Identification of 3-chymotrypsin like protease (3CLPro) inhibitors as potential anti-SARS-CoV-2 agents

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Running Title: Identification of potential inhibitors for SAR-CoV-2 3CLpro enzyme

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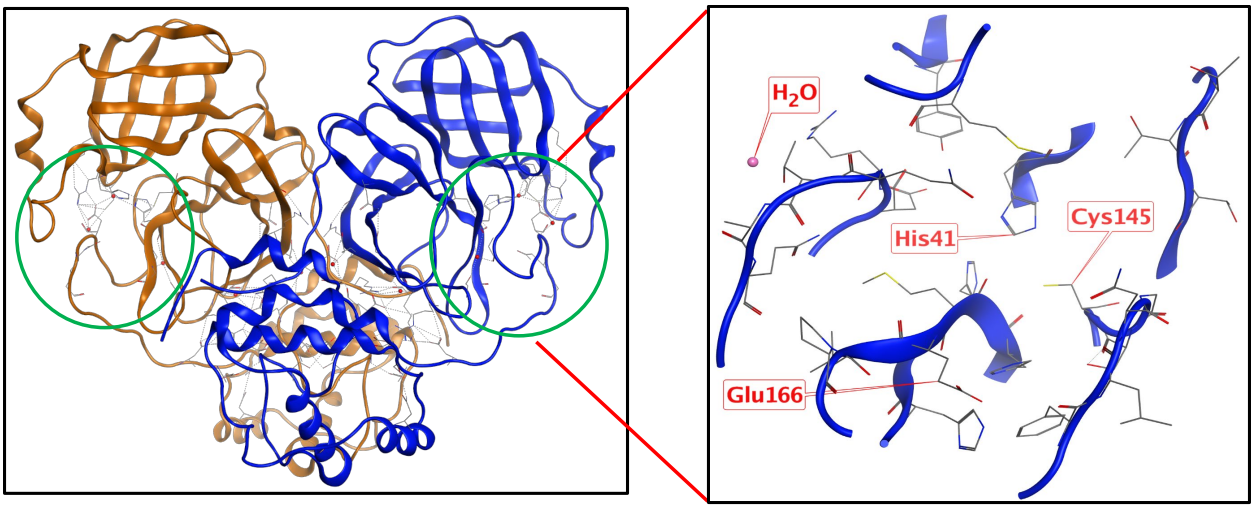
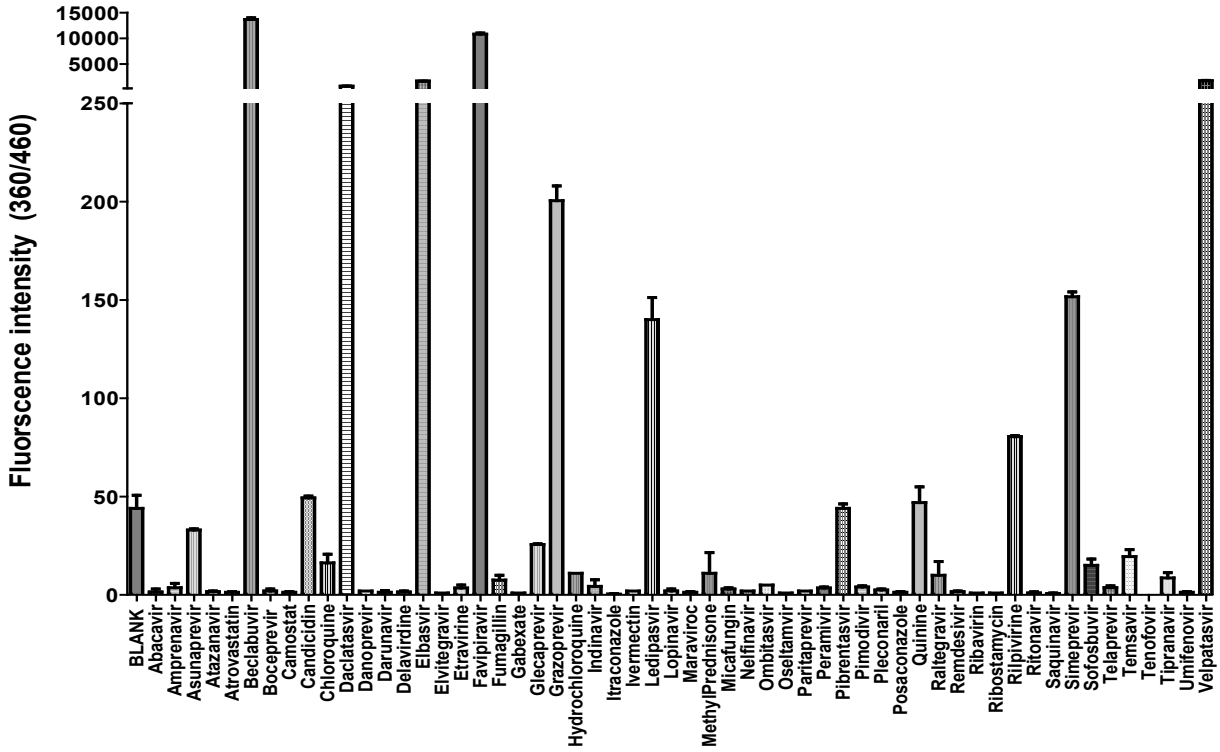
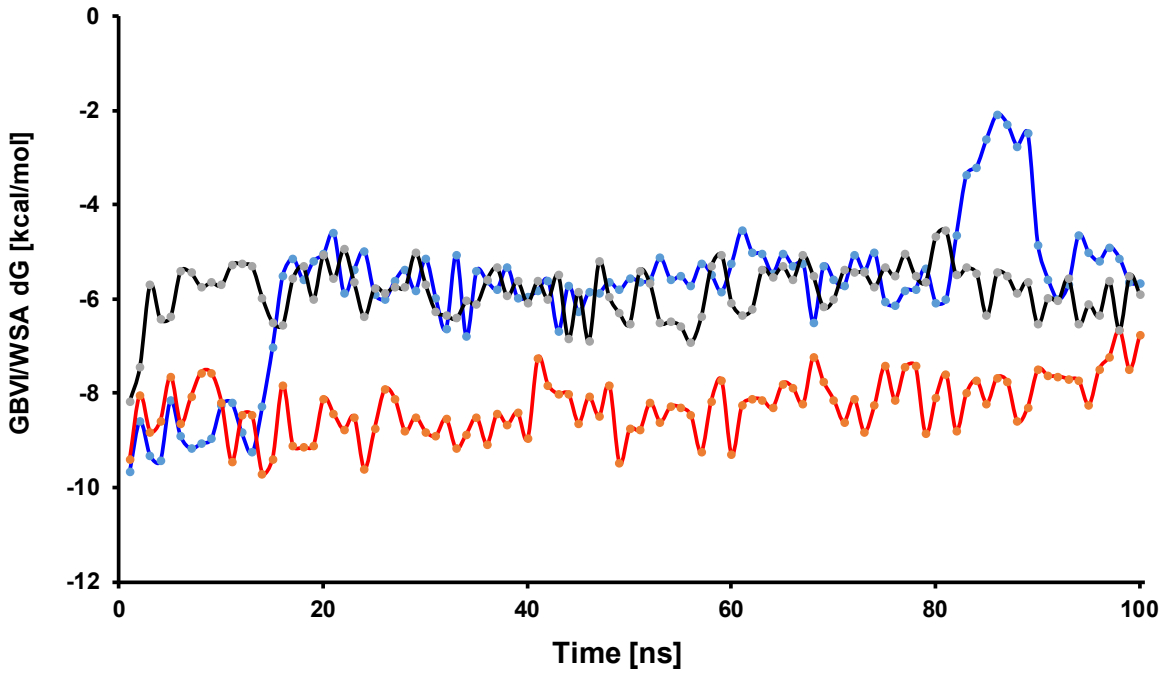


