Genetic algorithm-based docking of potent inhibitors against SARS-CoV-2 main protease: a comparison between natural products and synthetic drugs.

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Abstract

The Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has caused coronavirus disease-2019 (COVID-19) pandemic. Despite the intensive research currently, there are no therapeutics and vaccines available. As the main protease (M^{Pro}) plays a vital role in SARS-CoV-2, it is an attractive drug target. Herein we report, potential inhibitors form natural products and synthetic drugs against M^{Pro}. In detail, we studied the interaction of inhibitors (Curcumin, Theaflavin, Deserpidine, Betulinic acid, Sinigrin, Emodin, Leptodactylone, Synthetic drugs, Lopinavir, Ritonavir, Indinavir, Amprenavir, Darunavir, Nelfinavir, Remdesivir, Saquinavir, Sivelestat, Galidesivir, and Favipiravir) with the catalytic site of M^{Pro}. Lastly, ADME (Absorption, Distribution, Metabolism, and Excretion) properties of Natural products and synthetic drugs are explored. We identified eight potential inhibitors against M^{Pro}.

Introduction

Near the end of the year 2019, a new pulmonary disease-causing virus caused an outbreak in the Wuhan city of Hubei province of China, it is a new type of coronavirus and it spread quickly on a global scale[1,2]. As the RNA sequence of this new coronavirus genome is similar to that of SARS coronavirus (SARS-CoV) and as this virus also belongs to the lineage clade b of the genus *Betacoronavirus*, under subfamily *Coronavirinae*[1,2], thus the name Severe acute respiratory syndrome-coronavirus 2 (SARS-CoV-2)[3]. Coronavirus disease (COVID-19) is a disease triggered by SARS-CoV-2. In 11th March 2020, the world health organization (WHO) declared the COVID-19 outbreak as a pandemic. On the 21st June of the year 2020 there were >8.8M confirmed cases globally and >410,000 confirmed cases in INDIA. Currently, there are no proven drugs/treatments for the COVID-19, only early diagnosis, isolation, and supportive treatments are carried out to prevent the spread of the viruses. The SARS-CoV-2 is an enveloped virus, spherical, and possess single +ve sense RNA genome, the possible drug targets in the structure of this virus can be 3-chymotrypsin like protease ($3CL^{pro}$ or M^{pro}), Spike glycoprotein (S), papin-like protease (PL^{pro}), and RNA-dependent RNA polymerase. Interestingly, the main protease[4] M^{pro} due to its vital role in life cycle of SARS-CoV-2 and due to the absence of analogous sites in humans it an attractive candidate for drug discovery as the developed drug will not show any or show minimal adverse effect. At present no antiviral therapy and preventive vaccines are available for COVID-18 treatment, several repurposing methods are being trailed using FDA approved synthetic drugs. ClinicalTrials.gov contains a updated list of clinical trials planned or currently investigated for the treatment of COVID-19. The medicinal plants is as gift as a wisdom to humans, from the prehistoric time to now we have been using

them to treat diseases, the traditional medicine gave us insights and the derived natural products and further synthetic improvement has also improved the western medicine inventory[5]. So, along the treatment with synthetic drugs, purified natural products from the traditional herbal/ayurvedic medicine can also provide insight on novel broad-spectrum antiviral drug design and improved healthcare for COVID-19. As no potent inhibitors available currently to end this pandemic many patients seem to be leaning towards traditional medicine like Ayurveda and Chinese herbal medicine, along with western medicine. It was reported that almost 92% of the patients in the hospital in northeast Chonquing (china) along with western medicine received traditional medicine[6]. So as a stepping stone in this work we explored 18 potential inhibitors based on natural products (found in traditional medicine) and synthetic drugs, against SARS-CoV-2 main protease M^{pro}.

Methods.

Preparation of receptor and ligands.

All the ligand files were obtained from the PubChem database, the atomic coordinates of M^{Pro} ligated with a-ketoamide inhibitors was obtained from RCSB PDB database PDB ID - 6Y2F. Before docking, the hydrogen bonds are added to the protein and water molecules were deleted, the co-crystalized ligands were extracted from the 6Y2F using GOLD (Genetic Optimisation for Ligand Docking)[7] interface. The ligands were converted to Tripos molecule structure format (.mol2), using the CSD-Discovery package the optimized conformers were generated for each ligand.

Site-specific docking and visualization.

