Therapeutics targeting the main protease of SARS-CoV-2 for the treatment of COVID-19: A molecular modeling approach deciphering relative efficacies

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Abstract

The pandemic due to the novel coronavirus 2019, SARS-CoV-2, has led to a global health and economic crisis. The disease, named coronavirus disease (COVID-19), has already affected 3090445 and killed over 217769 people worldwide, as of April 30, 2020. So far, there is no specific effective medicine or vaccine against SARS-CoV-2. Several existing and approved drugs are under clinical studies for re-purposing. However, owing to the emergent situation and thereby to avoid time needed for de novo drug discovery, drug re-purposing remains to be the best option to find an effective therapeutic against the virus. Thus, the preset study was designed to evaluate potency of 82 compound/drugs in inhibiting the main protease (3CLPro) of SARS-CoV-2, using molecular docking tool. This protease is a vital enzyme for replication of the virus, and is thus a promising drug target. The analyzed compounds include 16 known protease inhibitors, two recently suggested α-ketoamides, 24 recently reported putative inhibitors, and 40 phytochemicals. The results indicate that Ritonavir, Indinavir, Montelukast, Nelfinavir, Candoxatril, Tigecycline and Lopinavir to be very potent protease inhibitors. Further, several other drugs and compounds, including phytochemicals, have been identified / predicted to be potent in inhibiting the enzyme. In addition, we hereby report relative efficacies of these compounds in inhibiting 3CLPro. Thus, the present study is significant in the therapeutic intervention of COVID-19.

1. Introduction

There have been three major outbreaks of severe acute respiratory syndrome (SARS) caused by coronaviruses (CoVs) in the current century. First, the SARS caused by SARS-CoV in 2002-03; second, the Middle East Respiratory syndrome (MERS) caused by MERS-CoV in 2012; and now a novel CoV emerged from Wuhan, China in December 2019 [1]. This novel CoV was initially named 2019-nCoV, and was subsequently re-named SARS-CoV-2 by viral taxonomists, owing to its similarity with the SARS-CoV. The World Health Organization (WHO) has named the disease coronavirus disease (COVID-19), the symptoms of which include cough, sneezing, dyspnea, fever, shortness of breath, pneumonia and in severe cases multi-organ failure occurs [2-4]. Initially, WHO declared the outbreak as Public Health Emergency of International Concern on 30 January, 2020. Subsequently, it was declared a pandemic on 11 March, 2020. The virus has already spread to more than 216 countries/area/territories across the globe, and affected 4761559 and killed over 317529 people, as of 20th May, 2020 (https://www.who.int/emergencies/diseases/novel-coronavirus-2019). The global humanity is currently in a grave crisis due to the pandemic, which has already caused great loss of life and property, and affected global economy severely.

SARS-CoV-2 is an enveloped virus containing plus-sensed single-stranded RNA genome. Since the virus shares 88% sequence identity with CoVs of bat and 50% identity with MERS-CoV [5], it is though that the virus has a zoonotic origin from bats. While the medical professionals are over-burdened by the increasing number of new cases and paucity of infrastructures, research community is eagerly searching

for therapeutic interventions. Since developing novel therapeutic agents against any pathogen is a time-consuming venture, and looking at the burgeoning crisis, one of the best strategies is re-purposing of existing drugs as a strategy to shorten time and reduce cost which occur in *de novo* drug discovery [6-7]. Thus, the search for potential therapeutics which can potentially inhibit replication of the virus continues [8].

Structural biology approaches, including crystallography, electron microscopy and homology modeling, have determined and revealed three-dimensional structures of several drug targets of SARS-CoV-2, including main protease (3-chymotrypsin-like protease; 3CLpro), papain-like protease, endoribonuclease, ADP ribose phosphatase, spike protein, nucleocapsid protein, RNA-dependent RNA polymerase (RdRp), etc. Li and De Clercq [9] suggested 3CLpro, papain-like protease, helicase, and RdRp and spike glycoprotein to be the five targets against the virus. So far, remdesivir, an antiviral drug targeting RdRp, has been suggested for use in COVID-19 patients [10]. Also, hydroxychloroquine in combination with remdesivir has been reported to be highly effective against SARS-CoV-2 *in vitro* [10-11]. Gao et al. [12] reported from clinical studies that chloroquine phosphate has efficacy in treating COVID-19 associated pneumonia. Further, nucleoside analogues, which are RdRp inhibitors, including favipiravir, ribavirin and galidesivir have been suggested for use in COVID-19. These are reported to be effective against several related viruses including influenza, Ebola, yellow fever, chikungunya, norovirus and enterovirus, and even SARS-CoV-2 [9,10,13].

