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Original article

Screening of drug databank against WT and mutant main protease of SARS-CoV-2: Towards finding potential compound for repurposing against COVID-19

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ABSTRACT

Although several pharmacological agents are under investigation to be repurposed as therapeutic against COVID-19, not much success has been achieved yet. So, the search for an effective and active option for the treatment of COVID-19 is still a big challenge. The Spike protein (S), RNA-dependent RNA polymerase (RdRp), and Main protease (Mpro) are considered to be the primary therapeutic drug target for COVID-19. In this study we have screened the drugbank compound library against the Main Protease. But our search was not limited to just Mpro. Like other viruses, SARS-CoV-2, have also acquired unique mutations. These mutations within the active site of these target proteins may be an important factor hindering effective drug candidate development. In the present study we identified important active site mutations within the SARS-CoV-2 Mpro (Y54C, N142S, T190I and A191V). Further the drugbank database was computationally screened against Mpro and the selected mutants. Finally, we came up with the common molecules effective against the wild type (WT) and all the selected Mpro. The study found Imiglitazar, was found to be the most active compound against the wild type of Mpro. While PF-03715455 (Y54C), Salvianolic acid A (N142S and T190I), and Montelukast (A191V) were found to be most active against the other selected mutants. It was also found that some other compounds such as Acteoside, 4-Amino-N- {4-[2-(2,6-Dimethyl-Phenoxy)-Acetylamino]-3-Hydroxy-1-Isobutyl-5-Phenyl-Pentyl}-Benzamide, PF-00610355, 4-Amino-N-4-[2-(2,6-Dimethyl-Phenoxy)-Acetylamino]-3-Hydroxy-1-Isobutyl-5-Phenyl-Pentyl}-Benzamide and Atorvastatin were showing high efficacy against the WT as well as other selected mutants. We believe that these molecules will provide a better and effective option for the treatment of COVID-19 clinical manifestations.

1. Introduction

First reported from Wuhan, China, Severe acute respiratory syndrome-Coronavirus-2 (SARS-CoV-2) is a new member of Coronavirus family, and is responsible for the highly contagious and infectious viral disease (COVID-19) (Zhu et al., 2020). Since the outbreak, World Health Organization (WHO) reported 74 million COVID-19 cases and 1.65 million COVID-19 cases-associated deaths worldwide (until mid-January 2020). Coronaviruses, (enveloped viruses belonging to coronaviridae family) are found in mammalian and avian species (Zhu et al., 2020). The coronaviruses are classified into different genera (α, β, γ and δ) (Kaul 2020). The causal agent of the current pandemic SARS-CoV-2 belongs to genera beta coronaviruses and is diligently linked to MERS-CoV and SARS-CoV, sharing > 79% of genomic sequence similarity. Owing to the human-to-human transmission of the virus, the re-emergence of SARS (2002–03 epidemic) and other coronaviruses may prove to be a discrete prospect and therefore there is an

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imperative need to comprehend these viruses and their coding proteins.

Amongst all the studied coronaviral drug targets, the main protease (Mpro) received major attention (Ziebuhr 2004; Ullrich and Nitsche 2020). The non-structural protein (nsp) of SARS-CoV-2, main protease (Mpro), sharing high sequence similarity with its SARS-CoV counterpart, and has emerged as a promising therapeutic target. Mpro gained major attention particularly in 2000s during the first SARS-CoV outbreak (Anand et al., 2003, Yang, Yang et al. 2003). This viral protease is well validated drug target and a number of drugs has been reported to be targeting this protease for the treatment of various diseases like HIV, HCV etc. (Agbowuro et al., 2018; Bafna et al., 2020; Mahdi et al., 2020). The Mpro of SARS-CoV-2 is reported to be proteolytically cleaving the overlapping pp1ab polyproteins into the functional proteins (Fig. 1). This cleavage of ppa1ab is a critical step involved in the viral replication. Mpro cleaves the large virion polyproteins (pp1a and b) at 11 sites, resulting in the release of 16 NSPs (Prajapat et al., 2020). Owing to the significance of viral replication cycle, Mpro has been anticipated as a target in the development of inhibitor against coronaviruses (Yang et al., 2003). In the last few years, especially since the start of this pandemic, the drug repurposing has emerged as a successful drug development alternative (Sleire et al., 2017; Cha et al., 2018; Chen et al., 2020). This alternative drug discovery approach has been proved very successful in the identification of effective drug candidates against hepatitis C, Ebola, and zika virus (Bai and Hsu 2019; Xie and Shi 2019). Though the Mpro, is known to be highly conserved across the coronaviridae family, but the mutations within this protease may pose a challenge (Bzowka et al., 2020). The aim of this study was to identify the prominent active site mutations within Mpro and to identify Mpro inhibitory molecules using virtual screening-based drug repurposing method. The molecules reported in this study were found to be effective against the WT as well as the mutant Mpro. The valuable information derived from this study will be useful in the repurposing of compounds as a selective and effective inhibitor of the SARS-CoV-2 Mpro and their mutants.