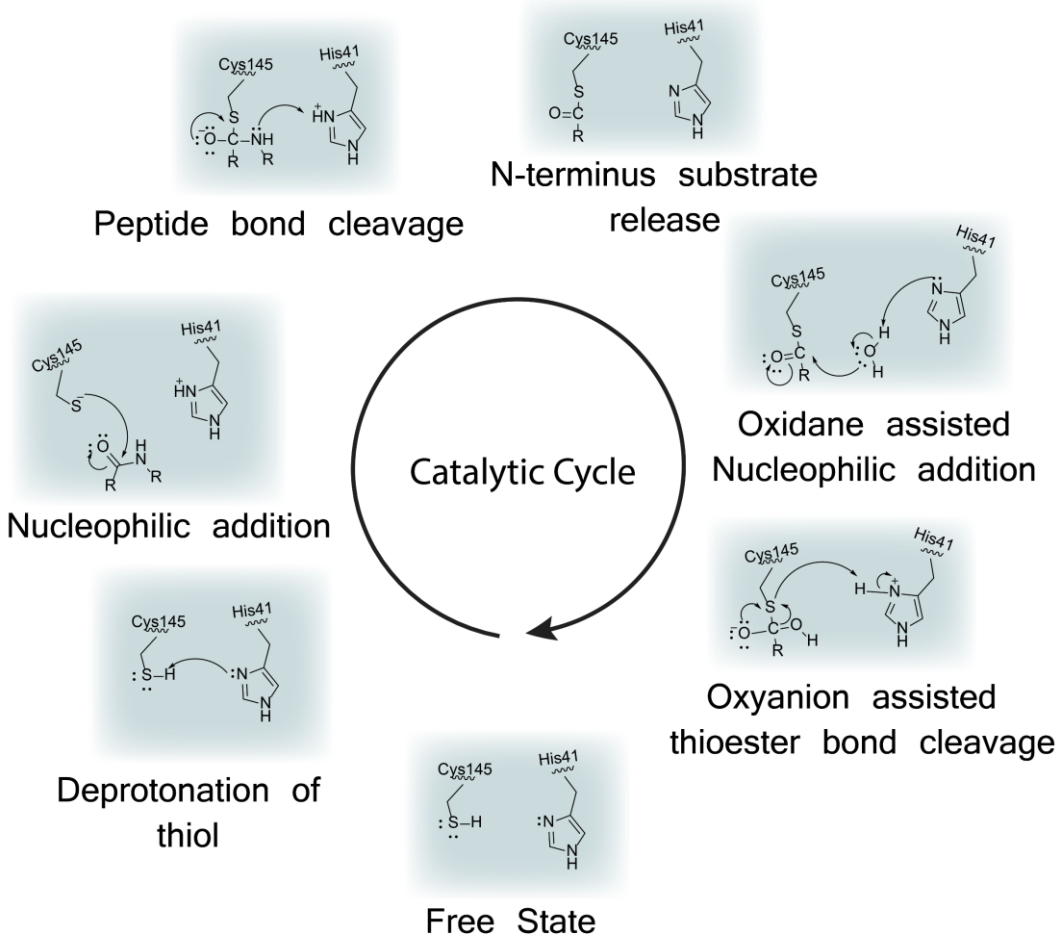
The molecular docking was carried out in GOLD which uses a genetic algorithm, after protein setup, we defined the binding site considering one or more ligands or cofactors. We selected A:O6K502 in the A chain of 6Y2F as our binding site. The optimized ligands were loaded as .mol2 format and each ligand was set to 20 GA runs, the threshold for the GA runs was set to terminate if the RMSD/solutions are within 0.9 Å. The CHEMPLP was used as a scoring function and the docking was initiated. The results were interpreted in Hermes and ligand-protein interaction was visualized using BIOVIA Discovery studio[8] and PyMOL GUI.

ADME prediction.

The canonical SMILES of selected Natural products and synthetic drugs were obtained from PubChem, then the compounds were calculated for Lipinski's rule of five, lipophilicity, and so on. The values of observation are presented in Table 1, the predictions were calculated using the SwissADME[9] server.

Results and Discussion

The SARS-CoV-2 main protease M^{pro} is a cysteine protease, they cleave no fewer than 11 cleavage sites in the polyproteins that are translated from viral RNA. The active site of the SARS-CoV-2 M^{pro} consists of Cys145 and His41 as a catalytic duo, where Cys145 is a nucleophile in the proteolytic pathway. In brief, His41 deprotonates the thiol group in Cys145 so that Cys145 can attack carbonyl carbon(of polyproteins) in nucleophilic addition, following this oxyanion initiates elimination resulting in cleavage of the peptide bond and N-terminal product accepts a proton from His41. Then hydrolysis of thioester bond occurs as His41 extracts a proton from oxidane which activates it for nucleophilic addition to attack the carbon of the thioester bond. Then another elimination is initiated by oxyanion which results in the cleavage of thioester bond, then the carboxylic acid is released. Finally, the free Cys145 now accepts a proton from His41, resting the enzyme to its native state as shown in **Scheme 1.** Thus, M^{pro} plays an essential role in the life of SARS-CoV-2, the active site comprising Cys145 and His41 is an ideal target for the inhibitor design/repurposing for the treatment against SARS-CoV-2 and other coronaviruses.



