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ORIGINAL RESEARCH ARTICLE

Identification of Cyanobacteria-Based Natural Inhibitors Against SARS-CoV-2 Druggable Target ACE2 Using Molecular Docking Study, ADME and Toxicity Analysis

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Abstract In 2019–2020, the novel "severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2)" had emerged as the biggest challenge for humanity, causing "coronavirus disease 19 (COVID-19)". Scientists around the world have been putting continuous efforts to unfold potential inhibitors of SARS-CoV-2. We have performed computational studies that help us to identify cyanobacterial photoprotective compounds as potential inhibitors against SARS-CoV-2 druggable target human angiotensin-converting enzyme (ACE2), which plays a vital role in the attachment and entry of the virus into the cell. Blocking the receptor-binding domain of ACE2 can prevent the access of the virus into the compartment. A molecular docking study was performed between photoprotective compounds mycosporine-like amino acids, scytonemins and ACE2 protein using AutoDock tools. Among sixteen molecularly docked metabolites, seven compounds were selected with binding energy < 6.8 kcal/mol. Afterwards, drug-likeness and toxicity of the top candidate were predicted using Swiss ADME and Pro Tox-II online servers. All top hits show desirable drug-likeness properties, but toxicity pattern analysis discloses the toxic effect of scytonemin and its derivatives, resulting in the elimination from the screening pipeline. Further molecular interaction study of the rest two ligands, mycosporine–glycine–valine and shinorine with ACE2 was performed using PyMol, Biovia Discovery studio and LigPlot+. Lastly biological activity of both the ligands was predicted by using the PASS online server. Combining the docking score and other studied properties, we believe that mycosporine–glycine–valine and shinorine have potential to be potent inhibitors of ACE2 and can be explored further to use against COVID-19.

Keywords SARS-CoV-2 · Angiotensin-converting enzyme-2 (ACE2) · Photoprotective compounds · Mycosporine-like amino acids · Scytonemin · Molecular docking

Introduction

Years 2020 and 2021 would register in human history as pandemic years, as an outbreak of "corona virus disease 19 (COVID-19)" caused by highly zoonotic "severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2)" that shook humanity, infecting 500 million people and claiming 6 million lives across 230 countries by 22 April 2022 [1]. Different regions of the world witnessed three waves of coronavirus and many more are expected in future. WHO has been monitoring the evolution of coronavirus with the collaboration of various researchers and national authorities to track the variants. It has declared delta and omicron variants of SARS-CoV-2 as a variety of concerns for India (October 2020) and several other countries (November 2021) respectively [2].

SARS-CoV-2 belongs to the genus beta coronavirus known to infect bats, humans and other mammals. Viral infections are associated with symptoms like cough, fever and difficulty in breathing which may progress to pneumonia and death if unattended [3]. The virus has a positive-sense single-stranded RNA genome encoding several structural and non-structural proteins (nsp) which are

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essential for viral attachment to the host cell, viral replication and subsequent infection [4]. The first and the most crucial step of infection involves the attachment and entry of the virus into the host cell. Viral envelope protein (spike glycoprotein) binds to specific receptors present on the host cell surface which facilitates its entry. Among all the receptors, the angiotensin-converting enzyme-2(ACE2)" receptor is one of the prime targets of the virus [5]. At subdomain-I of ACE2, the binding domain of the spike glycoprotein of SARS-CoV-2 binds and activates the membrane fusion process [6]. With this process the virus's genetic material (RNA) is released into the cytoplasm of the host cells [7].

Most human organs express ACE2 protein in different degrees. Type II alveolar epithelial cells of the respiratory system strongly express ACE2. The epithelial cell of the oral, nasal mucosa and nasopharynx shows a weak expression of ACE2, which proves that the lungs are the primary target for SARS-CoV-2 [8]. Myocardial cells, bladder cells and cells of the proximal tubule of the kidney express the ACE2 very strongly. Enterocytes of the small intestine (specifically the ileum) also copiously express this protein [8]. The blood circulatory system plays a significant role in transporting viral particles to those organs associated with the high expression of ACE2 [9]. According to the report of Zhou et al. (2020), SARS-CoV-2 is only able to affect cells with ACE2 receptors [10]. So blocking the ACE2 receptors using inhibitors will make the entry of the virus difficult and thereby reduce the spread. This makes ACE2 receptors a potential drug target for therapeutic remedies of SARS-CoV-2.

Since the emergence, the studies on SARS-CoV-2 have come to a very long way. Researchers are exploring effective therapeutic drugs and vaccine candidates against SARS-CoV-2 by identifying target sites in the host and virus [11]. U.S. Food and Drug Administration (FDA) approved numerous synthetic antiviral drugs such as lopinavir [12] and remdesivir for treating COVID-19. Antimalarial drugs such as hydroxychloroquine and chloroquine [13] are also available for treatment. However, various deleterious side effects of these synthetic drugs have been reported. Therefore, there is need to replace these synthetic drugs with naturally derived ones having no side effects.

Even though vast arrays of naturally derived metabolites exist, experimenting with drug discovery and running trails around each of them can be challenging. Medical chemistry needs to change for more productive and rapid solutions to changing medical requirements. "Computer-aided drug design (CADD)" has played a vital role in cutting the search short and it can help in understanding complex biological processes for improving drug discovery. Also, the additional benefits like cost-saving, time to market, insight knowledge of drug-receptor interaction, speed up in drug discovery and development increase its popularity in scientific research [14].

