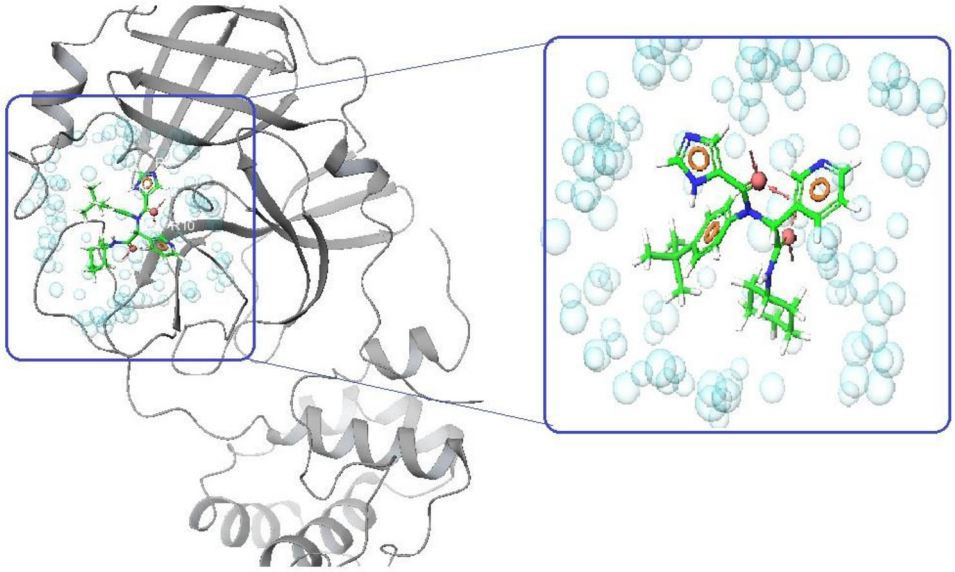


Figure 1. E-pharmacophore model for M^{pro}-X77 complex mapped to the bound inhibitor X77: The left panel shows the bound inhibitor X77 (ball-and-stick model) in the active site of M^{pro} (ribbon model). The E-pharmacophore features of the inhibitor are shown in red. The right panel is a zoomed in image of the inhibitor, X77 with the pharmacophore features marked in red.

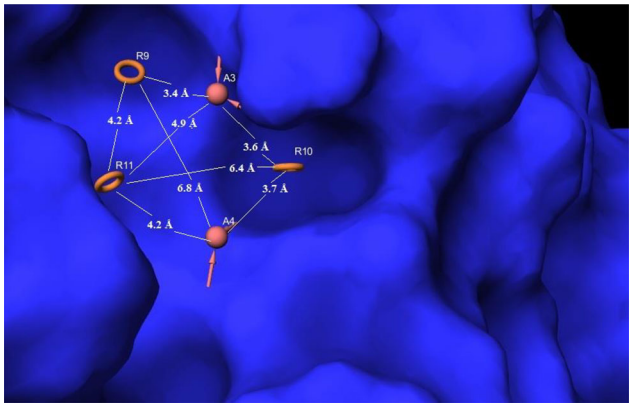


Figure 2. Energy optimised pharmacophore hypothesis AARRR. A3 and A4 are hydrogen bond acceptors; R9, R10 and R11 are aromatic rings. The distances between the pharmacophore features are also shown.

protonation states were assigned. The ligands were subjected to energy minimisation with OPLS3 force field using the ligprep module of Maestro 11.4. In order to generate a subset of drugs with the desired molecular features for optimal binding to M^{pro}, as mapped by the E-pharmacophore model, a pharmacophore based virtual screening was carried out using the phase module of Schrodinger suite. The fitness scores were used to select the best hits.

2.3. Structure based virtual screening

The initial screening using the E-pharmacophore model enabled the selection of 1000 drugs with a potential to make energetically favourable interactions with the active site of M^{pro}. Further, to identify the most promising candidate drugs form this subset, a structure based screening was performed on the selected drugs using Molecular Docking. The GLIDE (Grid-based Ligand Docking with Energetics, version 7.8) module of Maestro 11.4 was used to perform all the molecular docking studies. A receptor grid was generated by keeping the bound ligand in the crystal structure (X77) as the centre of the grid box. The size of the box was set to $15 \times 15 \times 15$ $Å^3$. GLIDE scores (g-scores) were used to rank the drugs based on its binding affinity (Friesner et al., 2006). The initial screening was performed using the High-Throughput Virtual Screening (HTVS) module of glide and 10% of top scoring compounds were subjected to standard precision (SP) docking. Finally, 50% of high scoring compounds from SP docking were subjected to extra precision (XP) docking to identify the best hits.

2.3.1. Validation of docking procedure

The docking procedure was validated by a control study. For this the bound ligand in the crystal structure was re-docked to the pre-processed and prepared protein keeping the same grid box. The glide score for this docking was used as a standard value against which the scores for the drugs were compared. The control docking was performed in the XP mode.