2. Material and methods

2.1. Sequence analysis and mutation identification

The sequence of protein of the SARS-COV-2 Mpro was extracted from the NCBI database (Coordinators 2016). BLAST (Boratyn et al., 2013) search was performed against the selected sequence and all the mutants were selected. To delineate and analyze the mutation across different countries, an in-house script written in Perl and Python was used (Khan et al., 2020). Further the classification was made on the basis of the active site mutation. A total of 4 different active site mutations were considered in this study (GenBank: QJD23268.1, QJC19621.1, QJA16866.1 and QIZ14843.1).

2.2. Structure modeling

The three-dimensional structures of all the mutants were generated using Modeller 9v23 (Webb and Sali 2016). The structure of selected mutants was modelled taking Crystal structure of the SARS-CoV-2 (COVID-19) main protease in complex with inhibitor UAW248 as a template (pdb id: 6XBI) (Sacco et al., 2020).

2.3. Virtual screening

The library of the compounds was downloaded from the drugbank (Wishart et al., 2008). The structures were subjected to dock against the WT as well as all the selected mutants. The screening was performed using CCDC Gold. The top scoring hits from each category was selected for further study.

2.4. Molecular dynamics

The complex of top scoring molecule against each protein was further subjected to Molecular dynamics simulation to examine the stability of the complex. All the molecular dynamic simulation studies were performed using Gromacs 2020 version 4 package (Hess et al., 2008; Pronk et al., 2013). Solvation was done in TIP3P

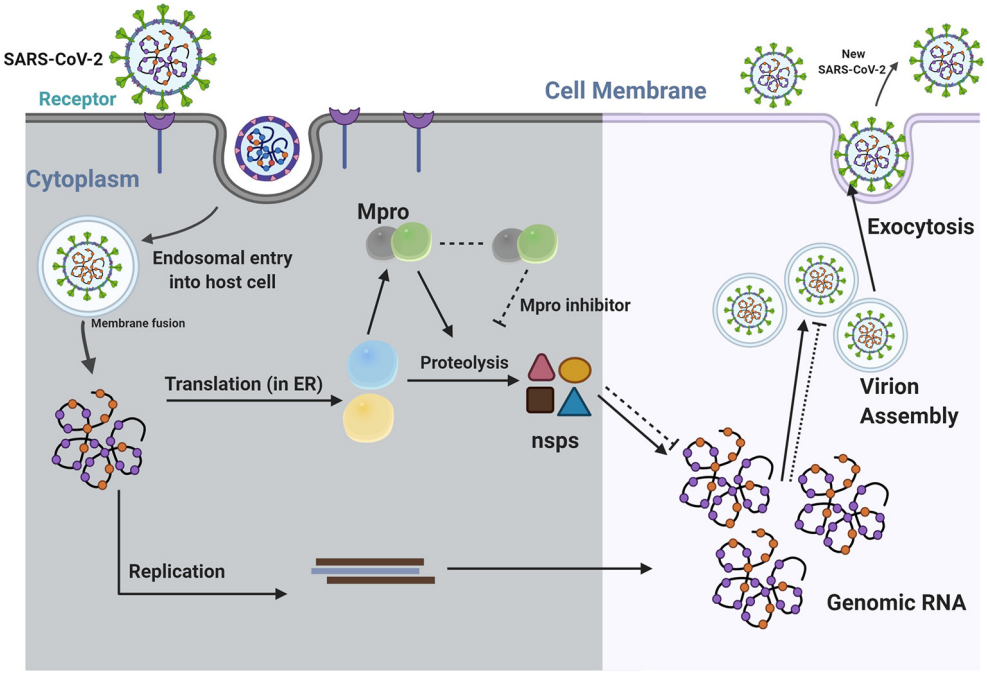

Fig. 1. The life cycle of SARS-COV-2 and the role of Mpro.

water model using cubic box model with a radius of 10 Å as its margin. System was made neutral using sodium ions. Protein-ligand complex along with water and ions using conjugate gradient minimization applying constant force for 50,000 steps. Isothermal and isochoric equilibration was done using Particle Mesh Ewald scheme for 150,000 steps. Production run was done for 50 ns using Verlet algorithm. Analysis and plots were built using Xmgrace and UCSF-Chimera.

