Flavonoids and Nucleotide Analogs Show High Affinity for Viral Proteins of SARS-CoV-2 by *in silico* Analysis: New Candidates for the Treatment of COVID-19.

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Abstract

The recent epidemic of COVID-19 caused by SARS-CoV-2 was declared by the World Health Organization as a public health emergency of international concern. The absence of an approved vaccine or a specific antiviral drug has made bioinformatic tools crucial for the identification of potential therapeutic targets and drugs for its control. As in other RNA viruses, the protease 3C-like and the RNA-polymerase are two of the SARS-CoV-2 targets to test drugs that can be analyzed in silico. In the present study, compounds derived from plants, fungi, and nucleoside 5'-triphosphate or uridine nucleotide analogs, with anti-DENV activity in vitro or in vivo, were analyzed by molecular docking as potential anti-SARS-CoV-2 drugs. Anthraquinone, with a DENV NS3 protease inhibitory activity; Balapiravir, Fisetin, Hyperoside, and Sofosbuvir, with a DENV NS5 RNA-polymerase inhibitory activity; and Quercetin, with both anti-NS3-NS5 activities, were tested against 3C-like protease and RNA-polymerase of SARS-CoV-2. All these drugs demonstrated a high affinity for the corresponding SARS-CoV-2 proteins, representing excellent candidates for the treatment of COVID-19. Therefore, in vitro or in vivo studies should be carried out using these compounds on models for SARS-CoV-2 infection.

Introduction

Coronavirus disease 2019 (COVID-19) is currently affecting millions of people worldwide. According to the World Health Organization (WHO), the COVID-19 pandemic has been declared as a public health emergency of international concern; causing more than 500,000 deaths worldwide [1,2]. Therefore; it is a priority to search antivirals that reduce or prevent COVID-19 mortality. COVID-19 is caused by the severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), a spherical virus with a positive-sense RNA genome [3]. SARS-CoV-2 viral proteins include the 3C- like protease (NSP5) and the RNA-polymerase (NSP12), two of the proposed RNA virus targets tested for different drugs [4–8]. The NSP5 protein is a chymotrypsin-like protease; which processes the polyproteins ppa1a and pp1ab; generating unique viral proteins [9]. It consists of three domains: domain I (8-100 aa), II (101-183), and III (200-302 aa). The protease catalytic site is located in the fissure between domains I and II; with a catalytic dyad of His41 and Cys145 [10]. On the other hand; the NSP12 protein is the RNA-dependent RNA polymerase (RdRp or RNA-polymerase), responsible for the replication and transcription of the genomic RNA [6,11]. This enzyme has the preserved architecture of viral RNA-polymerases. It is composed of the fingers; palm; thumb; NiRAN (nidovirus RdRp-associated nucleotidyltransferase), present only in nidoviruses such as SARS-CoV-2; and interface domains (Figure 1B) [6,11]. Therefore; it has been described that some antivirals, whose principal functions are to inhibit these two enzymes, may have a broad spectrum against RNA viruses [12,13].

Antiviral drugs have been developed to target either host cell proteins required for viral replication or viral proteins. The first approach may provide a broader spectrum of activity and less chance of developing resistance; however, inhibiting host cell proteins has a more significant impact on cell viability. For this reason, the use of antiviral drugs that target viral proteins is more specific and less toxic [14,15]. The recent closure of research laboratories worldwide as part of the containment measures to prevent the

spread of the SARS-CoV-2 virus has hindered the *in vitro* and *in vivo* trials to test the effectiveness of drugs [4]. However, the integration of computational and experimental strategies in pharmacology has been of great value in the identification and development of new antivirals. Particularly, the molecular docking analysis is a widely used bioinformatics tool in modern drug design [16].

Currently, there is no specific antiviral to content SARS-CoV-2 infection, and the expectation of an efficient vaccine continues to cause concern due to the time required for its approval and distribution. Therefore, testing the antiviral activity of FDA-approved drugs, using bioinformatic strategies, is today a promising tool for the selection of drugs that can potentially inhibit the replication of SARS-CoV-2 [7,8,17,18].

In recent years secondary metabolites of plants such as Quercetin, Fisetin, and Hyperoside flavonoids, have demonstrated antiviral effect *in vivo* and *in vitro* [19,20], in RNA virus-like dengue virus (DENV) by acting on NS5 RNA-polymerase. Moreover, its antiviral effects have also been observed in other viral infections such as with Enterovirus, Rhinovirus, Ebola virus, Chikungunya, and Zika virus [19,21–24]. On the other hand, Quercetin and Anthraquinone, secondary metabolites of plants and fungi, have demonstrated activity against the DENV NS3 protease [19,25]. Other examples of antiviral compounds are the nucleoside and nucleotide analogs such as Balapiravir and Sofosbuvir, respectively, alternative substrates for viral polymerases that competitively inhibit the synthesis of viral RNA in infections such as hepatitis C virus (HCV) and DENV [26,27] excellent candidates against the SARS-CoV-2.

In this study, we tested the affinity of Anthraquinone and Quercetin to the SARS-CoV-2 3C-like protease, and the affinity of Balapiravir, Quercetin, Fisetin, Hyperoside, and the previously tested Sofosbuvir for SARS-CoV-2 RNA-Polymerase using a molecular docking analysis. Our results showed that these drugs, previously used as antivirals for other RNA viruses, have an affinity for SARS-CoV-2 viral proteins and, therefore a potential inhibitory activity on viral replication.