In recent decades, the global pharmaceutical industry has relied heavily on natural products from microbial and plant sources to develop novel and effective therapeutics. High potential and lesser side effects make natural products a great candidate for drug discovery. Similar efforts were put in for finding natural drugs for SARS-CoV-2 as well. Secondary metabolites from cyanobacteria possess a wide range of biological activities such as antiviral, antifungal, anticancer, antibacterial, antimalarial and antitumor properties [15]. Also, in 2021 Naidoo et al. have shown the potential of cyanobacterial secondary metabolites against SARS-CoV-2 [16]. This research was the trigger point for us to find a similar trend among cyanobacterial photoprotective compounds such as mycosporine-like amino acids (MAAs) [17], scytonemin and its derivatives [18].

Cyanobacteria produce colorless and water-soluble MAAs having absorption maxima ranging from 309 to 362 nm. Structurally, MAAs consist of cyclohexenone or cyclohexinimine chromophore conjugated with the nitrogen substituent of an amino acid or its imino alcohol, having a molecular weight (MW) ranging from 188 to 1050 Da [17].

Unlike MAAs, scytonemin is a yellow–brown lipid-soluble compound primarily located in the cyanobacterial sheath. Scytonemin shows absorption maxima at 384, 300, 278 and 252 nm and exists in two forms i.e., oxidized (MW-544 kDa) and reduced (MW-546 kDa). The molecular structure is a dimer of indole and phenol subunits connected by an olefinic carbon atom, a unique natural product. The scytoneman skeleton is a novel ring system created due to the olefinic linkage in the scytonemin [18].

The bioactive compounds MAAs and scytonemin with UV-absorbing nature are considered natural photoprotectants and antioxidants can be exploited in various ways. Apart from the activities mentioned above, research has shown antimicrobial potential [18, 19] of both the compounds and their derivatives forming the base of this research to find their antiviral potential.

The present research employed a library of 16 biologically active and photoprotective metabolites (MAAs and scytonemin) from cyanobacteria to target the ACE2 receptors using a molecular docking approach. We undertook efforts to comprehensively analyze the physicochemical, drug-like features and antiviral activity of the cyanobacterial metabolites that displayed encouraging inhibitory potential.

Table 1 List of selected photoprotective compounds with their respective PubChem ID, molecular formula, chemical structure and respective binding energy

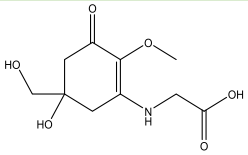
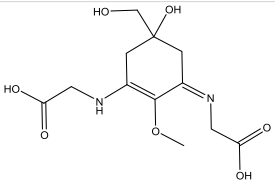
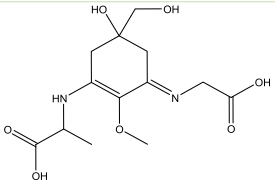
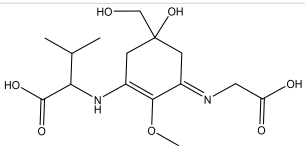
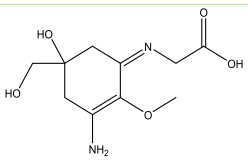
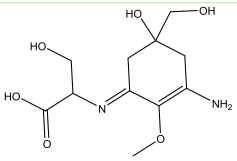
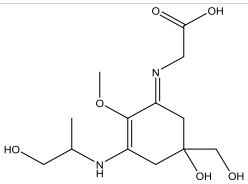
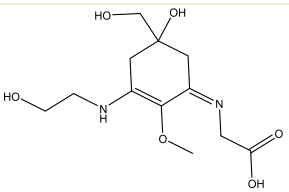
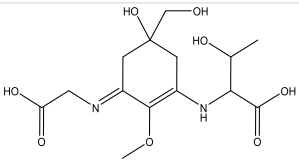
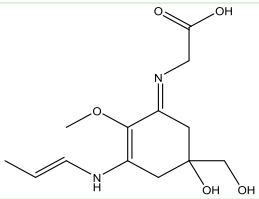
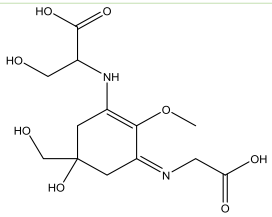
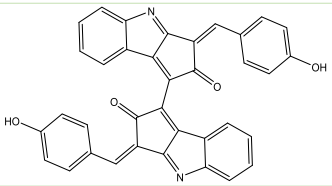
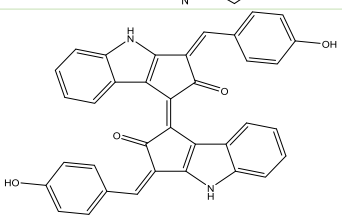
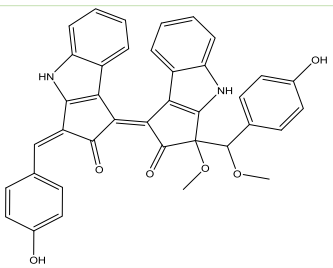
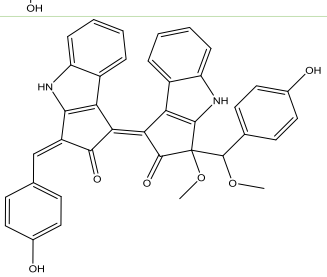
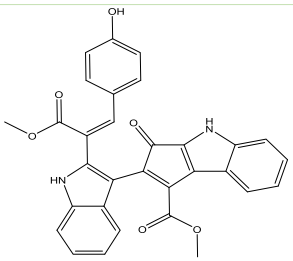
Cyanobacterial photoprotective compounds	PubChem Compound ID	Molecular formula	Chemical structure	Binding energy (kcal/mol)
Mycosporine-glycine	14444486	$C_{10}H_{15}NO_6$		-6.2
Mycosporine-2-glycine	23427657	$C_{12}H_{18}N_2O_7$		-6.7
Mycosporine-glycine-alanine	102110164	$C_{13}H_{20}N_2O_7$		-6.8
Mycosporine-glycine-valine	101016647	$C_{15}H_{24}N_2O_7$		-7.2
Palythine	16047608	$C_{10}H_{16}N_2O_5$		-6.4
Palythine-serine	1774828	$C_{11}H_{18}N_2O_6$		-6.4
Palythinol	9948334	$C_{13}H_{22}N_2O_6$		-6.4
Asterina-330	13194807	$C_{12}H_{20}N_2O_6$		-6.3
Porphyra-334	6857486	$C_{14}H_{22}N_2O_6$		-6.8