2.4. Estimation of binding free energy

The theoretical binding free energies of the potent inhibitors of M^{pro} were calculated using the prime module of Maestro 11.4 (Lyne et al., 2006). MM-GBSA is a popular method to calculate binding energy, which uses energy properties of free ligand, free receptor and receptor–ligand complex for binding affinity calculation. Binding energies were estimated for the 40 drugs selected based on the glide scores of XP docking, using the MM-GBSA method.

2.5. Molecular dynamics

The stability of binding of the selected drugs with M^{pro} in an explicit solvent system was determined by molecular dynamics (MD) using Desmond module of Schrodinger (Bowers

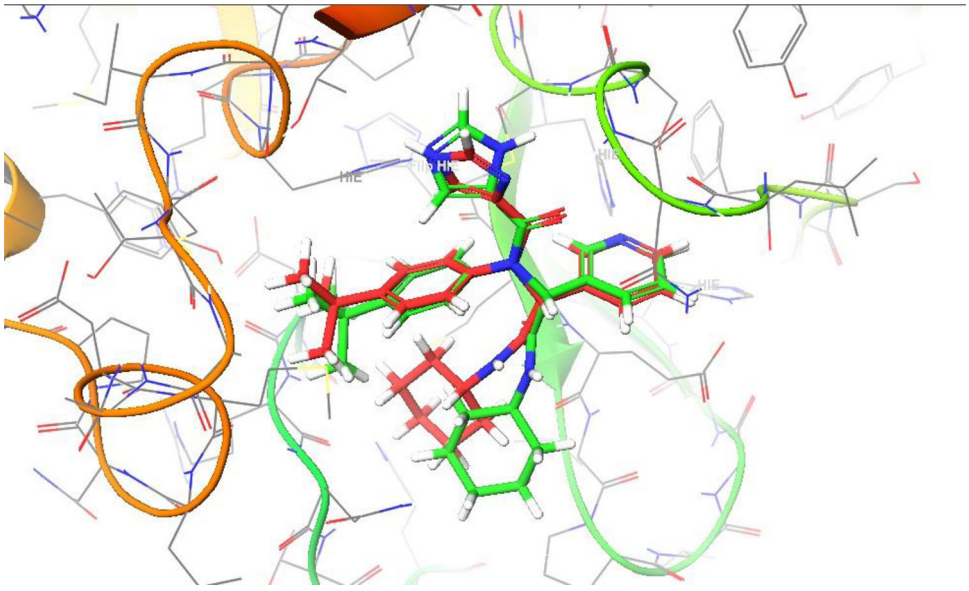
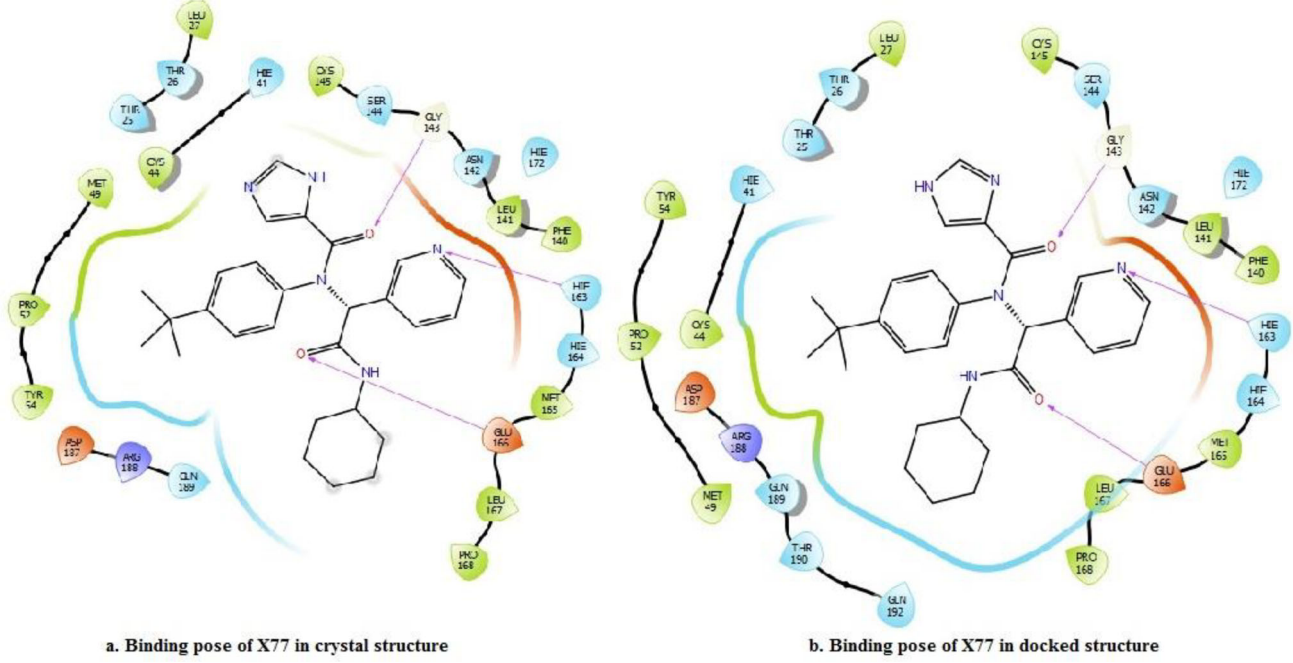