Zhang et al. [14] have suggested α-ketoamide derivatives for inhibiting the main protease. Using molecular modeling approaches, Wu et al. [15] have suggested a large number of compounds which can inhibit different drug targets of the virus. Their list includes known compounds as well as natural products against 3-CLpro, Spike, RdRp and papain-like protease. The present study endeavors to identify compounds which can potentially inhibit the main protease of SARS-CoV-2, 3CLpro, using molecular docking approaches. 3CLpro is essential for processing the polyproteins that are translated from the positive-sensed viral RNA genome upon infection of host cell, and is thus one of the most important drug targets against SARS-CoV-2 [14,16]. We selected 42 compounds which are known to be inhibitors of viral proteases, or which have been reported by others to have the potential to inhibit the enzyme. Further, we have used 40 plant derived natural products to test their efficacy in inhibiting the enzyme. The purpose of this study was to evaluate the relative efficacy or potential of different known/potential protease inhibitors, as well as determine the potential of some of phytochemicals in inhibiting the 3CLPro.

2. Methodology

2.1. The Drug target:

The three-dimensional structure of the SARS-CoV-2 main protease (3CLpro) was downloaded from the RCSB Protein data bank. The structure, bearing PDB id 6Y2F, is a crystal structure (monoclinic form) of the complex resulting from the reaction between SARS-CoV-2 (2019-nCoV) main protease and tert-butyl (1-((S)-1-(((S)-4-(benzylamino)-3,4-dioxo-1-((S)-2-oxopyrrolidin-3-yl)butan-2-yl)amino)-3-cyclopropyl-1-

oxopropan-2-yl)-2-oxo-1,2-dihydropyridin-3-yl)carbamate (alpha-ketoamide 13b). It was expressed in *E. coli*, determined using X-ray diffraction at resolution of 1.95 Å, R-Value Free of 0.219 and R-Value Work of 0.178 (https://www.rcsb.org/structure/6y2f). It was deposited in PDB on 2020-02-15 by Zhang et al. [14].

2.2. The Ligands:

Sixteen known protease inhibitors were included in the study to determine their relative inhibitory potential, and 24 compounds suggested by Wu et al. [15] from ZINC database, two α-ketoamide inhibitors suggested by Zhang et al. [14] and forty phytochemicals from our in-house database were included in the study (Table 1). Three-dimensional structures of all the compounds were downloaded from the NCBI PubChem compounds database, and their physico-chemical properties including molecular weight, numbers of hydrogen bond donor and acceptor groups, octanol/water oartition coefficient (LogP) and topological polar surface area (TPSA), were recorded (www.pubchem.ncbi.nlm.nih.gov/). For the two ketoamides, the structures were drawn in Mobyle@RPBS (https://mobyle.rpbs.univ-paris-diderot.fr/) to determine the physicochemical properties. For the ketoamides, and ritonavir, lopinavir, saquinavir and indinavir the three-dimensional structures were not available at NCBI database, and were thus drawn and converted, followed by energy minimization.

2.3. Molecular docking:

Molecular docking was performed using Molegro Virtual Docker 6.0 software, following standard procedures [17]. Briefly, the structure of the enzyme/receptor was loaded into the workspace, all water molecules were removed, and amino acids with invalid protonation were corrected. Following this, the ligands were loaded and docking was performed between the ligands and active site of the receptor. The bound co-crystallized ligand O6K_502[A], ketoamide, was selected as the reference for docking site (coordinates X: 10.93; Y: -0.46; Z: 20.84) and amino acids with 15 Å were included in the docking. MolDock scoring function was selected with Grid resolution of 0.30Å, 10 runs for each ligand, and 1500 iterations, following Mazumder et al. [18] and Mazumder and Choudhury [19]. The best poses of each ligand, in terms of Rerank score, were selected for further analysis. Limonene, although a plant-derived compound, has no hydrogen bond donor or acceptor group, and was included in the study for validation of the modeling.