Results

2.1 Validation of the three-dimensional structures and sequences of the SARS-CoV-2 3C-like protease and RNA-polymerase,

Previous to the molecular docking analyses, the crystalline structures of the SARS-CoV-2 3C- like protease (PDB ID: 6M2N) (Figure 1A) and RNA-polymerase (PDB ID: 6NUR) (Figure 1B) were validated by amino acid sequence comparison with those reported in the UniProtKB database (ID: P0C6×7). The comparison was performed using the EMBOSS Needle software (https://www.ebi.ac.uk/Tools/psa/emboss_needle/) [28], obtaining a sequence identity of 96.1% and 97.5%, respectively.

Furthermore, the quality of the protein structures was evaluated using ERRAT software (https://servicesn.mbi.ucla.edu/ERRAT/) which differentiates regions of protein structures based on atomic interaction, generating a structure quality factor in a percentage of 0-100% [29], and the Verify 3D software (https://servicesn.mbi.ucla.edu/Verify3D/) that determines the compatibility of the 3D atomic

model with its amino acid sequence and its atomic coordinates, obtaining a high score when the structure was correct [30].

The 3C-like protease structure of SARS-CoV-2 showed a score of 90.43% with the Verify3D software and a structured quality factor of 94.5% with the ERRAT software. The structure of the RNA-polymerase showed a quality factor of 96.68% with ERRAT software and 83. 81% with Verify3D. Once the three-dimensional structures of both proteins and their amino acid sequences were validated, the molecular docking assays were carried out with the candidate drugs.

2.2 Screening of DENV NS3 protease inhibitors (Quercetin and Anthraquinone) against the 3C-like protease of SARS-CoV-2

First, the molecular docking assays were validated by recreating the experimental data on the binding of the drug 5,6,7-trihydroxy-2-phenyl-4H-chromen-4-one (Baicalein) found in the same structure as the PDB ID: 6M2N with the 3C-like protease of SARS-CoV-2. The analysis found that the drug in both traditional and computational experiments joined in the same protease structure position (Figure 2A), validating our conditions. The binding energy of the drug and protease obtained from the computational data was -7.95 kcal/mol^{-1}.

Quercetin and Anthraquinone inhibitors of DENV NS3 protease bound with a high binding affinity (-6.5 and -7.95 kcal/mol^{-1}, respectively) to the 3C-like protease of SARS-CoV-2 (Figure 2B), both drugs bound to the active site of the protease, particularly to the HIS41 which belongs to the catalytic dyad. The affinity of the 3C-like protease for Quercetin and Anthraquinone was compared with the one for Baicalein, and the drug interactions with the highest affinity for the active site were graphed based on an RMSD <1 Å (Figure 4A). Baicalein had the higher affinity for the active site of SARS-CoV-2 3C-like protease (-7,946 ± 0.005 kcal/mol^{-1}) followed by Quercetin (-7,562 ± 0.243 kcal/mol^{-1}), and Anthraquinone (-6.5 ± 0 kcal/mol^{-1}).

2.3 Screening of DENV NS5 polymerase inhibitors against SARS-CoV-2 RNA polymerase

In the case of the SARS-CoV-2 RNA polymerase, a crystal structure coupled to an inhibitor was not found in the PDB database. Therefore, it was not possible to recreate the data obtained by traditional methods. However, Remdesivir has been described as an RNA-polymerase inhibitor and has been approved as a therapeutic drug against SARS-CoV-2 [31–33]. Therefore, to perform the Molecular Docking assays, we used Remdesivir as a control and then proceeded to test the drugs selected for our study. Fisetin, Quercetin, Hyperoside, Balapiravir, Sofosbuvir, and Remdesivir bound to two RNA-polymerase sites with high affinity, the RNA-polymerase active site, and the NiRAN-Fingers site (Figure 3). On the other hand, Quercetin, Fisetin, Balapiravir, and Sofosbuvir showed a higher affinity for the active site of the RNA-polymerase (-7.26, -7.08, -7.25 and -7.23 kcal/mol^{-1}, respectively), respect to the control drug Remdesivir (-6.81 kcal/mol^{-1}) (Figures 3A and 3B). Hyperoside showed a lower affinity (-6.68 kcal/mol^{-1}); however, it is not ruled out as an RNA- polymerase inhibitor, since in predicting the number and type of non-covalent

interactions using the PLIP software, it was found that Hyperoside has the maximum possible number of non-covalent interactions with RNA-polymerase (Table 2).

Besides, to the high affinity of the tested drugs to the active RNA polymerase site, a high affinity for the NiRAN sub-domain was also found. The NiRAN subdomain is present only in nidoviruses such as SARS-CoV-2, it has a nucleotidylation activity and is essential for viral replication; its mutation generates non-infective viral particles. It has also been involved as a protein primer for RNA synthesis, as the Vpg primer activity exhibited in Picornaviruses [34,35]; reasons why it might be an excellent target to inhibit viral replication.

Fisetin, Balapiravir, Quercetin, and Sofosbuvir exhibited a higher affinity (-8.1, -8.06, -7.79, -7.74 kcal/mol^{-1}) for the RNA polymerase NiRAN-Fingers site, compared to the Remdesivir control (-7.67 kcal/mol^{-1}) (Figure 3C and 3D). Hyperoside showed a lower ΔG (-7.43 kcal/mol^{-1}), compared to the control; however, it exhibits the highest number of non-covalent interactions (Table 2).