3. Results

The mutants selected in this study were the active site mutation reported from different regions. The important mutants investigated in this study are shown in Fig. 2. Virtual screening of drug-bank was performed against the SARS-CoV-2 Mpro using CCDC GOLD. Y54C, an Mpro mutant reported in March 2020 in Malaysia was considered in this study. Not many reports are available regarding the occurrence of mutation. N142S was another mutation considered in this study, has been reported 17 times from 5 different countries. T190I, a mutation occurring 110 times (0.03% of the sequenced NSP5) has been reported from 15 different countries (https://www.gisaid.org/). This mutation was also considered in this study. First reported in March 2020 from South Africa, this mutant has recently been reported in January 2021 in US. A191V, another active site mutation with occurrence rate of 0.30% of the total sampled sequence was also considered in this study. A191V has been reported so far in 34 countries. The library was also screened against the selected Mpro mutants as well (Fig. 3). The

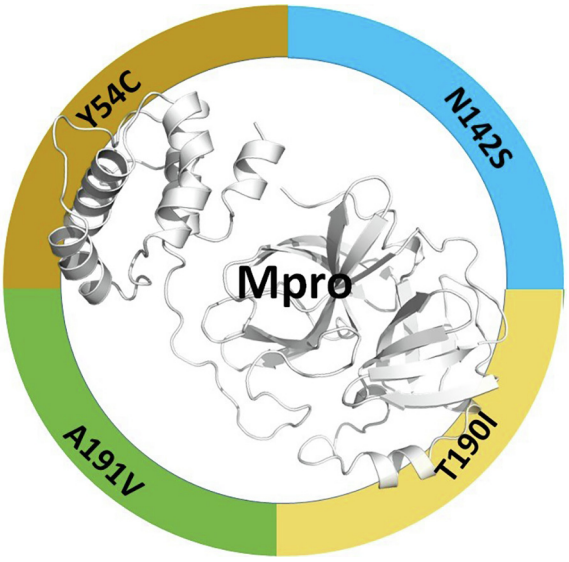

Fig. 3. Top selected mutant of Main Protease.

compounds demonstrating higher binding affinities (PLP Fitness) were selected. It was found that several compounds were showing higher binding affinity against all the selected Mpro mutants. Imiglitazar (Adeghate et al., 2011), a potent agonist for PPARα and PPARγ was found to be most effective against Mpro (WT) (Table 1). This compound was binding with the PLP fitness score

