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Identification of Potential Flavonoid Inhibitors of the SARS-CoV-2 Main Protease 6YNQ: A Molecular Docking Study



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ABSTRACT

Objective Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the causative agent for coronavirus disease 2019 (COVID-19), is responsible for the recent global pandemic. As there are no effective drugs or vaccines available for SARS-CoV-2, we investigated the potential of flavonoids against SARS-CoV-2 main protease 6YNQ.

Methods *In silico* molecular simulation study against SARS-CoV-2 main protease 6YNQ.

Results Among the 21 selected flavonoids, rutin demonstrated the highest binding energy (− 8.7 kcal/mol) and displayed perfect binding with the catalytic sites.

Conclusions Our study demonstrates the inhibitory potential of flavonoids against SARS-CoV-2 main protease 6YNQ. These computational simulation studies support the hypothesis that flavonoids might be helpful for the treatment of COVID-19.

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1 Introduction

The unprecedented coronavirus disease 2019 (COVID-19) outbreak has had a critical impact on countries across the globe and on people from every walk of life. As of the beginning of October 2020, the world has recorded 1 111 998 deaths due to COVID-19

and more than 39 944 882 confirmed cases ^[1]. The causative agent of COVID-19, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), belongs to the β -coronavirus group. Antiviral drugs can target diverse phases of viral infection. In the case of SARS-CoV-2, both structural and nonstructural proteins

have been identified as potential drug targets. The main proteases (M^{pro} or $3CL^{pro}$) of corona viruses tend to be highly conserved and are critical for viral replication. These proteins are responsible for the maturation of both nonstructural and structural viral proteins, making them a very attractive target for novel anti-coronavirus drugs. Thus, any inhibitor against these proteases (M^{pro} or $3CL^{pro}$) that can block the replication of SARS-CoV-2 would be effective for the development of therapeutic agents or antiviral drugs against SARS-CoV-2 [2].

The main protease of SARS-CoV-2, 6YNQ, is a homodimer bound to 2-methyl-1-tetralone, which has been expressed in *Escherichia coli* and has a known crystalline structure, comprising 306 amino acids [3]. One important finding is that there have been no mutations in this protein to date. This viral protease is a multifunctional protein involved in the transcription and replication of various viral RNAs and is responsible for the cleavage of the functional replicase polyproteins at 11 different sites. Additionally, 6YNQ [3] was shown to share 100% identity and similarity with 6LU7 [4] using blast-2-seq, Smith-Waterman and Needleman-Wunsch sequence alignments. In addition, Protein Data Bank (PDB) structures of 6YNQ demonstrate a better resolution (1.8 Å), which ensures better protein structure evaluations. Furthermore, R and R free, who assess the similarity between the calculated values and the observed structural factor amplitudes, were lower for these structures than 6LU7. Although the major protease, spike protein, RdRp, and the papain-like protease have been evaluated as antiviral targets, 6YNQ has not yet been evaluated for anti-COVID 19 activity. Co-crystallized ligand P6N (PDB ID 6YNQ) has been shown to interact with the binding site of M^{pro} , indicating its association with 6YNQ demonstrating the main interaction site for drug targeting [5]. Similarly, 6YNQ has been recently listed as a possible target for evaluating the efficacy of certain agents against COVID-19 [6]. Thus, 6YNQ with its superior protein structure predictions is far more likely to yield reliable docking results, which is essential in the drug discovery process.

Recently, the United States Food and Drug Administration approved the use of Gilead's Remdesivir, marketed under the brand name Veklury, for treatment of COVID-19 for hospitalized patients. This is the first and only drug approved for adults and pediatric patients (age 12 years and older, weighing at least 40 kg) in the treatment of COVID-19 patients requiring hospitalization [7, 8]. Hydroxychloroquine was under early investigation for use in COVID-19 treatment; however, initial clinical trials were withdrawn amid safety concerns raised by the WHO [9]. In order to identify any potential interactions with the SARS-

CoV-2 protease enzymes, many existing drugs such as Lopinavir, Oseltamivir, Ritonavir and Favipiravir using computational analysis have been evaluated [10, 11].

