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Research Paper

Phytocompounds as Potent SARS-CoV-2 Main Protease Inhibitors: A Molecular Docking Study

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ABSTRACT

A novel coronavirus (CoV) strain named SARS-CoV-2 identified in Wuhan, China has threatened millions of lives and with mortality of more than 6 million world-wide in the ongoing pandemic outbreak. Lack of specific treatment for coronavirus disease has motivated researchers globally for drug discovery and in the current investigation we have explored phytocompounds as potential SARS-CoV-2 main protease inhibitors, using a molecular docking study. SARS-CoV-2 6y84 and 6lu7 domains were docked with curcumin, phytol, ά-naphthoflavone and ursolic acid and the analysis was carried out. Hydroxychloroquine and Favipiravir were used as standards for comparison. The binding energies obtained from the docking of 6y84with native ligand curcumin, phytol, ά-naphthoflavone, ursolic acid, remdesevir and favipiravir were between -8.4 to -13.1 kcal/mol. Therefore, phytol, ursolic acid, curcumin and ά-naphthoflavone appeared to have the best potential to act as SARS-CoV-2 M^{PRO} inhibitors and may prove to be beneficial in the treatment of this corona virus disease.

1. Introduction

The world is currently under the grip of an outbreak which started in December 2019 from Wuhan, Hubei province of China as an unknown disease with pneumonia like symptoms.[1] As of 14th April, 2020, a total of more than 1.85 million of confirmed cases have been reported from 213 countries, among which 1,17,000 deaths have been confirmed.[2] The real cause of this mysterious disease was identified in later weeks of January 2010 as a novel virus.[3-6]. The virus has been assigned a name as Severe Acute Respiratory Syndrome Corona Virus 2 (SARS-CoV-2) and the related disease was termed by the World Health Organization as Corona Virus Disease 2019 (COVID-19).

Coronavirus (CoV) is a highly diverse, enveloped RNA virus which consists of a positive-sense single-stranded RNA (approximately 27–32 kb) and belongs to the family Coronaviridae. SARS-CoV-2 is a novel member of CoVs, has a crown like appearance with spherical external glycoprotein spike protein (S) on the envelope. Its form may be rounded or elliptical and most often pleomorphic with about 60–140 nm diameter. Though its source is yet to be understood, genomic studies propose that SARS-CoV-2 may probably be evolved from a strain found in bats based on the similarity of its genetic sequence to that of other CoVs.[5] Although SARS-CoV-2 has some genetic features comparable to that of CoV, but it has different gene sequences those are significantly dissimilar from previously reported CoVs.

As compared to other respiratory pathogens, the communication of SARS-CoV-2 is supposed to occur via respiratory droplets through coughing and sneezing. While the clinical presentation of COVID-19 may vary from asymptomatic to respiratory failure, common symptoms include fever, fatigue, dry cough, myalgia, and dyspnea.[7-9] Unusually, productive cough, headache, hemoptysis, and diarrhea may be associated[10]. Most of the patients suffering from COVID-19 are male and nearly half of them have more than one co-existing conditions such as high blood pressure, diabetes and cardiac disease. Mortality rate was seen to be higher among those with coexisting medical condition[10].

Until now, there is no effective antiviral treatment, or vaccine, which is commercially available for the treatment of COVID-19, and patients are treated symptomatically. With the increasing spread of the epidemic and rising number of fatalities, a sense of urgency for novel drug discovery has developed. One of the most important SARS-CoV-2 protein targets for drug discovery is the main protease (M^{PRO}) which is also known as 3C-like protease ($3CL^{PRO}$)[11] due to its essential role in functionalizing the polyproteins that are translated from the viral RNA.[12] $3CL^{PRO}$ is operated at minimum of 11 cleavage sites on the large polyprotein 1ab (replicase 1ab, ~790 kDa) and inhibition of this enzyme activity would essentially block the replication of the virus. Also, as none of the human proteases have a similar cleavage specificity, inhibitors are not likely to be toxic. Since the crystal structure of $3CL^{PRO}$ is now known, it will be time-saving for the researchers to discover antiviral drug for SARS-CoV-2.[13]

It was envisaged to find suitable natural compounds that may offer antiviral properties by blocking the 3C-like protease. Phytocompounds such as phytol, ά-naphthoflavones, curcumin and ursolic acid have been used in viral diseases earlier. Therefore, the present study was undertaken to evaluate protein-ligand interaction of 6y84 and 6lu7 domain of SARS-CoV-2 with natural compounds allicin, ajoene, ά-naphthoflavones, curcumin, and ursolic acid by *in silico* docking approach in order to identify potential inhibitors of SARS-CoV-2.