Finally, to compare the affinity of drugs for the RNA polymerase, the drug interactions with the highest affinity for the active and NiRAN sites were graphed based on an RMSD <1 Å (Figure 4B and 4C). Of the drugs analyzed, Fisetin and Quercetin have a higher affinity for the active site of the RNA polymerase (-7,042 ±0.035 and -7,038 ±0.304 kcal/mol^{-1} respectively) (Figure 4B), showing that both can be excellent candidates for binding the SARS-CoV-2 RNA polymerase. Similar affinities of Fisetin and Quercetin to both the RNA-polymerase active and NiRAN sites were found since no significant differences were obtained (Figure 4C).

2.4 Comparison of non-covalent interactions and ligand-binding energy, as well as drug legislation and their toxicity

Finally, a comparison of the different drugs analyzed as possible inhibitors of 3C-like protease and SARS-CoV-2 RNA-polymerase is shown in Table 1, including their PubChem access number, binding energy, toxicity reported by PubChem and the FDA legislation. Additionally, using the PLIP software that compares probable interactions according to distance thresholds and angles reported in the literature to give a probable report of non-covalent interactions between the target and the ligand [36]. We show the possible non-covalent interactions between the compounds and their targets (Table 2).

Table 1. Results of the screening and characteristics of the drugs against 3C-like protease and RNA-Polymerase of SARS-CoV-2. Ligand-target binding energy, toxicity, and drug legislation.

	PubChem CID	Name	Binding Energy (kcal/mol^{-1})		Toxicity (PubChem)	FDA Approved
3C-like protease	5281605	Baicalein (Reference PDB ID: 6M2N)	-7.95		Not available	Yes
	5280343	Quercetin	-7.62		Unlikely cause of clinically apparent liver injury	Yes
	6780	Anthraquinone	-6.5		Not described in human	Yes
			Binding Energy (kcal/mol^{-1})			
			Active site RdRp	NiRAN		
NSP12 RNA-polymerase	121304016	Remdesivir (Reference)	-6.81	-7.67	Not available	Yes
	5280343	Quercetin	-7.26	-7.79	Unlikely cause of clinically apparent liver injury	Yes
	11691726	Balapiravir	-7.25	-8.06	Not available	Yes
	45375808	Sofosbuvir	-7.23	-7.74	Unproven but suspected cause of clinically apparent liver injury in susceptible individuals	Yes
	5281614	Fisetin	-7.08	-8.1	Not described in human	Yes
	5281643	Hyperoside	-6.68	-7.43	Not available	Yes

Table 2. Non-covalent interactions of drugs and their targets. These were obtained by using the PLIP (Protein-Ligand Interaction Profiler) software.

	Drug	Number of interactions	Hydrogen Bonds	Hydrophobic Interactions	Π-Π Interaction	Salt Bridges
3C-like protease	Baicalein (Reference PDB ID: 6M2N)	5	ASN142, GLY143, GLU166 (2)	MET165		
	Quercetin	11	TYR54, GLU166 (2), ASP187, GLN192 (2)	HIS41, MET165, LEU167, GLN192		
	Anthraquinone	7	GLY143, SER144, CYS145, GLU166	PHE140, GLU166	HIS163	
NSP12 RNA Polymerase (Active site RdRp)	Remdesivir (Reference)	11	ILE494, ASN496 (2), ASN497, ARG569, GLN573, LEU576, LYS577, TYR689	ASN496		LYS577
	Quercetin	7	TYR619, LYS621, CYS622, ASP623 (2), ASP761, GLU811			
	Balapiravir	9	LYS551, ASP618 (2), LYS621 (2), SER795	VAL166, GLU167, PRO620		
	Sofosbuvir	9	TYR619 (3), LYS621 (2), CYS622, SER795 (2)			
	Fisetin	11	PHE442 (2), GLN444, ILE548, SER549, ARG553 (2)	LYS545, LYS551 (2), ARG553		
	Hyperoside	13	HIS439, SER549, ALA550, LYS551, GLN815 (2), ASP833, ARG836	ALA550, LYS551, ARG836		HIS439
NSP12 RNA Polymerase (NiRAN)	Remdesivir (Reference)	11	ARG349, THR394, PHE396, ASN459 (2)	THR319, PRO323, PHE396 (2), VAL675, PRO677		
	Quercetin	11	THR319, LEU460, THR462 (3), ASN (628), MET629	VAL315, THR319, ASN628		
	Balapiravir		PHE321, ARG349, ASN459, LEU460, ASN628	VAL315, PRO323, ARG349, ARG457, VAL675, PRO677		
	Sofosbuvir	8	ARG249 (2)	LEU172, THR246, LEU251, LEU316, THR319, LEU460		
	Fisetin	10	THR319, LEU460, THR462 (2), ASN628 (2), MET629	THR319, GLU350		
	Hyperoside	16	THR246, ARG249, LEU251, SER255, THR319 (2), ARG349 (2)	ARG249, PRO461		

Discussion

The World Health Organization has declared the recent epidemic of COVID-19 caused by SARS-CoV-2, as a public health emergency of international interest [2]. Approximately 17 million cases and more than 650,000 deaths for COVID-19 have been reported until mid-2020. America, the continent most affected had reported 7 million new cases in 24 hours [1]. Given this, it is crucial to find drugs as antivirals to inhibit viral replication. However, the closure of research laboratories to prevent the contagion and spread of the virus has made it difficult to conduct trials to test the effectiveness of drugs [37]. Therefore, an alternative strategy is a search for drugs against SARS- CoV-2 using computational techniques, whose *in vitro* and *in vivo* action could be later corroborated by traditional experimental methods.