Interestingly, many bioactive compounds derived from plants that are known to exert some antiviral effects, have attracted researchers' attention, with a large panel of these compounds under evaluation for SARS-CoV-2 [12, 13]. Among them, flavonoids have been revealed as the most promising antiviral agents [14]. Flavonoids are a large class of food additives that have a positive impact on health and which have been extensively evaluated against a wide range of DNA and RNA viruses. For example, the flavones apigenin, has been reported to exert an antiviral effect against picornavirus (RNA virus), while the flavonol quercetin-3- β -galactoside was found to competitively inhibit SARS-CoV $3CL^{pro}$ in an *in vitro* assay [15]. Additionally, an *in silico* docking simulation established that Biflavone adheres to the SARS-CoV $3CL^{pro}$ binding pocket [16]. The role of flavonoids and their interactions with diverse cellular targets and pathways involved in the viral life cycle have been widely demonstrated, and when these features are considered in conjunction with the structural diversity and degree of hydroxylation in flavonoids, it is obvious that these compounds could be used against SARS-CoV-2.

In this study, we assessed the docking of 21 flavonoids and evaluated their potential as inhibitory compounds against the SARS-CoV-2 main protease 6YNQ using the AutoDock Vina software. Furthermore, we confirmed our initial findings and evaluated the structural flexibility of the docked poses for the best flavonoids using CABS-flex 2.0 software [17].

2 Materials and Methods

2.1 Platform for molecular docking

The computational docking assessment of 21 flavonoid ligands for the SARS-CoV-2 main protease 6YNQ was performed using the AutoDock Vina software, and comparative docking was performed using Swiss dock (<http://www.swissdock.ch/>), an online server that uses EADock DSS software [18, 19].

2.2 Preparation of proteins and grids

In silico analysis of 21 flavonoids was performed using a 1.80 Å crystal structure of 6YNQ from the SARS-CoV-2 main protease in complex with an inhibitor 2-methyl-1-tetralone (PDB ID: 6YNQ, with a resolution < 2 Å, R-value free < 0.25, R-value work < 0.25), which was retrieved from the PDB (<https://www.rcsb.org>). The 6YNQ protein contains a chain with

(2-{S})-2-methyl-3,4-dihydro-2-{H}-naphthalen-1-one, which was used in the macromolecule preparation [4]. The protein preparation parameters of AutoDock were then used to prepare the whole structure by deleting water molecules, adding hydrogen, and assigning partial charges using Kollman and Gasteiger, and the binding sites were identified after deleting the ligand.

2.3 Ligand preparations

For this investigation, the ligand (2-{S})-2-methyl-3,4-dihydro-2-{H}-naphthalen-1-one and the structures of each of the flavonoids under investigation were retrieved from PubChem (<https://pubchem.ncbi.nlm.nih.gov/>) and saved in SDF. Since the PDB, Partial Charge (Q) and Atom Type (T) (PDBQT) formats can all be used as input in the AutoDock Vina software, Open Babel (version 3.0.0) 21 was used to convert these SDF files to PDB [20]. A total of 24 compounds were selected to target the main protease of SARS-CoV-2, including two approved anti-RdRp compounds (Remdesivir) [21] and a recently identified compound used to treat a mild to moderate cases of coronavirus (Favipiravir) [22].

2.4 Determining compound active sites

The active sites were defined as the coordinates of the ligand in the original target protein grids, and these active binding sites in the target protein were identified using the computed atlas for surface topography of proteins (CASTp) [23] and Biovia Discovery Studio 4.5 [24]. The amino acids in the active site were used to evaluate the Grid box and docking evaluation results. PyMol software (version 1.7.4) [25] and the Protein-Ligand Interaction Profiler (PLIP) web server [26] were used to profile the interactions between the ligand-protein complexes showing the lowest binding score and RMSD < 2.0 Å.

2.5 Protein ligand docking and visualization

AutoDock Vina was used in all the docking experiments, using the optimized model as the docking target. Computational docking is executed to generate a population of promising orientations and conformations of the ligand within the binding site. The grid center for docking was set at X = 3.789, Y = -1.789, and Z = 18.846, with the grid box set to 40 Å × 40 Å × 40 Å. Flavonoids were individually evaluated in the molecular docking, and prior to their first interactions, the classical MM2 force field was applied to optimize the structures of these small molecules, ensuring that their active sites were rigid. After validation of the docking protocol, virtual

screening was accomplished using rigid molecular docking into the active site of the partner proteins. Throughout the virtual screening, both the macromolecule and the ligands were kept rigid. Finally, the binding energy limits were removed from the software, and the investigation of the 2D hydrogen-bond interactions could be completed using the Biovia Discovery Studio 4.5 program. This analysis produces a graphical output describing the hydrophobic bonds, hydrogen bonds, and their bond lengths in each docking pose.