Table 1 continued

Usujirene	15847474	$C_{13}H20N_2O_5$		-6.8
Shinorine	10471931	$C_{13}H_{20}N_2O_8$		-7.0
Scytonemin	135473381	$C_{36}H_{20}N_2O_4$		-11.1
Reduced scytonemin	1C01366214	$C_{36}H_{22}N_2O_4$		-13.3
Dimethoxyscytonemin	10461341	$C_{38}H_{28}N_2O_6$		-12.8
Tetramethoxyscytonemin	10327140	$C_{40}H_{34}N_2O_8$		-10.4
Scytonin	10256051	$C_{31}H_{22}N_2O_6$		-10.8

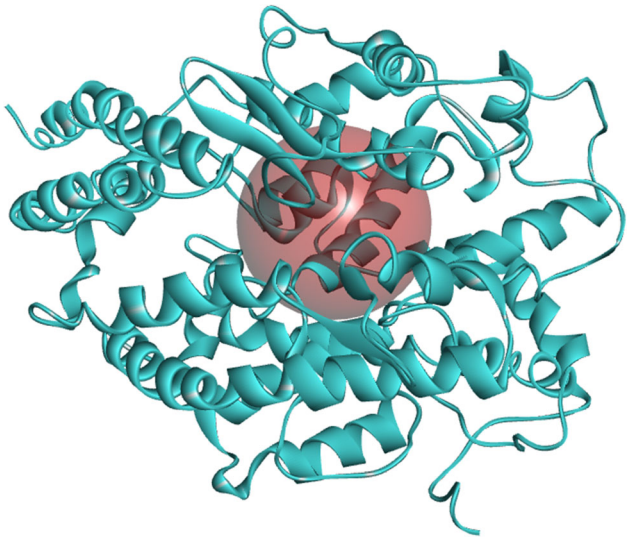


Fig. 1 Cartoon representation of chain A of ACE2 protein structure with their pocket binding site (represent by maroon sphere)

Materials and Methods

Preparation of Target Protein

The crystal structure coordinates of human ACE2 protein (PDB_ID: 1R4L) in the bound state with inhibitor MLN-4760 determined by X-ray diffraction were obtained from the protein data bank (https://www.rcsb.org/) in PDB format. It was made sure that the protein is in 3-Dimensional confirmation without any protein break. AutoDockTools 1.5.6 available at https://ccsb.scripps.edu/mgltools/ programs were used at ubuntu 19.10 linux platform for the preparation of target. The protein structure was prepared for docking analysis by removing water molecules, attached ligands and ions. Polar hydrogen atoms and Kollman charges were added to the structure. Chain A of human ACE2 was selected for docking study as the previously known inhibitor MLN-4760 is located in chain A [20]. The binding site of ACE2 around the MLN-4760 was defined using Biovia Discovery studio.

Determination and Preparation of Ligands

Through an extensive literature survey, several bioactive compounds like MAAs (mycosporine–glycine, mycosporine-2-glycine, mycosporine–glycine–alanine, mycosporine–glycine–valine, palythine, palythine–serine, palythinol, asterina-330, porphyra-334, shinorine, usujirene) and scytonemin (reduced scytonemin, dimethoxyscytonemin, tetramethoxyscytonemin and scytonin) from cyanobacteria were selected for docking study. All the selected compounds are well known for their photoprotective nature, anti-inflammatory action, antioxidant and medicinal properties. The ligands of interest with their 3-Dimensional structure were sourced from PubChem database in SDF format. All the 3-Dimensional structures were then converted into PDB format using the online SMILES Translator and Structure File Generator provided by the National