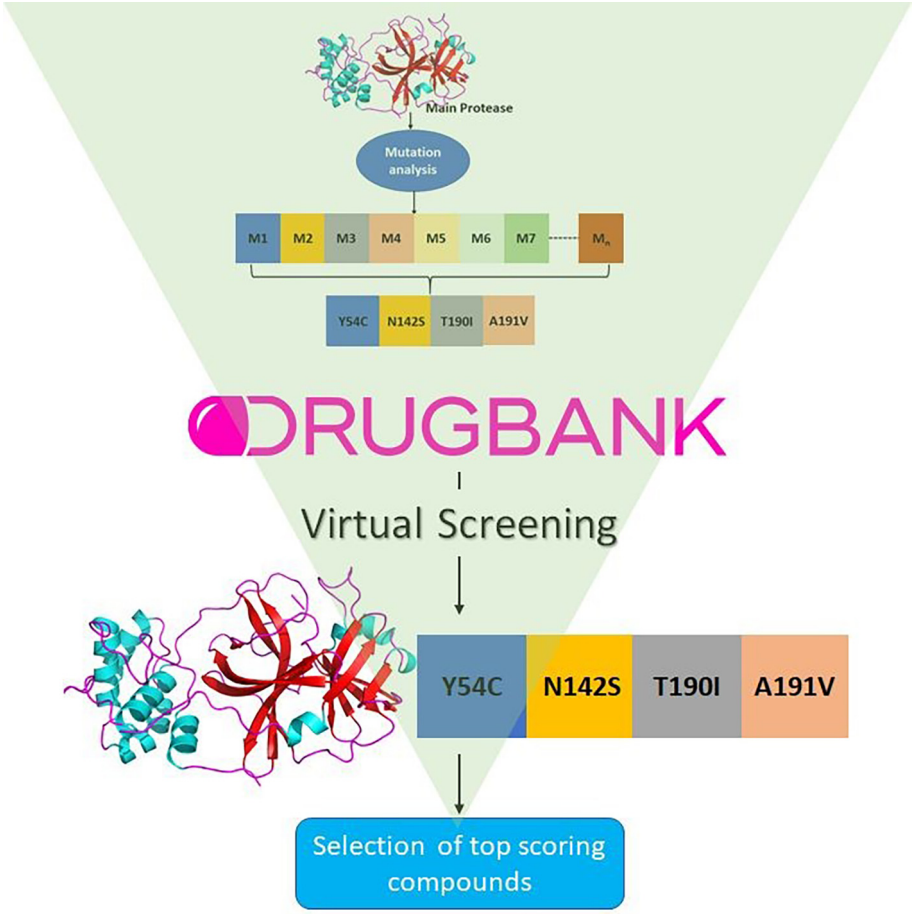

Fig. 2. Workflow of the virtual screening.

Table 1
Binding details of the top selected compounds against all mutants.

		Residues Involved	
		Hydrogen Bond	Other interacting residues
Mpro (WT)	Imiglitazar	T26, E166	T25, L27, H41, C44, T45, S46, M49, C145, M165, E166, L167, P168
	Acteoside	T26, F140, S144, H163, Q189	T25, L141, C145, M165, E166, P168, Q189, A191
	PF-00610355		T25, L27, H41, S46, M49, C145, M165, E166, L167, P168
Y54C	PF-03715455	T190	H41, M49, L50, C145, M165, E166, P168, Q189, A191
	Glesatinib	T26, F140, E166	T25, T26, L27, H41, M49, S139, L141, M165, E166
	3,8-Diamino-6-Phenyl-5-[6-[1-[2-[(1,2,3,4-Tetrahydro-9-Acridinyl)Amino]Ethyl]-1h-1,2,3-Triazol-4-Yl]Hexyl]-Phenanthridinium	L141, G143, S144, C145, E166	T25, H41, S46, M49, L141, N142, C145, M165, E166, D187, R188
N142S	Salvianolic acid A	Y54, L141, G143, S144, C145, R188, Q189, T190, Q192	H41, M49, L141, N142, C145, M165, E166, P168, D187, R188, Q189
	[2,4,6-Triisopropyl-Phenylsulfonyl-L-[3-Amidino-Phenylalanine]]-Piperazine-N'-Beta-Alanine	S142, G143, Q192	T25, M49, F140, L141, H163, M165, E166, L167, H172, R188, Q189
	Montelukast	T26, E166, Q189	T25, H41, M49, G143, C145, M165, E166, P168, Q189, A191
T190I	Salvianolic acid A	L141, H163, D187, I190, Q192	M49, L141, M165, E166, P168, Q189
	Acteoside	T25, H41, V42, C44, H163, Q192	T25, H41, C44, M49, N142, C145, H163, M165, E166, P168, A191
	PF-00610355	T26, H164	T26, M49, L141, N142, C145, M165, E166, R188, Q189
A191V	Montelukast	T25, C44, E166	T25, H41, M49, C145, M165, E166, D187, R188, Q189
	Acteoside	T25, C44, L141, S144, E166, Q189, Q192	H41, M49, N142, C145, M165, E166, P168, H172
	PF-03715455	T25, N142	T25, C44, M49, L141, N142, G143