2.6 Physicochemical properties

Lipinski's rule was used to assess the physicochemical properties of all the selected flavonoids and predict their drug-like properties, and the Swiss ADME (<http://www.swissadme.ch/>) was used to compute the SMILES structures of each compound [27].

2.7 Molecular dynamics simulations

Molecular dynamics simulations were performed using the CABS Flex 2.0 server and were based on the coarse-grained simulations of protein motion [28] over 50 cycles and 50 trajectory frames of 10 ns each with some additional distance restraints including a global weight of 1.0 applied. These were built with Poisson-Boltzmann/Generalized Born (PB/GB) molecular mechanics, and the solvent probe radius was set to 1.4 Å, the minimum atomic radius was 1 Å, the salt radius was 2 Å, the ionic strength was 0.15, and the temperature of the simulation was 1.4. These restraints allowed us to analyze the conformational stability of the receptor-ligand complex system.

3 Results

3.1 Molecular docking

The results of the molecular docking study using AutoDock Vina revealed the binding energies of the selected compounds, inhibitors and reference molecules (Table 1). Additionally, these results were compared with SwissDock. The algorithm consisted of several steps, including the generation of as many binding modes in the local docking as possible, estimating CHARMM energies on the grid, the evaluation of the most favored binding modes using FACTS, and then clustering evaluation. Cluster "0" has the best full fitness (FF) score. We submitted both our protein and phytochemicals one by one. After each docking run, the output clusters were identified and the individual conformer from each cluster with the most favorable binding mode and most negative FF score were chosen for further evaluation as these had the best fit.

Table 1 Ligands with their binding energy and Lipinski rule parameters from the PDB for 6YNQ from SARS-CoV-2

Sr. No.	Ligand	Docking score (kcal/mol)	MW (g/mol)	Rotatable bonds	H-bond acceptors	H-bond donors	TPSA	LOGP	Follow lipinski	Violations
1	Inhibitor	-5.6	160.21	0	1	0	17.07	2.11	YES	0
2	Favipiravir	-5.5	157.10	1	4	2	88.84	0.39	YES	0
3	Remdesivir	-8.6	602.58	14	12	4	213.36	3.40	NO	2
4	Rutin	-8.7	610.52	6	16	10	269.43	0.46	NO	3
5	Baicalein	-7.9	270.24	1	5	3	90.90	2.43	YES	0
6	Fisetin	-7.3	286.24	1	6	4	111.13	1.50	YES	0
7	Astragaln	-8.5	448.38	4	11	7	190.28	1.29	NO	2
8	Chrysin	-7.1	254.24	1	4	2	70.67	2.27	YES	0
9	Epigallocatechin	-6.8	306.27	1	7	6	130.61	0.98	YES	1
10	Icaritin	-6.3	368.38	4	6	3	100.13	3.26	YES	0
11	Isoquercetin	-8.1	464.38	4	12	8	210.51	0.94	NO	2
12	Isorhamnetin	-7.4	316.26	2	7	4	120.36	2.35	YES	0
13	Isovitexin	-7.5	432.38	3	10	7	181.05	1.97	YES	1
14	Kaempferol	-6.6	286.24	1	6	4	111.13	1.70	YES	0
15	Laricitrin	-7.8	332.26	2	8	5	140.59	2.24	YES	0
16	Laricitrin 3 glucoside	-8.4	494.4	5	13	8	219.74	1.89	NO	2
17	Luteolin	-6.9	286.24	1	6	4	111.13	1.86	YES	0
18	Myricetin	-7.1	318.24	1	8	6	151.59	1.08	YES	1
19	Quercetin	-7.2	302.24	1	7	5	131.36	1.63	YES	0
20	Quercetin 7 galactoside	-7.8	464.38	4	12	8	210.51	1.84	NO	2
21	Quercitrin	-8.3	448.38	3	11	7	190.28	1.60	NO	2
22	Syringetin	-7.3	346.29	3	8	4	129.59	1.77	YES	0
23	Trifolin	-8.5	448.38	4	11	7	190.28	1.44	NO	2
24	Vitexin	-8.0	432.38	3	10	7	181.05	1.63	YES	1

