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Research Article

Screening of Preferential Binding Affinity of Selected Natural Compounds to SARS-CoV-2 Proteins Using in Silico Methods

Nikolina Tomic,[1] Lejla Pojskic,[1] Abdurahim Kalajdzic,[1] Jasmin Ramic,[1] Naida Lojo Kadric,[1] Tarik Ikanovic,[2] Milka Maksimovic,[2] Naris Pojskic[1]

[1]University of Sarajevo – Institute for Genetic Engineering and Biotechnology, Sarajevo, Bosnia and Herzegovina
[2]Faculty of Science-University of Sarajevo, Sarajevo, Bosnia and Herzegovina

Abstract

Objectives: The global burden of the current severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) causing the corona virus disease-19 (COVID-19) is enormous. No definitive treatment and prophylactic guidelines for COVID-19 currently exist except for physical distancing and aerial barriers between individuals. This work explored the natural compound-binding efficiency of SARS-CoV-2 proteins essential for host cell interaction and infection.

Methods: The binding activity of artemisinin to SARS-CoV-2 spike glycoprotein (Protein Data Bank (PDB) ID: 6VYB), SARS-CoV-2 main protease (3C-like main protease (3CLpro); PDB ID: 6Y84) and SARS-CoV-2 papain-like protease (PLpro; PDB ID: 6W9C), were tested using in silico methods. Moreover, chloroquine and hesperidin were used as the positive control of binding affinity and proven therapeutic effect, respectively.

Results: The highest affinities for binding to all tested SARS-CoV-2 proteins are observed for hesperidin (−5.8,−10.0, and −8.1 kcal/mol), then for artemisinin (−4.8,−8.3, and −6.0 kcal/mol), and the lowest for chloroquine (−4.1,−8.2, and −4.8 kcal/mol). Artemisinin, hesperidin, and chloroquine had similar positioning toward targeted proteins at specific sites when these interactions were visualized.

Conclusion: This study shows that artemisinin has the potential to bind and inhibit the SARS-CoV-2 spike protein, the 3CLpro main protease, and PLpro proteinase similar to hesperidin and chloroquine that have been proven as antivirals in previous preclinical and clinical studies.

Keywords: Artemisinin, molecular docking study, SARS-CoV-2 proteins

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Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has spread rapidly since its first identification in patients with severe pneumonia in China causing the pandemic coronavirus disease (COVID-19). As of August 2020, more than 22 million cases have been confirmed worldwide with an estimated mortality risk of 1%–4% (https://covid19.who.int). Consequently, several vaccines and treatments are registered as in progress at the clinical trials stage (clinicaltrials.gov).

Screening and repurposing of available drugs registered for human use are major challenges associated with the search for COVID-19 treatment. In that respect, antiviral drugs with defined safety profiles are of special interest.[1, 2] Also, small-molecule agents that are available and safe for human use are being extensively studied for their application in anti-SARS-CoV-2 treatments.[2] However, the reproducibility of curative effects for many tested antiviral drugs remains elusive and will probably need extended trials.[3] Notably, several studies explored the use of bioactive and antiviral compounds in herbal preparations in the treatment of specific COVID-19 symptoms, but additional clinical evaluations are needed to validate their recommended use.[4] Their curative, antiviral activity would depend upon its ability to inhibit viral attachment to human cell receptors or biosynthesis of viral

Address for correspondence: Lejla Pojskic, Prof. Dr. University of Sarajevo, Institute for Genetic Engineering and Biotechnology, Zmaja od Bosne 8 (Kampus),71000 Sarajevo, Bosnia and Herzegovina
Phone: +38733220926 **E-mail:** lejla.pojskic@ingeb.unsa.ba / lejla.pojskic@gmail.com
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proteins in the host cell. In silico methodology proved as an indispensable approach in the identification of natural compounds as molecular candidates with known structure and safety profiles that interact with viral proteins and as such may serve as prophylactic or adjuvant support in combating severe respiratory and associated symptoms in COVID-19.[5]

Several in silico studies showed that hesperidin is capable of binding, with low binding energy, to the key SARS-CoV-2 proteins such as the main protease (Mpro or 3C-like main protease (3CLpro)) and spike glycoprotein.[6-11] In addition, hesperidin is among the compounds with the lowest binding energy and maximum affinity to SARS-CoV-2 proteins, suggesting that it could act as an effective antiviral agent.[12] Chloroquine is also one of the most mentioned substances in studies of anti-SARS-CoV-2 agents. Many contrary results exist but docking studies on chloroquine mostly showed prominent binding interaction with SARS-CoV-2 proteins.[13]

