Docking and *in silico* toxicity assessment of *Arthrospira* compounds as potential antiviral agents against SARS-CoV-2

Lena PETIT
Algama https://orcid.org/0000-0003-1315-5821
Lea VERNES
Algama https://orcid.org/0000-0002-8115-2609
Jean-Paul CADORET (jeanpaul.cadoret@algamafoods.com)
Algama https://orcid.org/0000-0001-7542-5474

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Abstract

A course is currently being launched as a result of the international health situation. This race aims to find, by various means, weapons to counter the Covid-19 pandemic now widespread on all continents. The aquatic world and in particular that of photosynthetic organisms is regularly highlighted but paradoxically little exploited in view of the tremendous possibilities it offers. Computational tools allow not only to clear the existence and activity of many molecules, but also to model their relationships with receptors identified in potential hosts. On a routine basis, our laboratory carries out a research activity on functionalities of molecules derived from algae, using *in silico* tools. We have implemented our skills, in algae biology and in modeling, as tests in order to identify molecules expressed by the genus *Arthrospira* showing an antiviral potential and more particularly anti SARS-CoV-2. Using Autodock Vina, we were able to identify 3 molecules: phycocyanobilin, phycoerythrobilin and folic acid. These 3 compounds showed binding energies able to compete with the SARS-CoV-2/ACE2 complex. Toxicity prediction as well as current regulations support their use as potential candidates for the fight against Covid-19.

Introduction

Covid-19 is probably the most written, published acronym in this year 2020. This is for objective and obvious reasons. Covid-19 is the pandemic caused by SARS-CoV-2 (Severe acute respiratory syndrome coronavirus 2) and it has overturned the entire planet as few pandemics have done, and this, in a modern era which has deployed modern and high-level medicine. Research on these pandemics takes multiple paths whether on technical, chemical or biological levels. We then attack the biology of viruses or the defense mechanisms of their hosts. Different methods can be used for antiviral research to act against SARS-CoV-2. We can first target structural proteins in view to inhibit virus entry in human host cells or looking for functional internal viral protein inhibitors to block for example its replication. A third method would be to target human receptor for virus entry prevention. In view to limit the impact on the human metabolism of such antivirals, we rather focused on the first critical step for the virus, its recognition by the host cell, mediated via specific structural proteins, spike proteins. As a food tech company, Algama produces innovative food ingredients made with microalgae. As a part of our daily research, we developed a tool enabling to select the most relevant molecules from microalgae for different food applications according to various criteria (bioavailability, safety, drug interaction…). In the context of the Covid-19 health crisis and to contribute to the collective effort, we tested our methodology towards antiviral compound identification from microalgae. Based on a literature framework about antivirals from *Arthrospira sp*, the aim of this study was to identify the most relevant SARS-CoV-2 antiviral molecules thanks to docking, and to conduct an *in silico* toxicity assessment of the most efficient antiviral compounds. The first part of this work focuses on the description of the virus and presents a state of the art of *Arthrospira* use as an antiviral agent. The second part of the article deals with the evaluation of the anti-SARS-CoV-2 activity by docking *Arthrospira* compounds. Finally, an *in silico* study of the selected molecules toxicity will be proposed.

Sars-cov-2

SARS-CoV-2 belongs to the coronavirus family, composed of enveloped virus with positive strain of RNA. Its genome is contained in a capsid formed by nucleocapsid proteins, itself included in an envelope. Three structural proteins are characteristic of coronavirus: membrane protein, envelope protein, and spike protein (S), a glycoprotein responsible for virus host cell attachment (Li 2016). Coronavirus took their name from “corona” (crown in Latin), formed by S protein protuberances on their surface.

The spike (S) glycoprotein ectodomain consists in a trimeric structure. Each monomer is composed of two subunits: S1, involved in the host cell receptor recognition and S2, responsible for the membrane-fusion mechanism. S1 subunit contains two major domains, N-terminal domain (S1-NTD) and C-terminal domain (S1-CTD) (Li 2016) (Fig. 1). The latter constitutes the receptor binding domain (RBD) and is composed of two subdomains: a core structure and a receptor-binding motif (RBM) (Li et al. 2005).

Zhou et al. (2020) recently determined the ability of SARS-CoV-2 to use human angiotensin-converting enzyme 2 (ACE2) as receptor to engage virus attachment to the host cell via its RBD, as for SARS-CoV. However, they excluded binding with other coronavirus receptors such as APN and DPP4 (Li 2016). Angiotensin-converting enzyme 2 (ACE2) identification as main receptor of SARS-CoV-2 in human has been confirmed shortly after by two other research teams (Ou et al. 2020; Hoffmann et al. 2020).

ACE2 is a metallocarboxypeptidase involved in arterial pressure system via cleavage of angiotensin peptides (Donoghue et al. 2000). It has been shown to be expressed in lung alveolar epithelial cells, in enterocytes of small intestine and in vascular endothelium (Hamming et al. 2004), explaining the location of Covid-19 pathologic symptoms. ACE2 binding site to SARS-CoV-2 was found to be within its extracellular peptidase domain (Yan et al. 2020).

Once coronavirus RBD is linked to ACE2 peptidase domain, the prefusion S protein becomes unstable and is submitted to a first proteolytic activation via a host cell protease, between S1 and S2 subunits (Walls et al. 2017). In addition to S1/S2 cleavage, a second site, critical for viral fusion, has been determined within the S2 domain, on the S2' site (Belouzard et al. 2009). This second proteolysis results in the shedding of S1 subunit and exposes an internal fusion peptide. This one is essential for fusion of the virus and host membrane via S2 subunit, leading to the viral genome release in ACE2. This peptide is highly conserved among coronavirus (Madu et al. 2009) and composed of serine residues 798 to phenylalanine 815 in SARS-CoV.

TMPRSS2 was identified in lung cells as the main human protease used by SARS-CoV-2 for S protein modification (Hoffmann et al. 2020). TMPRSS2 belongs to the Type II transmembrane serine proteases (TTSPs) which have been involved in the spread of various respiratory viruses (Choi et al. 2009). Expressed in airway epithelial cells (Böttcher et al. 2006), Shulla et al. (2011) further pointed the colocalization of TMPRSS2 and ACE2 during an immunoprecipitation assay. In this context, SARS-CoV-2 using the same receptor (ACE2) and host cell protease (TMPRSS2) than SARS-CoV, this colocalization appears to sorely facilitate its entry and spreading in human host cells.

Gui et al. (2017) determined different conformational prefusion states of SARS-CoV S protein. Each Spike RBD on the S1 subunit of SARS-CoV can be found in two conformational states, one "up" and one "down". In down position, SARS-CoV RBD was reported as inaccessible for ACE2 recognition, due to steric clashes. On the contrary, when a trimer is in "up" position, RBD is then exposed, allowing its binding with host cell ACE2 (Gui et al. 2017; Yuan et al. 2017).

At this day, two conformational states of the S protein have been found in SARS-CoV-2 (Wrapp et al. 2020; Walls et al. 2020). As for SARS-CoV, one presents all RBD in down state (not completely closed according the Cryo-EM assays) and the second shows one of the three RBD exposed in an open state. Like for its predecessor, SARS-CoV-2 RBD binding with ACE2 seems to be impossible when the Spike trimer is entirely in down conformation. On the contrary, with one RBD in up position, no steric clash with S protein was detected, suggesting that an open state is required for Spike protein binding with its receptor (Yan et al. 2020).

As crystal structure of RBD has been defined with a better resolution than the open state of the SARS-CoV-2 Spike ectodomain structure, and as no complete complex of Spike protein with ACE2 was available, RBD section of the S protein was chosen for this work. This further ensured to use a valid conformational state of the RBD.

Key binding residues involved in the Spike/ACE2 link were elucidated in several papers. Final consensus sequence for binding site on ACE2 was established from residues highlighted by the several teams (Yan et al. 2020; Lan et al. 2020; Shang et al. 2020; Wang et al. 2020): S19, Q24, F28, D30, K31, H34, E35, D38, Y41, Q42, L79, M82, Y83, K353, D355, R357. Regarding Spike glycoprotein, consensus for ACE2 binding residues has been established has followed: K417, G446, Y449, Y453, L455, A475, F486,N487, Y489, Q493, G496, Q498, T500, N501, G502 (Yan et al. 2020; Walls et al. 2020; Lan et al. 2020; Shang et al. 2020; Wang et al. 2020) (Table 1).