Scheme 1. Mechanism of substrate(polyproteins) hydrolysis in M^{pro} by Cys145 and His41 at the active site

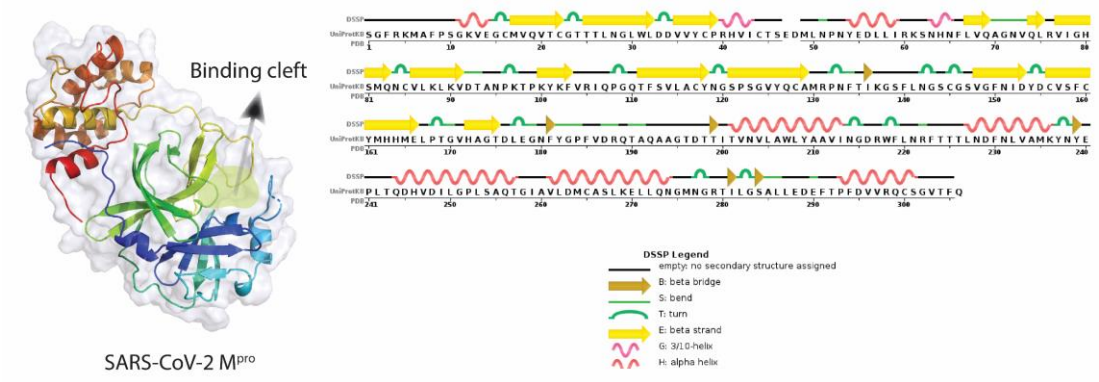


Figure 1| Structure of a protomer form SARS-CoV-2 main protease M^{pro} (highlights binding site where Cys145 and His41 resides) and description of its amino acid sequence (PDB ID: 6Y2F).

The structure of SARS-CoV-2 M^{pro} with PDB ID 6Y2F is illustrated in figure 1, the complete structure of M^{pro} is a dimer containing protomer A and protomer B. Here in figure 1 Illustrates the structure of catalytically competent Protomer A and its detailed amino acid sequence[10], also the highlighted area is where the active site of this