2. Experimental

2.1 Protein Macromolecule

SARS-CoV-2 $3CL^{PRO}/M^{PRO}$ (PDB ID: 6LU7 and 6Y84) (COVID-19 main protease with unliganded active site) structures were found from RCSB protein data bank (https://www.rcsb.org/) in .pdb format. PDB is an archive for the crystal structures of biological macromolecules worldwide. 6LU7 and 6Y84 are SARS-CoV-2 M^{PRO} structure with unliganded active site.[14] Currently, there are no targeted therapeutic agents for targeting these proteins, and effective treatment options remain very limited.

2.2 Ligands

The 3D structures of the compounds were found as .sdf format from the PubChem website which is a chemical-cum-biological substance activities repository containing substance, compound, and bioassay databases.

2.3 In silico analysis

The protein-ligand interaction of phytol, ά - naphthoflavones, curcumin and ursolic acid with the Novel COVID-19 6y84 domain was analyzed by molecular docking approach. Hydroxychloroquine and Favipiravir[15] were taken as standard drugs for comparison. The protein Domain-Ligand interaction was analyzed by in silico docking approach. The MARVIN DRAW software (ChemAxon, Budapest, Europe) was acclimated to draw the molecular structure of ligands and that made to dockable PDB structure by Accelrys Discovery Studio 2016. All the water molecules were abstracted, and polar hydrogen atoms were integrated to the targeted protein moiety by Accelrys Discovery Studio 2016. The grid box was allocated felicitously in targeted protein to include the active residue center, and the grid map was yare by MGL Tools 1.5.6. The ligand– protein interaction was calculated and architecturally prepared by AUTODOCK VINA[16,17], and the affinity of binding in kcal/mol was resolute.[18] The highest negative value was considered as the best soothsaid possible binding ligand–protein.[19] The output pdbqt file were analysed and the Protein-Ligand Intricate PDB file was made by utilizing PyMol softwere. The interacting pocket a, bonds and the length were analysed by Accelrys Discovery Studio 2016.

The protein-ligand interaction was carried out and the affinity of protein to ligand binding (in kcal/mol) was calculated. The lowest digit value was

taken as the most relevant predicted value for binding energy of protein-ligand interaction. The genetic sequence of the SARS-CoV-2 6y84 Domain used in this molecular docking study was:
"SGFRKMAFPSGKVEGCMVQVTCGTTTLNGLWLDDVVYCP
RHVICTSEDMLNPNYEDLLIRKSNHNFLVQAGNVQLRVIG
HSMQNCVLKLKVDTANPKTPKYKFVRIQPGQTFSVLACYN
GSPSGVYQCAMRPNFTIKGSFLNGSCGSVGFNIDYDCVSFCY
MHHMELPTGVHAGTDLEGNFYGPFVDRQTAQAAGTDTTI
TVNVLAWLYAAVINGDRWFLNRFTTTLNDFNLVAMKYNY
EPLTQDHVDILGPLSAQTGIAVLDMCASLKELLQNGMNGR
TILGSALLEDEFTPFDVVRQCSGVTFQ".
"SGFRKMAFPSGKVEGCMVQVTCGTTTLNGLWLDDVVYCP
RHVICTSEDMLNPNYEDLLIRKSNHNFLVQAGNVQLRVIG
HSMQNCVLKLKVDTANPKTPKYKFVRIQPGQTFSVLACYN
GSPSGVYQCAMRPNFTIKGSFLNGSCGSVGFNIDYDCVSFCY
MHHMELPTGVHAGTDLEGNFYGPFVDRQTAQAAGTDTTI
TVNVLAWLYAAVINGDRWFLNRFTTTLNDFNLVAMKYNY
EPLTQDHVDILGPLSAQTGIAVLDMCASLKELLQNGMNGR
TILGSALLEDEFTPFDVVRQCSGVTFQ"
which were retrieved from the Protein Data Bank.