Algae and antiviral activities

The term "algae" actually includes a wide variety of organisms with a complex evolutionary history. These organisms have features in common such as the ability to do photosynthesis and being predominantly aquatic. We can distinguish two main categories of algae: macroalgae, multicellular eukaryotes and microalgae, in the largest sense of the word, including unicellular eukaryotes and prokaryotes. Most macroalgae are found in seawater and are called "algae", but some can also be found in freshwater (e.g.: *Oedogonium*, *Cladophora* and *Spirogyra*). Microalgae are found in both environments, but the most consumed species (*Arthrospira*, *Klamath* and *Chlorella*) generally grow in freshwater.

These aquatic plants are known to be rich in proteins and lipids, but also offer a wide range of immunostimulatory, antioxidant and antiviral properties. Numerous publications have reviewed the generic properties of algae in these areas (Mimouni et al. 2012). For example, the immune response in the cytokines which is tested by Talukdar et al. (2020) with astaxanthin. Griffithsin, a lectin isolated from red algae *Griffithsia* has shown activity on protein S of MERS-CoV (Middle East Respiratory Syndrome Coronavirus)(Millet et al. 2016). As for microalgae, the very numerous studies have been compiled by de la Jara et al. (2018) and Perumal and Sundararaj (2020).

Antiviral compounds from *Arthrospira* state of the art (tables)

Arthrospira gender refers to prokaryotic filamentous cyanobacteria. The best-known species, *Arthrospira platensis* and *Arthrospira maxima*, are mostly used as food supplement due to their nutritional properties: high protein content, vitamins, minerals… Moreover, *Arthrospira* also contains various metabolites with beneficial health properties such as polyphenols, carotenoids, sterols. Due to drug-resistant virus strains emergence, and because of adverse effects caused by usual synthetic antiviral treatments, increasingly researchers are looking into more natural approaches. This explains a growing interest in antivirals from natural source, in algae in general and in *Arthrospira* for example in particular. Hence, *in vitro* and *in vivo* studies have been conducted to evaluate *Arthrospira* compounds as antiviral against various viruses such as Influenza, Herpes simplex virus (HSV), Hepatitis C (HCV)…as presented in Tables 2 and 3.

Data from scientific literature were critically reviewed in order to highlight results of the most rigorous studies: presence of controls, replicates, measurement of toxicity, and inhibition efficacy. The following information were collected and analyzed: CC_{50}, cytotoxic concentration of the compounds decreasing *in vitro* cell viability to 50%; EC_{50}, effective concentration needed to inhibit 50% of virus; and therapeutic index (TI or SI), referring to the ratio CC_{50}/EC_{50}. A high therapeutic index (TI), or selectivity index (SI), is preferable for a drug to have a favorable safety and efficacy profile.

Radonic et al. (2010) evaluate the effect of sulphur-containing exopolysaccharides from *A. platensis* (TKV3). According to their study, anionic polysaccharides from *Arthrospira* showed antiviral activity against enveloped virus such as VACV (EC_{50} (TK V3) = 0.78 µg.mL^{-1}) *via* interactions with viral membrane glycoproteins and thus inhibition of virus binding to host cells. In the work of Chen et al. (2016), *Arthrospira* extract was capable of inhibiting *in vitro* influenza viral replication and plaque formation (Table 2), by targeting hemagglutinin, an influenza virus surface glycoprotein.

Abdo et al. (2012), El-Baz et al. (2013), Deyab et al. (2020), and Hetta et al. (2014), focused on *A. platensis* antiviral effect on adenovirus and Coxsackievirus (CV). Most of them evaluate *Arthrospira* methanol extract activity on Hep 2 cell *in vitro* model. In all cases, *Arthrospira* appears as a good antiviral agent: reducing virus titer from 50%, preventing virus attach to host cell receptor when used as pre-treatment with TI(CVB3) = 30 and TI(RV)= 45 (Deyab et al. 2020). Efficiency of methanolic extract is attributed to the presence of polar compounds binding to viral capsid. Moreover, El-Baz et al. (2013) found that *Arthrospira* ethanolic extract was active against non-enveloped RNA and DNA viruses. However, authors did not compare *Arthrospira* effect with a control drug since there is no available drug against enteric virus according to them.

Elsewhere, Rechter et al. (2006) compared spirulan-like substance to ganciclovir (GCV), a reference drug used as anti-herpesvirus. According to their *in vitro* experiments, preincubation of substances enhances antiviral activity *via* virus entry and replication inhibition: EC_{50} (TK-V3a) = 1.4 ± 0.3 µg.mL^{-1}, close to drug reference EC_{50} = 0.7 ± 0.1 µg.mL^{-1} in post incubation (Table 2).

Due to the emergence of drug-resistant *Herpes simplex* strains, several studies have focused on the search for alternatives to synthetical acyclovir in the prevention and treatment of *Herpes Simplex* Virus (HSV) 1 and 2. For instance, hot water extract of *A. maxima* provided EC_{50} of 0.069 $mg.mL^{-1}$ against HSV-2. The Authors then attributed this antiviral activity to high polar compounds (Hernández-Corona et al. 2002). In a second study, phosphate buffer extract of *A. fusiformis* showed a viral infection inhibition almost as high as acyclovir (85% against 99%) during the intracellular replication period (Schnitzler et al. 2013). In the same way, Chirasuwan et al. (2009) evaluated in vitro *A. platensis* extract as potential HSV antiviral agent to propose an alternative to the synthetic anti-herpes drug acyclovir. Sulfoquinovosyl diacylglycerol (SQDG) was responsible for antiviral activity of *A. platensis* lipid fraction (EC_{50} = 6.8 $\mu g.mL^{-1}$) with an effect comparable to the reference drug (EC_{50} = 1.5 $\mu g.mL^{-1}$), without toxicity for cells. This activity is due to DNA polymerase inhibition thanks to SQDG interaction with different regions of the enzyme. Furthermore, Lee et al (2007) highlighted antiviral potency of spirulan molecules from *A. platensis*. Best *in vitro* results were obtained when compounds were added to the medium during HSV-1 and 2 infections. Na-Sp then showed the highest selectivity indexes of 13,000 and 17,000, with very low ECs_{50} (0.63 and 0.41 $\mu g.mL^{-1}$). Otherwise, Shalaby et al (2010) attributed the observed Hepatitis-A (RNA virus) and HSV-1 antiviral effect of aqueous and phosphate buffer extracts to the sulphated polysaccharides and tannins. According to them, these compounds may interfere at different viral stage such as attachment and penetration of the virus.

In the case of Kok et al. (2011) study, different extracts from microalgae were compared to conventional drugs (acyclovir & foscarnet) for their antiviral capacity against Epstein-Barr Virus (EBV). Methanol extract from *A. platensis* reduced the cell-free EBV DNA load in B95-8 cells with an EC_{50} of 0.021 $\mu g.mL^{-1}$ with an CC_{50} of 166 $\mu g.mL^{-1}$ (TI=7905) (Kok et al. 2011).

Peptides were also evaluated as antiviral agent. Indeed, Jang and Park (2016) found that SM peptide, isolated from *A. maxima*, displayed in vitro antiviral activity since it inhibited reverse transcriptase activity in HIV-1 infected cell by 90% compared to the no-peptide assay. Moreover, 0.75 $mg.mL^{-1}$ of SM peptide inhibited HIV-1 p24 antibody production by more than 95%, without toxicity for the cells. Similarly, Ayehunie et al. (1998) evaluate HIV antiviral activity of *Arthrospira*. According to their results, both polysaccharide fraction and the fraction depleted in polysaccharide and tannin of *A. platensis* inhibited *in vitro* HIV-1 replication. Preincubation of polysaccharide fraction extract with cells showed better results due to polysaccharides binding to CD4 receptors, thus preventing virus attachment to the host cell through its gp120 envelope glycoprotein.

According to *in vitro* studies presented above, *Arthrospira* has antiviral properties against different types of viruses: *Herpes simplex*, hepatitis, vaccinia, coxsakievirus…. It has no, or very little, cytotoxicity to cells, and low doses (EC_{50}) are sufficient to induce an effect (1-10 $\mu g.mL^{-1}$) with high therapeutic indices. It seems that *Arthrospira* compounds act during the early stages of viral infection. According to the main hypotheses evoked, compounds of polar nature and mainly sulphated polysaccharides and peptides are responsible for interactions with viral envelope glycoproteins, thus preventing binding to the host cell. Calcium spirulan, a sulfated polysaccharide from *Arthrospira*, is the most cited compounds with antiviral activity against various enveloped virus. The sulfate groups as well as the carboxyl groups of polysaccharides, such as Ca-Sp isolated from the extract, have negative charges which can react with the basic amino acids of viral proteins and block the interaction with cellular receptors.