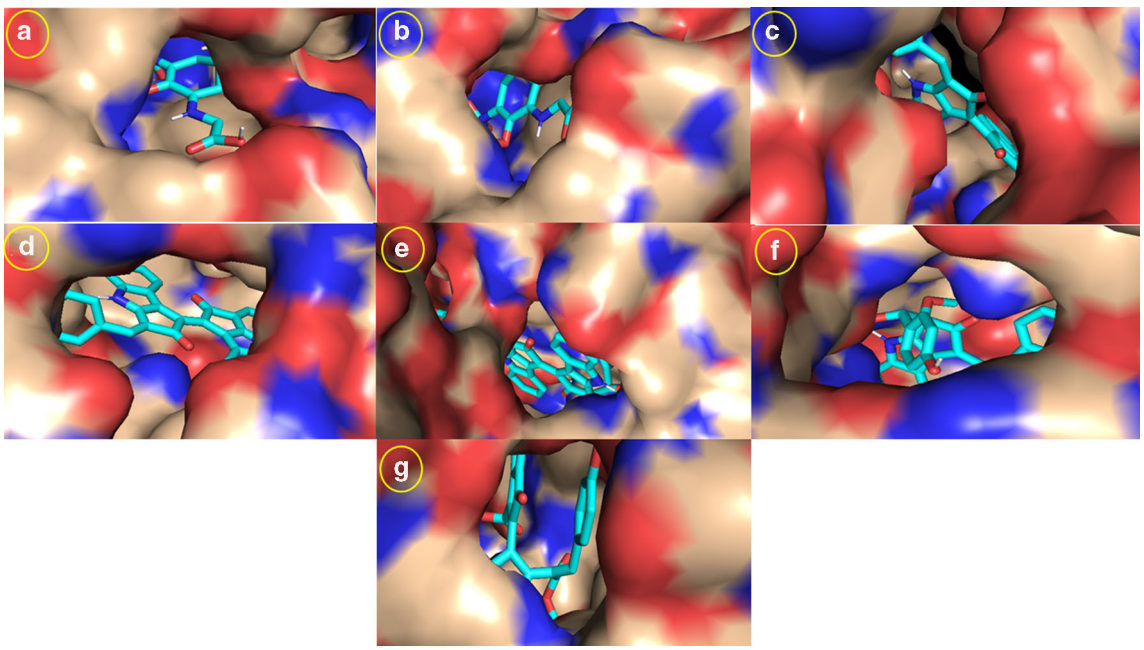


Fig. 2 In silico docked complexes of all the ligands (ball and stick) with binding energy less than − 6.8 kcal/mol with active site pocket of ACE2 (Molecular surface representation) by PyMol **a** Mycosporine–glycine–valine **b** Shinorine, **c** Scytonemin, **d** Reduced scytonemin, **e** Dimethoxyscytonemin, **f** Tetramethoxyscytonemin, **g** Scytonin

Cancer Institute (https://cactus.nci.nih.gov/translate/) and saved in the pdbqt formats. A list of all the selected ligands along with their structure and PubChem ID has been provided in Table 1.

Molecular Docking Study

An in silico approach for ligand and protein docking analysis was performed to inspect the structural complex of 1R4L with cyanobacterial bioactive compounds. Docking study was performed indigenously by docking one ligand at a time to the protein manually using AutoDock Vina 1.1.2 [21]. The default setting of Vina was used as the scoring matrix in this program is stochastic and each run uses a random seed position except for the grid box which was adjusted with extended grid dimension (center_x = 40.425, center_y = 1.218, center_z = 23.783 and size_x = 40, size_y = 40, size_z = 40) in the binding cavity of the target molecule. In the study, ligands were kept flexible allowing free rotation of all bonds, whereas receptors were considered rigid. The outcome was analyzed according to their docking score/binding energy.

Drug-Likeness, ADME, Toxicity Properties Prediction

Absorption, distribution, metabolism, excretion (ADME) and drug-likeness are crucial factors determining the destiny of many potent therapeutics agents whether they will be able to reach the clinical trials. Metabolites that displayed binding energy ≤ -6.8 kcal/mol with ACE2 protein were further subjected to the Swiss ADME online server [22] for prognosis of their physiological and drug-likeness (according to Lipinski rule of five) features [23]. Swiss ADME is a free web tool that provides free access to fast and robust models to compute the pharmacokinetics properties, drug-likeness and therapeutics chemistry of a molecule [22]. ProTox-II web server was used to detect carcinogenicity, mutagenicity, cytotoxicity, neurotoxicity and lethal dose 50 (LD_{50}) value [24].

Visualization of Protein–ligand Interactions

Topmost binding modes based on lowest binding free energy for two screened ligands were selected. Protein–ligand interactions were further visualized by PyMol [25], Biovia Discovery Studio and LigPlot+ [26]. A diligent examination of each protein–ligand cluster was carried out to find out interacting amino acids, hydrogen bonds (H-bonds) and the individual atoms involved in each ligand cluster (Fig. 1).

PASS Prediction for Antiviral Activity

Prophecy of cyanobacterial photoprotective compounds for antiviral activity was created with the assistance of the PASS web server (http://way2drug.com/PassOnline/) [27]. PASS is a computer system-based program employed to predict a compound's various physiological activities and biological potential based on its structure. The estimated activity of a substance was predicted as probable activity (Pa) and probable inactivity (Pi). The compound with a higher Pa value than Pi was considered feasible for a particular medicinal activity [27].