Supplementary Figure 1: Structure of 3CLpro showing Cys^{145}, His^{41} and water molecules that are required for the catalytic function. a) Structure of 3CLpro protein, green circle indicates the presence of Cys-145, His-41 and water (H_2O) molecule at the active site. **b)** Expanded region of active site region that exhibits the molecules (Cys^{145}, His^{41} and Glu^{166}) required for catalytic function.



Supplementary Figure 2: Drugs that exhibited autofluorescence at excitation/emission wavelength of 360/460nm. Before performing the *in vitro* enzymatic assay for the selected drug candidates, the autofluorescence activity of all the drugs was examined. 10µl of 250µM of drugs was mixed with 40µl of assay buffer and read at 360/460nm (excitation/emission). Assay buffer with substrate served as blank. The final concentration of drugs and substrate was 50µM. Drugs exhibiting more than 200 fluorescence unit was eliminated from the enzymatic studies as the DMSO control samples fluorescence was around 200 (considered as 100% activity). The data were representative of two individual experiments in triplicates.



Supplementary Figure 3: 100ns molecular dynamic (MD) simulation studies for ivermectin and Micafungin. The S-score distribution vs time scale (ns) for ivermectin with monomer and homodimer form of 3CLpro, and micafungin with monomer form of 3CLpro.

Supplementary table 1: List of all the viral protease, non-viral protease inhibitors and off target drugs with the mechanism of action.