The inhibitor 2-methyl-1-tetralone binds to the active site of 6YNQ with the lowest binding energy (-5.6 kcal/mol), where CYS A: 145 and HIS A: 163 represent the catalytic residues (Figure 1A). Favipiravir (binding energy was -5.5 kcal/mol) docked with a confirmation that forms conventional hydrogen bonds with HIS A: 163, GLU A: 166, MET A: 165 and ASN A: 142 along with pi donor hydrogen bonds with CYS A: 145 and LEU A: 141 (Figure 1B), suggesting its interaction with the catalytic residues. In contrast, Remdesivir had the lowest binding energy of -8.6 kcal/mol, more than the inhibitor and Favipiravir, but failed to make hydrogen bonds with the catalytic residues and rather formed bonds with GLU A: 166, HIS A: 41, and THR A: 25 along with pi alkyl

interactions with PRO A: 168 (Figure 1C).

Among the 21 flavonoids, rutin was shown to bind with the lowest binding energy, -8.7 kcal/mol, which was close to the Remdesivir binding energy, forming conventional hydrogen bonds with the catalytic active site residues CYS A: 145, HIS A: 163, SER A: 46, THR A: 45, HIS A: 41, GLU A: 166, and GLN A: 189 and additional Van der Waals interactions with ASN A: 142. In addition, it produced a pi alkyl interaction with MET A: 49, re-enforcing its binding (Figure 2). However, the other flavonoid baicalein demonstrated a binding energy of -7.9 kcal/mol and formed hydrogen bonds with the active site HIS A: 163 and GLU A: 166, a pi alkyl interaction with the other catalytic residue CYS A: 145, various Van der Waals interactions

with LEU A: 141, and a pi bond with MET A: 49 and HIS A: 41 (Figure 3). Fisetin presented with a binding energy of -7.3 kcal/mol and was shown to interact with various amino acid residues producing hydrogen bonds with active site HIS A: 163, LEU A: 141, GLU A: 166, ARG A: 188, GLN A: 192, THR A: 190, PRO A:

168 and GLN A: 189, and pi alkyl interactions with the other catalytic residues CYS A: 145 and PRO A: 168 (Figure 4). The other flavonoids presented with binding energy values ranging from -8.4 kcal/mol to -5.6 kcal/mol, although there were no hydrogen bonds with the catalytic site.

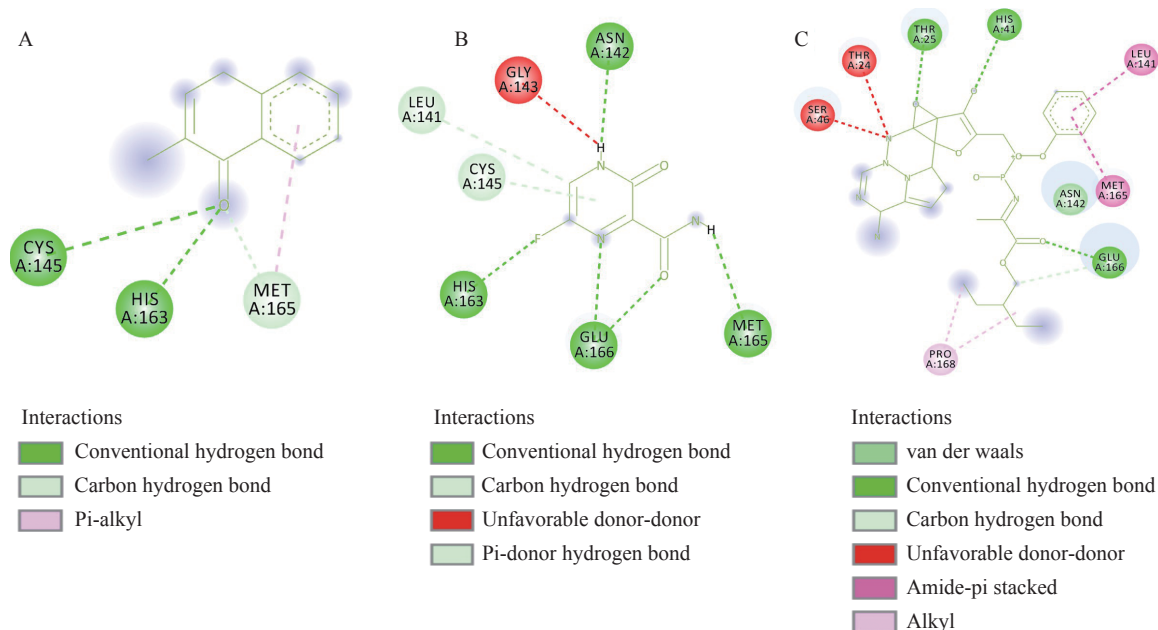


Figure 1 Docking Interactions of reference compound and inhibitors with SARS-CoV-2 main protease 6YNQ A, 2 methyl 1 tetralone. B, favipiravir. C, remdesivir.