of 101.3 against Mpro. Imiglitazar was found to be moderate effective against other mutants as well. Acteoside, and PF-00610355 were found to be other most active compound against WT Mpro (PLP Fitness score: 95.4569 and 94.2133). PF-03715455, a p38 inhibitor administrated for the treatment of chronic obstructive pulmonary disease (Norman 2015) was found to be most effective against Y54C Mpro. This compound also demonstrated high binding affinity against N142S, T190I and A191V, where it was binding with the PLP fitness score of 94.66, 88.58 and 92.62, respectively (Table 2). Salvianolic acid A, an antioxidant molecule extracted from *S. miltiorrhiza* (Ho and Hong 2011) was found to be most effective against the N142S and T190I. This compound known for its chemo preventative and cardioprotective properties was found to bind with the PLP fitness score of 97.85 and 94.2 against N142S and T190I, respectively (Table 1 and 2). This antioxidant demonstrated high binding efficacy against other Mpro mutants as well (Table 2). Montelukast (Jarvis and Markham 2000), was found to be the most active compound against A191V. This selective cysteinyl leukotriene receptor antagonist was found to be very effective against T190I and N142S as well (Table 2). In the present study we focused on the top 10 scoring compounds against each mutant and WT Mpro. Here we found that there were several compounds showing very high efficacy against all or most of the selected mutants (Table 2). PF-00610355 (Diderichsen et al., 2013), a molecule used for the treatment of Asthma, Pulmonary Disease, and Bronchial Diseases was found to be very effective against all the WT and other selected mutants. Likewise, 4-Amino-N- { 4-[2-(2, 6-Dimethyl-Phenoxy)-Acetylamino]-3-Hydroxy-1-Isobutyl-5-Phe nyl-Pentyl}-Benzamide, an anti-malarial compound known for its inhibitory activity against Plasmepsin-2 (Asojo et al., 2002) was found to be very active against all the selected Mpro mutants. The study also revealed that the active site residues playing very prominent roles in accommodating the compounds within the active site of Mpro and its mutants (Fig. 4). In the WT as well as other selected mutants T25, T26, H41, C145, M165, E166, P168, D187, R188, and Q189 were found to be very prominently involved in accommodating the compounds within the binding pocket (Table 1). It was also observed that the binding was dominated by the presence of hydrogen bonds. MD studies for the top-scoring molecule from each category in complex with their respective target (Imiglitazar-WT, PF-03715455-Y54C, Salvianolic-A-N142S, Salvianolic-A-T190I and Montelukast-A191V) were performed. The MD analysis provided a valuable insight on the stabilities of these selected compounds in complex with their receptors. Trajectory analysis and the RMSD plot analysis indicated that the compounds namely Imiglitazar, PF-03715455 and Montelukast were stable within the binding pocket of the receptor with after the initial 20 ns run and deviations of less than 3 Å were observed for the rest of the time-frame of the simulation (Fig. 5A). Salvianolic-A was found to be highly stable with T190I mutant, and a deviation of less than 2 Å was observed, while this compound was found to be unstable with the N142S mutant (Fig. 5A). It indicated the deviations upto 4 Å with constant variation in its bound conformation especially in the dihydroxyphenyl-ethenyl group within the binding cavity (Fig. 5A). Hydrogen bond analysis of the trajectory indicated that the ligands namely PF-03715455, Montelukast and Imiglitazar were having constant hydrogen bonds with their respective receptor proteins (Fig. 5B). While Salvianolic-A was not forming the constant hydrogen bonds with the N142S mutant, while for the T190I mutant the hydrogen

Table 2
The compounds demonstrating high affinity against all the mutants.

Compounds	Mutants	Score
Acteoside	**WT**	95.4569
	Y54C	79.32
	N142S	90.29
	T190I	93.72
	A191V	94.36
PF-00610355	**WT**	94.2133
	Y54C	89.8851
	N142S	83.28
	T190I	91.09
	A191V	85.93
PF-03715455	**WT**	86.6998
	Y54C	100.0499
	N142S	94.66
	T190I	88.58
	A191V	92.62
Salvianolic acid A	**WT**	85.6004
	Y54C	91.606
	N142S	97.85
	T190I	94.2
	A191V	86.71
3,8-Diamino-6-Phenyl-5-[6-[1-[2-[(1,2,3,4-Tetrahydro-9-Acridinyl)Amino]Ethyl]-1h-1,2,3-Triazol-4-Yl]Hexyl]-Phenanthridinium	**WT**	89.0458
	Y54C	93.1382
	N142S	91.27
	T190I	88.97
	A191V	84.87
Montelukast	**WT**	86.8348
	Y54C	85.4632
	N142S	96.57
	T190I	89.14
	A191V	95.33
4-Amino-N-{4-[2-(2,6-Dimethyl-Phenoxy)-Acetylamino]-3-Hydroxy-1-Isobutyl-5-Phenyl-Pentyl}-Benzamide	**WT**	89.555
	Y54C	85.8951
	N142S	85.8951
	T190I	77.8
	A191V	88.68
Atorvastatin	**WT**	91.3016
	Y54C	79.88
	N142S	85.1
	T190I	82.86
	A191V	89.67

bonds were mostly constant throughout the simulation (Fig. 5B). The RMSD analysis indicated that the backbone of all the selected structures were stable throughout the simulation time period (Fig. 5C). A slight fluctuation (~0.5 Å) was noticed in the A191V (Fig. 5C). The RMSF analysis highlights that the residues involved around the binding cavity were mostly stable (Fig. 5D).