In order to confirm *in vitro* results, a second state of the art was carried out focusing on human clinical studies involving the use of *Arthrospira* as an antiviral agent (Table 3).

Most of the *in vivo* studies focused on *Arthrospira* supplementation effect on HIV-infected patients. Tpday, there is no treatment capable of eradicating AIDS. HIV-infected patients still carry the virus and they can spread it to others. So far, there are five major classes of anti-HIV drugs that targets distinct steps in the virus life cycle: reverse transcriptase inhibitors, protease inhibitors, fusion inhibitors, integrase inhibitors, and multidrug combinations (Jang and Park 2016). To improve therapeutic potential of existing medicines, research on *Arthrospira*-based novel treatment have been conducted.

In this context, Winter et al. (2014) conducted an *in vivo* study on 73 HIV-infected women to evaluate *Arthrospira* supplementation effect. The study was a three-month pilot, randomized, double-blind and placebo-controlled intervention. No significant clinical effect of *Arthrospira* supplementation was observed regarding viral load and CD4 cells; nevertheless, *Arthrospira* supplementation showed a positive effect on weight stabilization and protection against opportunistic infections. Similarly, Teas and Irimeh (2012) found that *Arthrospira* strengthen immune system of affected patients. Unfortunately, sample size of selected patients for this study was too small (11) to conclude that there were significant effects of *Arthrospira* supplementation. Ngo-Matip et al. (2015) conducted a single-blind, randomized, multicenter study on 320 HIV-1 ARV-naïve participants for 12 months to analyze *Arthrospira* supplementation effect compared to local diet, both with standard HIV therapy. This work confirmed previous results, showing that *Arthrospira* supplementation intake positively and significantly stimulated the immune system and inhibited virus replication of HIV-infected subjects. As in the previous cases, *Arthrospira* contributed to the proper functioning of immune system and thus helped to limit the appearance of opportunistic diseases. Similar results were also obtained by Azabji-Kenkack et al. (2011), indicating that *Arthrospira* supplementation helps HIV-infected and malnourished adults to improve their immune defenses (Azabji-Kenfack et al. 2011). For this experiment, authors compared effects of *Arthrospira* and soya beans supplementation. Like other studies, *Arthrospira* supplement improved general state of health by promoting weight gain of malnourished adults infected with HIV. Regarding the baseline and the results obtained with soya beans, *Arthrospira* consumption reduced significatively viral load and increased CD4 count (Azabji-Kenfack et al. 2011).

Two *in vivo* studies listed in table 2 focused on *Arthrospira* effect in the case of hepatitis C-infection (HCV). In the work of Gomaa et al. (2017), *Arthrospira* was added to usual thalassemic drug for 6 months to evaluate the effect on thalassemic HCV-infected children. As patients are suffering from 2 different pathologies, it was difficult to affirm that *Arthrospira* was responsible for health improvement. However, authors conclude that *Arthrospira* stimulated immune system of thalassemic children infected with HCV since CD4 and CD8 number was increased after 6 months of *Arthrospira* intake. They also state that phycocyanin from *Arthrospira* stimulated hematopoiesis, by inducing erythropoietin hormone (EPO) release, thus helping thalassemia children. Moreover, Yakoot and Salem (2012) conducted an *in vivo* study comparing the effect of *Arthrospira* and *Silymarin* against hepatitis C. For this study, sixty-six patients with chronic hepatitis C virus infection had been randomized, double-blind, and treated with *Arthrospira* or *Silymarin* for a period of six-months treatment. According to their results, *Arthrospira* helped to improve general well-being of patients and has led to a loss or reduction of detectable hepatitis C virus RNA for 6 patients. Compared to the second group treated with *Silymarin*, these results were no significant (0.12) (Table 2). Nevertheless, this study did not include a placebo control since *Silymarin* possess itself some benefits in the treatment of viral hepatitis. Thus, it does not allow to conclude on these results.

According to these *in vivo* studies, it appears that *Arthrospira* improves the general health of AIDS and HCV-infected patients by contributing to restore their body's defense mechanism against infectious immune system disorder, thus limiting opportunistic diseases. According to these results, *Arthrospira* may significantly affect virus progression according to some studies. However, most studies conducted *in vivo* on human patients have evaluated the effect of daily *Arthrospira* supplementation in addition to the usual antiviral treatment. These studies were mostly randomized and double-blind, but the study duration was often short with a small number of patients and did not always present a placebo control. It could be interesting to evaluate the effect of *Arthrospira* alone, without other antiviral treatment, in a rigorous long-term study to confirm its antiviral potential.

According to this literature review, *Arthrospira* acts as antiviral against various type of viruses. Indeed, significant antiviral effects were observed *in vitro*, and *in vivo* studies confirmed that *Arthrospira* is well-tolerated by patients and even improved their general state of health by increasing immune response. *Arthrospira* can thus be administered in addition to current gold standard therapy in order to improve its effects, or it can also be considered as an interesting alternative to certain drugs with serious adverse effects. Many compounds such as sulfated polysaccharides (Calcium-spirulan), fatty acids (Hetta et al. 2014) and proteins (C-phycocyanin, cyanovirin-N, microvirin) have been identified as responsible for this antiviral activity. Indeed, these compounds can bind to glycoprotein virus envelope, and thus hinder virus attachment to its host cell. Nevertheless, according to Ayehunie et al. (1998), it appears that other compounds than polysaccharides and tannins, present in the

aqueous extract of *Arthrospira*, may also have an antiviral activity. To go further, it might therefore be interesting to evaluate the antiviral potential of other *Arthrospira* metabolites.

Few studies have been identified in the literature as dealing with the antiviral potential of microalgae compounds against SARS-Cov-2. For instance, astaxanthin as well as linolenic acid (C18:00) have been mentioned as potential candidates for the treatment or the prevention of Covid-19. (DOI: 10.31219/osf.io/yahd4; URL: https://preprints.aijr.org/index.php/ap/preprint/view/36). The use of docking in order to test *Arthrospira* as a candidate against SARS-CoV-2 is original. A pre-published work publicly available, highlighted *Arthrospira* potential on two viral proteins. However, it was based on SARS-CoV-1 database, and it is pending for publication (DOI: 10.26434/chemrxiv.12051927) These are pre-published studies and the confirmation of the robustness will be given upon the final peer reviewed publications. Moreover, very few authors have taken an interest in the specific case of *Arthrospira*, and most of the articles present a literature survey without *in silico*, *in vitro* or *in vivo* evaluation.

In order to propose new antiviral leads from *Arthrospira*, this study evaluates *in silico* the antiviral potential of *Arthrospira* metabolites using an updated database specific to SARS-coV-2 thanks to docking tools. Molecular docking is a computational method which simulates different orientations of a protein and a ligand to predict the most stable complex, with a minimal free energy. Based on algorithms, best binding-conformations, as well as binding affinity are defined.

In order to contribute to the collective effort to find a solution to the current global Covid-19 crisis and to further explore the antiviral potential of *Arthrospira*, this article investigates antiviral activity of *Arthrospira* molecules from Algama internal database. This database is composed of 51 high value molecules from *Arthrospira* genus.

Materials And Methods

Protein-protein docking

Two crystal structures of the receptor-binding domain (RBD) of SARS-CoV-2 Spike protein bound to the cell receptor ACE2 have been determined (Lan et al. 2020; Wang et al. 2020) and deposed on the Protein Data Bank under the respective codes 6M0J and 6LZG. Both structures were used as experimental controls by submitted them to Prodigy tool with a temperature set to 25°C (Vangone and Bonvin 2015; Xue et al. 2016) for protein-protein binding affinity prediction.

Protein-ligand docking

All the docking assays were executed with the firstly deposed crystal structure of the complex, corresponding to the 6LZG identifier (Wang et al. 2020). RBD part was extracted from this crystal complex using Discovery Studio Visualizer, thus expecting having the most relevant conformation of the RBD in the Spike/ACE2 complex (Forli et al. 2016). Receptor structure was then prepared, heteroatoms (included water) were deleted, all the hydrogens were added, and the file was saved as PDB file.