Viral proteins such as 3C-like protease and RNA-polymerase of SARS-CoV-2 are two of the proposed targets for preventing viral replication [4,5]. 3C-like protease (NSP5) is a chymotrypsin- like protease, which processes the polyproteins ppa1a and pp1ab, generating unique viral proteins involved in the first steps of the virus replication [9]. The RNA-polymerase (NSP12) is responsible for the replication and transcription of the genomic RNA, together with the NSP7 and NSP8 cofactors required to stimulate its polymerase activity [11]. Given the critical functions of both proteins, they have been widely proposed as targets for pharmacological action.

Remdesivir is a drug that inhibits the action of different viral RNA polymerases, and Inhibitory activity against SARS-CoV-2 has been demonstrated. It has been tested as a treatment option in phase III clinical trials with patients, showing an improvement; however, there is still mortality of 13% of the patients, and in 60% of those treated, side effects such as liver problems, diarrhea, skin rash, kidney failure, and hypotension occurred [38,39].

Therefore, the search for new effective treatments for COVID19 disease with fewer side effects, which can be used in severe patients and which are available to the entire population, must be studied.

In this work, we analyzed utilizing molecular docking techniques, drugs with a previous in vitro, or in vivo anti-DENV activity. Anthraquinone with a DENV NS3 protease inhibitory activity, and Balapiravir, Fisetin, Hyperoside, and Sofosbuvir with a DENV NS5 RNA-polymerase inhibitory activity. Also, Quercetin with anti-DENV NS3 and NS5 activity was tested [40,41]. Here, the ability of these compounds to bind to SARS-CoV2 NSP5 protease and RNA-polymerase NSP12 were tested.

Fisetin, Quercetin, Hyperoside, and Anthraquinone are drugs derived from plants and fungi. Fisetin and Quercetin are the drugs that exhibited the highest affinity for the SARS-CoV-2 RNA- polymerase active site (-7.08 kcal/mol^{-1} and -8.1 and -7.26 kcal/mol^{-1} respectively) and the NiRAN- Fingers domain, and concerning Remdesivir control (-6.81 kcal/mol^{-1} for the active and 7.67 kcal/mol^{-1} and the NiRAN-sites), suggesting that so they may be good anti-SARS-CoV-2 drug candidates.

Fisetin and Quercetin are flavonols and secondary metabolites from a diversity of plants (e.g., *Rhus cotinus* and *Quercus* spp.), with an antibiotic, antiviral, antioxidant, anti-inflammatory, and anti-cancer

activities [42,43]. The DENV2 activity of Quercetin was demonstrated in experimental studies with a half-maximal inhibitory concentration (IC50) of 28.9 μg/ml^{-1} and the half-maximal cytotoxic concentration (CC50) of 252.6 μg/ml^{-1} in Vero cells [19]. In DC-SIGN infected cells, Quercetin showed an IC50 of 24.5 μM and a CC50 of 340 μM. It was also found that both Quercetin and Fisetin inhibit DENV2 and DENV3 infection in the absence or presence of antibodies (anti- DENV) and negatively regulate the production of pro-inflammatory cytokines induced during severe DENV infection [44]. Quercetin has been described as a candidate in the prevention and treatment of COVID-19, due to its efficacy in other antiviral diseases, low cost, bioavailability, and low toxicity [45]. Our study would reinforce the experimentally use of this drug in patients with COVID-19.

Hyperoside showed the greatest number of interactions with the NiRAN domain and the active site of RNA-polymerase (Figure 3 and Table 1B); this metabolite comes from the extract of *Houttuynia cordata*, which is consumed in Thailand. It has shown an anti-DENV activity by slowing down viral RNA synthesis and inactivating infection by blocking the virus entry into HepG2 cells. In DENV-infected LLC-MK2 cells, an EC50 = 0.8 μg/ml and a CC50 of 1.24 mg/ml were obtained [46,47]

In this study, the drug with the lowest binding score to the NSP5 protease was Anthraquinone with -6.5 kcal/mol^{-1}; however, this binding energy is comparable to that exhibited by Remdesivir (-6.4 kcal/mol^{-1}) in other studies, using Autodock Vina [48]. Therefore, its use and effectiveness for Covid-19 cannot be ruled out. Anthraquinones are plants (*Rheum palmatum*, *Cassia obtusifolia*, *Morinda panamensis*, etc.) [49] and fungal (*Monascus* spp.) metabolites with pharmaceutical, food, and clothing dye use [50]. Experimental studies with both computational and traditional DENV2 have shown that it reduces viral replication by a concentration of 4.2 μM (EC50) and a CC50 of 69 uM in LLC-MK2 cells [25]. Anthraquinone also has been tested on DENV infected Huh7 cells, where an EC50 was of 2.69 μM, and a CC50 of 106.6 μM were found, in which there was an absence of cytotoxicity during three days of incubation [51]. Besides, anthraquinone derivatives have been shown to have anti-Herpetic, anti-DENV, anti-human immunodeficiency virus (HIV), and antineoplastic effects [41,52,53].