4. Discussion

Declared as pandemic by World Health Organization (WHO), COVID-19 is the most threatening viral disease witnessed in the last several decades (Ashour et al., 2020; Xu et al., 2020). Though several vaccines are available for emergency use or in the last phase of human trails, still there is an uncertainty (Polack et al., 2020; Sharma et al., 2020; Singh and Upshur 2020; Knoll and Wonodi 2021). Efforts are continuously being made towards the characterization of pivotal molecular targets for the development of anti-viral drugs. Of several structural and non-structural SARS-COV-2 protein, Mpro is designated as a potential therapeutic target for therapeutics development (Chen et al., 2020). The inhibition of Mpro is a checkpoint preventing the viral replication and thereby making it an important therapeutic target constitutes one of the potential anti coronaviral strategies (Shawky et al., 2020). Recently, a large number of studies have focused on the repurposing of old drugs as a plausible treatment for COVID-19 (Loucera et al., 2020; Mohapatra et al., 2020; Singh et al., 2020; Krishna et al., 2021). The success of drug repurposing has gained large research interest (Cusinato et al., 2020; Rubin et al., 2020). Remedesivir, an antiviral which was originally developed to treat respiratory syncytial virus (RSV), a hepatitis C and later investigated during the Ebola outbreak (Pardo et al., 2020). During the COVID-19 outbreak, remedesivir becomes the first drug to receive emergency use authorization by US-FDA (Lamb 2020). Likewise, there are several other drugs, which have been found to show inhibitory effect against SARS-CoV-2 (Gao et al., 2020; Watashi 2020). To continue this effort here we have screened the drugbank against the SARS-CoV-2 Mpro of. The novelty of this work lies in the identification of some important active site mutations in Mpro and thereby screening the drug databank against these mutants as well. Acteoside also known as Verbascoside, a phenylpropanoid glycoside with reported antimicrobial, anti-inflammatory, antioxidant, and antitumor activity (Shawky et al., 2020) was found to be showing high binding affinity against the WT and all the selected mutants (Table 2). The binding potential of this compound against Mpro has also been discussed in previous studies as well (Shawky et al., 2020). Salvianolic acid A, a phenolic acid extracted from sage (*Salvia officinalis*) (Ma et al., 2019) was another compound demonstrating high binding affinity against all the selected Mpro mutants. This compound has also been previously reported to carry inhibitory potential against Mpro (Ibrahim et al., 2020). 3,8-Dia mino-6-phenyl-5-[6-[1-[2-[(1,2,3,4-tetrahydro-9-acridinyl)amino] ethyl]-1H-1,2,3-triazol-4-YL]hexyl]-phenanthridinium also known as TZ4, a widely reported inhibitor of acetylcholinesterase was also found to be very effective against the WT and all selected Mpro mutants (Table 2). Belonging to the quinolines, though there are no available reports showing the linkage of this compound with COVID-19 or its role in suppression of any viral progression, but several molecules under this class have been reported to carrying wide range of antiviral activity (Alexpandi et al., 2020). PF-03715455, a p38 kinase inhibitor (Norman 2015), was another compound screened as a potential inhibitor showing high binding affinity against all the selected mutants (Table 2). The findings strongly support the previous reports demonstrating the role of p38 inhibitors in COVID-19 and related viral infections (Grimes and Grimes 2020; Hemmat et al., 2021). Along with, there are several p38 inhibitors under clinical trials to be used as a therapeutic option in serious COVID-19 infection (Grimes and Grimes 2020). PF-00610355, a β_2 adrenoreceptor agonist, investigated for the treatment of COPD and asthma (Diderichsen et al., 2013) demonstrated notable binding efficacy against WT and all the selected mutants. Studies have reported β_2 adrenoreceptor agonist like Epinephrine, formoterol, dobutamine to be carrying therapeutic potential against COVID-19 or other viral infections (Arya et al. 2020; Bolelli et al., 2020; Derakhshan et al., 2020). There were several other compounds reported in this study, which may be further investigated as a potential agent to be repurposed as a therapeutic candidate for the treatment of COVID-19. Atorvastatin was another compound found to be demonstrating high affinity against the selected mutants. Atorvastatin, belonging to the statin class of medications, has been well reported to lower the severity in COVID-19 infection (Tan et al., 2020). Currently Atorvastatin is under Phase 2 trials for its use as Adjunctive Therapy in COVID-19 (ClinicalTrials.gov Identifier: NCT04380402). Montelukast, an antagonist for cysteinyl leukotriene (cysLT) receptor (Ihaku et al., 1999) was also found to be demonstrating high binding affinity against the selected mutants. Montelukast, widely reported to be carrying anti-inflammatory effects has also been found to be carrying antiviral activity as well (Chen et al., 2019; Park et al., 2020). This immune modulatory has been found to be limiting the progression of COVID-19 infection. Currently this drug is under Phase 3 clinical trial to be used as COVID-19 treatment (ClinicalTrials.gov Identifier: NCT04389411) (Fidan and Aydogdu 2020). The study