Ligands were divided into two categories: small molecules (mainly secondary metabolites) and peptides. Small molecule structures were obtained from Pubchem and downloaded as SDF format. Peptide sequences were taken from literature and converted in SMILES format via NIH Cactus translator, and then in SDF format in Open Babel (O'Boyle et al. 2011). All the 51 ligands from Algama database were energy minimized before being finally converted in PDBQT format on PyRx (Dallakyan and Olson 2015).

Docking was executed with Autodock Vina (Trott and Olson 2010) on the PyRx software (Dallakyan and Olson 2015). The grid box was manually adjusted around active residues identified above, with following coordinates: X:-37.348; Y: 29.6843; Z: 3.1984 and dimensions : X:29.0036 Å; Y: 56.1876 Å; Z: 24.9881 Å. Exhaustiveness was set on 10, equivalent to the *short* mode, since no statistically significant difference in accuracy was detected using the *short*, *medium* or *long* mode of Autodock Vina (Nguyen et al. 2020).

Binding affinity result files were exported as CSV for analysis and docked structures were visualized in Discovery Studio Visualizer in view to obtain a complete structure of each complex. Ligand interactions were determined with this same tool and analyzed to target better candidates for SARS-CoV-2 RDB binding. Those with both a relevant binding affinity with the receptor, low RMSD values, and favorable interactions with residues involved in the RBD/ACE2 link were selected for deeper analysis.

In silico toxicity assessment

Toxicity of each selected molecules was assessed via the AdmetSAR 2 prediction tool (Yang et al. 2019), using canonical smiles from Pubchem for compound identification.

Acute oral toxicity was determined through toxicity class. Carcinogenicity, mutagenicity, and hepatotoxicity were also predicted. In view to avoid interaction with other potential treatment, it was needed to study the main cytochrome as well as P-glycoprotein to avoid drug interactions.

Results

Docking

Both RBD/ACE2 complexes docked as control provided similar binding affinity around -12 kcal.mol^{-1}. As comparison, entire Spike glycoprotein complexed with ACE2 has been submitted to the same analysis by another team, still using Prodigy, after docking between ACE2 (PDB code: 1R42) and Spike protein isolated from the PDB structure 6ACK (Ortega et al. 2020). Binding energy was then established at -15.7 kcal.mol^{-1}, a result quite lower, probably explained by the low resolution of the Spike portion (4.50 Å).

After docking the 51 molecules from Algama internal database with Spike RBD in Autodock Vina, the first three modes were selected for each compound, corresponding to the best three binding affinities (Table 6 Supplemental data). Only values less than -7 kcal.mol^{-1} were kept, in view to be the most competitive as possible with the binding energy from the RBD Spike/ACE2 complex. Out of the 153 conformations presented here, 23 fulfilled this criterion. To ensure stability of selected conformations, we selected those with both their RMSD/ub and RMSD/lb lower than 2 Å (Trott and Olson 2010). 13 conformations were then obtained, corresponding to 11 compounds (Table 4): catechin, epicatechin, dieckol, apigenin, β-carotene, rutin, astaxanthin, rosmarinic acid, phycobilin, phycoerythrobilin and folic acid. After comparison of number and localization of H-bonds, unfavorable interactions, total and nature of interactions, and number of key residues involved in those interactions, 3 compounds were finally identified: phycobilin, phycoerythrobilin and folic acid. Despite its high binding affinity, dieckol was not selected because it only had one H-bond and included two unfavorable interactions which will tend to break the link with RBD.

Phycocyanobilin

Phycocyanobilin / Spike RBD complex is composed of 5 Van der Waals interactions, with residues ARG403, TYR453, LEU492, GLN493 and ASN501 (Fig. 2). Five π-Alkyl bonds are involved between phycocyanobilin and Spike RBD, distributed between TYR449, TYR495, PHE497 and TYR505, consolidated by a H-bond on TYR449. Three other H-bonds engage SER494, GLY496 and GLN498. Finally, GLY496 is also linked to phycocyanobilin via a π-Donor hydrogen bond. Binding affinity for this compound reaches -7.2 kcal.mol^{-1} and is not impeded by unfavorable strength, highlighting a good potential for antiviral activity.

Phycoerythrobilin

Phycoerythrobilin binds to Spike RBD via 9 Van der Waals interactions, with following residues: GLU406, GLN409, LEU455, GLN493, TYR495, PHE497, GLN498, ASN501, TYR505. Additionally, 3 alkyl and π-alkyl interactions engage LYS417, TYR449 and TYR453. ARG403 is involved in two different kinds of bonds: one π-cation and one carbon-hydrogen bond. A second carbon-hydrogen bond links SER494 to Spike RBD, as well as one H-bond. The two last H-bonds are located on the GLY496 residue (Fig. 3). Thus, with four key binding amino acids involved in different interactions including two H-bonds on them,

phycoerythrobilin shows an interesting binding affinity of -7.3 kcal.mol^{-1}. These results provide us good arguments for deeper research on a phycoerythrobilin based antiviral.

Folic acid

Folic acid presents 4 van der Waals interactions with RBD Spike, engaging LYS417, SER494, PHE497 and TYR505 residues. 3 π-cation and one π-anion interactions are detected on the AR403 and the ASP405. One π- π stacked interaction engages the aromatic group of folic acid with TYR453. Three carbon hydrogen bonds are highlighted on two residues, GLU406 and TYR495. Despite an unfavorable donor-donor interaction on the arginine 408, 6 conventional hydrogen bonds are predicted on ASP405, GLU406, GLN409, GLY496, GLN498 and ASN501 (Fig. 4). With a binding energy of -7.4 kcal.mol^{-1} and 6 hydrogen bonds of which 3 are engaged with residues directly involved in Spike/ACE2 link (GLY496, GLN498 and ASN501), folic acid is suggested as a great mid-competitive inhibitor to block the virus attachment and entry via ACE2 human receptor.

Both three selected compounds presented competitive binding energies regarding the control complex. This later reached -12.4 kcal.mol^{-1} whereas phycocyanobilin, phycoerythrobilin and folic acid presented binding energies of respectively -7.2, -7.3 and -7.4 kcal.mol^{-1}. These values, added to favorable solid interactions, suggest these three molecules could constitute mid-competitive inhibitors for the Spike / ACE2 link. Such inhibition could limit the hanging of SARS-CoV-2 on the human host receptor ACE2 and thus contribute efficiently to the fight against Covid-19.

Toxicity assessment

After toxicity prediction with AdmetSAR 2 tool, each selected molecule was analyzed regarding specific toxicity criteria (Table 5). None of the tested compounds present neither mutagenicity nor carcinogenicity. All of them possess acute oral toxicities included in the third class of toxicity. This later corresponds to LD_{50} comprised between 500 and 5000 mg.kg^{-1}, a low toxicity level, equivalent to the 4th class of toxicity in the Hodge and Sterner class. Phycoerythrobilin and folic acid showed no potential inhibition of following cytochromes: CYP1A2, CYP2C19, CYP2C9, CYP2D6 and CYP3A4. Regarding phycocyanobilin, two inhibitions against CYP1A2 and CYP2C9 were detected with a probability of 0.52. As this value is below 0.70, it was considered as not enough statistically significant. Nevertheless, *in vitro* and *in vivo* studies remain essential to warrant these results. The three compounds respond positively to the hepatotoxicity criterion but with quite low probability for phycocyanobilin and phycoerythrobilin. The risk is higher for folic acid, with a probability reaching 0.80, it is thus necessary to remain vigilant regarding this parameter. Phycocyanobilin and phycoerythrobilin may cause P-glycoprotein inhibition, a major actor in drug metabolism (Rautio et al. 2006). Such inhibition may enhance bioavailability of drugs by accumulation in cells which, if it is not the wanted effect, could lead to toxic effects. Thus, a particular attention should be paid regarding drug interactions with these two relevant compounds.

Discussion

In the global context of Covid-19 pandemic, joint advances in research are essential. Spike protein, a major structural protein of the SARS-CoV-2 constitutes a privileged target for antiviral research, as the first interactions between virus and human host cell take place via the Spike protein RBD, interacting with human ACE2 receptor.

Taking advantage of our skills with *in silico* studies, we choose to apply this knowledge as a test in the Covid-19 frame. Using largely widespread docking software, three *Arthrospira* molecules among 51 were identified as candidates for antiviral development. Folic acid, phycocyanobilin and phycoerythrobilin presented binding energy of respectively -7.4, -7.2 and -7.3 kcal.mol^{-1}. Regarding the complex Spike RBD / ACE2, with a binding energy of -12.4 kcal.mol^{-1}, the three compounds could represent serious mid-competitive inhibitors, helping in the fight against Covid-19 pathology.