Figure 3. The docked pose of X77 (red) superimposed on the crystal structure (green).



a. Binding pose of X77 in crystal structure

b. Binding pose of X77 in docked structure

Figure 4. Interactions between standard inhibitor X77 and M^{pro} active site residues. (a) X77-M^{pro} interactions in the crystal structure. (b) X77-M^{pro} interactions in the docked structure.

et al., 2006). The docked poses of protein ligand complexes were used as input structures and each complex was prepared by system setup option in Desmond module. In the first step the complexes were solvated with TIP3P water model and the solvated system was neutralized by adding Na^+/Cl^- ions. After the system generation, minimization and relaxation of the protein-ligand complexes under NPT were carried out using default protocol of Desmond module. MD simulations were conducted with the periodic boundary conditions in the NPT ensemble using OPLS3 force field. The temperature and pressure were kept at 300 K and 1 atmosphere respectively using Nose-Hoover temperature coupling and isotropic scaling. The operation was followed by 100 ns NPT production run. The binding energy of each complex was determined after the run from selected MD trajectory frames by using MM-GBSA method.

3. Results and discussion

3.1 E-pharmacophore hypothesis

Pharmacophore is defined as 'an ensemble of steric and electronic features that is necessary to ensure the optimal supramolecular interactions with a specific biological target and to trigger (or block) its biological response' (Proekt & Hemmings, 2019). Pharmacophore model based screening has evolved as a key tool in computer aided drug discovery because of its ability to screen large libraries for potent hits

Table 1. Drugs with highest binding energies towards M^{pro}.

Drug	Binding free energy (kcal/mol)	Fitness score	H- bonded residues	Primary target/activity
Binifibrate	−69.04	1.3	Gly143, His 163, Glu 166	Peroxisome proliferator-activated receptor agonist/ Hypolipidimic agent
Macimorelin acetate	−64.25	1.296	Gly 143, His 164, Glu 166, Thr 190	Agonist of Growth Hormone (GH) secretagogue receptor
Bamifylline	−63.19	1.534	Gly143, Glu 166, Gln 189	Selective A1 adenosine receptor antagonist.
Rilmazafone	−61.37	1.409	Thr 26, Gly 143, His 163, Glu 166	GABA-A receptor agonist/ Non-benzodiazepine sedative
Afatinib	−60.89	1.169	Gly 143, Glu 166	Tyrosine kinase inhibitor
Ezetimibe	−60.21	1.175	Glu 166	Inhibits intestinal cholesterol absorption by physical interactions with Niemann-Pick C1-Like 1 (NPC1L1) transporter

Table 2. Energy contributions from different components to the total binding energy (kcal/mol)*.

Drug	Coulomb	dG_Bind _Covalent	vdW	Lipo	Solv_GB	Hbond	Packing
Binifibrate	−23.14	11.67	−61.20	−20.31	27.42	−1.74	−1.73
Macimorelin acetate	−50.03	10.22	−49.21	−16.17	47.52	−2.30	−4.26
Bamifylline	−27.93	1.45	−41.84	−13.22	23.41	−2.09	−2.96
Rilmazafone	−21.88	1.34	−53.12	−14.58	29.69	−2.09	−0.72
Afatinib	−29.13	8.70	−59.76	−13.28	38.17	−2.29	−3.28
Ezetimibe	−11.76	1.88	−43.56	−18.75	15.88	−1.18	−2.71

*Coulomb = Electrostatic, dG Bind Covalent = Covalent, vdW = van der Waals, Lipo = Lipophilic, Solv_GB = Generalized Born electrostatic salvation, Hbond = Hydrogen bonding, Packing = pi-pi packing.

within a short period of time and minimal computational capacities. Energy optimised pharmacophore models tries to combine the stereo-electronic features of the ligand with the energetics of its interactions with the protein structure (Muthusamy et al., 2013). In the present study an E-pharmacophore hypothesis was generated to screen for the inhibitors of M^{pro} protein of SARS-CoV-2 using its crystal structure in complex with a strong, broad spectrum non-covalent inhibitor. The bound inhibitor, an imidazole carbaximide derivative dubbed X77, interacts strongly with the active site amino acid residues. Based on the ligand-protein complex an energy optimised five-featured pharmacophore hypothesis, AARRR was obtained. The generated E-pharmacophore model contains two hydrogen bond accepters (A) and three aromatic rings (R) (Figure 1). Figure 2 shows the planar representation of the pharmacophore hypothesis with distances between the features. The hypothesis AARRR was used as a 3 D search query to screen 4600 drugs from the SuperDRUG2 database to identify drugs with comparable pharmacophore features. During the screening, the phase module analyses the fitness of compounds with the query hypothesis and ranks the compounds on the basis of fitness scores. One thousand compounds were selected in this way and used for the molecular docking based screening.