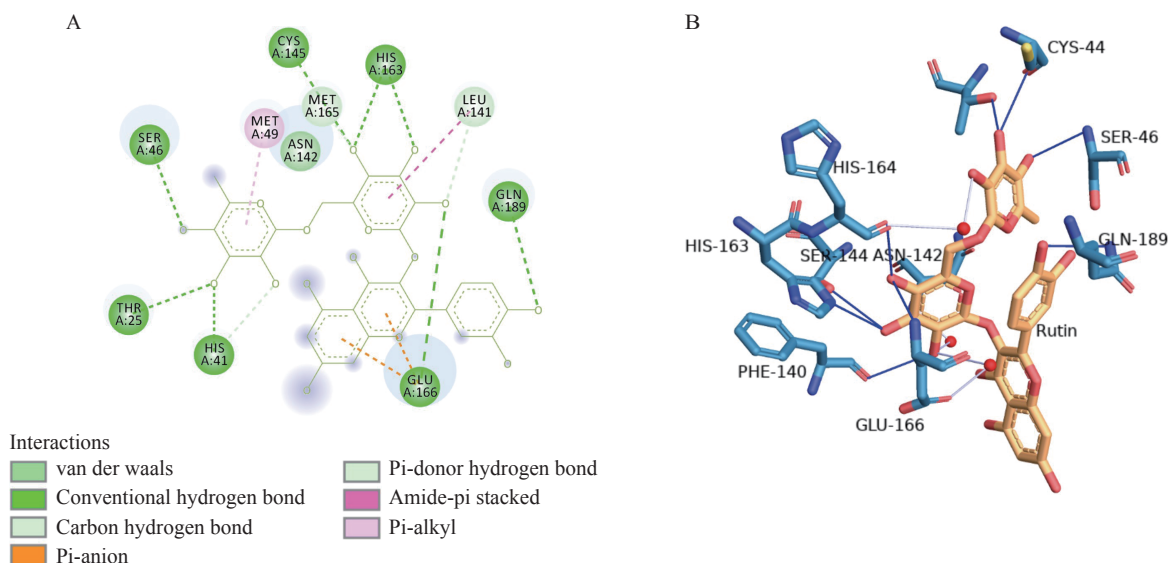


Figure 2 Interactions after docking SARS-CoV-2 main protease 6YNQ with rutin A, 2D image. B, 3D image.

3.2 Physicochemical characterization

Furthermore, the physicochemical properties of the compounds were studied to predict the pharmacokinetics of the drugs, using Lipinski's rule. Lipinski's

rules describe orally active drug compounds as having a molecular weight (MW) of < 500 Da, an octanol-water partition coefficient ($\text{Log } P$) of < 5 , a polar surface area (PSA) of $< 150 \text{ \AA}^2$, number of hydrogen bond donors (HBDs) < 5 , number of