The chemical structure and application of artemisinin have been widely explored placing this compound in the focus of pharmacological research. It is a natural compound of *Artemisia annua* L., sweet wormwood, a highly aromatic herb of Asian and Eastern European origin that is nowadays naturalized worldwide.[14, 15] By chemical nature, artemisinin is a sesquiterpene lactone endoperoxide present in the aerial parts of this one of the most widespread industrial plants in the world.[16] Moreover, artemisinin has shown an antimalarial, antiparasitic, and anti-inflammatory effect in various experimental designs. However, it should be used with caution due to its side effects.[17] Although in vitro studies exist in Vero E6 cells that confirmed the antiviral activity of physiologically active concentrations of artemisinin in combined treatments, some authors still support that additional trials are needed to test the utility of artemisinin as an inexpensive and widely available antiviral bioactive compound against SARS-CoV-2.[18-20] Additional trials may be helpful to elucidate if a plausible risk exists for the development of tolerant Plasmodium strains within the population treated with artemisinin and other antimalarials.[21]

The binding characteristics of artemisinin to SARS-CoV-2 spike glycoprotein, SARS-CoV-2 main protease (Mpro), and SARS-CoV-2 papain-like protease (PLpro) were tested to explore the potential mode of the inhibitory activity of artemisinin toward SARS-CoV-2. The drugs that showed better noncovalent interaction and binding energy, chloroquine and hesperidin, were selected further for cross-validation in AutoDock Vina1.1.2.

Methods

The three-dimensional structures of the two target proteins SARS-CoV-2 spike glycoprotein (Protein Data Bank (PDB) ID: 6VYB), SARS-CoV-2 main protease (PDB ID: 6Y84) and SARS-CoV-2 papain-like protease (PDB ID: 6W9C), were retrieved from the Protein Data Bank in PDB format (https://www.rcsb.org/). The PDB formats of ligands structures, artemisinin (accession no: DB13132), chloroquine (accession no: DB00608), and hesperidin (accession no: DB04703), were obtained from the DrugBank database (https://www.drugbank.ca/). The target proteins and ligands were prepared for docking analysis and converted into PDBQT format using AutoDock tools (MGLTools 1.5.6.). AutoDock Vina 1.1.2 was used for testing the receptor–ligand docking following the standard procedure.[22] The docking position was defined at the sites and used reports available in the literature, which were relevant for protein function. A gridbox (30 Å × 30 Å × 30 Å), for docking with SARS-CoV-2 Mpro, was defined and centered at 9.204, −4.557, and 19.602 based on previously reported methodology and identification of catalytic His41, His164, and Cys145.[23] The catalytic site of SARS-CoV-2 PLpro (Cys111,His272, and Asp286) was identified and the grid box (30 Å × 30 Å × 30 Å) was centered at −36.007, 24.121, and 34.327 for the x,y, and z coordinates, respectively.[24] For docking with SARS-CoV-2 spike glycoprotein, the AutoDockVina gridbox (30 Å × 30 Å × 30 Å) was set to cover the residues in RBD which are emphasized in the literature and was centered at 212.138, 192.303, and 256.433.[25-28] Dial spacing (angstrom), in each analysis, was set to 1.0 which is the scaling factor (http://vina.scripps.edu/tutorial.html). The configuration file was typed into the notepad and the Vina program was run through the command prompt. Docking was done using an exhaustiveness value of 8. Every compound (ligand) was independently tested against each protein (receptor). PyMOL package was used to visualize the binding interactions between each ligand and 3D model of each target protein.[29]

Results

After the successful docking of selected substances into the SARS-CoV-2 main protease and SARS-CoV-2 spike glycoprotein, AutoDock Vina produced various modes of ligand–protein interactions (nine binding modes for every tested ligand) with a particular docking score (binding energy). The binding mode with the least binding energy is the most stable for the ligand and was therefore regarded as the best mode of binding. The best binding modes and corresponding affinities are summarized in Table 1. All the docked structures were visualized in PyMOL2.3 (Figs. 1-3).

A similar SARS-Cov-2 main protease binding characteristics of artemisinin were observed to that of chloroquine (Asn 142) and hesperidin (Thr 24) Fig. 1b–d). The highest affinity for SARS-Cov-2 main protease was found for

Table 1. Target proteins (receptor) and the tested compounds (ligand) undergoing docking analysis with their best docking score (lowest binding energy) and zero distance from the best mode (rmsd l.b. 0.000; rmsd u.b. 0.000)

Receptor	Ligand binding score affinity (kcal/mol)		
	Artemisinin	Hesperidin	Chloroquine
SARS-CoV-2 Mpro	-4.8	-5.8	-4.1
SARS-CoV-2 PLpro	-8.3	-10.0	-8.2
SARS-CoV-2 spike glycoprotein	-6.0	-8.1	-4.8

hesperidin (–5.8 root-mean-square deviation (rmsd) l.b. 0.000; rmsd u.b. 0.000) and slightly lower for artemisinin (–4.8 rmsd l.b. 0.000; rmsd u.b. 0.000) and chloroquine (–4.1 rmsd l.b. 0.000; rmsd u.b. 0.000).