The RNA-polymerase inhibitors Balapiravir and Sofosbuvir demonstrated a high affinity for the active and the NiRAN sites of SARS-CoV-2 RNA-polymerase, even higher than the drug Remdesivir, suggesting they can be excellent candidates for the treatment of COVID-19.

Balapiravir is a nucleoside 5'-triphosphate analog and Sofosbuvir is a uridine nucleotide analog, which are alternative substrates for viral polymerase and competitively inhibits viral RNA synthesis [54,55].

Balapiravir was developed for the treatment of hepatitis C virus (HCV) and was tested in humans with chronic phase 1b HCV infection, exhibiting a viral suppression of 3.7 log(10) in 14 days of treatment at its highest dose of 4500 mg, where reversible hematological changes occurred in the patients. The tolerable dose of 300 mg reduced the infection by 2.6 log(10) [56]. Because of its similarity between the NS5 polymerases of both HCV and DENV, Balapiravir was tested in phase II human trials, in which it showed no effect on viremia or fever elimination [57]. However, another study reported that Balapiravir in its

triphosphate form acts during DENV infection, but cell activation may decrease the conversion of the drug by requiring a host enzyme and losing its activity [58]. Therefore, the conversion and effectiveness of the drug in infections with SARS-CoV-2 should be analyzed.

As Balapiravir, Sofosbuvir is a prodrug that must be triphosphorylated in the cell for its action. Clinical studies have been done in phases I and II, and it currently has an FDA approval for the treatment for chronic HCV infections [55]. In Huh7 cells infected with DENV2, Sofosbuvir has been shown to have an affinity for NS5 polymerase, with an EC50 of 4.9 μM and a CC50> 100 μM [59]. Currently, it has been proposed as a drug for the treatment of COVID-19, using a comparative analysis of the binding site in the structure of the RNA-polymerase of SARS-CoV2 and that of HCV [60]. The results presented here and in those previous results from Jácome et al., 2020, promote the study and use of Sofosbuvir, as well as plant and mushroom derivatives Quercetin, Fisetin, Anthraquinone and Hyperoside as a COVID-19 treatment.

Materials And Methods

4.1 Structure of NSP5 (3C-like protease) and NSP12 (RNA-polymerase) from SARS-CoV-2

The structure of 3C-like protease (X-Ray diffraction, 2.20 Å resolution) and RNA polymerase (Electron Microscopy, 3.10 Å resolution) of SARS-CoV-2 were obtained from the Protein Data Bank library (RCSB PDB) with PDB ID: 6M2N and 6NUR, respectively. The quality of the protein structures and the sequence was evaluated using ERRAT [29], Verify 3D [30], and EMBOSS Needle [28] software.

4.2 *Drugs candidates tested*

Six drug candidates with inhibitory activity against 3C-like protease and SARS-CoV-2 RNA- polymerase were selected through literature reviews of drugs with inhibitory activity of viral proteins against DENV [40,41]. They were selected based on *in vitro* and/or *in vivo* experimental background, in addition to previous Food and Drug Administration (FDA) development and approval processes.

The selected drugs were obtained from the PubChem database, and are listed below with their PubChem CID: Anthraquinone (6780) against 3C-like protease and Balapiravir (11691726), Fisetin (5281614), Hyperoside (5281643) against RNA-polymerase from SARS-CoV-2. Quercetin (5280343) was tested against both viral proteins from SARS-CoV-2, as it showed inhibitory activity against NS3 protease and NS5 RNA-polymerase from DENV.

4.3 *Molecular docking of drugs against 3C-like protease and RNA polymerase of SARS-CoV-2*

The Autodock4 and AutoGrid4 software [61–63], were used for the molecular docking analysis of drug candidates against 3C-like protease and RNA-polymerase from SARS-CoV-2.

Previously, the crystalline structures of 3C-like protease and RNA-polymerase were obtained from the PDB and minimized with the PyMOL 2.3.3 software [64] and the text editor Kate 2.2 [65], removing water and

other molecules associated with the PDB file [66].

Then, the ligand and target structures were prepared with the AutoDockTools 1.5.6 software, adding polar atoms and charges [62]. The grid box parameters used were 126 Å, 126 Å and 126 Å (X, Y, and Z), grid spacing of 0.375 Å and center grid box (X, Y, and Z) of -35.667 Å, -47.402 Å and

37.332 Å (3C-like protease) and 97.115 Å, 97.809 Å and 94.063 Å (RNA-polymerase). The genetic algorithm parameters used were: Number of GA Runs 100, Population size 150, Maximum Number of evals medium 10000000, the other values were used by default. The best interaction model was chosen based on the lowest calculated ΔG.

The validation of the molecular docking was based on recreating the experimental data of the crystalline structures of 3C-like protease (PDB ID: 6M2N) (Su, H.X., et al, to be published) and RNA polymerase (PDB ID: 6NUR) [11] linked to its inhibitor 5,6,7-trihydroxy-2-phenyl-4H-chromen-4-one and Remdesivir, respectively.

4.4 Visualization of molecular docking results

The molecular docking results were analyzed with the AutoDockTools 1.5.6, PyMOL 2.3.3, and UCSF Chimera 1.14 software [62,64,67], to visualize the best target-ligand bond formation with lower ΔG. The number and type of non-covalent interactions between the drug and the viral protein were obtained using the PLIP software [36].