3.2. Structure based virtual screening

The best hits obtained in the E-pharmacophore based screening ($n = 1000$) were further screened using molecular docking. The docking study analyses the molecular interactions of the different plausible geometries of the drugs (poses) with the surrounding active site residues of the SARS-CoV-2 M^{pro} and ranks them on the basis of binding scores. The docking processes were carried out using three different approaches: HTVS, SP and XP methods; by filtering the outputs after each stage based on glide scores. The docking studies also revealed the atomic level interactions between the drugs and the protease. Re-docking the crystallographic ligand X77 (N-(4-tert-butylphenyl)-N-[(1R)-2-(cyclohexylamino)-2-oxo-1-(pyridin-3-yl)ethyl]-1H imidazole-4-carboxamide) to the active site of the protease using the same protocol resulted in the binding of the ligand in the same position and orientation (Figure 3) and this verified that the docking parameters chosen were optimal. Analysis of the crystal structure of M^{pro}-X77 complex showed that the binding of the ligand were stabilized through three hydrogen bonds with the active site residues, viz, Gly 143, His 163 and Glu 166. The *in silico* docked structure also exhibited these three hydrogen bonds (Figure 4).

Based on HTVS and SP docking, 40 drugs were selected for XP docking. The g-score calculated for the crystallographic ligand, X77 was -8.243 kcal/mol. Seventeen of the 40 drugs used for XP docking showed g-scores comparable to that of the standard inhibitor used in the study (g-scores better than -7.0 kcal/mol) (supplementary data, S1). Three highest scoring drugs, viz, Hidrosmin (-12.689 kcal/mol), Diosmin (-11.409 kcal/mol) and Monoxerutin (-10.745 kcal/mol) are flavanoids with similar pharmacological properties. They are used as vaso-protectives and capillary stabilising agents. Remikirin (-9.429 kcal/mol) is an interesting hit because it is a well-known inhibitor of Renin, an aspartyl endoprotease which acts as the primary enzyme in the renin-angiotensin system (Himmelmann et al., 1996). Doxorubisin (-9.16 kcal/mol), an anthracyne class antineoplastic used as an anti-cancer drug and Fluvastatin (-8.346 kcal/mol), an inhibitor of hydroxymethylglutaryl-coenzyme A (HMG-CoA) reductase, used as an antilipemic agent also showed high binding score to the protease. Of note, Doxorubisin and its derivatives had been previously shown to be effective *in vitro* against viruses like HIV, HSV, Dengue virus, Yellow Fever Virus, Rauscher leukemia virus and avian myeloblastosis virus (Kaptein et al., 2010; Ash & Diekema, 1987; Jeyaseelan et al., 1996; Papas & Schafer, 1977). Although the drug targets in each case vary, the drugs were able to reduce virus replication *in vitro*. Statins, in general,