Phycocyanobilin and phycoerythrobilin are phycobilins of the respective phycobiliproteins C-phycocyanin and phycoerythrin. The tetrapyrrolic structure of these chromophores is involved in light harvesting of cyanobacteria such as *Arthrospira* (Akimoto

et al. 2012; Rodrigues et al. 2018). Phycocyanin and phycoerythrin are authorized by both FDA and European Commission and are usually used as food coloring (Bratinova 2015).

No clinical trial on the website Clinicaltrials.com directly reported the use of those compounds with the aim to cure or prevent viral infections, thus opening a new way of research. Phycocyanins are mainly known for their great antioxidant properties (Zhou et al. 2005; Wu et al. 2016). *In vitro* study showed that phycocyanobilin represents the main *Arthrospira* compound responsible for its antioxidant effect (Hirata et al. 2000). Phycocyanobilin was shown to have a similar structure to a biliverdin, an NADPH inhibitor (Zheng et al. 2013). Phycocyanobilin is converted into phycocyanorubin by biliverdin reductase (Terry et al. 1993) with a structure close to bilirubin (McCarty 2007). In other *in vivo* studies, both phycocyanin and phycocyanobilin were able to normalize oxidative stress markers and expression of components from NADPH pathway (Zheng et al. 2013). Mc Carty (2010) suggested NADPH inhibition as a way of treatment in Influenza viral infection. Indeed, it has been identified as the main oxidative stress source in lung epithelial cells, participating in viral symptoms. Thus, phycocyanobilin and phycoerythrobilin present additional arguments to support their antiviral potential.

Folic acid, also known as vitamin B9 is a largely used molecule, approved and under health claims by both FDA and EFSA (FDA 2020; Barroso 2014). Folic acid is usually used during pregnancy to prevent the risk of neural tube defects (Greenberg et al. 2011). It is also widely used as concomitant treatment with Methotrexate to offset its side effects. Indeed Methotrexate (an antirheumatic drug) tends to exert a competitive activity against folic acid, necessitating a substantial supplementary administration of this folic acid (Shea et al. 2013). In this context, folic acid has been included in two clinical studies: one in HIV infected patients taking both Methotrexate and folic acid (Clinical trial n°NCT01949116) and a second one on hepatitis C infected patients (Clinical trial n°NCT02150291). This later studied how folic acid could act as prophylactic treatment in a viral infection to counteract side effects of the main treatment. These arguments are in favor of a safety and well-known consumption of folic acid which thus can be seriously considered as a candidate in the fight against Covid-19.

Toxicity parameters have been conducted *in silico* to check safety of these compounds. No carcinogenicity or mutagenicity was seen together with a safe high LD_{50}, comprised between 500 and 5000 $mg.kg^{-1}$. No significant interaction with the main cytochromes and P-glycoprotein was predicted, suggesting no drug interaction. While a low hepatotoxicity risk has been detected, folic acid, phycocyanobilin and phycoerythrobilin remain known and authorized compounds, both in USA and Europe. These molecules also possess specific biological activities such as antioxidant which may further increase the relevance of their utilization as an antiviral treatment for Covid-19.

Except preprint publications, few papers refer to *in silico* research of Spike protein ligand. Sinha et al. (Sinha et al. 2020) studied interactions of saikosaponins, bioactive molecules from Traditional Chinese Medicine plants, with the entire Spike protein in open conformation. Their results highlighted three saikosaponins (V, U, C), showing binding energies comprised between -7.2 and -8.4 $kcal.mol^{-1}$. Excepting saikosaponin V which interacted with one key binding residue of the Spike protein/ACE2 link (475), none of the two others interacted with key binding residues involved in this critical bind. Another publication presented only hesperidin as potent inhibitor of Spike/ACE2 interface despite a quite large list of compounds with high binding energies (Wu et al. 2020).

On the basis of these results, our work provides a significant stone on this subject in view of the few results published to date. It will be more interesting to follow the future work that will deal with the interaction of our 3 molecules in viral episodes and further *in silico* studies will improve and guide bioactive molecule selection for antiviral compound research.

Declarations

Competing interests:

The authors declare no competing interests.

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Tables

Table 1 *Binding residues involved in Spike RBD / ACE2 link; green residues correspond to consensus sequences*

ACE2	References
S19, Q24, K31, E35, D38, L79, M82, Y83, K353.	Shang *et al.*, 2020
Q24, D30, H34, Y41, Q42, M82, K353, R357.	Yan *et al.*, 2020
S19, Q24, F28, D30, K31, H34, D38, Y41, Q42, L79, M82, Y83, K353, D355.	Wang *et al.*, 2020
Q24, T27, **F28, D30, K31, H34, E35,** E37, **D38, Y41, Q42, L79, M82, Y83,** N330, **K353,** G354, **D355, R357,** R393.	Lan *et al.*, 2020
SPIKE RBD	References
L455, F486, Q493, S494, **N501.**	Shang *et al.*, 2020
K417, Y453, Q474, **F486, Q498, T500, N501.**	Yan *et al.*, 2020
K417, G446, Y449, Y453, A475, E484, F486, N487, Y489, G496, Q498, T500, G502.	Wang *et al.*, 2020
T402, Y436, N439, Y440, **L455,** N473, **F486,** G488, Y491, **Q493, Q498, N501.**	Walls *et al.*, 2020
K417, G446, Y449, Y453, L455, F456, **A475, F486, N487, Y489, Q493, G496, Q498, T500, N501, G502,** Y505.	Lan *et al.*, 2020

Table 2 *In vitro evaluation of Arthrospira antiviral activity: state of the art*

MODEL	TARGETED VIRUS	EXTRACT/COMPOUND	ANTIVIRAL ACTIVITY	CONTROL	REFERENCE
Hep-2 cells	VACV	TK V2 (intracellular), Exopolysaccharides TK V3	Decrease of viral hepatic replication. Replication inhibition: EC_{50}(TK V3) = 0.78 µg.mL^{-1}	Untreated control	(Radonic et al. 2010)
MDCK cells	Influenza	Cold water extract of *A. platensis*	Inhibition of viral plaque formation (EC_{50} = 0.58 ± 0.02 mg.mL^{-1})	Untreated control	(Chen et al. 2016)
Hep 2 cells	Adenovirus type 40	Methanolic extract of *A. platensis*	50 % reduction of viral titer (EC_{50} = 0.8-3.1 mg.mL^{-1})	NA	(Abdo et al. 2012)
Hep 2, BGM, and MA104 cell lines	Adenovirus type 7, CVB4, astrovirus type 1, RV Wa strain, & adenovirus type 40	Ethanolic extract of *A. platensis*	53.3%, 66.7%, 76.7%, 56.7%, and 50% reductions of viral titer response; dose: 1.6 – 1.9 mg.mL^{-1}	Untreated control	(El-Baz et al. 2013)
Vero cells	CVB3 & RV	Methanolic extract of *A. platensis*	TI(CVB3) = 30, TI(RV) = 45	Untreated control	(Deyab et al. 2020)
MA104, Hep-2 and BGM cell lines	RV Wa strain, adenovirus type 7, adenovirus type 40, CVB4	70% methanol & n-hexane extract of *A. platensis*	Inhibition of 56.7% and 66.7% against RV Wa strain; 60% and 63.3% against adenovirus type 7; 53.3% and 50% against adenovirus type 40 respectively and 50% for both extracts against CVB4 (0.5 mg.mL^{-1})	Untreated control	(Hetta et al. 2014)
HFFs	HCMV	Polysaccharide fractions isolated from *A. platensis*(spirulan-like molecules)	HCMV Inhibitory effect: EC_{50} = 1.4 ± 0.3 µg.mL^{-1} with preincubation, EC_{50} = 93.3 ± 0.1 µg.mL^{-1} with post-incubation	Reference drug: ganciclovir (GCV)	(Rechter et al. 2006)
Vero cells	HSV 1 & 2	Partial desulfated and oversulfated sodium spirulan (Na-SP) from *A. platensis*	HSV-1: EC_{50} (Na-SP) = 0.63 µg.ml^{-1}, EC_{50}(Os-SP-2) = 0.46 µg.mL^{-1}, HSV-2: EC_{50}(Na-SP) = 0.41 µg.ml^{-1}; EC_{50} (Os-SP-2) = 0.46 µg.mL^{-1}	Reference drug: acyclovir	(Lee et al. 2007)
RC-37 cells	HSV	Cold & hot water (2.5 mg/mL), phosphate buffer (10 mg.mL^{-1}) extracts of *A. fusiformis*	Virus infectivity reduction of 54.9%, 64.6%, and 99.8%	Untreated control & Reference drug: acyclovir	(Schnitzler et al. 2013)
Vero cells	HSV-2	Hot water extract of *A. maxima*	Adsorption and penetration inhibition: selectivity index of 128, EC_{50} = 0.069 mg.mL^{-1}	Uninfected cells treated with the same extract	(Hernández-Corona et al. 2002)
Vero cells	HSV-1	Sulphoquinovosyl diacylglycerol isolated from *A. platensis*	HSV-1 inhibition: EC_{50} = 6.8 µg.mL^{-1}	Untreated control & Reference drug: acyclovir	(Chirasuwan et al. 2009)
Vero cell & HepG2 cells	HAV-MBB strain, HSV-1	Phosphate buffer and water extract of *A. platensis*	60% inhibition of hepatis A virus with 50 µg.mL^{-1} water extract; 98% inhibition of HSV-1 with 50 µg.mL^{-1} of water & phosphate buffer extract	0.02 M NaCl	(Shalaby et al. 2010)
Burkitt's lymphoma (BL) cell lines: Akata, B95-8, and P3HR-1	EBV	Methanolic extract of *A. platensis*	Effect on virus load: EC_{50} = 0.021 µg.mL^{-1}, CC_{50} = 166 µg.mL^{-1}, TI = 7905 in the case of B95-8 cells	Untreated control & Reference drugs: acyclovir, foscarnet	(Kok et al. 2011)
human T cell line MT4	HIV-1	Peptide isolated from Spirulina maxima (SM-peptide)	Inhibit induced cell lysis: EC_{50} = 0.475 mg.mL^{-1}, CC_{50} = 1.457 mM; Inhibition of HIV-1 reverse transcriptase (0.75 mg.mL^{-1}) & p24 antigen production >95%	Untreated control	(Jang and Park 2016)
Human T-cell lines, peripheral blood mononuclear (PBMC) & Langherans cells (LC)	HIV-1	Aqueous extract of *A. platensis*	Reduce viral production in PBMCs: EC_{50}= 0.3-1.2 µg.mL^{-1}	Uninfected cells treated with the same extract	(Ayehunie et al. 1998)