4.5 *Statistical analysis*

The higher affinity interactions between the candidate drugs directed to the active sites or the NiRAN domain of 3C-like protease and the RNA-polymerase of SARS-CoV-2, with RMSD <1 Å, was statistically analyzed using ordinary one-way ANOVA with Dunnett's multiple comparisons test to determine significant differences among ΔG (kcal/mol) means ± standard deviation among the control drugs (Remdesivir and Baicalein) and the candidate drugs (Fisetin, Quercetin, Hyperoside, Balapiravir, Sofosbuvir, and Anthraquinone) using the GraphPad Prism software version 6. The results were considered statistically significant when p values were < 0.05.

Declarations

Author Contributions: LADJ-G, JFO-R, and JMR-R collaborated in generating the experimental data. LADJ-G, JFO-R, JMR-R, CNF-M, SNP-R, CDC-R, AMH-M, ALG-E, and RMdÁ, collaborated equally in the manuscript writing. ALG-E and RMdÁ, coordinated and edited the manuscript. All authors have read and agreed to the published version of the manuscript.

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Figures

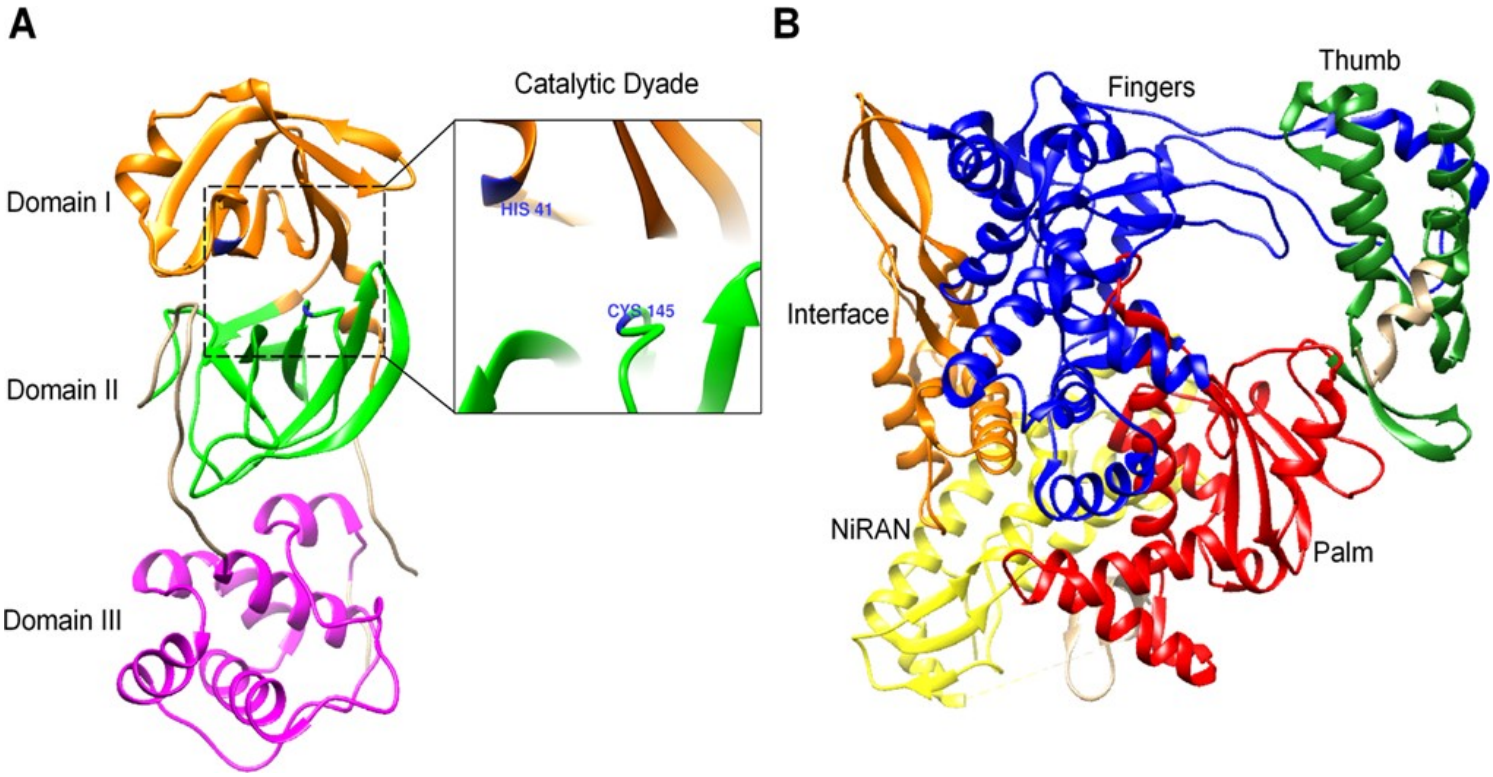

Figure 1

Overview of the structure of NSP5 (3C-like protease) and NSP12 (RNA-Polymerase) from SARS-CoV-2. A) Structure of the 3C-like protease (Doman I-orange, Domain II-green, Domain III-purple) and its catalytic dyad (blue). B) Structure of RNA-Polymerase and its different domains (Fingers-blue, Thumb-green, Palm-red, Interface-orange, NiRAN-yellow).

