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RESEARCH ARTICLE

Molecular Docking Identifies Novel Phytochemical Inhibitors Against SARS-COV-2 for Covid-19 Therapy

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ABSTRACT:

SARS-CoV-2 the new strain of SARS corona virus is an RNA virus that inflicts acute respiratory distress syndrome due to infection of the alveolar epithelial cells, its primary target. No effective drug is currently available to treat this viral infection. Therefore, we focused on identifying inhibitors of the main viral protease domain (Mpro) which plays important role in the virus life cycle. Two tired computer-aided drug discovery approach were adopted for screening of novel inhibitors against Mpro, the target protein. First, based on their ADME/T properties, phytochemicals as well as synthetic drugs six compounds were selected from the available database. In second screening by molecular docking based on binding affinity and molecular interactions of these compounds with Mpro led to the identification of the best phytochemical and synthetic compound against Mpro. The result of docking complex showed that, interacting residues for myricetin are continuous while, in case of fosamprenavir, these are non-contiguous. Both molecules interact with the residues in the active site occupying the site for the catalytic activity indicate possible competitive inhibitors of the Mpro.

KEYWORDS: COVID-19, Phytochemical, Anti-viral, therapeutic, docking, ADME/T.

INTRODUCTION:

Since the outbreak of novel corona virus SARS-CoV2 infection or the disease COVID-19 in December 2019, millions of people in 150 countries are affected and thousands succumbed to acute respiratory disorder that followed SARS in 2002 and Middle East Respiratory Syndrome corona virus (MERS-CoV) in 2012 [1].

The corona virus- classified into α- and β-subtypes that infect mammals, and γ- and δ-subtypes that infect birds and pigs- was first reported by Tyrell and Bonne in 1996 from the patients suffering common cold [2-4] . The two-thirds of its 26-32 kb genome encodes viral polymerase, RNA synthesis materials, and two large non-structural polyproteins whereas one-third of the genome codes for the envelope, membrane, nucleocapsid (N), and helper proteins by proteolysis of a common polypeptide chain[3-6]. Proteases thus play important roles in viral replication and therefore identifies itself as a possible target for anti-COVID-19 inhibitors[7].

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Main protease domain (Mpro) is a shared conserved domain in its subfamily papain-like protease (PLP) and 3C-like protease (3CL or Nsp5) but differs from that of human proteases[5]. Many covalent Mpro inhibitors were developed against SARS-CoV but may associated with side effects and toxicity. By contrast, since ancient time natural compounds proved an alternative source of treatment with less side effects, as many natural compounds have served humans as cheaper and safer drug candidates against several diseases [7-11]. Till today wide range of phytochemicals such as flavonoids, terpenoids etc have proved therapeutic agents against various diseases. These phytochemicals and their synthetic derivatives have been identified by a combination of in vitro and in vivo studies in different biological assays[10]. Computational drug discovery method is very useful tool for designing new drug candidate. So, the present study using molecular docking approach is focused on screening phytochemicals as well as synthetic compounds database for identification of potent inhibitors to target SARS-CoV2 Mpro.

MATERIALS AND METHODS:

In silico molecular modeling investigation was carried out on machine with computational specifications (Intel(R) Core(TM) i3-3210M CPU @ 2.50 GHz, 5Core(s) processor with 4.00 GB RAM and 64-bit Windows-7 Operating System).

Ligand database arrangement:

The ligand dataset is divided into categories containing 10 drug molecules each. The 3D PDB structures of selected antiviral ligand molecules were downloaded from Pubchem database[11] and 2D structure was drawn using Marvin sketch[13]. Phytochemical based drug molecule especially flavonoids were retrieved from NPACT database[14]. All ligands were optimized using Discovery studio.

Absorption, distribution, metabolism, excretion and Toxicity (ADME/T) studies of compounds:

ADME/T study is an essential and primary step of pharmacological drug screening. It includes properties of structural analogues, predicting both physically significant descriptors and pharmaceutically relevant properties. It also calculates the analogues depending upon Lipinski's rule of 5 (Lipinski 2001), which is important step for rational drug design. As synthetic drug were selected from FDA approved list, ADME screening was carried out for all selected drug molecules. The properties were predicted using SWISS ADME tool [14].

Retrieval of SARS-CoV2 main protease structure:

3-D COVID-19 main protease (PDB:6lu7) was obtained from the Protein Data Bank (http://www.rcsb.org) with 2.16 Å resolution. The available crystal structure was bound with inhibitors and water molecules. The refining of co-crystal structure was carried out in Discovery Studio by removing water molecules and existing inhibitor.