3. Results

3.1. Inhibition of the 3CLpro:

The molecular modeling approach was validated owing to the fact that the co-crystallized ligand (Fig. 1A) and the same ligand when docked (Fig. 1B) have the same docking pose and site of binding. Further, the compound Limonene (which has no hydrogen bond forming group) shows zero hydrogen bond score (Table 1), which further validates the modeling. The molecular docking revealed that the compounds studied have the potential to interact with the active site of the protease. All the compounds bind to the target at the same active site as that of the bound ketoamide (Fig. 1).

3.2. Relative potential of the inhibitors:

One of the mottos of the present study was to identify compounds with high potency in inhibiting 3CLpro. Taking the docking score (Rerank score) as the criterion and indicator of better inhibition of the drug target, the analysis was performed. The docking scores signify the amount of energy liberated when a ligand binds with the active site of a receptor/enzyme [20-22]. Higher, i.e. more negative the docking score stronger is the interaction between the two, and hence more is the inhibitory potential of the compound. The results of the present study indicate that Ritonavir is the best inhibitor with MolDock score of -191.681, Rerank score of -145.68 and hydrogen bond score of -7.6833 (Table 1). Taking the ketoamide [14] as reference, the Rerank score (inhibitory potential) of Ritonavir, Indinavir, Montelukast, Acteoside, Nelfinavir, Rutin, Candoxatril, Tigecycline, Lopinavir and Almitrine were found to be higher by 1.295-, 1.173-, 1.158-, 1.126-, 1.1-, 1.088-, 1.062-, 1.057-, 1.044- and 1.026-fold respectively. Among all these 10 compounds, while Lopinavir had lowest hydrogen bind score (a positive value), while Acteoside showed highest hydrogen bond score of -16.9199 (Table 1). Hydrogen bond score is essentially dependent on the number of hydrogen bond donor and acceptor groups in a compound [17,18], and thus Acteoside with a large number of such groups showed highest Hydrogen bond score.

Among the compounds suggested by Wu et al. [15], Montelukast was found to be the best inhibitor, and Candoxatril, Tigecycline and Almitrine were also highly potent inhibitors of the enzyme. Among phytochemicals, while Acteoside was found to be the best inhibitor of the enzyme, Rutin is also a highly potent inhibitor. All these compounds have inhibitory potentials better than the ketoamides suggested by Zhang et al. [14]. Nevertheless, the docking score of ketoamide 14b ranks eleventh.

4. Discussion

Computational molecular modeling approaches, including Molecular docking, are amongst the most vital tools in identifying novel therapeutics against known drug targets. Use of such approaches is highly valuable in the present global crisis due to COVID-19 pandemic, where the research community is meticulously searching for therapeutics against SARS-CoV-2. While the three-dimensional crystal structures of different drug targets have been made available, studies performed on other related CoVs have been of great significance in understanding the replication of this novel virus. Computational studies are being performed to identify known compounds which may potentially interact with and inhibit these targets so as to prevent replication of the virus in human cells.

In our study, we have used 16 known viral protease inhibitors, two ketoamides as suggested by Zhang et al. [14], 24 compounds suggested by Wu et al. [15], and 40 plant-based compounds, and determined their potency in interacting with the active site of 3CLPro of SARS-CoV-2. The docking score, Rerank score, has been considered as a parameter to determine the relative efficacy of the compounds in inhibiting the target. Ritonavir was found to be the best inhibitor of the enzyme, followed by Indinavir. Lopinavir and Nelfinavir. Lopinavir was suggested as a therapeutic option against SARS-CoV-2 by Khan et al. [23], since the compound was found to be effective against others CoVs including MERS-CoV and SARS-CoV [24]. A