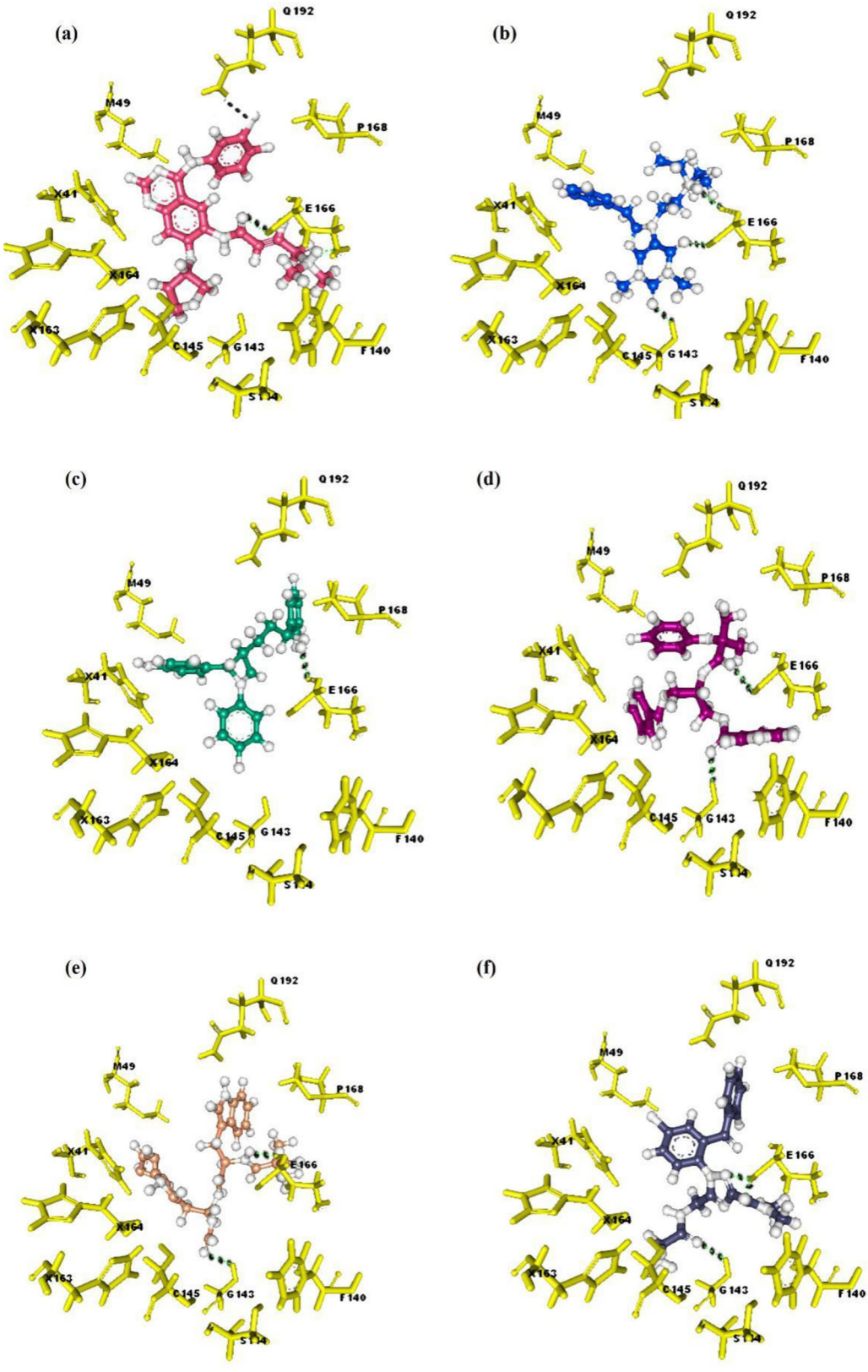


Figure 5: Conformation of drugs with highest binding energies bound in the active site of M^{pro} and surrounding protein residues. (a) Afatinib (b) Bamifylline (c) Ezetimibe (d) Binifibrate (e) Macimorelin acetate (f), Rilmazafone.

are known to inhibit the replication of many enveloped viruses by the inhibition of cholesterol/isoprenoid pathway (Shrivastava-Ranjan et al., 2018). Fluvastatin was earlier shown to have an inhibitory effect on *Heamophilus influenza* replication *in vitro* (Peng et al., 2014). Thus, the present study identifies the M^{pro} of SARS-CoV-2 as a novel targets for the