In the case of the papain-like protease of SARS-CoV-2, all compounds had high binding scores. Hesperidin had the highest (–10.0 rmsd l.b. 0.000; rmsd u.b. 0.000), followed by the very similar affinity of artemisinin (–8.3 rmsd l.b. 0.000; rmsd u.b. 0.000) and chloroquine (–8.2 rmsd l.b. 0.000; rmsd u.b. 0.000). Visualized interactions showed similarity in artemisinin and chloroquine binding modes to PLpro close to the position of Glu 161 (Fig. 2b, d).

The highest affinity for binding to SARS-CoV-2 spike glycoprotein (P1 and P2) (Fig. 3a) was observed for hesperidin (–8.1 rmsd l.b. 0.000; rmsd u.b. 0.000), followed by artemisinin (–6.0 rmsd l.b. 0.000; rmsd u.b. 0.000), and lowest for chloroquine (–4.8 rmsd l.b. 0.000; rmsd u.b. 0.000). Hesperidin and artemisinin have been found to have similar positioning toward targeted protein at Phe 456 and Phe 490 when these interactions were visualized (Fig. 3b, d).

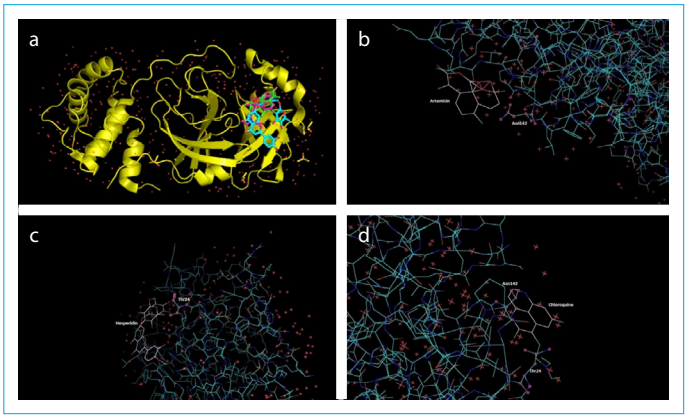

Figure 1. Visualisation of the SARS-CoV2 main protease **(a)** (PDB ID: 6Y84) and its best mode binding interactions with **(b)** artemisinin, **(c)** hesperidin, and **(d)** chloroquine.

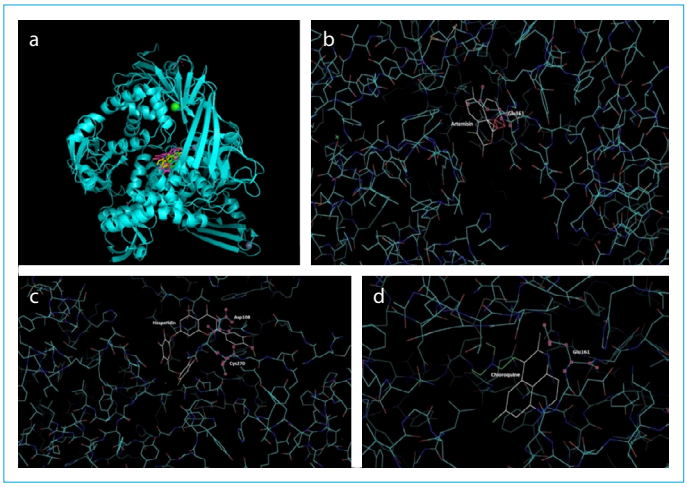

Figure 2. Visualisation of the SARS-CoV2 papain-like protease **(a)** (PDB ID: 6W9C) and its best mode binding interactions with **(b)** artemisinin, **(c)** hesperidin, and **(d)** chloroquine

Discussion

Based on coronavirus mechanism of infections, several possible targeted therapies were described as acting: (a) on enzymes or functional proteins that are essential to the virus