Vaccinia Virus (VACV); Coxsackievirus (CV); Rotavirus (RV); Human cytomegalovirus (HCMV); Hepatitis-A-virus-type-MBB (HAV-MBB); Epstein-Barr virus (EBV); Vero cells = African green monkey kidney; Human Immunodeficiency virus (HIV); NA : Not Available

Table 3 *Clinical study evaluation of Arthrospira antiviral activity: state of the art*

MODEL	TARGETED VIRUS	EXTRACT/ COMPOUND	ANTIVIRAL ACTIVITY	BENEFICIAL EFFECT	DURATION	STUDY PARAMETERS	REFERENCE
73 HIV-infected adult females	HIV	5 g.day^{-1} of *A. platensis*	No effect on the viral load and/or the CD4 T-cells	Improvement of anemia status, good nutritional rehabilitation effects	3 months	Placebo, randomized, double-blind	(Winter et al. 2014)
11 antiretroviral-naïve	HIV-1	5 g.day^{-1} of dried *A. platensis*	No significant effect on CBC, metabolic & lipid panel; stable CD4 & virus load	Clinically significant improvement in CD4 (>100 cells.mL^{-1}), decreased HIV viral load of 0.5 log10 for 1 subject	3 months	Placebo, randomized	(Teas and Irhimeh 2012)
320 naïve HIV-1 patients	HIV-1	10 g.day^{-1} of dried *A. platensis*	Significant increase of CD4 count cells, decrease of viral load level & higher hemoglobin level	Improve immune system & prevent opportunistic diseases	12 months	Randomized, single-blind, control without spirulina	(Ngo-Matip et al. 2015)
52 HIV-infected	HIV-1	0.2 to 0.37 g.kg^{-1}.day^{-1} of *A. platensis*	Significantly lower viral load, higher CD4 count, increase in hemoglobin level (1.6 g.dL^{-1})	Increase quality of weight gain	3 months	Randomized, single-blind, control group	(Azabji-Kenfack et al. 2011)
25 thalassemic children HCV-infected	HCV	250 mg.kg^{-1}.day^{-1} of *A. platensis*	Significant increase of CD4 (from 19.56 ± 7.8 to 32.2 ± 13.5 x10^3 cells.100 mL^{-1}) & CD8 (from 17.68 ± 6.88 to 26.44 ± 9.08 x10^3 cells.100 mL^{-1}) after 6 months	Immune stimulation	6 months	NA	(Gomaa et al. 2017)
66 HCV-infected	HCV	3x 500 mg.day^{-1} of *A. platensis*	No significant effect on virus load; ALT, CLDG & ASEX improved by spirulina	Loss or reduction of detectable hepatitis C virus RNA for 6 patients	6 months	Randomized, double-blind, control group (3x 140 g of Silymarin/day)	(Yakoot and Salem 2012)

Hepatitis C (HCV); Human Immunodeficiency virus (HIV); CBC: complete blood count; ALT: alanine transaminase; CLDG: Chronic Liver Disease Questionnaire; ASEX: e Arizona Sexual Experiences Scale; NA: Not Available

Table 4 *Docking results for small molecules vs Spike RBD of SARS-CoV-2; Residues in red reflect unfavorable interactions between the molecule studied and the spike RBD, those in bold are directly involved in the link between ACE2 and spike RBD. Stars were added to amino acids involved in H-bond*

Molecules	Pubchem ID	Binding affinity (kcal.mol^{-1})	RMSD/ub	RMSD/lb	Binding residues
Catechin	9064	-7.2	0	0	ARG403, GLU406*, **TYR453**,**GLY496**, **ASN501***, TYR505
Epicatechin	72276	-7.1	0	0	ARG403*, TYR453, **GLY496*,**TYR505
Dieckol	3008868	-8.5	0	0	ARG403, GLU406, ARG408,**LYS417**, **TYR449, TYR453, GLN498***
Apigenin	5280443	-7.1	0	0	ARG403*, **TYR453**, **GLY496,**TYR505*
Beta-carotene	5280489	-7.1	0	0	**TYR449,** LEU452, PHE490, TYR505
Rutin	5280805	-7.4	0	0	**GLN493***, **GLY496**, **ASN501*,**TYR505*
Astaxanthin	5281224	-7.1	0	0	**TYR449**, LEU452, TYR505
Rosmarinic acid	5281792	-7.1	0	0	ARG403*, **TYR449,GLN493***, PHE497, TYR505*
Phycobilin	6438349	-7.2	0	0	ARG403*, **TYR449,** SER494*, TYR495, **GLY496***, PHE497,**GLN498***, TYR505
		-7.2	1.997	1.607	**TYR449***, SER494*, TYR495, **GLY496*,** PHE497, **GLN498***, TYR505
Phycoerythrobilin	6443764	-7.3	0	0	ARG403, **LYS417, TYR449,TYR453,** SER494*, **GLY496***
Folic acid	135398658	-7.4	0	0	ARG403, ASP405*, GLU406*, ARG408, GLN409*, **TYR453**, TYR495, **GLY496*, GLN498***, **ASN501***
		-7.3	1.921	1.536	ARG403, ASP405*, GLU406*, **LYS417**, **TYR453**, SER494, TYR495, TYR505*

Table 5 *Toxicity parameters of phycocyanobilin, phycoerythrobilin and folic acid. Orange data highlights positive results with a probability > 0.70*

	Phycocyanobilin		Phycoerythrobilin		Folic acid	
	Value	Probability	Value	Probability	Value	Probability
Ames mutagenesis	-	0.71	-	0.67	-	0.80
Acute Oral Toxicity (c)	III	0.59	III	0.58	III	0.62
Carcinogenicity (binary)	-	0.87	-	0.90	-	0.87
CYP1A2 inhibition	+	0.52	-	0.58	-	0.93
CYP2C19 inhibition	-	0.73	-	0.78	-	0.92
CYP2C9 inhibition	+	0.52	-	0.53	-	0.91
CYP2D6 inhibition	-	0.87	-	0.88	-	0.94
CYP3A4 inhibition	-	0.74	-	0.78	-	0.91
Hepatotoxicity	+	0.67	+	0.57	+	0.80
P-glycoprotein inhibition	+	0.77	+	0.78	-	0.87