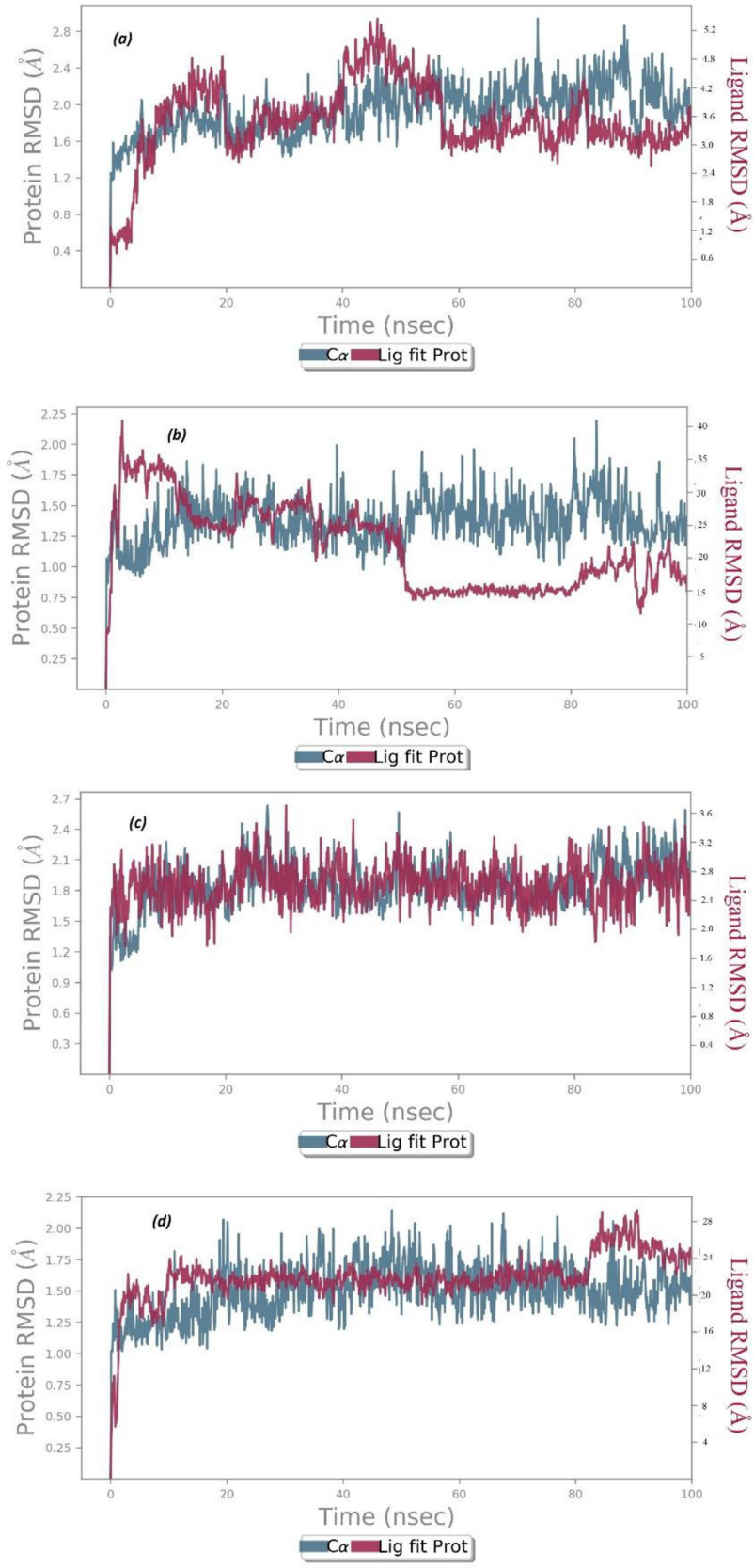


Figure 6. Protein and ligand RMSD of SARS-CoV-2 M^{pro} in complex with (a) Binifibrate, (b) Macimorelin acetate, (c) Bamifylline and (d) Rilmazafone as observed in the 100 ns MD simulations.

known antivirals, Doxorubisin and Fluvastatin. Fluvastatin is an interesting hit considering the fact that the SARS-CoV-2 is also an enveloped virus. The drug might have a cumulative inhibitory effect on the propagation of the virus, if it can inhibit both the M^{pro} and the cholesterol synthesis pathway.