combination of Ritonavir and Lopinavir is has been approved by the FDA for treatment of HIV-1 [25], and were used against SARS [26,27] and MERS [28,29]. It was earlier suggested for COVID-19 as well [10]. While Ritonavir is a viral protease inhibitor, it also inhibits the host cytochrome P450 enzyme which metabolizes lopinavir [30]. Thus a combination of the two drugs is given his to enhance bioavailability of lopinavir [31]. Lopinavir has also been found to be effective against SARS-CoV and MERS-CoV [27,32]. Choy et al. [33] reported antiviral potential of lopinavir against SARS-CoV-2 *in vitro.* Cao et al. [34] reported from randomized, controlled, open-label trial with 199 COVID-19 positive patients, 99 of whom received Lopinavir/Ritonavir therapy, that the treatment did not improve mortality or detectable viral RNA at different time points. However, in their modified intent-to-treat analysis, there was a reduction in the median time of clinical improvement by 1 day. Nevertheless, although gastro-intestinal adverse events were more common in the treatment group (which may be attributed to side effects of the treatment), severe gastro-intestinal complications were more common in patients not given the antiviral treatment. Further, there was some evidence that the treatment reduced mortality rate as well as shortened ICU stays. Yan et al. [35] performed a retrospective study with 120 hospitalized patients, and reported that the patients who started the therapy within 10 days of onset of the COVID-19 symptoms had a shorter duration of virus shedding, and thus recommended that the therapy should be initiated within 10 days of symptom onset. Thus, it is recommended that large scale clinical trials are conducted using these 3CLPro inhibitors alone or in combination.

Among the compounds suggested by Wu et al. [15], Montelukast was found to be the best inhibitor, and Candoxatril, Tigecycline and Almitrine were also highly potent inhibitors of the enzyme. This is further supported by the findings of Wu et al. [15]. Montelukast is used in the treatment of asthma, whereby the drug reduces bronchial constriction by preventing inflammation [36]. Thus, the drug may reduce respiratory distress in COVID-19 patients as well. Among phytochemicals, while Acteoside was found to be the best inhibitor of the enzyme, Rutin was also a highly potent inhibitor. All these compounds have inhibitory potentials better than the ketoamides suggested by Zhang et al. [14]. Acteoside is a well-known antioxidant, anti-inflammatory, hepatoprotective, cell and immune-regulatory properties [37]. Rutin is a low molecular weight polyphenol, and has several pharmacological activities including antibacterial, antiinflammatory, antiallergic, antiviral, cytoprotective and antihypertensive [38-39]. Among plant secondary metabolites, Acteoside and Rutin were found to be the best inhibitors of the enzyme, and thus may investigated as potential lead molecules against the virus.

Thus, the present study identifies the existing protease inhibitors as antivirals against SARS-CoV-2. These drugs may therefore be tried for clinical studies against COVID-19. Nevertheless, while clinical trials with different antivirals are ongoing, we have identified several plant-based natural products as potent inhibitors of the 3CLpro, which may emerge as future therapeutics. The benefit of using such natural products is that most of these are very potent antioxidants and anti-inflammatory compounds. Since COVID-19 is associated with extreme rise in the levels of cytokines including IL2, IL7, IL10, GCSF, IP10, MCP1, MIP1A, and TNFα [40], use of anti-inflammatory drugs remains to be one of the most important strategy in preventing injury to organs [41-44]. Thus, natural products with known anti-inflammatory properties would be highly beneficial in protecting the organs and fatalities in COVID-19 patients. In a

case study, Ni et al. [45] reported use of traditional Chinese medicine along with Western medicine for the effective treatment of COVID-19. Likewise the plant-based compounds may be used for better management of the disease, which may confer dual benefit. However, this will require *in vitro* and *in vivo* studies to ascertain their efficacies.

5. Conclusion

In the present global crisis of the COVID-19 pandemic, the world awaits therapeutics against the novel coronavirus, SARS-CoV-2. However, owing to the emergent situation and thereby to shorten time, drug repurposing remains to be the best option to find an effective therapeutic. In this juncture, computational modeling, using molecular docking, is one of the best tools in hand. In the present study, 82 compounds/drugs were tested for their potential in inhibiting the main protease of SARS-CoV-2. Among all the compounds studied, antiviral drugs including Ritonavir, Indinavir, Nelfinavir and Lopinavir were found to be potent in inhibiting the 3CLPro. Other potent 3CLPro inhibiting drugs / compounds include Montelukast, Acteoside, Rutin, Candoxatril, Tigecycline and Almitrine. It is therefore suggested that large scale clinical studies are initiated using the drugs. Further, the secondary metabolites, including Acteoside and Rutin, may serve as lead molecules for development of future therapeutics. Thus, the present study is of immense importance in the therapeutic intervention of COVID-19.