Figures

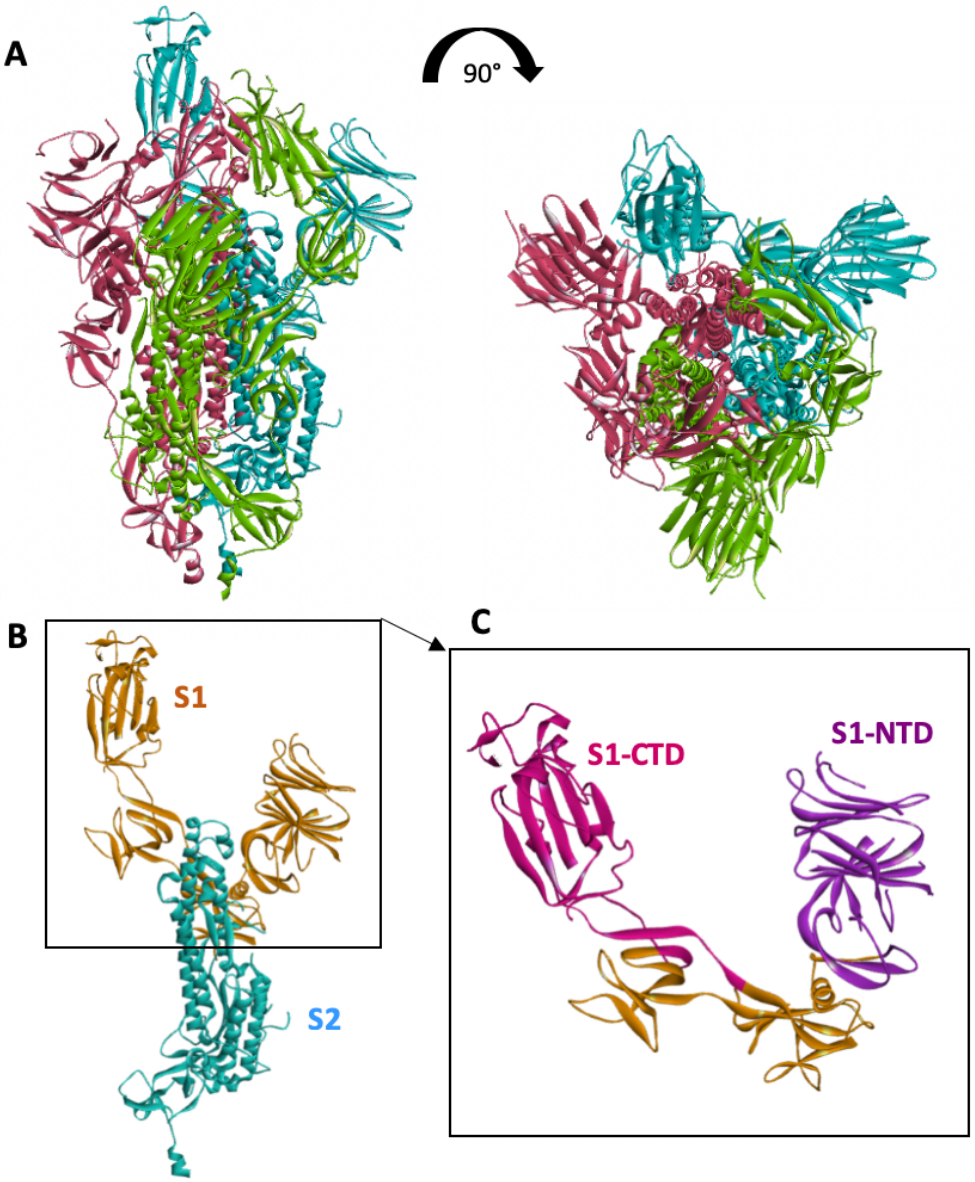

Figure 1

A. Cryo-electron microscopy structure of prefusion trimeric SARS-CoV-2 spike protein (from PDB: 6LZG). Three monomers are identified (pink, green and blue) from two angles of view. B. Monomer structure of SARS-CoV-2 spike protein, subunit S1 is represented in orange and S2 in blue. C. RBD localization in S1 subunit: S1 C-terminal domain (S1-CTD = RBD) appears in pink and S1 N-terminal domain (S1-NTD) in purple

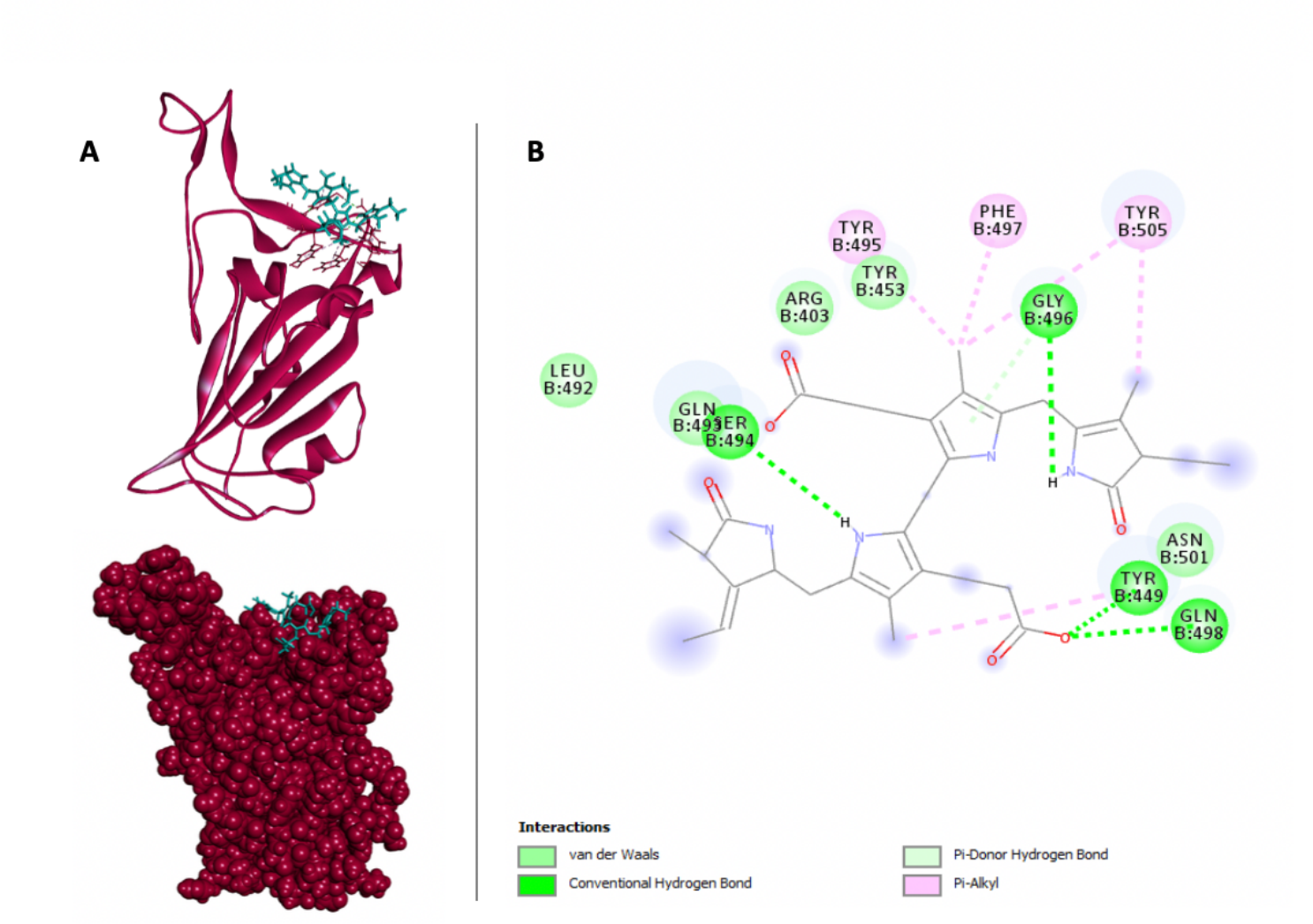

Figure 2

Phycobilin docked with spike RBD of SARS-CoV-2. A. 3D structure of the complex, phycobilin (ligand) is represented in green, Spike RBD (receptor) is in dark pink B. Interaction map of the ligand/receptor complex