Results

Molecular Docking

AutoDock vina [28] is considered as one of the fastest and most widely used docking program, which uses binding free energy evaluation to find the best binding mode. All the 16 selected compounds were individually docked to the active site of ACE2 protein. The lowest binding energy pose (present at topmost position) was selected among 9 docking poses. The binding free energies of topmost docking poses for all the ligands are listed in Table 1. The topmost binding energy of all the 16 compounds falls between the ranges of − 13.3 kcal/mol to − 6.2 kcal/mol (Table 1). A commercial inhibitor of ACE 2, chloroquine phosphate, shows binding energy of − 6.8 kcal/mol with ACE2 [29]. Taking this data as a reference, we have selected those compounds with binding energy less than − 6.8 kcal/mol with receptor to be considered as better representatives than chloroquine phosphate to inhibit receptor protein. Out of 16 compounds, only seven compounds were screened as per the criteria where reduced scytonemin exhibits the lowest binding energy of − 13.3 kcal/mol. Rest of the screened compounds such as dimethoxyscytonemin, scytonemin, scytonin, tetramethoxyscytonemin, mycosporine–glycine–valine and shinorine show moderate interaction with ACE2 with binding energy of − 12.8 kcal/mol, 11.1 kcal/mol, − 10.8 kcal/mol, − 10.4 kcal/mol, − 7.2 kcal/mol and -7.0 kcal/mol respectively (Table 1). Comparatively, scytonemin and its derivatives show lower binding energy than MAAs. In silico docked complex of ligands (ball and stick representation) with ACE2 (surface representation) are shown in Fig. 2.

Physicochemical and Drug-Likeness Properties

Among the selected compounds subjected to the swiss ADME server for physicochemical properties prediction,

Table 2 Physicochemical properties of the active compounds in accordance with the rule of drug-likeness

Ligands	MW (g/mol)	Log P	HBA	HBD	AMR	TPSA	AlogS	Solubility	nRB	Number of violation of Lipinski rule	LogKp	Bioavailability	BBB	GI absorption	PAINS alert
Mycosporine–glycine–valine	344.36	− 0.56	8	5	85.11	148.68	− 2.43	Soluble	8	0	− 8.56	0.56	No	Low	0
Shinorine	332.31	− 1.89	9	6	76.66	168.91	− 0.77	Very soluble	8	1	− 9.92	0.11	No	Low	0
Scytonemin	544.55	5.29	6	2	170.46	99.32	− 7.74	Poorly soluble	3	1	− 5.45	0.55	No	Low	0
Reduced scytonemin	546.57	5.6	4	4	166.1	106.18	− 8.57	Poorly soluble	2	1	− 4.99	0.55	No	Low	1
Dimethoxyscytonemin	608.64	5.07	6	4	176.65	124.64	− 7.98	Poorly soluble	5	1	− 6.04	0.55	No	Low	1
Tetramethoxyscytonemin	670.71	4.61	8	4	187.21	143.1	− 7.39	Poorly soluble	8	1	− 7.08	0.55	No	Low	0
Scytonin	518.52	4.43	6	3	147.99	121.48	− 7.62	Poorly soluble	7	1	− 6.79	0.55	No	Low	0

MW molecular weight, *HBA* hydrogen bond acceptor, *HBD* hydrogen bond donor, *AMR* atom molar refractivity, *TPSA* topological polar surface area, *nRB* number of rotatable bonds, *BBB* blood brain barrier, *GI* gastrointestinal, *PAINS* pan-assay interference compounds

Table 3 Results of toxicity prediction using ProTox- II, showing lethal dose 50 (LD50) value, toxicity class, toxicity end points for organ toxicity, carcinogenicity, immunotoxicity, mutagenicity, cytotoxicity and prediction accuracy for selected cyanobacterial photoprotective compounds

Cyanobacterial photoprotective compounds	LD_{50} (mg/kg)	Toxicity class	Toxicity end point predicted by ProTox II										Prediction accuracy
			Organ toxicity		Carcinogenicity		Immunotoxicity		Mutagenicity		Cytotoxicity		
			Prediction	Probability	Prediction	Probability	Prediction	Probability	Prediction	Probability	Prediction	Probability	
Mycosporine–glycine–valine	300	Class-III	Inactive	0.85	Inactive	0.69	Inactive	0.99	Inactive	0.7	Inactive	0.67	54.26%
Shinorine	300	Class-III	Inactive	0.85	Inactive	0.71	Inactive	0.99	Inactive	0.64	Inactive	0.66	23%
Scytonemin	350	Class-IV	Active	0.51	Active	0.59	Inactive	0.85	Active	0.53	Inactive	0.56	54.26%
Reduced scytonemin	3000	Class-V	Active	0.51	Active	0.5	Inactive	0.6	Active	0.5	Inactive	0.7	54.26%
Dimethoxyscytonemin	2800	Class-V	Inactive	0.51	Active	0.52	Active	0.99	Active	0.51	Inactive	0.67	54.26%
Tetramethoxyscytonemin	530	Class-IV	Inactive	0.54	Active	0.5	Active	0.73	Inactive	0.51	Inactive	0.63	54.26%
Scytonin	1760	Class-IV	Inactive	0.53	Active	0.51	Active	0.86	Active	0.53	Inactive	0.67	54.26%