Binding site prediction:

Active site analysis calculates the number, boundary of mouth openings of every pocket, molecular reachable surface and area. Binding site analysis provides a significant insight to recognize the surface structural pockets, active site, shape and volume of every pocket, internal cavities of proteins. The probable binding site was searched based on structural association of template, PDBsum [15].

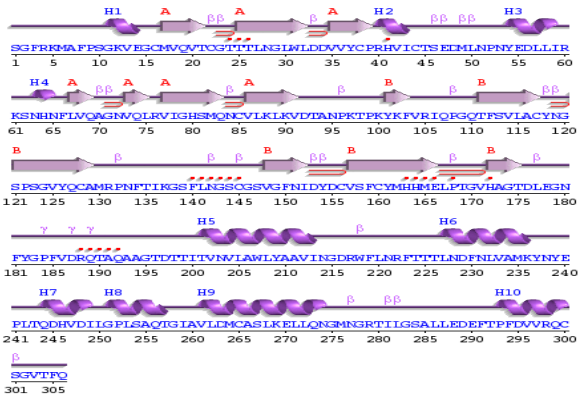
Molecular Docking study:

Molecular docking is performed in Autodock 4.2 for selected ligand molecule against main protease[16]. Ten best docked poses were generated through applying a scoring function and genetic algorithm. Among these, best interacting ligands were screened based on RMSD (Root-Mean-Square Deviation), binding energy and ligand efficiency, usually measured in Angstrom (Å) and docking score.The binding interactions in the docked complexes obtained by tool were analyzed by Discovery studio visualizer[17].

RESULT:

3-D structure of protein:

The crystal structure of Mpro complexed with an inhibitor at a resolution of 2.16^{0}A is already reported (PDB ID :LU7). The Mpro is composed of 306 amino acids, with molecular weight of 34.5kDa. It is made of single A chain, contains 2 beta sheets, 7 beta hairpin, 10 helices and 16 beta turns in its secondary structure (Figure 1). Mpro 3-D structure shows 3 domains-Domain I (residues 8–101) and domain II (residues 102–184) with an antiparallel β-barrel structure; and domain III (residues 201–303). The Cys–His catalytic dyad, and the substrate-binding site of this protein is located in a cleft between Domain I and II.



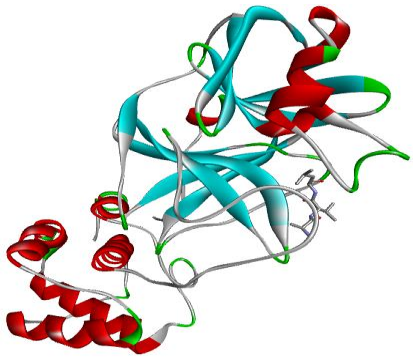


Figure 1. The primary (left) and the Secondary (right) structures of main protease (Uniprot : P0DTD) with the complexed inhibitor (PDB ID 6LU7).

Binding site prediction:

The binding site information of target protein was predicted by performing PDBsum and literature survey. The ligand plot obtained from PDBsum showed binding site region of TG2 contains 13 amino acid residues, *viz.*, GLY143, SER144, HIS163, HIS164 and GLU166 of chain A; these residues lined the inhibitor binding site. are used for setting the grid for molecular docking.

ADME/T studies of compounds:

ADME/T analysis of selected compounds predicted for Adsorption, Distribution, Metabolism and Excretion property by using SWISS ADME software. All molecules passed Lipinski rule of five showing zero violation (Table 1) and may therefore be used for developing new drug.

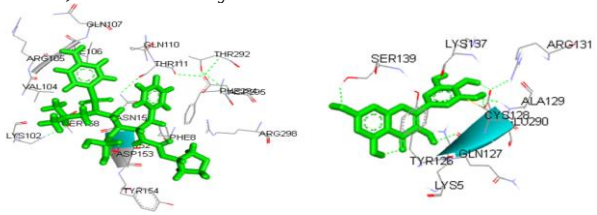
Table 1: Selected probable inhibitory molecules against Mpro.

Category	Compound	Molecular weight g/mol	ADME
Phytochemical	Myricetin	318.23	Yes
	Cyanidin	287.24	Yes
	Europinidin	331.3	Yes
	Delphinidin	338.69	Yes
	Curcumine	368.4	Yes
	Eucalyptol	154.25	Yes
	Euparin	216.23	Yes
	Quercetin	302.23	Yes
	Nobiletin	402.4	Yes
	Vinacamine	354.44	Yes
	Fosamprenavir	585.6	Yes
	Emtricitabine	247.25	Yes
Synthetic Drug	Ganciclovir	255.23	Yes
	Lamivudine	229.26	Yes
	Abcavir	286.33	Yes
	Nervirapine	266.3	Yes
	Amprenavir	505.6	Yes
	Ritonavir	720.9	Yes
	Nelfinavir	567.8	Yes
	Tipranavir	602.7	Yes