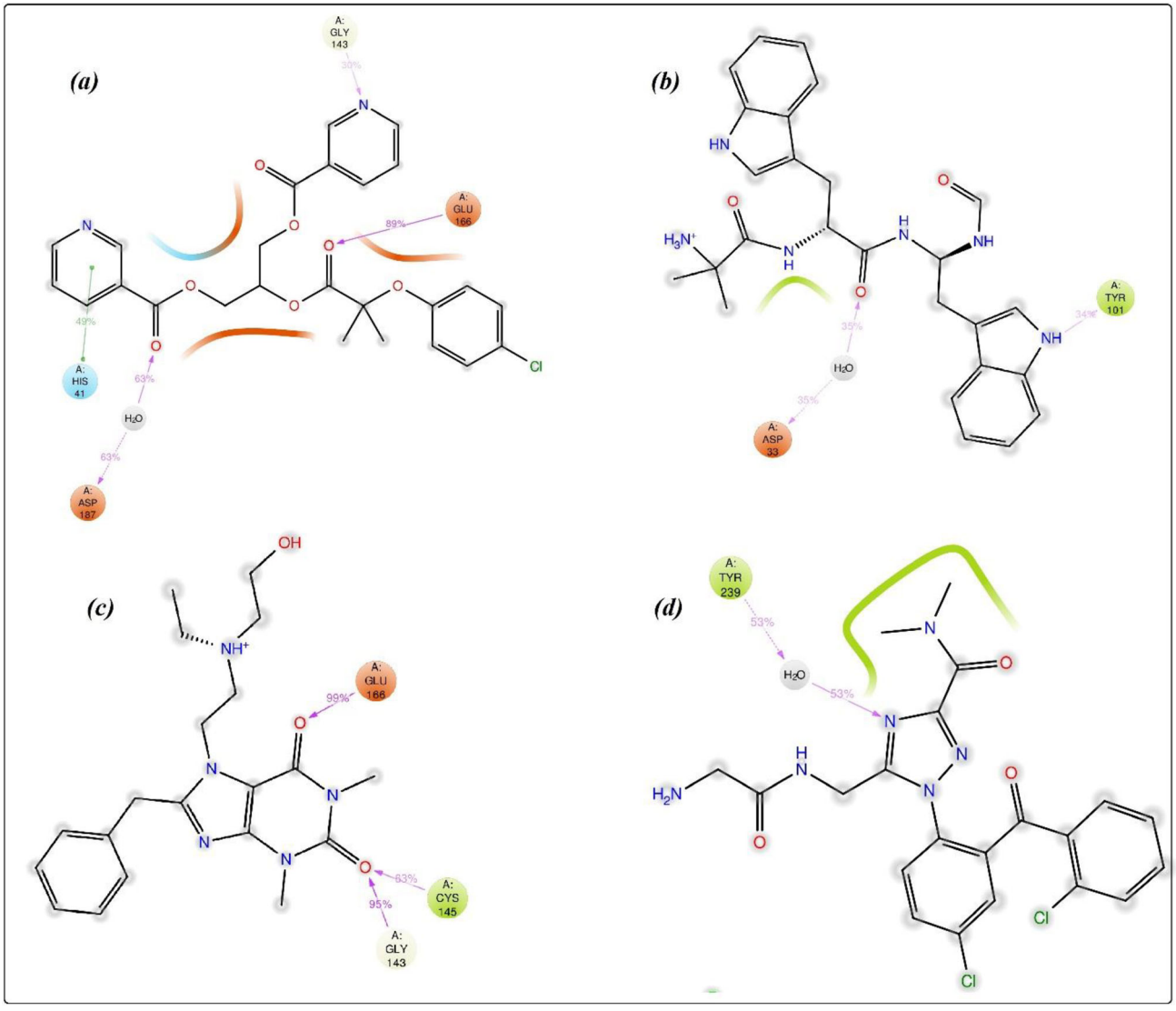


Figure 7. Schematic diagram of the ligand interactions with surrounding protein residues. (a) Binifibrate, (b) Macimorelin acetate, (c) Bamifylline and (d) Rilmazafone during the course of MD simulation.

Table 3. Binding free energy values of the drugs with SARS-CoV-2 M^{pro} after MD.

Drug	Binding energy after MD simulation kcal/mol
Binifibrate	−67.78 ± 5.97
Macimorelin acetate	−17.36 ± 14.53
Bamifylline	−65.24 ± 6.20
Rilmazafone	−39.05 ± 6.15

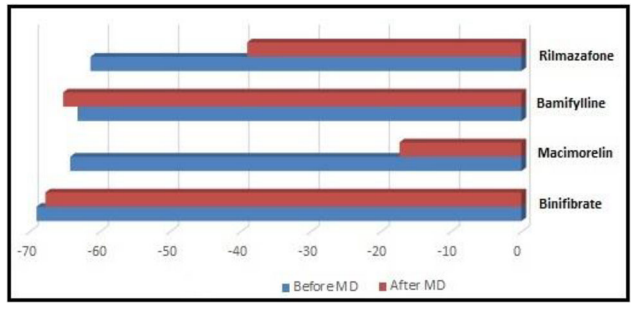


Figure 8. The comparison of binding energy values before and after MD simulation.

3.3. Estimation of binding energies of docked drugs

Drugs which showed high glide score was further subjected to binding energy calculation using the MM-GBSA method. The binding energy of crystallographic ligand, X77 as determined by MMGBSA was -73.68 kcal/mol. Six drugs with binding free energies better than -60 kcal/mol were selected from the pool of 40 drugs using molecular docking. These drugs were Binifibrate, Macimorelin acetate, Bamifylline, Rilmazafon, Afatinib and Ezetimibe. Except Bamyfilline (g-score; −6.61 kcal/mol) all these drugs had shown g-scores better than -7 kcal/mol in the docking studies. However few drugs that showed glide scores comparable to the standard inhibitor showed lower binding energies. Binifirbate, a hypolipidaemic drug showed a very high binding energy (-69.04 kcal/mol) similar to that of the standard inhibitor, X77. Binifibrate forms hydrogen bonds with three active site residues, viz, Gly 143, His 163, and Glu 166. The binding free energies and protein residues that interact with the drugs were shown in Table 1. The primary targets/activities of the drugs were also shown in the table. Energy contributions from different components like, Electrostatic, Covalent, Van

Table 4. Energy contributions from different components to the total binding energy (kcal/mol) after MD simulations.*.

Drug	Coulomb	dG_Bind _Covalent	vdW	Lipo	Solv GB	Hbond	Packing
Binifibrate	−15.21	4.87	−61.37	−15.94	24.36	−1.25	−3.23
Macimorelin acetate	−21.69	0.30	−14.26	−4.63	25.50	−1.17	−1.42
Bamifylline	−38.72	2.22	−44.07	−11.24	32.35	−2.27	−3.51
Rilmazafone	−13.71	2.01	−32.14	−11.35	17.09	−0.41	−0.54

*Coulomb = Electrostatic, dG_Bind_Covalent = Covalent, vdW = van der Waals, Lipo = Lipophilic, Solv_GB = Generalized Born electrostatic salvation, Hbond = Hydrogen bonding, Packing = pi-pi packing.

der Waals, Lipophilic, Generalized Born electrostatic solvation, Hydrogen bonds and Pi-Pi packing towards the total binding energy were given in Table 2. From the results presented in Table 1 it can be seen that the hydrogen bonding interactions with Gly 143, His 163, and Glu 166, the three polar amino-acid residues in the active site of the protease are critical in the high affinity binding of the drugs to the protein. Figure 5 shows the conformation of the bound drugs in the active site of the enzyme and surrounding protein residues.