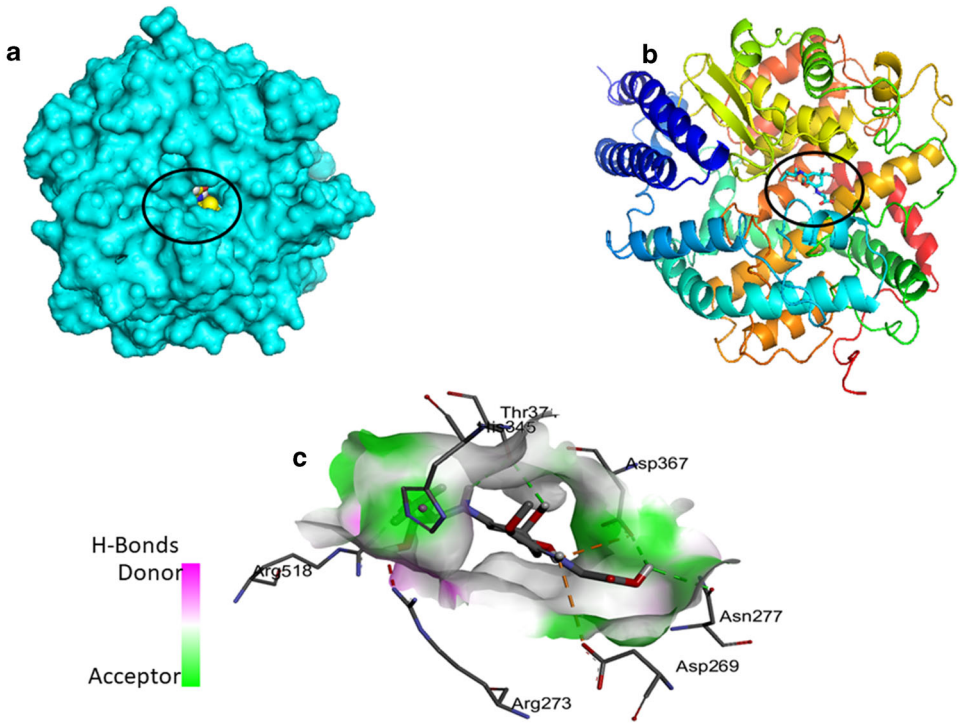


Fig. 3 Molecular docking interaction of mycosporine–glycine–valine with chain A of ACE2 receptor protein **a** Molecular surface map built in PyMol, cyan surface represent chain A of ACE2 and orange sphere represent mycosporine–glycine–valine, **b** Docked complex map built in PyMol, **c** 3-Dimensional contact map based on hydrogen bond donor and acceptor characteristics of amino acid residue built in Biovia Discovery Studio

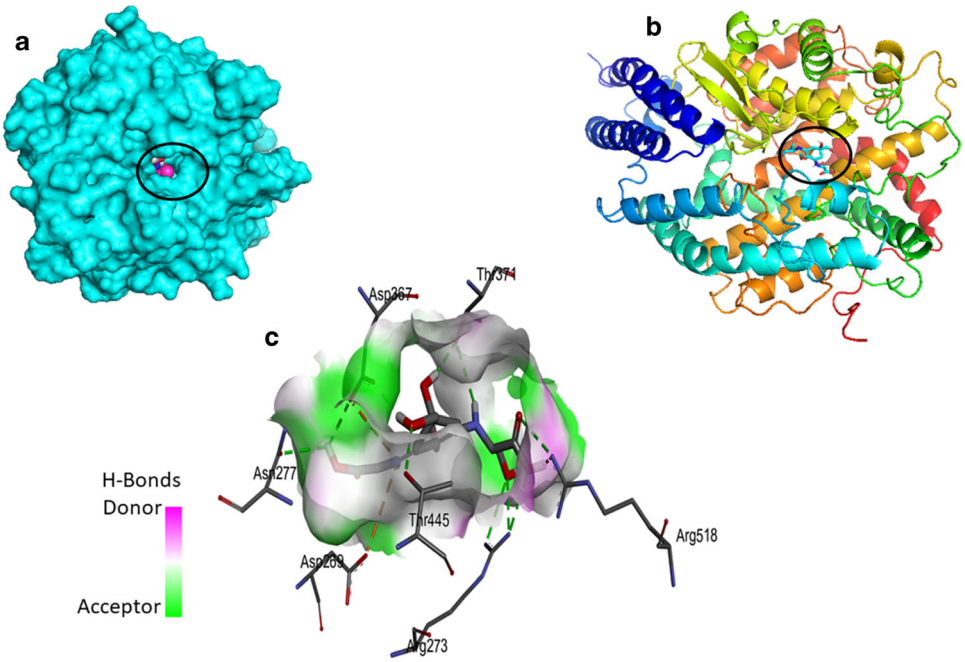


Fig. 4 Molecular docking interaction of shinorine with chain A of ACE2 receptor protein. **a** Molecular surface map built in PyMol cyan surface represent chain A of ACE2 and pink sphere represent shinorine, **b** Docked complex map built in PyMol **c** 3-Dimensional contact map based on hydrogen bond donor and acceptor characteristics of amino acid residue built in Biovia Discovery Studio