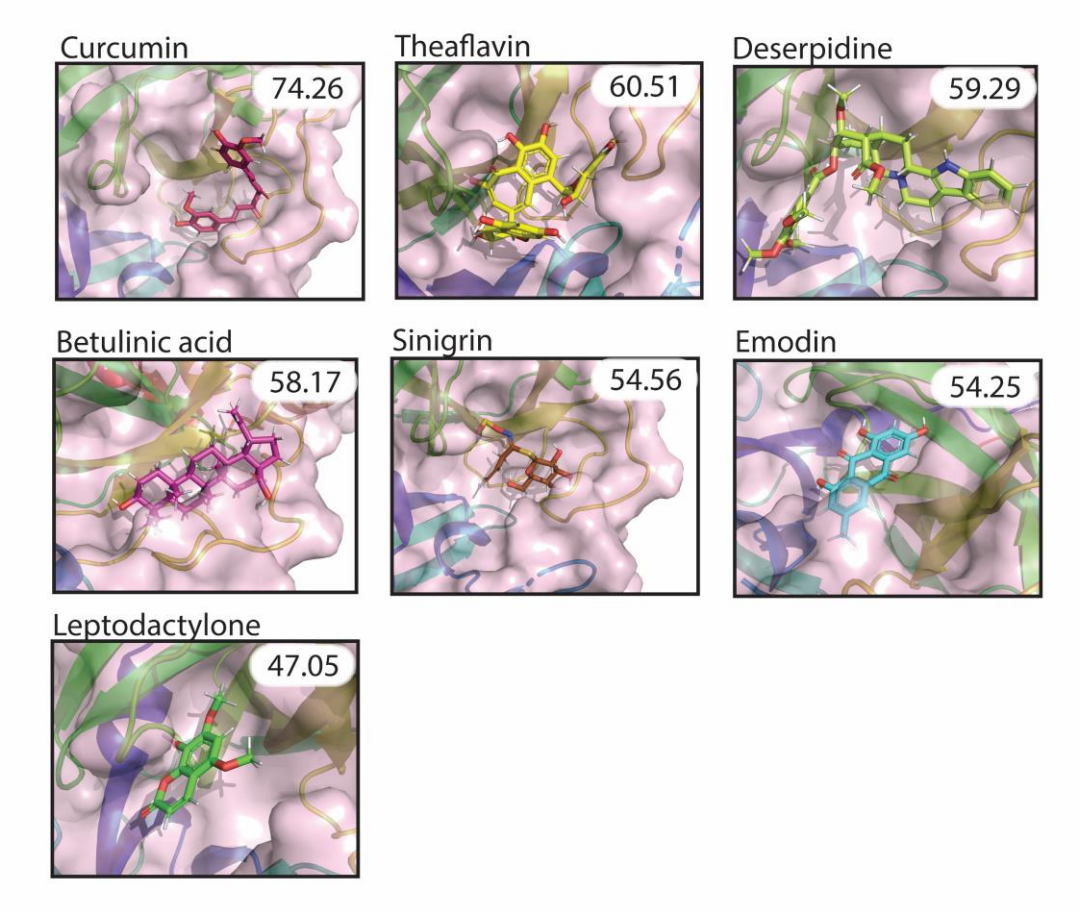


Figure 2| Docking analysis and visualization of 6Y2F inhibition by Natural products and their ChemPLP ranking.

protease is and it has Cys145 and His41. This is a preferred binding target for the inhibitor and drug design. So, in our study, we have screened our selected natural products and synthetic drugs through molecular docking using genetic algorithms (i.e. to say 'evolve' 'mutate' to generate low energy conformation). We implemented

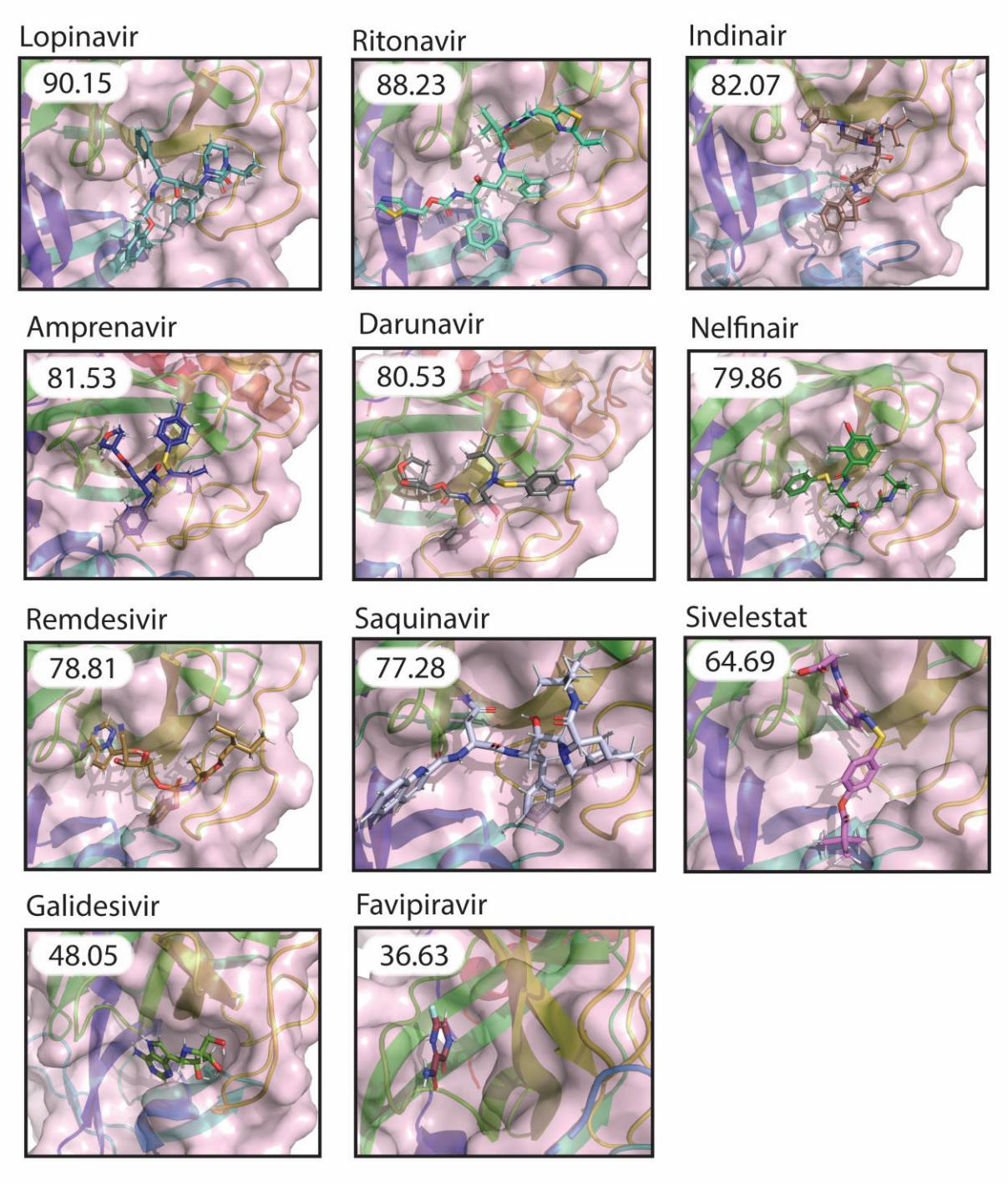


Figure 3| Docking analysis and visualization of 6Y2F inhibition by Synthetic drugs and their ChemPLP ranking.