Molecular Docking study:

Binding energy suggests the ligands affinity and strength of interaction with the target protein, a compound with a lower binding energy is preferred as a possible drug candidate because that will generate a competitive and reversible inhibitor. So, molecular docking is carried out to identify the effect of 10 phytochemicals and synthetic compounds on COVID-19 (Table-2 and Figure 4). Out of 10 synthetic molecules, 6 compounds show better activity (Fosamprenavir, Emtricitabine, Ganciclovi, Lamivudine, Abcavir and Nevirapine). Among these compounds, Fosamprenavir showed best docked complex score with binding energy -5.28 kcal/mol and it interacts with GLN107, THR111,GLN110,THR292 and lys102 amino acid resides in the active site of target protein. Usually nevirapine is used as an anti-HIV drug and showed capacity to inhibit replication of the virus .

From the phytochemical catogery, four bioactive compounds exhibited good activity- Myricetin, Europinidin, Eucalyptol and Euparin. Myricetin showed highest binding affinity with -4.05kcal/mol and interacted with SER139, LYS137, TYR126, GLN127, ARG131. Myricetin belongs to flavonoid class of polyphenolic compound, which is predominantly found in tomato, oranges and red wine. In present study, it proved as a potential antiviral agent against Mpros protein, but its activity awaits validation.



A **B**

Figure 2: Molecular docking analysis between 6LU7 A) Fosamprenavir B) Myricetin

Table 2. Molecular docking analysis of selected compounds against Major Protease (6LU7).

Category	Compound	Binding energy	Ligand effecency	Inhibition constant	Amino acid
Phytochemical	**Myricetin**	-4.05	-0.18	1.07	SER139,LYS137, TYR126,GLN127, ARG131.
	Cyanidin	-5.7	-0.25		ARG105, ILE106, GLN107,
	Europinidin	-3.5	-0.45	1.00	VAL104, ARG105, ILE106
	Delphinidin	-7.5	-	-	LYS102
	Curcumine	176.34	6.53	-	-
	Eucalyptol	-3.6	-0.34	1.96	GLY143
	Euparin	-3.22	-0.2	1.18	LYS102, PHE103, VAL104, ARG105,ALA120
	Fosamprenavir	-5.28	-1.45	675.60	GLN107, THR111, GLN110, THR292 , LYS102
	Emtricitabine	-0.23	-0.01	682.39	LYS137
	Ganciclovir	-3.25	-0.18	4.13	GLN127,HIS163

Antiviral	Lamivudine	-3.89	-0.26	1.4	TYR126, SER139 VAL135
	Abcavir	-0.46	-0.22	388.94	LYS137
	Nervirapine	-2.64	-0.26	134.59	GLN127, ARG4, GLY143, LYS137, TYR126, GLY138, TYR126, SER139 VAL135

DISCUSSION:

COVID-19 is a major ongoing global threat to human health. Therefore, identifying a possible inhibitor becomes imperative[18]. We argued that the Main protease domain (Mpro) is an important target for its functional contribution to the proteases which are critical for viral replication and thereby the spread of the infection. Natural products, in particular, flavonoids, already known to be effective anti-viral drugs and some synthetic antiviral molecules were therefore targeted as Mpro inhibitors using drug discovery approach. Many phytochemicals have been proven effective antiviral, through blockage of cellular receptors, inhibition of viral antigenic determinants, loss of enzymatic function and inhibition of particle biosynthesis[19-21].

The present data have shown that Myrecetin and Fosamprenavir showed better docking activity than other molecules. These identified molecules can bind to the substrate-binding pocket of Mpro. Both Fosamprenavir and Myrecetin are competitive inhibitors and follow Michelis-Menten equation in α-Amylase and HIV protease inhibition, respectively[22-24]. Myricetin exhibited antiviral activity against a number of viruses including Moloney murine leukemia virus, Rauscher murine leukemia virus, and the HIV[25]. The study suggested that Fosamprenavir inhibited the virus by impairing the protease activity that eventually affected reverse transcription[25]. As both molecules interact with the residues in the active site occupying the site for the catalytic activity, these molecules are likely to be effective competitive inhibitors of the protease. These compounds imitate the exact structure of substrate and show an almost equivalent affinity towards the binding site, eventually neutralizing the enzymatic action .

CONCLUSIONS:

The use of computer-based drug designing and high-throughput screening may prove to be a promising method to find out new drug target against COVID-19. Results identify a drug-worthy binding affinity of Myricetin and Fosamprenavir for Mpro and a likely therapeutic. candidate against COVID-19, albeit contigent upon the validation in infections models.

CONFLICT OF INTERESTS:

The authors have no conflict of interest to declare.

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