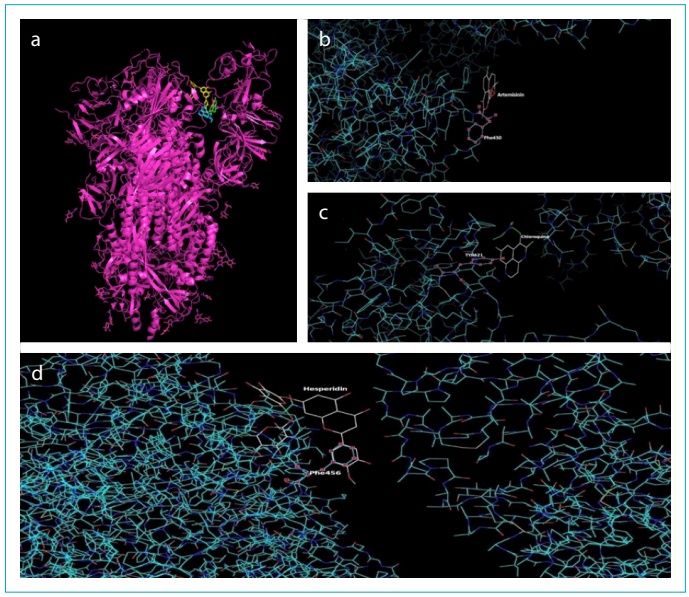

Figure 3. Visualisation of the SARS-CoV2 spike glycoprotein **(a)** (PDB ID: 6VYB) and its best mode binding interactions with **(b)** artemisinin, **(c)** hesperidin, and **(d)** chloroquine.

(linked to RNA synthesis and replication); (b) on structural proteins of the virus, blocking virus from completing multiplication and spreading; (c) on the production of virulence factor to restore the host's innate immunity; and (d) on the host's specific receptors or enzymes, preventing the virus from entering into the host's cells. In a study by Canrong et al.,18 possible viruses and two hosts (human) proteins (ACE2 and TMPRSS) were used as targets to identify ligands from a virtual database including natural bioactive compounds that could be considered as potential candidates for extended in vitro and in vivo testing for use in COVID-19 treatment.[6] Many studies that employed virtual screening strategies have detected hesperidin *(Citrus aurantium)* and its derivatives to be the only reactive with viral structures at various levels. Moreover, the structural proteins S1 and S2 of SARS-CoV-2affect the structural integrity and cleavage activation which have key roles in virus invasion and virulence; PL-pro, 3CLpro, N-terminal MBD, and helicase domain; and high binding affinity to host binding protein-ACE2.[30] In many studies, a panel of available drug candidates has been tested for binding affinity to various structural and nonstructural proteins with comparison to a reference drug usually confirmed for antiviral or antimalarial activity.[30, 31] In this study, hesperidin was used as a positive reference and showed the highest binding affinities for all SARS-CoV-2 proteins included which confirmed the previous findings of the authors. In addition, artemisinin has slightly lower binding scores compared to hesperidin, while chloroquine has the lowest when compared to these two.

The targeted anti-SARS-CoV-2 activity via the main viral proteases was screened for several antiviral and antimalarial drugs for their potential rational use in anti-COVID-19 treatments using in silico methodology.[32] The range of the binding energies obtained for quinine, artesunate, clotrimazol, artemether, quercetin, mefloquine, ciprofloxacin, clindamycin, cipargamin, and SJ-733 was between −7.0 and−9.6 kcal/mol. This work observed that chloroquine, artemisinin, and hesperidin was a bit lower at −4.1, −4.8, and −5.8 kcal/mol, respectively. A much higher binding affinity of tested compounds was observed for SARS-CoV-2 PL-Pro of −8.2 to −10.0 kcal/mol suggesting that this protein may be a better-fitting target for antiviral activity of tested compounds.

The safety and applicability of the different topical application of antiviral compounds have been explored in several placebo-controlled clinical studies, showing that adequate formulation is safe and efficient in the reduction of symptoms of common cold and viral load in nasal lavages.[33, 34] This suggests that nasal, oral, or other topical application of anti-SARS-CoV-2 should be further explored in the development of prophylactic measures with special attention and additional trials to explore potential adverse effects in developing antimalarial resistance.

Conclusion

The public availability of SARS-CoV genome and protein information has enabled extensive research in the identification of potential targets and mechanisms of action of new anti-COVID-19 treatments and prophylaxis. In that respect, medicinal plants and bioactive compounds represent an indispensable resource of low-cost and widely accessible therapeutics. For example, artemisinin is a metabolic product isolated from one of the most widespread industrial plant in the world (*A. annua L.*). Its antiviral and antimalarial effects have been thoroughly explored and proven. The molecular docking methods employed in this work showed a high binding affinity of artemisinin to SARS-CoV-2 proteins. The results of this study showed that artemisinin has the potential to inhibit the main viral proteins of the SARS-CoV-2-spike protein (S), 3CLpromain protease, and PLpro proteinase similar to hesperidin and chloroquine compounds with proven antiviral effects. These results paved the road to computational drug discovery of highly accessible (natural or synthetic) compounds such as artemisinin and indicate multiple functional potentials for their simultaneous, inmixture pharmacological use with other known compounds (i.e., hesperidin and chloroquine) to suppress SARS-CoV-2 infection, replication, and spread as well as associated symptoms of this possibly lethal viral disease.

Disclosures

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