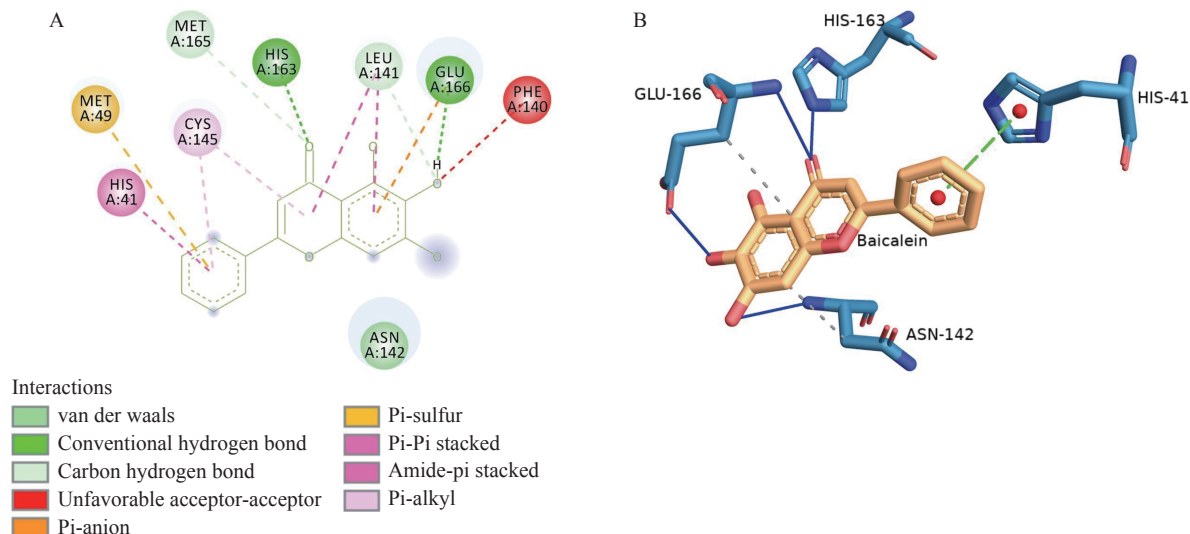


Figure 3 Interactions established after docking SARS-CoV-2 main protease 6YNQ with baicalein
A, 2D image. B, 3D image.

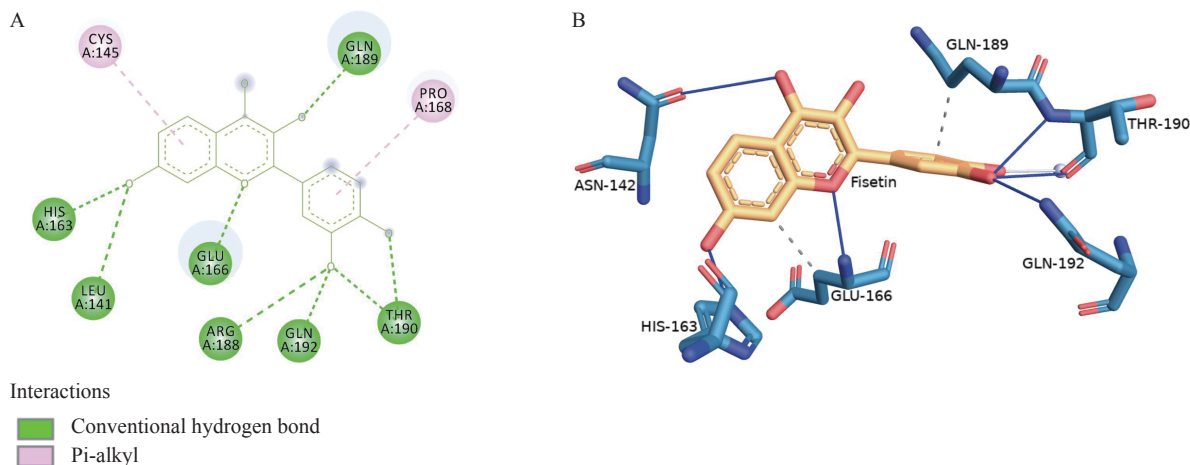


Figure 4 Interactions established after docking SARS-CoV-2 main protease 6YNQ with fisetin
A, 2D image. B, 3D image.

hydrogen bond acceptors (HBAs) < 10, and number of rotatable bonds (RBs) < 10 [29]. The Lipinski values for each of the selected compounds are listed in Table 1.

3.3 Molecular dynamics

The structural flexibility of the best three phytoconstituents in complex with 6YNQ was evaluated using CABS-flex 2.0. To validate the docking results, the structural PDB file was provided to the server with default parameters to obtain the maximum simulation output [30]. The root mean square fluctuation (RMSF) values (Figure 5) explain the fluctuation of each amino acid residue in the best docked ligand in order to validate the conformational stability of the protein-ligand docked complexes (Figure 6).