3.4. Molecular dynamics

MD simulations were performed on the top 4 hits selected based on the binding free energies, namely, Binifibrate, Macimorelin Acetate, Bamifylline and Rilmazafon in complex with M^{pro}. The conformational changes of the protein and the ligand from the initial structure during the simulation can be expressed in terms of Root Mean Square Deviation (RMSD). Any RMSD within 3 Å is perfectly acceptable for globular proteins. Larger deviations indicate that the protein is undergoing large conformational changes during the simulation and it means that the protein-ligand complex is unstable. The four complexes that were subjected to MD simulations showed deviations in protein RMSD is below 3 Å when compared to initial frame (Figure 6). The overall values of Root Mean Square Fluctuation (RMSF), (which specifies the flexibility of protein residues during the interaction with the drug) falls within 3 Å and hence indicates that the protein is stable while in complex with the drugs in the active site. Analysis of the ligand RMSD shows that Binifibrate and Bamifylline do not exhibit large deviations from the initial position indicating that their binding is stable. However, Macimorelin acetate and Rilmazafone displayed larger RMSDs during the MD run indicating that they have less stable binding. Taken together, it is clear that Binifibrate and Bamifylline possess better binding affinity towards SARS-CoV-2 M^{pro} active site compared to the other two drugs. The interactions of drugs with specific residues of M^{pro} during the MD run were shown in Figure 7. The major hydrogen bonds which stabilize the binding of Binifibrate (Gly 143 and Glu 166) and Bamifylline (Gly143, and Glu 166) were persistent during the entire course of the MD simulations. The hydrogen bonding interactions established by Macimorelin acetate (with Gly 143, His 164, Glu 166, Thr 190) and Rilmazafone (with Thr 26, Gly 143, His 163, Glu 166) were eventually lost during the course of the simulation and that accounts for the lower stability of the binding.

After the MD run, binding energy of the selected frames from MD trajectory was calculated by MM-GBSA. For this, the structure of each 10 ns frame from the 100 ns (total 10 structures) MD trajectory was exported and binding energy was calculated for each complex. The binding energies obtained were averaged and standard deviations were determined (Table 3). Binifibrate showed highest average binding energy during the MD run (-67.78 ± 5.97 kcal/mol) and the energy value was similar to the binding energy before the MD run (-69. 04 kcal/mol). The binding energy values of Bamifylline before and after the MD run (-63.19 kcal/mol and -65.24 ± 6.20 kcal/mol respectively) were also similar indicating that the drug interacts with the M^{pro} active site with high stability. Rilmazafone which had an initial binding free energy of -61.37 kcal/mol showed a significantly lower energy (-39.05 ± 6.15 kcal/mol) after the MD run. Likewise Macimorelin acetate showed a large deviation in binding energy before (-64.25 kcal/mol) and after the MD run (-17.362 ± 14.53 kcal/mol). Also, the RMSD of Macimorelin acetate showed a huge variation (15-40 Å). The MD simulation indicates that binding of Macimorelin to M^{pro} was not stable. Figure 8 summarises the comparison of binding energy values before and after MD simulation and Table 3 summarises the contribution of each energy parameters to the binding energy of the simulated systems. The MD data indicates that the drugs Binifirbate and Bamifylline might bind to the active site SARS-CoV-2 M^{pro} and inhibit its activity. Hence, these drugs have the potential to be repurposed against COVID-19. However, Binifibrate, a hypolipidemic fibrate drug co-administered along with statins, has been presently withdrawn from the market. The reason cited for the withdrawal is that the risks associated with the drug outweighs its efficacy as an agent that reduces triglycerides and increases high density lipoprotein-C (HDL-C) (Abb Vie Inc, 2016). A rational assessment of the benefits of using binifibrate against COVID-19 and the potential side effects should be made before considering it for drug repurposing against COVID-19.

4. Conclusion

Drug repurposing is perhaps the best way to combat the medical emergency poised by the SARS-CoV-2 infections that grows in magnitude with the passing of each day. Repurposing involves screening and identification of known bio-actives against specific therapeutic targets in SARS-CoV-2. Repurposed drugs gets to the market at relatively lesser time periods and costs compared to novel drugs. The M^{pro} of SARS-CoV-2 is involved in the proteolytic processing of viral polyproteins to form key non-structural components involved in viral multiplication and hence is an attractive target for drug development. In the present study using a combination of E-pharmacophore and structure based virtual screening

followed by binding energy estimation; a subset of known drugs from the superDRUG2 database was repurposed against COVID-19. Out of 4600 drugs from the database, after a series of screening, four drugs were selected for molecular dynamic simulation studies to check their binding stabilities in the active site of SARS-CoV-2 M^{pro}. Based on the MD results, it was found that Binifibrate and Bamifylline may bind to the SARS-CoV-2 M^{pro} active site and inhibit its activity. Hence, Binifibrate and Bamifylline are two drugs that could be repurposed against COVID-19. However, it is important to note that Binifibrate is currently withdrawn from the drug market because of its side effects. It is suggested that a rational risk-benefit analysis should be performed before the drug can be used for repurposing against COVID-19. To conclude, Bamifylline is proposed as potential drug worthy of testing against COVID-19 based on the *in silico* studies described here.

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Disclosure statement

The authors declare no conflicts of interest

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