only mycosporine–glycine–valine obeyed all the Lipinski's rules (Table 2). Scytonemin, reduced scytonemin, dimethoxyscytonemin, tetramethoxyscytonemin and scytonin violate Lipinski's first rule as they have a molecular weight above 500. Shinorine with more than five hydrogen bond donors violates Lipinski's third rule (Table 2). The entire compound shows a higher value of Topological Polar Surface Area (TPSA) and Atom Molar Refractivity (AMR) (Table 2). Tetramethoxyscytonemin shows the highest AMR value (187.21), and shinorine shows the highest TPSA value (168.91). Pan Assay Interference Compound (PAINS) for all the 7 compounds is given in Table 2. Reduced scytonemin and dimethoxyscytonemin were predicted with one PAINS alert [23].

Blood–brain barrier permeability for all the selected compounds was nil as well as the gastrointestinal

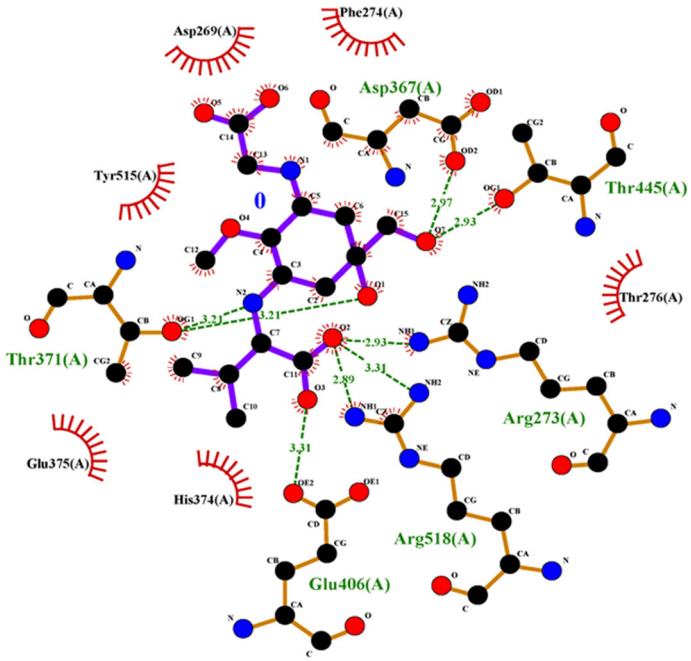


Fig. 5. 2-Dimensional representation of molecular docking interaction between mycosporine–glycine–valine with chain A of ACE2 receptor protein, hydrogen bond in the respective figures are shown in green dotted line with their bond length 2-D contact map built in LigPlot+

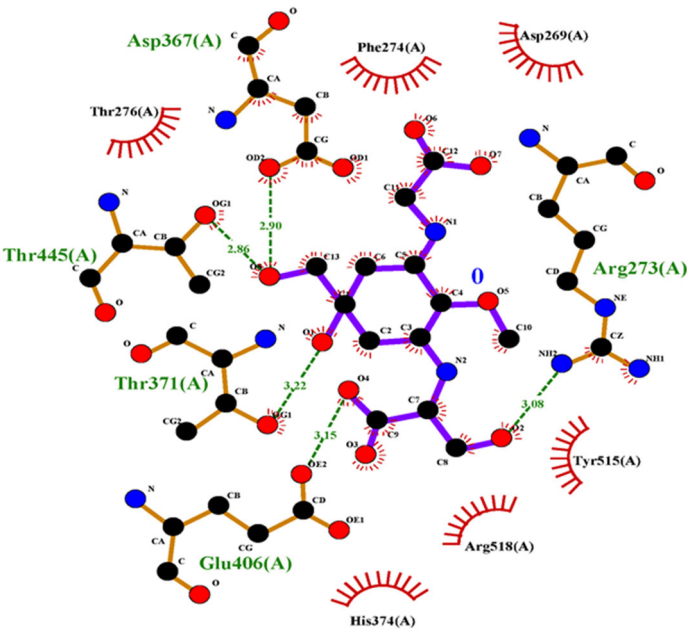


Fig. 6. 2-Dimensional representation of molecular docking interaction between shinorine with chain A of ACE2 receptor protein, hydrogen bond in the respective figures are shown in green dotted line with their bond length 2-D contact map built in LigPlot+

Table 4 Interacting amino acid residue of ACE2 protein with screened ligands at receptor site

Name of the ligand	Binding energy(kcal/mol)	Numbers of hydrogen bond interaction	Interacting residue of target along with their bond length
Mycosporine–glycine–valine	− 7.2	8	Arg 273 (2.93 Å), Asp 367 (2.97 Å), Thr 371 (3.21 Å), Glu 406 (3.31 Å), Thr 445 (2.93 Å),Arg 518 (2.89 Å,3.81 Å)
Shinorine	− 7.0	5	Arg 273 (2.93 Å), Asp 367 (2.90 Å), Thr 371 (3.22 Å), Glu 406 (3.15 Å),Thr 445 (2.86 Å)