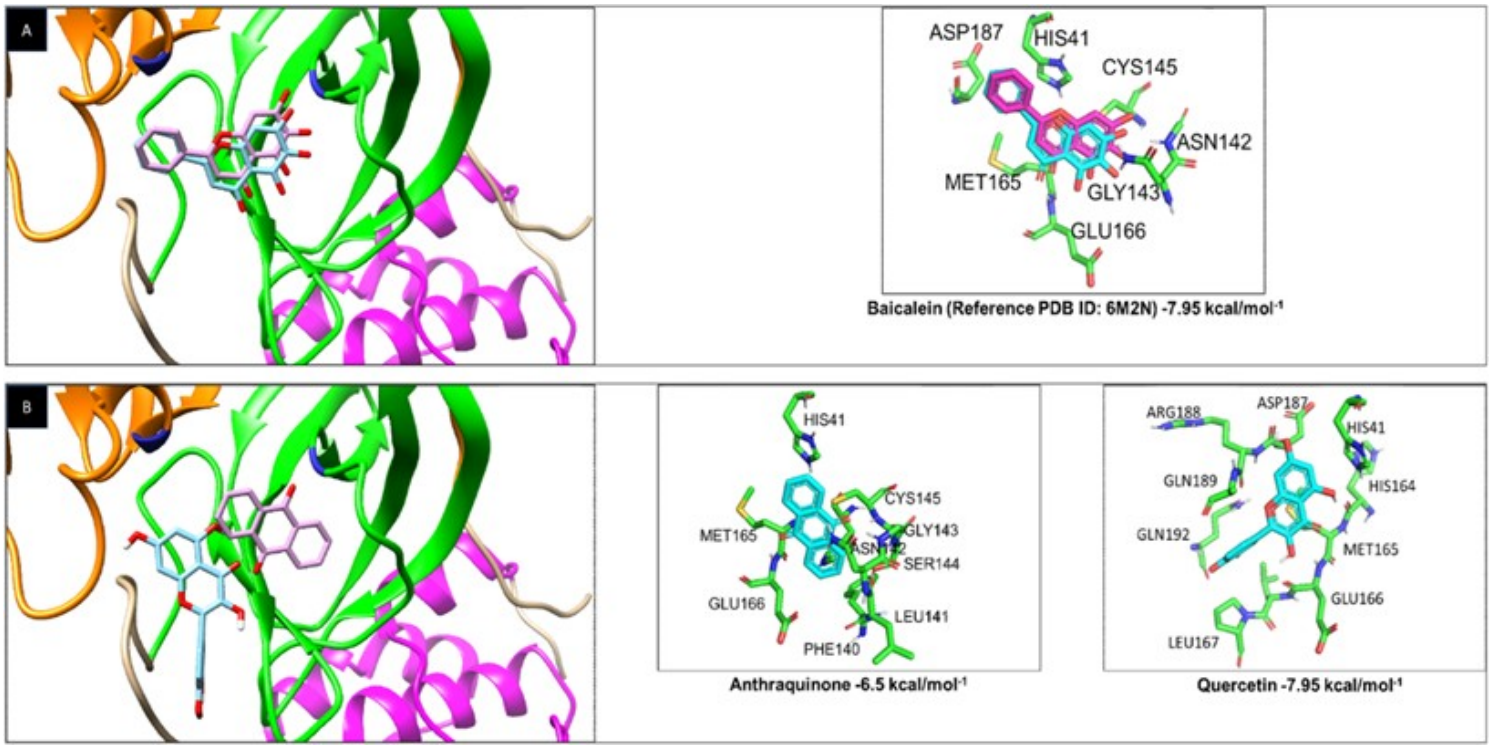

Figure 2

Baicalein, Quercetin, and Anthraquinone bind with a high affinity to the active site of the 3C-like protease of SARS-CoV-2. A) Recreating experimental results by Molecular Docking (Rosa. Experimental reference by crystallography assays of the binding of 5,6,7-trihydroxy-2-phenyl-4H- chromen-4-one (PDB ID: 6M2N) to the active site of the 3C-like protease; Blue. Binding results by Molecular Docking Assays.). B) Molecular Docking of Anthraquinone and Quercetin to the active site of the protease. In both cases, the domains I (orange), II (green), III (purple), and the catalytic dyad (blue) of the 3C-like proteases are shown. Besides, Amino acids are shown to interact with different drugs and biding energy.