Declarations

Conflict of interest: None declared.

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Ethical disclosure: The present study is a molecular modeling work, and thus does not require ethical approval from any agency.

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Table

Table 1: Table showing the list of compounds / drugs used in the present study, their physicochemical properties and docking scores. The docking scores were obtained following docking using Molegro Virtual Docker 6.0 software.

Name of compound / inhibitor	PubChem ID	MolDock Score	Rerank Score	HBond	Remarks
Ritonavir	392622	-191.681	-145.68	-7.68331	Known inhibitor
Indinavir	5362440	-169.518	-131.986	-7.07843	Known inhibitor
Montelukast	5281040	-171.568	-130.33	-10.8343	Wu et al. 2020
Acteoside	5281800	-142.815	-126.769	-16.9199	Phytochemical
Nelfinavir	64143	-156.375	-123.755	-13.4594	Known inhibitor
Rutin	5280805	-146.196	-122.459	-12.3004	Phytochemical
Candoxatril	5362417	-164.386	-119.547	-8.08428	Wu et al. 2020
Tigecycline	54686904	-130.209	-118.945	-11.3303	Wu et al. 2020
Lopinavir	92727	-172.448	-117.421	0.06577	Known inhibitor
Almitrine	33887	-141.513	-115.477	-3.4736	Wu et al. 2020
14b*	-	-150.217	-112.525	-6.76198	Ketoamide inhibitor
Curcumin	969516	-129.902	-112.396	-4.43329	Phytochemical
Rosmarinic acid	5281792	-131.984	-111.052	-15.3093	Phytochemical
Bis-demethoxycurcumin	5315472	-132.107	-110.963	-5	Phytochemical
Saquinavir	441243	-166.614	-110.767	-3.17374	Known inhibitor
Pleconaril	1684	-135.398	-108.824	-8.21897	Known inhibitor
Cilastatin	6435415	-130.666	-108.738	-4.60117	Wu et al. 2020
Quercetin-3-β-D-glucoside	5280804	-119.668	-108.271	-15.4112	Known inhibitor
Amprenavir	65016	-142.83	-108.185	-4.77329	Known inhibitor
Demethoxycurcumin	5469424	-130.989	-107.374	-4.83556	Phytochemical
Cefpiramide	636405	-163.523	-107.077	-9.71581	Wu et al. 2020
Pioglitazone	4829	-132.337	-107.07	-8.57175	Wu et al. 2020
Withaferin A	265237	-125.824	-104.903	-5.64581	Phytochemical

Puerarin	5281807	-119.323	-104.881	-12.7102	Phytochemical
11r*	-	-165.451	-103.461	-4.55018	Ketoamide inhibitor
Alfuzosin	2092	-124.293	-101.327	-10.9508	Wu et al. 2020
Carvedilol	2585	-138.689	-101.257	-7.08229	Wu et al. 2020
Isobavachalcone	5281255	-124.512	-101.216	-6.4969	Known inhibitor
Paeoniflorin	442534	-130.31	-99.7539	-13.5334	Phytochemical
Phenethicillin	272833	-127.122	-98.6297	-5.13691	Wu et al. 2020
Carminic acid	10255083	-100.751	-96.9069	-18.7213	Phytochemical
Sesamin	72307	-130.68	-96.4424	0	Phytochemical
Quercetin	5280343	-106.728	-95.9808	-13.4112	Phytochemical
Chrysotoxine	5315860	-111.147	-95.5632	-5.71678	Phytochemical
Famotidine	5702160	-106.463	-92.5482	-7.82187	Wu et al. 2020
Catechin	9064	-108.479	-92.4243	-12.6705	Phytochemical
Helichrysetin	6253344	-110.409	-92.2142	-13.0345	Known inhibitor
Kaempferol	5280863	-102.499	-91.2825	-10.9606	Phytochemical
Sesaminol	94672	-134.694	-91.2395	-2.68177	Phytochemical
Resveratrol	445154	-108.239	-90.5939	-7.26707	Phytochemical
Dronabinol	16078	-108.491	-89.155	-4.94748	Phytochemical
Nordihydroguaiaretic acid	4534	-106.585	-88.9944	-10.6845	Phytochemical
Rosmanol	13966122	-99.2136	-88.7562	-8.59965	Phytochemical
Demeclocycline	54680690	-91.3397	-88.658	-4.20822	Wu et al. 2020
EGCG	65064	-131.494	-88.4334	-13.1813	Phytochemical
Telmisartan	65999	-196.847	-88.0605	-3.37205	Wu et al. 2020
Tenuigenin	12442762	-121.5	-87.2238	-3.51168	Phytochemical
Withanolide A	11294368	-104.876	-85.7868	-0.815134	Phytochemical
Herbacetin	5280544	-97.3802	-85.702	-15.6582	Known inhibitor
Nicardipine	4474	-145.672	-81.5043	-1.77899	Wu et al. 2020