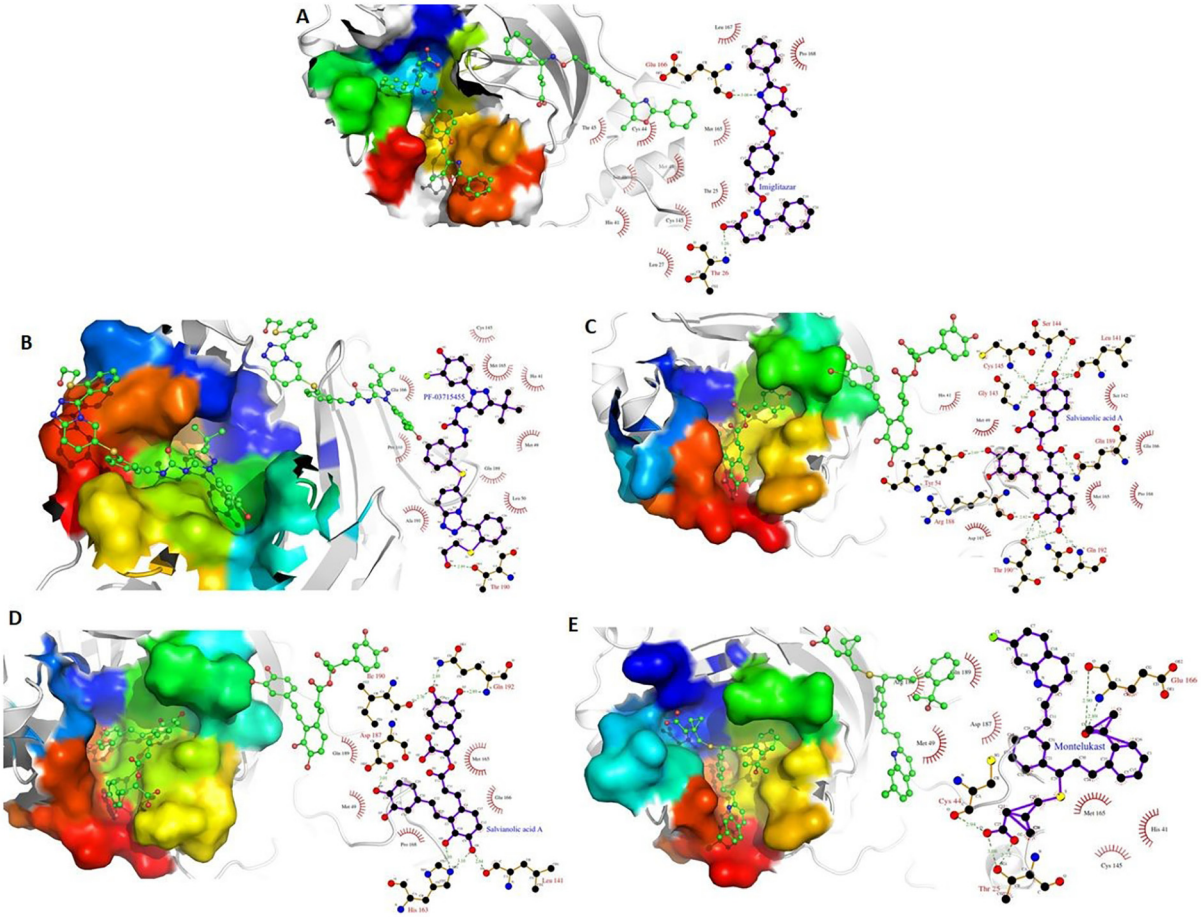

Fig. 4. The binding of top scoring compounds (A) Binding of Imiglitazar within the active site of Mpro (WT) (B) Binding of PF-03715455 within the active site of Mpro (Y54C) (C) Binding of Salvianolic acid A within the active site of Mpro (N142S) (D) Mpro (T190I) (E) Binding of Montelukast within the active site of Mpro (A191V).