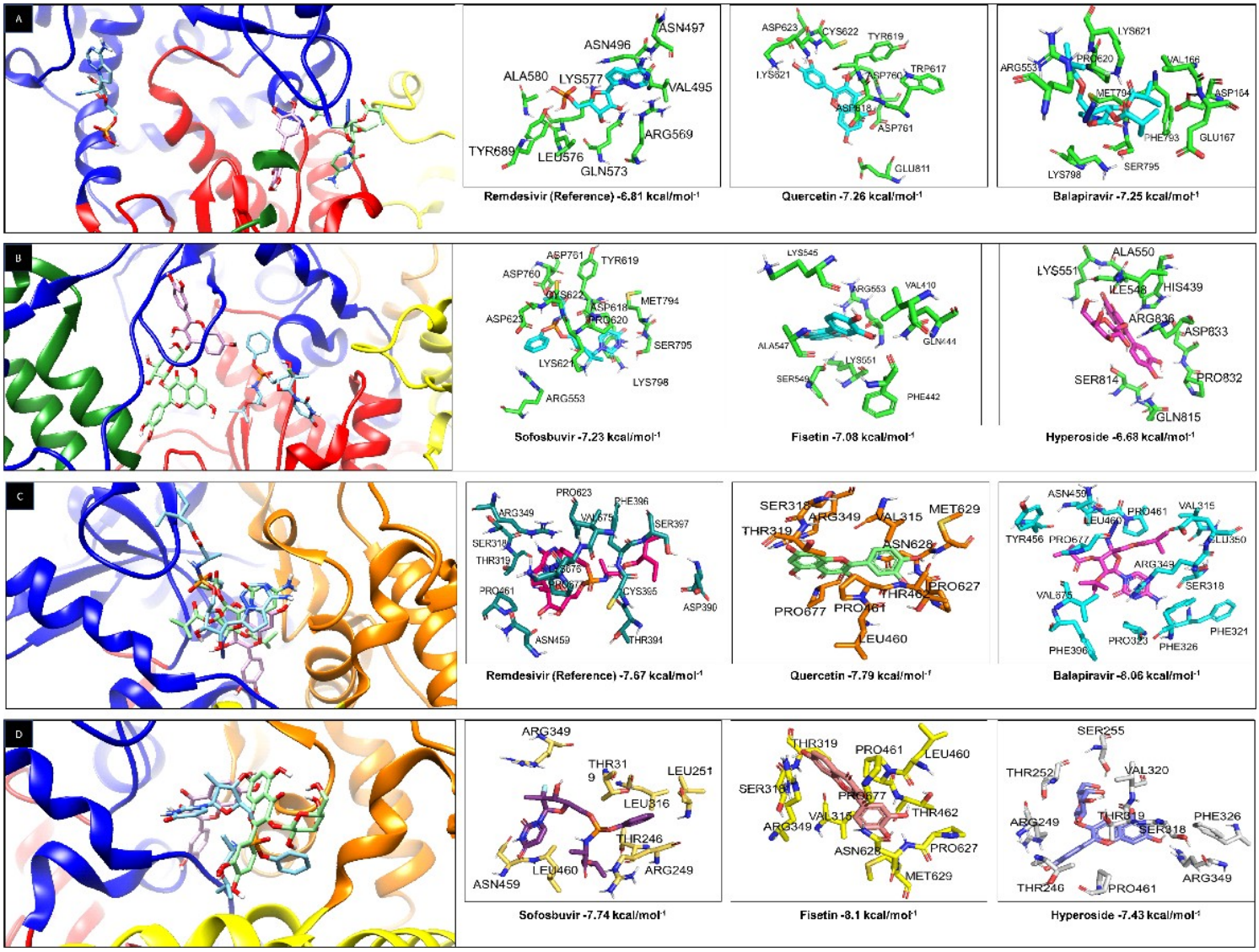

Figure 3

Remdesivir, Quercetin, Fisetin, Hyperoside, Balapiravir, and Sofosbuvir bind with high affinity to the active site and the NiRAN domain of the SARS-CoV-2 RNA-polymerase. A) Molecular Docking of Remdesivir (blue), Quercetin (pink), and Balapiravir (green) to the active site of the RNA- Polymerase. B) Molecular Docking of Sofosbuvir (blue), Fisetin (pink), Hyperoside (green) to the active site of the RNA-Polymerase. C) Molecular Docking of Remdesivir (blue), Quercetin (pink), and Balapiravir (green) to the NiRAN domain of the RNA-Polymerase. D) Molecular Docking of Sofosbuvir (blue), Fisetin (pink), Hyperoside (green) to the RNA-Polymerase NiRAN domain site. RNA-Polymerase Fingers domain (blue), Thumb (green), Palm (red), Interface (orange), and NiRAN (yellow) are shown. Also, amino acids are shown to interact with different drugs and binding energy.

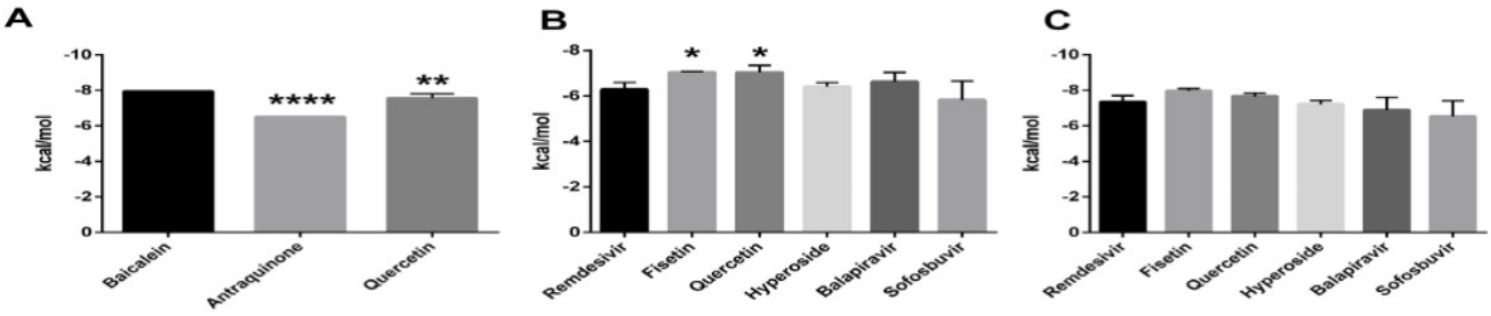

Figure 4

Comparison of drug affinities by 3C-like protease and SARS-CoV-2 RNA polymerase. A) Drug affinities by the active site of the 3C-like protease. B) Drug affinities for the active site of RNA polymerase. C) Affinities of drugs by the NiRAN site.