flexible ligand and rigid receptor model using GOLD and CSD-Discovery package, then we screened natural products (Betulinic acid, Curcumin, Theaflavin, Deserpidine, Emodin, Leptodactylone, and Sinigrin) and Synthetic drugs (Amprenavir, Darunavir, Favipiravir, Galidesivir, Indinavir, Lopinavir, Nelfinavir, Remdesivir, Ritonavir, Saquinavir, and Sivelestat) at the binding cleft of M^{pro} (see methods). The results of the natural products and synthetic drugs are shown in figure 2 and figure 3 respectively, the scoring function ChemPLP in the GOLD interface was used to rank the ligands. According, to the overall best ranking ligand poses are arranged in from high to low rank on figures 2 and 3. Then individually form all the ligands examined we took the top three ligand poses and inspected if any interaction exists with the catalytically active residues Cys145 and His41 on the binding pocket (see Table 1).

Table 1. Analysis of docked ligand for Cys145 and His41 interactions.

Natural Products	Top 3 poses	Interaction with Cys145	Interaction with His41	List of residues interacted from the binding site
Curcumin	74.26	No	Yes	Thr190, Gln192, Glu166, His164, Gln189, Pro168, Met165, His41, Leu167
	71.83	Yes	No	Gly143, Cys145, Leu141, Thr190, Asn142, Pro168,
	68.63	No	Yes	Thr190, Gln192, His164, Gln189, His41, Leu167, Met165, Pro168
Theaflavin	60.51	Yes	No	Cys145, Glu166, Leu141, Thr26, Val186, Arg188, Asn142, Asp187, Met165
	60.45	Yes	No	Cys145, Glu166, Leu141, Thr26, Val186, Arg188, Asn142, Met165
	58.7	Yes	No	Cys145, Glu166, Leu141, Thr26, Arg188, Asn142, Met165
Deserpidine	59.29	Yes	No	Cys145, His164, Asn142, Met165
	58.53	Yes	No	Gly143, Cys145, His164, Asn142, Met166
	57.84	Yes	No	Gly143, Cys145,Glu166, Thr26, Thr25, His164, Asn142, Met165
Betulinic acid	58.17	No	Yes	Gln189, His164, His41, Met165, Pro168, Leu167, Met49
	56.57	No	Yes	Gln189, Thr190, His164, His41, Met165, Pro168, Leu167, Met49
	52.23	Yes	Yes	Gly143, Arg188, Asn142, Cys145, Met165, Met49, His41
Sinigrin	54.56	No	Yes	Glu166, Val186, Arg188, His164, Asn142, Asp187, His163, His41

	53.69	Yes	Yes	Glu166, Gln192, Val186, Arg188, His164, Asn142, Asp187, His163, Cys145, His41
	53.17	Yes	Yes	Cys145, His164, Glu166, His164, Met165, His41
Emodin	54.25	Yes	Yes	Glu166, His41, Phe140, Leu141, Ser144, His172, His163, Met49, Cys145
	53.17	Yes	Yes	Gly143, Ser144, Glu166, His41, Phe140, Asn142, His172, Cys145, His143, Met49
	51.16	Yes	Yes	Gly143, Ser144, Glu166, His41, Phe140, Asn142, His163, Met49, Cys145
Leptodactylone	47.05	Yes	No	Gly143, Ser144, Cys145, Leu141, His163,
	46.15	Yes	No	His163, Phe140, Cys145
	46.04	Yes	No	Leu141, Ser144, His163, Phe140, Cys145

Synthetic drugs	**Top 3 poses**	**Interaction with Cys145**	**Interaction with His41**	**List of residues interacted from the binding site**
Lopinavir	90.15	Yes	Yes	Gln89, Glu166, Cys44, Met49, Cys145, His41, Pro168, Met165
	84.32	Yes	Yes	Glu166, Thr26, Thr25, Cys145, His41, Leu27, Met165
	83.38	No	No	Glu166, His164, Arg188, Asn142, Met49, Gly143, Met165, Cys44
Ritonavir	88.23	No	Yes	Glu166, Met165, Thr26, Met49, His41, Pro168, Leu167, Cys44
	87.03	Yes	Yes	Glu166, Gln189, His41, Pro168, Met165, Cys145
	84.63	No	Yes	Thr26, Thr24, Glu166, Met165, His41, Pro168, His163, Met49, Cys44
Indinair	82.07	No	Yes	Glu189, Asn142, Glu166, Met165, His41, Met49
	75.39	No	Yes	Gln189, Asn142, Glu166, Met165, His41, Met49
	73.98	Yes	No	Glu166, Thr190, Gln192, His164, Met49, Cys145, Leu141, Asn142, Pro168, Met165
Amprenavir	81.53	Yes	Yes	Glu166, His164, Leu167, Phe140, His163, Leu141, His41, Cys145, Met165, Pro168, Met49
	75.19	No	Yes	Glu166, Pro168, Met165, His163, His41, Leu167, Met49, Met165
	75.05	No	Yes	Glu166, Leu167, Pro168, Met165, Gln189, His41, Met49