4 Discussion

Molecular docking studies of flavonoids with SARS-CoV-2 main protease 6YNQ exhibited promising results based on their binding energies, as determined by AutoDock Vina. In this study, some known antiviral and other flavonoids were selected for targeting SARS-CoV-2 main protease 6YNQ, and molecular docking studies were carried out to assess their potential antiviral effect. To evaluate the binding between the flavonoids and the targets, we selected 21 flavonoids against 6YNQ, along with their known inhibitor 2-methyl-1-tetralone, and reference compounds Remdesivir and Favipiravir. Our results suggest that most of the ligands present with nearly the same score in either docking method, with a corresponding correlation coefficient of 0.7527 between docking scores obtained using AutoDock vina and

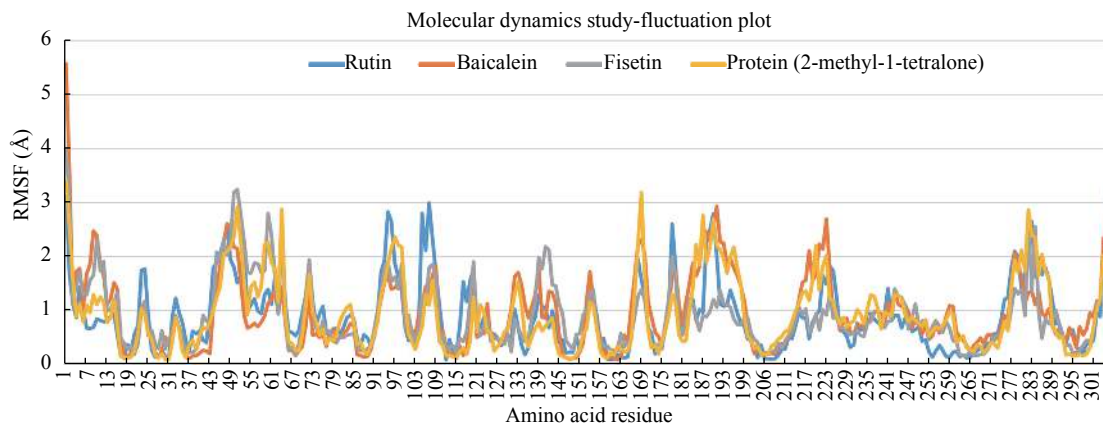


Figure 5 Root mean square fluctuations in protein structures in response to specific substrates

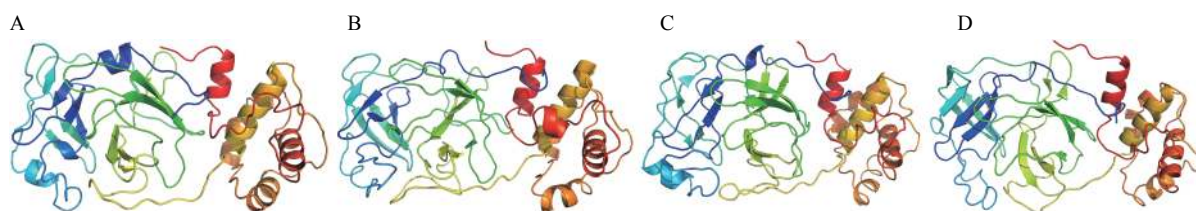


Figure 6 Stable protein structures generated following molecular dynamics simulations

A, 2-methyl-1-tetralone. B, rutin. C, baicalein. D, fisetin.

Delta G by SwissDock, supporting the accuracy of the AutoDock vina predictions. Based on these results, three flavonoids, rutin, baicalein and fisetin, should be considered potential inhibitors of SARS-CoV-2 main protease 6YNQ acting via M^{pro} 6YNQ inhibition.

Rutin demonstrated strong inhibition of 6YNQ, forming conventional hydrogen bonds with the catalytic active site residue CysA 145 and having the lowest binding energy (-8.7 kcal/mol) of any of the compounds. Taken together, this suggests that it exhibits the strongest and most stable binding. This result is in agreement with previously published data that suggest that rutin (docking score: -9.16 kcal/mol) is the most potent inhibitor for 6LU7 [31]. Other researchers have also reported that rutin is an effective inhibitor of various targets of the SARS-CoV-2 proteases [32]. These studies have confirmed that CysA 145 is a critical residue within the binding pocket of these proteases falling within a 6 \AA radius around the catalytic center of these proteins [33, 34], and support the application of rutin as a competitive SARS-CoV 3CL^{pro} inhibitor that interacts via hydrogen bonding with the catalytically active residue CysA 145.