Sl No.	Drug	Mechanism of action	Clinical application	Reference
Viral protease inhibitors (PIs)				
1	Amprenavir	Inhibitor of HIV-1 protease thereby prevents the processing of viral gag and gag-pol polyprotein precursors.	HIV-Infections	[1]
2	Asunaprevir	NS3 protease inhibitor	Hepatitis-C infection	[2]
3	Atazanavir	Inhibitor of HIV-1 protease thereby prevents the processing of viral gag and gag-pol polyprotein precursors.	HIV-Infections	[3]
4	Boceprevir	NS3–4A serine protease	HCV-infections	[4]
5	Camostat	Serine protease inhibitor	SARS-CoV and MERS	[5]
6	Danoprevir	selective inhibitor of Hepatitis C Virus (HCV) NS3/4A protease	Hepatitis-C infection	[5]
7	Darunavir	Protease inhibitor	HIV-Infections	[6]
8	Gabexate	Protease inhibitor		[7]
9	Glecaprevir	NS3/4A protease inhibitor	Hepatitis-C infection	[8]
10	Indinavir	Comptetive protease inhibitor	HIV-Infections	[9]
11	Lopinavir	HIV-1 Protease Inhibitor	HIV Infections	[10]
12	Nilfinavir	Binds to active site HIV Proteases	HIV Infections	[11]
13	Paritaprevir	Inhibits protease activity of nonstructural protein 3 and 4A (NS3/4A)	Hepatitis-C infection	[12]
14	Ritonavir	HIV – 1 Protease Inhibitor	HIV Infections	[10]
15	Saquinavir	HIV protease inhibitor	HIV Infections	[13]
16	Sofosbuvir	NS5B polymerase, thus inhibiting the HCV-RNA synthesis by RNA chain termination	HCV Infection	[14]
17	Tipranavir	Binds to active site of HIV proteases	HIV-Infections	[15]
Viral Non-Protease Inhibitors (VNIs)				

18	Abacavir	Nucleoside reverse transcriptase inhibitor (NRTI)	HIV infection	[16]
19	Delavirdine	Non-nucleoside HIV-1 reverse transcriptase inhibitor	HIV	[17]
20	Elvitegravir	Integrase inhibitor	HIV	[18]
21	Etravirine	Nonnucleoside reverse transcriptase inhibitors	HIV	[19]
22	Ombitasvir	Targets the nonstructural proteins (NS5A) and inhibits the viral replication and assembly	Hepatitis-C infection	[20]
23	Oseltamvir	inhibits the active site of the neuraminidase enzymes	Influenza viral infection	[21]
24	Peramivir	Neuraminidase inhibitor	Influenza viral infection	[22]
25	Pibrentasvir	NS5A inhibitor		[8]
26	Pimodivir	Inhibits RNA-dependent RNA polymerase subunit 2 (PB2) to prevent replication	Influenza-A infection	[23]
27	Pleconaril	Prevents viral replication by inhibiting viral uncoating and blocking viral attachment to host cell receptors	Enteroviruses and rhinoviruses infections	[24]
28	Raltegravir	Integrase inhibitor	HIV	[18]
29	Remdesivir	RNA Dependent RNA Polymerase inhibitor	COVID-19	[25]
30	Ribavirin	Competitive inhibition of viral RNA polymerase	Hepatitis-C infection	[26]
31	Temsavir	Binds to gp120 and blocks CD4-induced conformational changes required for viral entry.	HIV Infections	[27]
32	Telaprevir	Protease inhibitors	Hepatitis-C infection	[28]
33	Tenofovir	Inhibits HIV-1 reverse transcriptase activity	HIV infections	[29]
34	Umifenovir	Inhibits of membrane fusion virus and host plasma membranes.	Broad Spectrum	[30]
Off Target Drugs (OTDs)				
35	Atrovastatin	HMG-CoA Reductase Inhibitor	Lipid Lowering Agent	[31]
36	Candicidin	polyene antibiotic	Fungal infections	[32]

37	Chloroquine Phosphate	Inhibition of heme detoxification	Uncomplicated non-falciparum malaria infection	33,34
38	Fumagillin	RNA synthesis, but may also act by inhibiting a key proteinase, type 2 methionine aminopeptidase	Parasitic infection	35,36
39	Hydrochloroquine	Inhibition of heme detoxification	Uncomplicated non-falciparum malaria infection	34
40	Itraconazole	inhibits ergosterol synthesis,	Fungal infections	37
41	Ivermectin	Inhibits chloride channels that are required for neuromuscular transmission in parasitic worms	Nematode infections	38
42	Maraviroc	CCR5 antagonist	HIV infection	39
43	MethylPrednisone	Anti-inflammatory	Inflammatory disorders	40
44	Micafungin	Inhibits β-1-3 gluacan synthase	Broad spectrum of fungal infection	41
45	Posaconazole	Inhibits 14-α-demethylase	Fungal infections	42
46	Quinine	Intercalation with parasite DNA, interrupting replication and transcription. Interaction with erythrocyte fatty acids, promoting hemolysis and preventing schizont maturation. Alkalinization of parasite digestive vacuoles, interfering with hemoglobin degradation.	Antimalarial	35
47	Ribostamycin sulfate	binding to the bacterial 30S ribosomal subunit, causing misreading of mRNA and leaving the bacterium unable to synthesize proteins vital to its growth.	Antibacterial	43

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