Darunavir	80.53	Yes	Yes	Glu166, Ala191, Gln192, His163, His41, Cys145, His163, Pro168, Met165
	79.2	No	Yes	Glu166, Pro168, Met165, Gln189, Asn142, Met49, Met165, His41, HIs163
	76.52	No	No	Glu166, Pro168, His163, Met49, Met165, Arg188, Gln189
Nelfinavir	79.86	Yes	Yes	Glu166, Arg188, Met49, Met165, His41, Cys145
	73.26	Yes	Yes	Glu166, Cys145, Met49, Met165, Pro168, His41
	72.64	Yes	Yes	Gly143, His41, Cys145, Met165, Met49, Glu166
Remdesivir	78.81	Yes	Yes	Glu166, Gln189, Phe140, Met49, Met165, His41, Asp187, Pro168, Cys145
	77.59	Yes	Yes	Glu166, Gln192, His164, Met165, Arg188, Cys145, Leu141, Asn142, Cys44, Met49, His41, Pro168
	75.79	Yes	Yes	Pro168, Glu166, Cys145, His163, Leu167, Met165, His41
Saquinavir	87.28	Yes	Yes	Gly143, Cys145, Glu166, His164, His163, Leu141, Glu166, Ser144, Met165, His41
	84.04	Yes	Yes	Asn142, Leu141, Gln189, Glu166, Cys145, Met49, Met165, His41
	80.13	No	Yes	Glu166, Met49, Met165, His41
Sivelestat	64.69	Yes	Yes	Asn142, Phe140, Leu141, Cys44, Met49, His41, Cys145
	64.38	Yes	Yes	Phe140, Leu141, Asn142, Cys44, Met49, His41, Cys145, Glu166
	62.2	Yes	Yes	Phe140, Leu141, Asn142, Cys44, Met165, Met49, His41, Cys145, Glu166
Galidesivir	48.05	No	Yes	Gln192, Arg188, Thr190, Glu166, His41, Met165
	47.87	No	No	Arg188, Gln192, Glu166, Met165
	47.68	No	Yes	Gln192, Arg188, Glu166, His41, Met165
Favipiravir	36.63	Yes	No	Gly143, Ser144, Cys145, His163, Asn142
	35.08	Yes	No	Gly143, Ser144, Cys145
	34.95	Yes	No	Gly143, Ser144, Cys145, Asn142

Figure 4 presents the 2D interaction map and 3D visualization of observed Lopinavir and curcumin interactions with Cys145 and His41 from molecular docking analysis. Curcumin showed a higher binding affinity of 74.26 amongst Natural products and Lopinavir showed a higher binding affinity score of 90.15 among synthetic drugs;