Conivaptan	151171	-145.915	-79.269	-0.776034	Wu et al. 2020
Disulfiram	3117	-97.9229	-79.2022	0	Known inhibitor
Ginkgolide A	9909368	-118.068	-78.8825	-7.90312	Phytochemical
Carnosol	442009	-94.8403	-77.9083	-7.08391	Phytochemical
Morin	5281670	-93.8023	-77.1992	-11.1263	Phytochemical
Flavin mononucleotide	643976	-118.384	-77.0244	-13.4506	Wu et al. 2020
Estradiol valerate	13791	-110.47	-75.9955	-2.47672	Wu et al. 2020
Epigallocatechin	72277	-99.5012	-74.8905	-12.0698	Phytochemical
Nepafenac	151075	-87.9547	-73.6417	-7.97907	Wu et al. 2020
Mimosine	3862	-81.0206	-69.8286	-8.63722	Wu et al. 2020
Withanone	21679027	-94.6249	-69.416	-9.39424	Phytochemical
L-theanine	439378	-79.1674	-67.8436	-7.45009	Phytochemical
Zingerone	31211	-81.7395	-67.6862	-2.54689	Phytochemical
Genistein	5280961	-90.1996	-67.3383	-9.76587	Phytochemical
Carnosic acid	65126	-83.2482	-66.0006	-8.34816	Phytochemical
Caffeic acid	689043	-77.1921	-63.2921	-5.05571	Known inhibitor
Thymoquinone	10281	-71.1736	-61.7502	-4.5178	Phytochemical
Isatin	7054	-71.2818	-61.5046	-1.78669	Known inhibitor
Favipiravir	492405	-66.9781	-60.5145	-7.16454	Known inhibitor
Ellagic acid	5281855	-78.964	-59.3876	-6.94904	Phytochemical
Gallic acid	370	-67.2234	-58.4605	-12.0867	Phytochemical
p-Coumaric acid	637542	-74.1294	-58.413	-3.06399	Phytochemical
Sesamol	68289	-66.0625	-51.9265	-4.40782	Phytochemical
Limonene	22311	-61.1149	-50.8082	0	Phytochemical
Diisopropylfluorophosphate	5936	-61.8131	-49.1607	-0.823786	Known inhibitor
Doxycycline	54671203	-97.647	-47.021	-6.0814	Wu et al. 2020
Oxytetracycline	54675779	-96.0528	-42.9165	-7.84098	Wu et al. 2020

Chlorhexidine	9552079	-124.171	-36.8801	-11.4681	Wu et al. 2020
Limecycline	54675776	-94.2957	-33.9643	-5.55622	Wu et al. 2020
Lutein	5281243	-119.461	1.62241	0	Wu et al. 2020
Garcinol	5281560	-131.293	5.40054	-13.0189	Phytochemical
Lycopene	446925	23.4536	160.484	0	Phytochemical

***13r and 14b are α-ketoamides which have been suggested by Wu et al. (2020) as inhibitors of the main protease of SARS-CoV-2.**