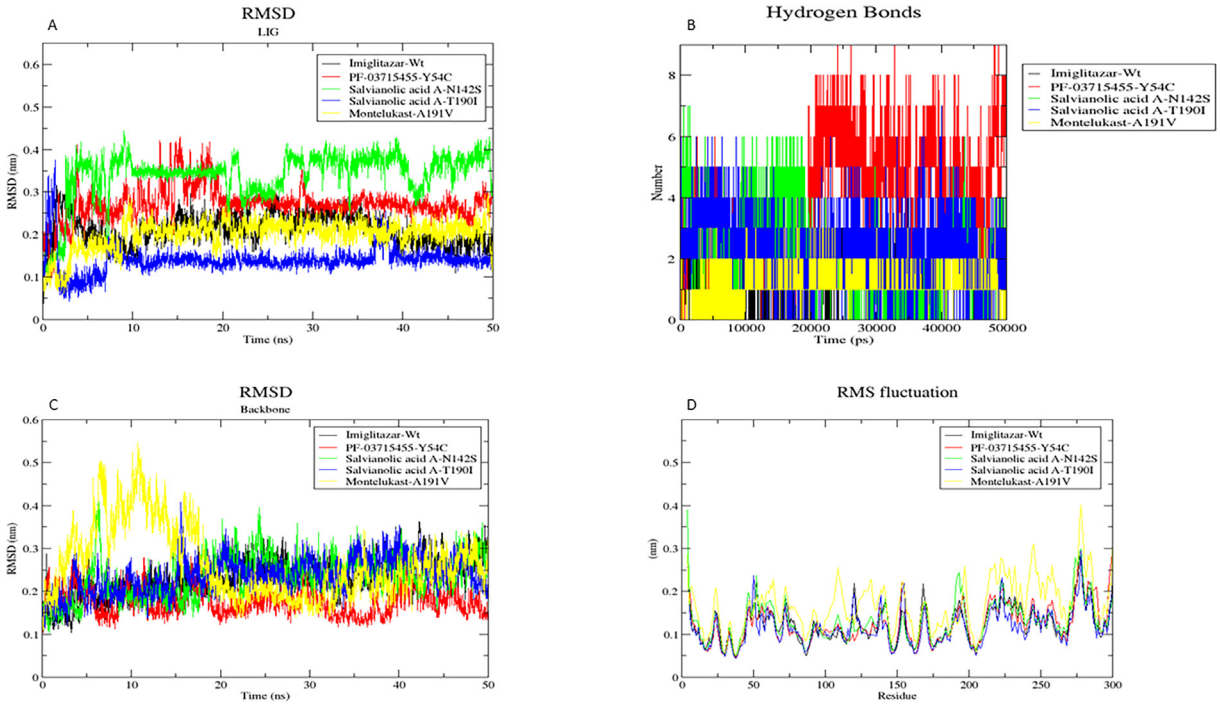

Fig. 5. Molecular dynamics results of the Imiglitazir-WT Mpro complex (Black), PF-03715455-Y54C (red), Salvianolic acidA-N142S (green), Salvianolic acidA-T190I (blue) and Montelukast-A191V (Yellow). (A) The ligand RMSD selected compounds (B) The backbone RMSD of Mpro (WT) and mutants in complex the selected compounds (C) The intermolecular hydrogen bond formations (D) The RMSF plot of the WT and mutant Mpro.

highlighted the important role of catalytic residues (H41, C145) and other active site residues. The role of the highlighted residues has been well reported in previous studies (Nukoolkarn et al., 2008; Bello et al., 2020; Shitrit et al., 2020). Overall, the findings of this study will be helpful in shortlisting of compounds to be repurposed as a therapeutic candidate against Mpro and its mutants.

5. Conclusion

This study reports the list of compounds showing high binding affinity against the SARS-CoV-2 Main protease as well as its mutants. These compounds may open a new therapeutic option for the repurposing of existing compounds against SARS-CoV-2. The study highlights several compounds like Acteoside, Salvianolic acid A, PF-03715455, Montelukast, 4-Amino-N-{4-[2-(2,6-Dimethyl-Phenoxy)-Acetylamino]-3-Hydroxy-1-Isobutyl-5-Phenyl-Pentyl}-Benzamide etc. to be very effective against the WT and most of selected Mpro mutants. We believe the use of these compounds may offer significant anti-COVID-19 treatment option.

Declaration of Competing Interest

The authors declared that there is no conflict of interest.

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