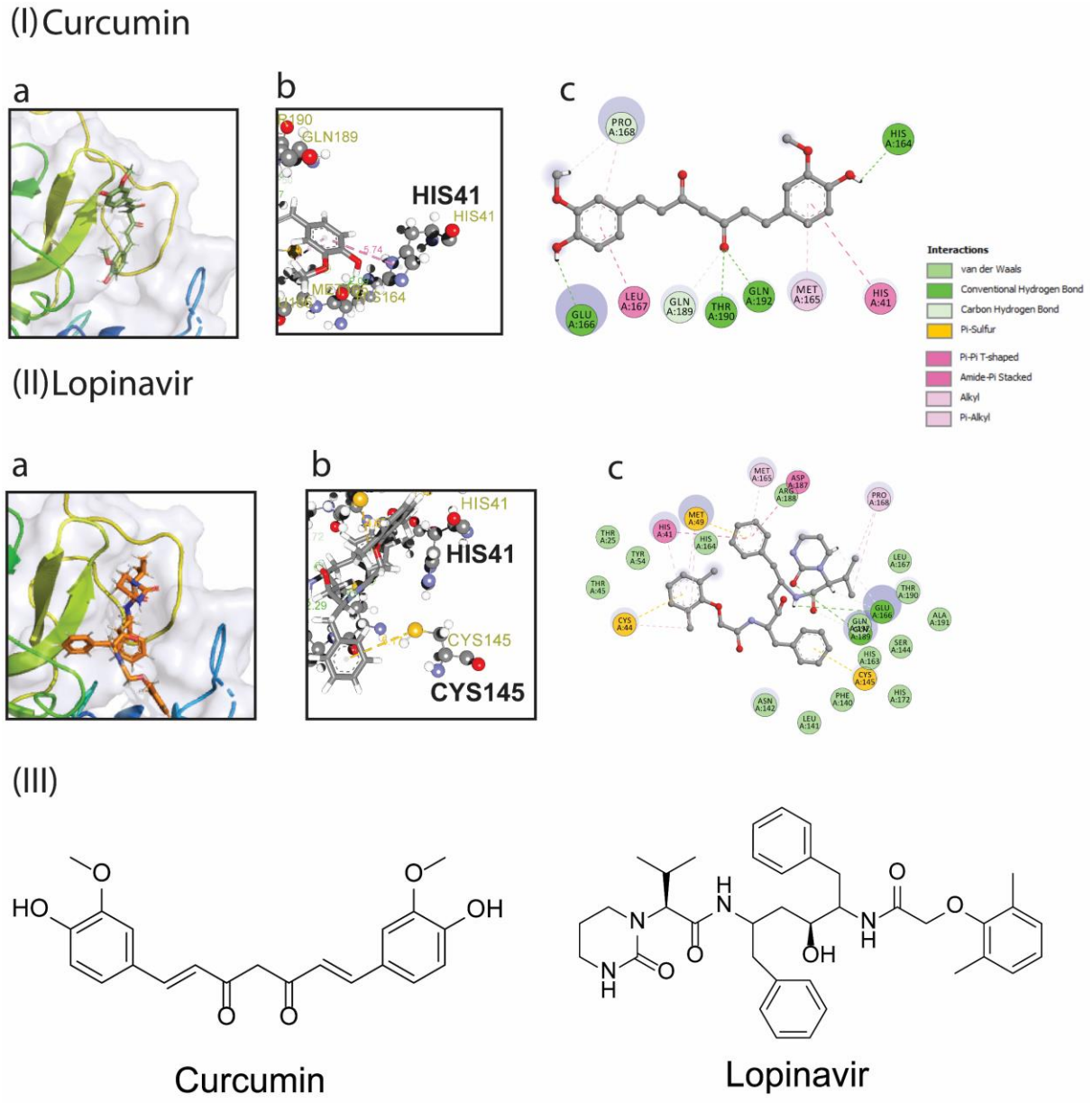


Figure 4| Result of highly scored protein-ligand complex form molecular docking **(I) a**, Curcumin ligated SARS-CoV-2 M^{pro}. **b,** protein-ligand interaction. **c,** 2D protein ligand interactions. **(II) a,** Lopinavir ligated SARS-CoV-2 M^{pro}. **b**, protein-ligand interaction. **c**, 2D protein ligand interactions. **(III)** 2D representation of Curcumin and Lopinavir.

against main protease M^{pro}. The top two poses of Lopinavir showed signs of possible inhibition as both Cys145 and His41 interaction were observed (Figure 4 (II)). But in the case of curcumin (Figure 4 (I)), the top two poses show only one inhibition either Cys145 or His41 we did not observe both interactions (see table 1). The molecular docking analysis suggests curcumin and Lopinavir as a top candidate for inhibitors. Although Emodin is ranked low in ChemPLP score of 54.25 in comparison to curcumin

Table 2. Pharmacokinetic properties and oral bioavailability of Natural products and synthetic drugs.

Molecule	MW	Lipinski (Yes/No)	TPSA	Consensus Log P	GI absorption	BBB permeant	P-gp substrate
Betulinic acid	456.7	Yes; 1 violation	57.53	6.11	Low	No	No
Curcumin	368.38	Yes; 0 violation	93.06	3.03	High	No	No
Theaflavin	564.49	No; 3 violations	217.6	1.31	Low	No	No
Deserpidine	578.65	Yes; 1 violation	108.55	3.65	High	No	Yes
Emodin	270.24	Yes; 0 violation	94.83	1.87	High	No	No
Leptodactylone	222.19	Yes; 0 violation	68.9	1.5	High	Yes	No
Sinigrin	359.37	Yes; 0 violation	199.79	-1.16	Low	No	Yes
Amprenavir	505.63	Yes; 1 violation	139.57	2.5	Low	No	Yes
Darunavir	547.66	Yes; 1 violation	148.8	2.45	Low	No	Yes
Favipiravir	157.1	Yes; 0 violation	88.84	-0.27	High	No	No
Galidesivir	265.27	Yes; 1 violation	140.31	-1.55	Low	No	No
Indinavir	613.79	Yes; 1 violation	118.03	2.76	High	No	Yes
Lopinavir	628.8	Yes; 1 violation	120	4.37	High	No	Yes
Nelfinavir	567.78	Yes; 1 violation	127.2	4.33	Low	No	Yes
Remdesivir	602.58	No; 2 violations	213.36	1.5	Low	No	Yes
Ritonavir	720.94	No; 2 violations	202.26	5.04	Low	No	Yes
Saquinavir	670.84	No; 2 violations	166.75	3.17	Low	No	Yes
Sivelestat	434.46	Yes; 0 violation	147.25	2.34	Low	No	No

the score of 74.26 among natural products, it is interesting to observe that all the top 3 poses in Emodin show signs of inhibiting Cys145 and His41; suggesting this could also be a potential candidate.