3. Results

Table 1 presents the medicinal properties of studied phytocompounds and their sources. Binding affinities and major interactions between SARS-CoV-2 M^{PRO} structure and phytochemicals like Ajoene, Allicine, Curc-umin, Theoflavin, Urosolic acid, and α- naphthof-lavone were obtained using Autodock Vina. Binding affinities were compared with those obtained with Favirapir, Remdesivir as inhibitor of the SARS-CoV-2 M^{PRO} structure. The binding affinity obtained from 6LU7 and 6Y84 binding with the Favirapir, Remdesivir and the phytochemicals are shown in Table 2. The binding affinity values vary from – 3.3 to – 10.5 kcal/mol. Discovery Studio 2020 Client software was used to illustrate the molecular interactions between active site SARS-CoV-2 M^{PRO} structure and the ligands. The ligands showed the expected interactions with the amino acids present in active site of protein, which implies powerful antagonistic properties towards SARS-CoV-2 M^{PRO} structure. Exhibiting the highest binding affinity Ursolic acid with a value of – 10.5 kcal/mol and -13.1 kcal/mol respectively (Fig. 1a and 1b) followed by Theaflavin with a binding energy value of – 9.1 kcal/mol and -10.8 kcal/mol. The ligands with the lowest values obtained in this study correspond to Allicine and Ajoene with binding affinity that – 3.3 and -5.1 kcal/mol and – 4.1 and -4.3 kcal/mol respectti-vely. In general, the binding affinity values of Ursolic acid and Theafalvin can be used as potential inhibitors of SARS-CoV-2 M^{PRO} structure.

Table 1 General information about studied phytocompounds

Phytocompounds	Sources	Medicinal Properties
Phytol	Natural linear diterpene alcohol	Antibacterial, Antiviral, Anticonvulsant, Anti-epileptic, Anti-inflammatory, Adjuvant [20-22]
ά-naphthoflavones	Fruit, Vegetables, Grains, Barks, Roots, Stems, Flowers, Tea	Antioxidant, Antiviral, Carcinostatic[23]
Curcumin	Turmeric	Antioxidant, Anticancer, Antiviral, Antidiabetic, Antihypertensive, Antiinflammatory, Antimicrobial, Antipsoriatic, Antiscleroderma[24-26]
Ursolic acid	Leaves of various plants (Rosemary, Marjoram, Lavender, Thyme, and Organum), Fruits (Apple fruit peel), Flowers, and Berries.	Anti inflammatory, Anti-oxidant, Antiviral Anti-carcinogenic , Antiobesity, Anti-diabetic, Cardioprotective, Neuroprotective, Hepatoprotective, Thermogenic effects[27,28]

Table 2 Results of compounds docking in 6LU7 and 6Y84 target

Receptor Protein	6LU7_COVID19			6Y84_ COVID19		
Ligand	**Affinity (kcal/mol)**	**Binding Site**	**Length distance (Å)**	**Affinity (kcal/mol)**	**Binding Site**	**Length distance (Å)**
Ajoene	-4.1	PHE-223 - With S	5.39	-5.1	LYS-5	4.23
			5.01		LYS-5	4.9
		LEU-271	4.42			
		TRP-298	4.96			
Allicine	-3.3	ASP-153	3.39	-4.3	LYS-5	4.77
		ILE-106	5.11		LYS-137	4.05
		VAL-104	4.23			
Curcumin	-8.4	LYS-137	4.33	-9.8	SER-139	3.5
		ASP-197	4.82			
		ASN-238	3.34			
		TYR-239	5.57		TYR-126	4.06
		LEU-287	4.62			
		LEU-286	4.22		LEU-286	4.96
		LEU-272	5.3			

Theaflavin	-9.1	MET-166	5	-10.8	VAL-125	3.14
			3.87		LYS-5	4.88
						4.16
		CYS-145	3.75		GLU-290	2.8
			3.24		GLN-127	3.24
		HIST-164	3.18		CGLU-288	4.33
			3.12		GLY-283	3.1
		MET-165	4.78			
		GLN-189	3.04		ARG-4	2.79
		PRO-168	4.77			
Ursolic acid	-10.5	PRO-9	4.45	-13.1	TYR-126	4.93
		VAL-125	5.13		LYS-5	4.52
						4.83
						5.08
					LYS-137	4.84
						5.13
					ARG-131	4.68
alpha-naphthoflavone	-7.3	LYS-102	3.04	-9.3	ALA-173	4.14
					LEU-167	4.8
		VAL-104	4.84		PRO-184	4.26
			4.23		VAL-186	4.44
		ILE-106	5.25			
Favipiravir	-4.2	PHE-294	5.63	-4.8	LYS-5	3.98
					TRP-207	2.95
					Pi-alkyl bond	4.23
Remdesivir	-9.5	ALA-193	3.71	-11	HIS-172	5.01
		ALA-194	4.52			
		VAL171	5.27		LYS-137	4.52
		ASP-197	3.3		SER-139	4.32
		LEU-287	3.45			