One of the most studied flavonoids, baicalein, also forms hydrogen bonds with these proteins, targeting the other catalytic residue, i.e., histidine. Numerous studies have reported that baicalein and its analogs are strong inhibitors of SARS-CoV-2 3CL^{pro} and helicase, suggesting that baicalein is a potential candidate for combating coronavirus disease [35, 36]. In

addition, a traditional Chinese medicine formulation containing baicalein was evaluated in a neutralization study using a fRhK4 cell line infected with 10 strains of SARS-CoV-2 from 10 different patients and shown to effectively neutralize these viruses, supporting the potential clinical application of this product [37]. The flavonol fisetin also produced both hydrogen and pi alkyl bonds with the catalytic center of 6YNQ, although its binding energy was somewhat lower than rutin and baicalein. Other studies have reported binding of fisetin with 6LU7 [38]. Given these results, we propose that a combination of rutin, baicalein and fisetin may produce a synergistic inhibition of both catalytically active residues in 6YNQ, improving its overall inhibition.

Lipinski's rule is a major deciding factor when evaluating the potential of drug candidates and is often used to determine whether a compound with particular pharmacological or biological actions possesses the necessary physical and chemical properties for administration in humans. Evaluation of the molecular properties of the compounds based on the computed partition coefficient (Log *P*) demonstrated that these compounds have relatively good lipophilicity, as the Log *P* values were less than 5 [39, 40]. These results also demonstrated that both baicalein and fisetin strictly followed Lipinski's rule with zero violations, indicating that both compounds are likely to possess active drug characteristics

Low RMSF values imply limited motion within a system, while high values in the molecular dynamics

simulations reflect more flexibility [41]. The results of the molecular dynamics simulations show that there are appropriate secondary structure residues with the α -helix and β -sheet of the protein that present with minimal fluctuation when evaluated using efficient constraints in the all-atom molecular dynamics algorithm, a classical simulation approach for proteins. Rutin, baicalein and fisetin were shown to maintain their molecular interactions with the target protein under all of these conditions, confirming their likely interaction.

Interestingly, these three flavonoids are nutraceuticals and act as vital nutritional component of various fruits and vegetables. Thus, we anticipate that this nutraceutical has the potential to enhance immunity and inhibit COVID-19 infections in the population [42]. Furthermore, combination therapy of synthetic drugs with flavonoids often results in superior outcomes for antiviral treatments [43, 44]. Most flavonoid evaluations for COVID-19 have focused on 3CL as the main viral protease [45]; however, our study demonstrates the potential for flavonoid treatments to affect other targets including 6YNQ of SARS-CoV-2.

Conclusions

Human health and safety is intrinsically linked with the need to find and test novel interventions for COVID-19 (SARS-CoV-2), making any study related to these endeavors critical to global concerns. Here, we have used computational docking studies of various flavonoids against the SARS-CoV-2 main protease 6YNQ to help identify novel therapeutic effectors. We evaluated a library of 21 flavonoids and revealed that rutin, baicalein and fisetin bind the target efficiently and may have value as potential inhibitors. Thus, we conclude that these phytochemicals can be used as potential antiviral candidates and suggest that further *in vitro* or *in vivo* experiments may provide better insight into the optimal flavonoid structure for preventing and treating COVID-19.

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Competing Interests

The authors declare no conflict of interest.

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新型冠状病毒主要蛋白酶 6YNQ 黄酮类化合物抑制剂的鉴定： 一项分子对接研究

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【摘要】目的 新型冠状病毒肺炎 (COVID-19) 的病原体新型冠状病毒 (SARS-CoV-2) 是导致最近疫情全球大流行的原因, 由于目前尚无针对 SARS-CoV-2 的有效药物或疫苗, 我们研究了黄酮类化合物对抗 SARS-CoV-2 主要蛋白酶 6YNQ 的潜力。**方法** 对 SARS-CoV-2 的主要蛋白酶 6YNQ 进行计算机模拟研究。**结果** 所选的 21 种黄酮类化合物中, 芦丁结合能 (-8.7 kcal/mol) 最高, 与催化位点结合良好。**结论** 黄酮类化合物对 SARS-CoV-2 主要蛋白酶 6YNQ 有抑制作用, 这些计算模拟研究支持了黄酮类化合物可能有助于 COVID-19 治疗的假设。

【关键词】 新型冠状病毒肺炎; 新型冠状病毒; 蛋白酶 6YNQ; 计算机预测; 分子模拟; 虚拟药物筛选; 类黄酮