Conclusively, the molecular docking analysis based on the docking scores suggests Curcumin and Lopinavir as the potential inhibitor. If a rule of lead optimization is set stating that 'if two docking poses ligand show interaction with both Cys145 and His41 = potential inhibitor'. Then from natural products Sinigrin and Emodin are potential

inhibitors and on the other hand from synthetic drugs Lopinavir, Nelfinavir, Remdesivir, Saquinavir, and Sivelestat are potent inhibitors.

Then to study the oral bioavailability and pharmacokinetics of the Lead optimized compounds in human body ADME (Absorption, Distribution, Metabolism, and Excretion) analysis was carried out. The results are presented in Table 2, one of the major criteria for drug likeliness is Lipinski's rule of five[11,12]. Form our Molecular docking analysis we suggested curcumin, sinigrin, and emodin as potential natural product-based inhibitors, all three of these ligands follow Lipinski rules. In the case of synthetic drugs remdesivir and Saquinavir do not follow Lipinski's rule, the other three lead optimized ligands (Lopinavir, Nelfinavir, and Sivelestat) follows Lipinski. Sivelestat no Lipinski violation but Lopinavir and Nelfinavir showed 1 violation: MW>500.

Passive diffusion plays a major role in the permeation of drugs through the human body, to undergo such passive diffusion the molecule must have favorable physiochemical properties. Many essential molecules for life do not have such properties of passive diffusion, hence many transmembrane transporters help enhance their permeability. For example, there are uptake transporters such as glucose transporters (GLUT1), Vitamin transporters, and so on, then there are Efflux transporter such as P-glycoprotein (Pgp, MDR1) and Breast cancer resistance protein (BCRP). They affect drug pharmacokinetic properties; this happens when the structural elements of drug facilitate binding to a transporter. The function of P-glycoprotein is to remove the toxic compound from the cells and it is abundant in cell barriers such as liver, kidney, pregnant uterus, adrenal gland, blood-brain barrier, and intestine. So, we also monitored Gastrointestinal (GI) tract barrier absorption and P-glycoprotein binding. In the case of Natural products, curcumin and Emodin shows high gastrointestinal absorption but it is not a P-glycoprotein substrate, on the other hand, sinigrin shows the opposite. In the case of lead optimized synthetic drugs, Sivelestat does not interfere with the transporters and has low GI absorption. Nelfinavir, Remdesivir, and Saquinavir have low GI absorption but their structural elements suggest they are Pgp substrates. Finally, Lopinavir has high GI absorption and it is a Pgp substrate. These are the prediction observed through ADME analysis for lead optimized natural products and synthetic drugs. Also, it should be noted that the former analysis is for oral bioavailability, as oral dosing is the economical, safe, most convenient, and non-invasive route of administration. Here we aim to reach the catalytic active site of SARS-CoV-2 main protease M^{pro} to inhibit catalytic Cys145 and His41. So different strategies of the formulation can improve solubility, permeability, and metabolic stability of the drug. Also, different routes of drug administration can be trailed such as for example Intravenous (IV) where the dosing has a rapid onset and complete bioavailability and Intraperitoneal (IP) where high concentrations of the drug can be achieved locally while minimizing adverse effects.

Conclusion.

Repurposing the existing inhibitors is a rapid and most appropriate approach to find a therapeutic solution for the COVID-19 pandemic. In this work, we screened 18 potential inhibitors and optimized through a detailed analysis of ligand poses and interaction profiles with the active site of M^{Pro}. Our observations suggest that Curcumin, Sinigrin, and Emodin from natural products; and Lopinavir, Nelfinavir, Remdesivir, Saquinavir, and Sivelestat from synthetic drugs are potent inhibitors against M^{Pro}. Following this, we understood the Pharmacokinetic properties of their inhibitors through ADME analysis. Although this study shows eight potent inhibitors, it is also clear that different strategies in the formulation should be considered to improve bioavailability. Also, form this preliminary study we believe that with assistance from traditional medicine, recovery of infected patients can be accelerated. We anticipate that these perspectives and analyses will be useful to medicinal scientists targeting M^{Pro} and to identify novel therapeutics for SARS-CoV-2.

Conflict of Interest.

The authors have not conflict of interest

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