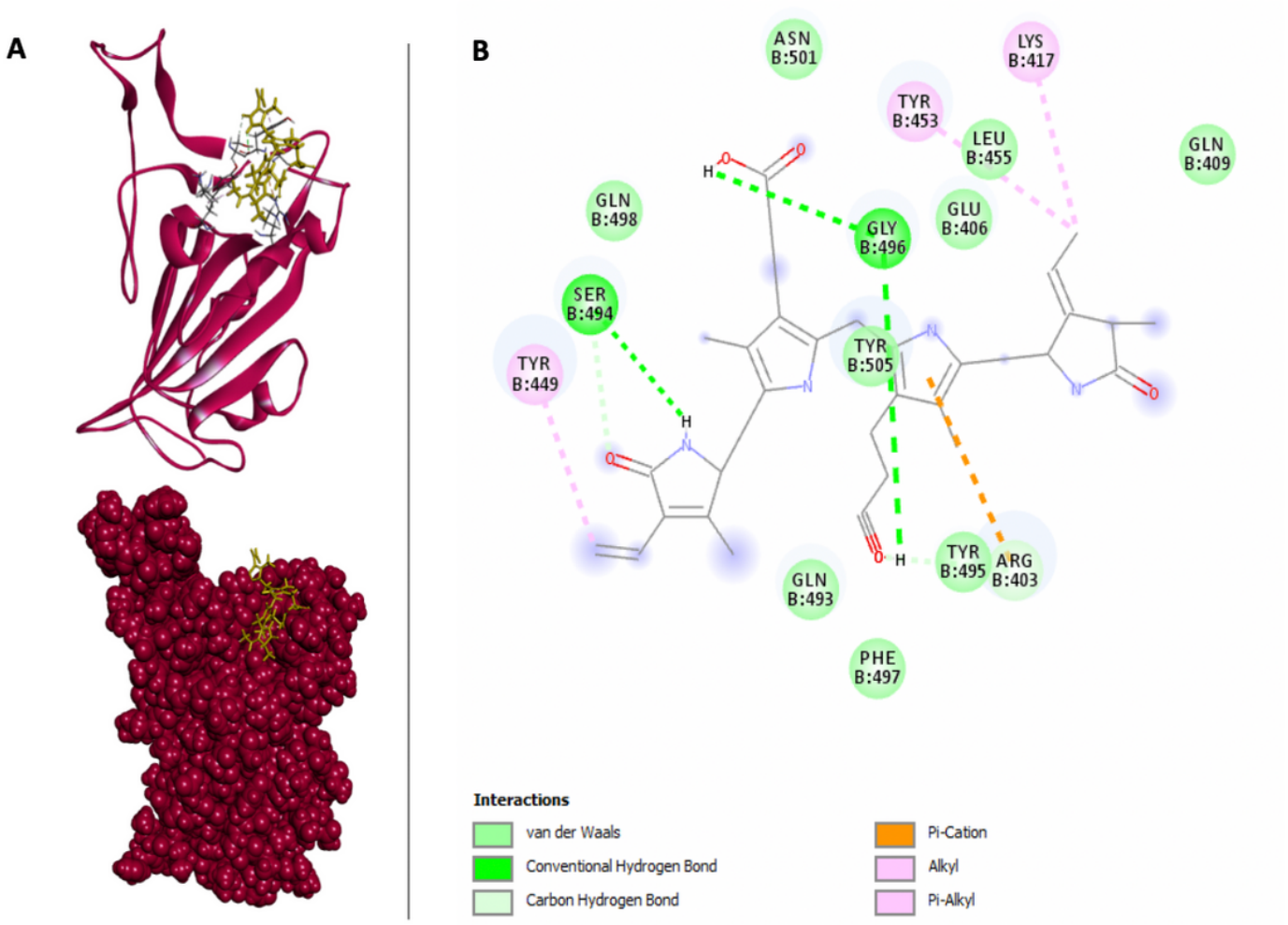

Figure 3

Phycoerythrobilin docked with spike RBD of SARS-CoV-2. A. 3D structure of the complex, phycoerythrobilin (ligand) is represented in green, Spike RBD (receptor) is in dark pink B. Interaction map of the ligand/receptor complex

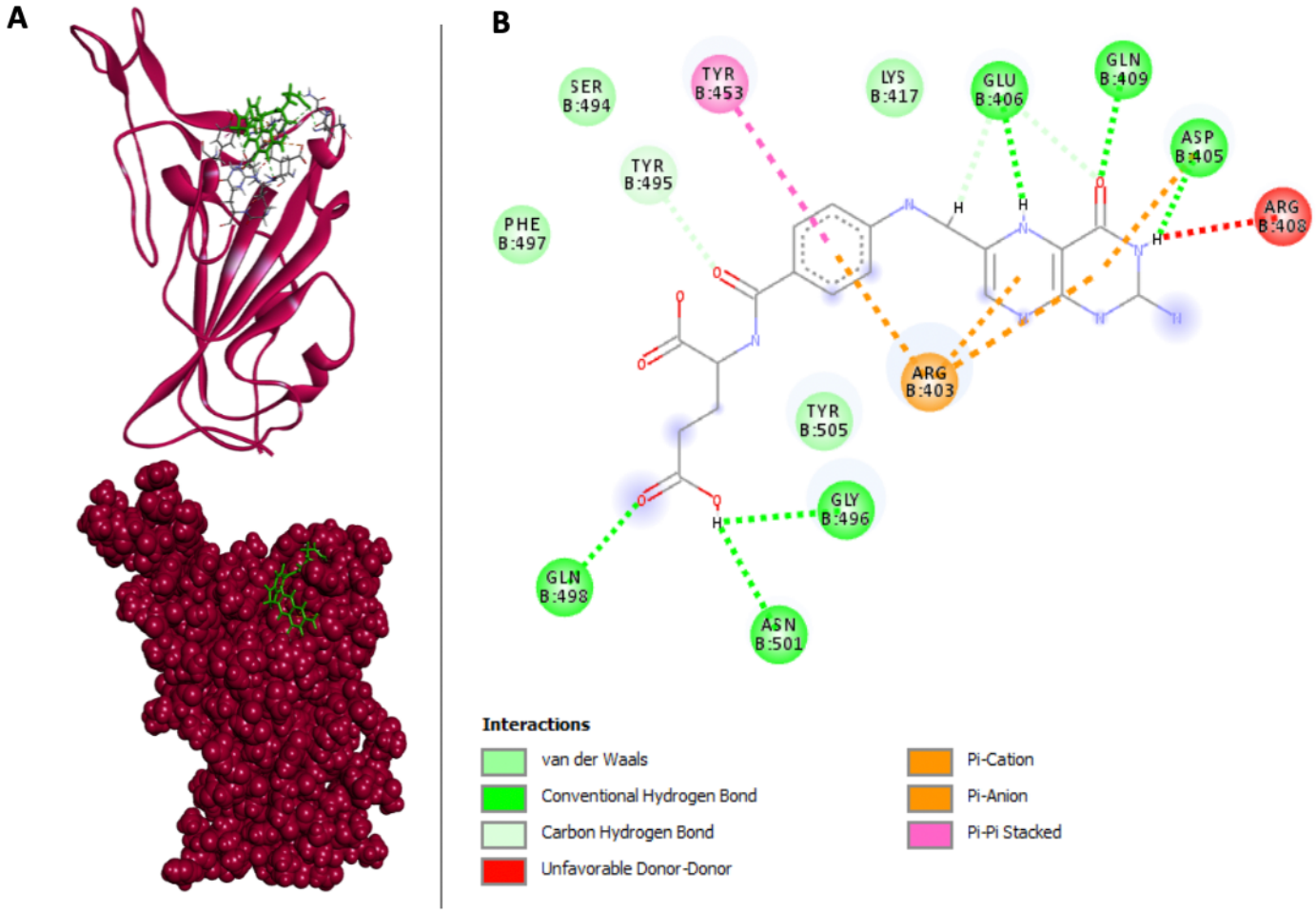

Figure 4

Folic acid docked with spike RBD of SARS-CoV-2. A. 3D structure of the complex, folic acid (ligand) is represented in green, Spike RBD (receptor) is in dark pink B. Interaction map of the ligand/receptor complex

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementarydataTable6.docx