Figures

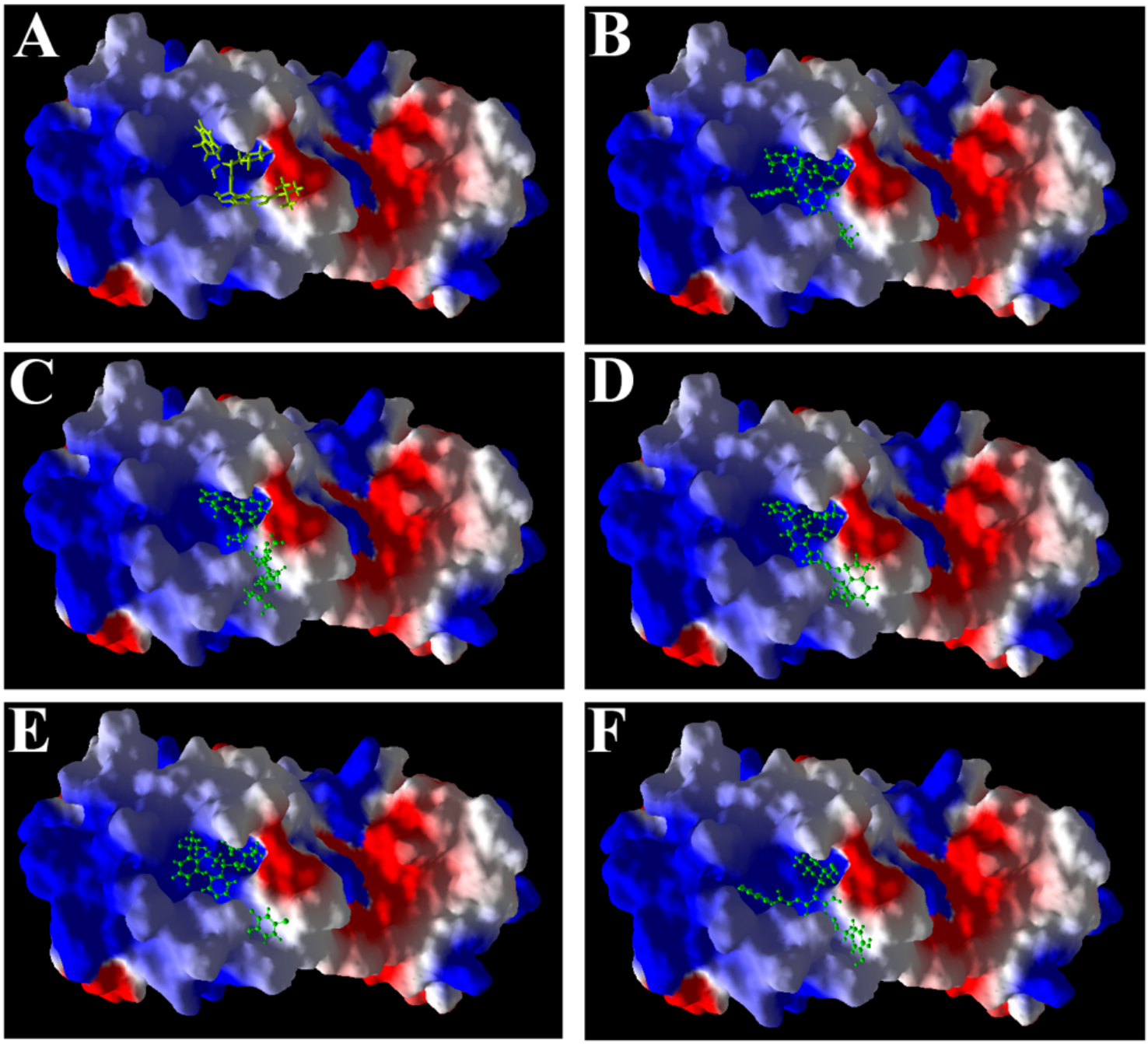

Figure 1

Docking poses of different compounds under investigation at the active site of 3CL protease (main protease) of SARS-CoV-2. (A) Pose of original co-crystallized ketoamide, and Docked poses of: (B) Co-crystallized ketoamide, (C) Ritonavir, (D) Indinavir, (E) Montelukast and (F): Acteoside. All these ligands bind to the same active binding site of the co-crystallized ketoamide. The poses were developed following docking using Molegro Virtual Docker 6.0 software. The pose of the co-crystallized compound (ketoamide) and the pose of the same compound when docked are same, which validates the docking study.