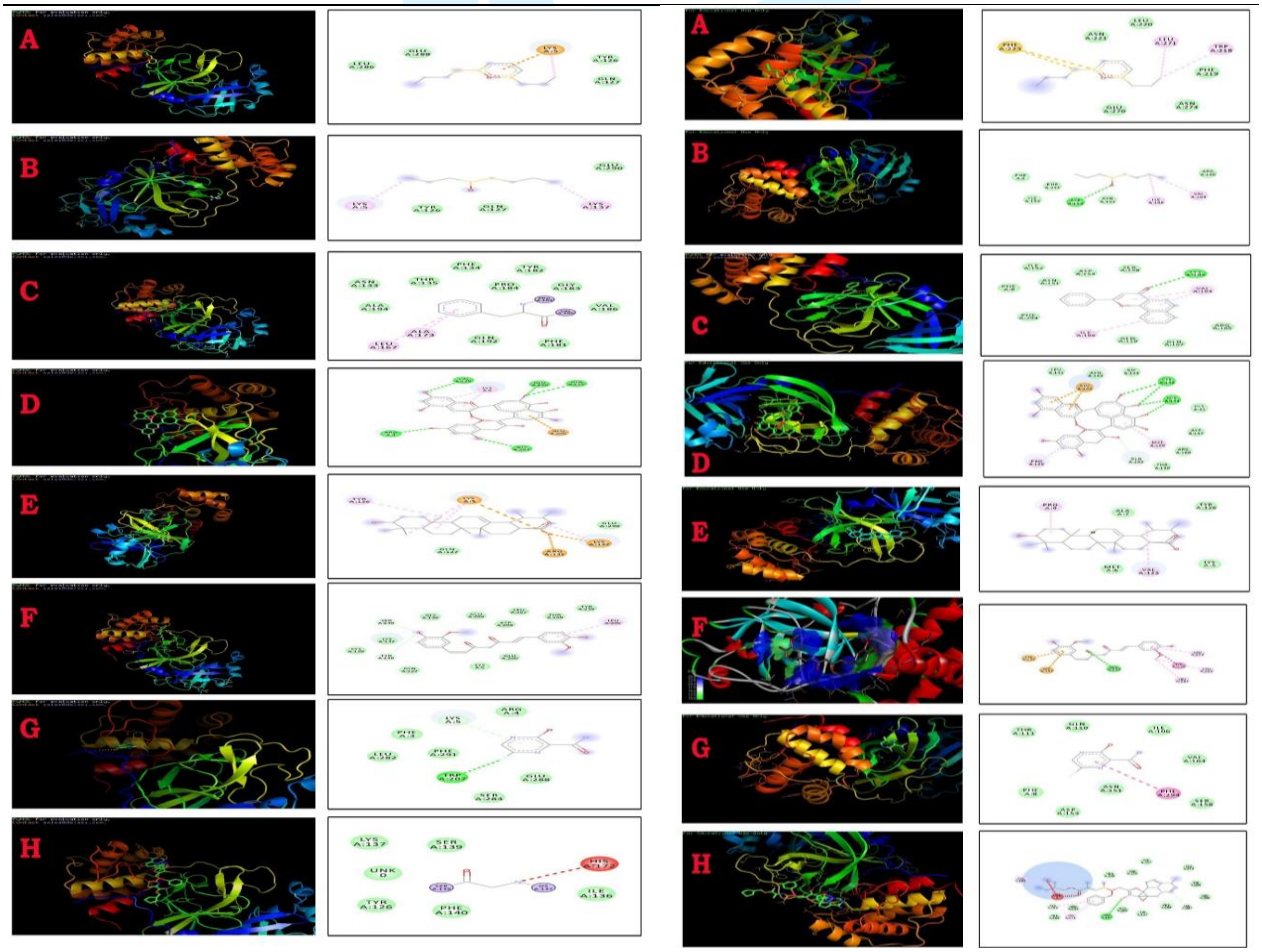

Fig. 1 a: *In silico* interaction of ligand 6lu7 with A. Ajoene B. Allicin C. ά-naphthoflavones D. Theoflavin E. Urosolic Acid F. Curcumin G. Favirapir H. Remdesevir b. *In silico* interaction of ligand 6y84 with A. Ajoene B. Allicin C. ά-naphthoflavones D. Theoflavin E. Ursolic Acid F. Curcumin G. Favirapir H. Remdesevir fgfg

4. Conclusion

Overall, in an exploration to find phytocompounds which could act as inhibitor of main protease of recently uploaded structure of SARS-CoV-2, our study manifested a promising antiviral drug for SARS-CoV-2 with presentation of strong binding energy activity of Ursolic acid >Theaflavin > ά-naphthoflavones > Curcumin. Information obtained by this study will be used in screening of inhibitors of the SARS-CoV-2 6y84 and 6lu7 domain protein and can be further used for drug design. This information may serve as a stepping point for future research in discovery of a suitable drug in the treatment of COVID-19 and salvage the humankind from the onslaught of deadly virus.

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