Table 5 Result of PASS calculation for antiviral activity of mycosporine–glycine–valine and shinorine Pa: Probable activity, Pi: probable inactivity (- represents absence of respective antiviral activities)

Antiviral activity prediction by PASS online	Mycosporine–glycine–valine		Shinorine	
	Pa	Pi	Pa	Pi
Antiviral (Rhinovirus)	0.364	0.318	0.353	0.126
Antiviral (Picornavirus)	0.490	0.055	0.451	0.075
Antiviral (Poxvirus)	–	–	0.326	0.047
Antiviral (influenza)	0.227	0.159	0.227	0.159
Antiviral (Influenza A)	0.233	0.131	0.224	0.154
Antiviral (Adenovirus)	0.373	0.041	0.391	0.033
Antiviral (Herpes virus)	0.232	0.145	0.318	0.079
Antiviral (CMV)	0.231	0.109	0.223	0.0124
Antiviral (Hepatitis B)	0.203	0.084	0.228	0.064

absorption was low for all the ligands. Shinorine and mycosporine–glycine–valine show high solubility, whereas scytonemin and its derivatives show lower solubility (Table 2). All the selected compounds show a decent bioavailability score (Table 2). Log Kp value (skin permeability coefficient) of all the selected compounds is also shown in Table 2. Values of Kp were linearly correlated with molecular size and lipophilicity, with a more negative value of Log Kp molecule is supposed to be less skin permeable [23].

The result of LD_{50} value, toxicity class and toxicity endpoint for organ toxicity, carcinogenicity, immunotoxicity, mutagenicity and cytotoxicity of chosen compounds have been interpreted and analyzed through the Pro Tox-II online server and depicted in Table 3. The LD_{50} value of reduced scytonemin was highest i.e., 3000 mg/kg followed by dimethoxyscytonemin and scytonin with LD_{50} of 2800 mg/kg and 1760 mg/kg respectively. In the toxicity study scytonemin and its derivatives are found to be active for carcinogenicity, mutagenicity and cytotoxicity (Table 3) which makes them unfavorable drugs candidates. Thus we they got eliminated in this step of screening.

Molecular Interactions Studies

Screened compounds mycosporine–glycine–valine and shinorine with low binding energy and without any harmful activity were investigated further to determine interacting residues of the target protein. 3-D docked complexes of screened hits mycosporine–glycine–valine and shinorine accommodated in the binding pocket are shown in Figs. 3 and 4 respectively. The hydrogen bond interaction pattern generated by LigPlot+ illustrated the details of interactions between the target and ligand molecule. Mycosporine–glycine–valine interacts with the target molecule by forming hydrogen bond (marked by green dotted lines) with Arg 273 (2.93 Å), Asp 367 (2.97 Å), Thr 371 (3.21 Å), Glu 406 (3.31 Å), Thr 445 (2.93 Å) and Arg 518 (2.89 Å, 3.31 Å) residue of the target molecule (Fig. 5). Similarly, hydrogen bond interaction between shinorine and the target protein molecule is illustrated in Fig. 6. Interacting residue are Arg 273 (3.08 Å), Asp 367 (2.90 Å), Thr 371 (3.22 Å), Glu 406 (3.15 Å) and Thr 445 (2.86 Å) (Fig. 4). Table 4 shows both the safe and screened ligand, their binding affinity, number of hydrogen bond with target along with their bond length.

Binding residues of both the ligands were also compared with the binding residue of known inhibitor MLN-4760. MLN-4760 interacts with Arg273, His345, His505 and Thr371 residue of chain A of ACE2 [20]. Here we have found that Arg273, and Thr 371 are common interacting residues for mycosporine–glycine–valine, shinorine and MLN-4760.

PASS Prediction for Antiviral Activity

Biological activity spectra of selected cyanobacterial bioactive compounds were procured through the online PASS version. All the predictions were analyzed, interpreted and used flexibly and given in Table 5.

Discussion

Coronavirus has a long history of infecting humans and animals causing respiratory diseases. Clinical management, infection prevention, control measures and development of reliable therapeutics and vaccines are primary focuses to control the spread of disease. Currently, many drugs and vaccines are approved by the WHO to treat and prevent disease. However, measure drawbacks lie in the harmful side effects of the drugs and vaccines. Thus it is high time to identify and characterize new drug candidates to overcome this pandemic.

ACE2 protein has provided outstanding possibilities to recognize the drug candidates that can inhibit the protein

and viral interaction and thus be an effective therapy against SARS-CoV-2. Natural products have been in high demand due to their potent antimicrobial activities in recent decades. Considering the above aspects and the immediate need for therapeutics against the virus, we have virtually screened 16 different photoprotective compounds as novel drug leads against ACE2 protein.

After the virtual screening, lead compound recognition focuses on binding affinities, molecular interaction and pharmacokinetic characterization. Molecules with considerable binding affinities, intense hydrogen and hydrophobic interaction and good ADMET properties can be considered as a lead for drug development. Therefore, out of 16 compounds, the top seven molecules were chosen for further studies based on their high binding affinities (< -6.8 kcal/mol) and good poses on active site pockets. The least binding energy with the target protein was shown by reduced scytonemin i.e., -13.3 kcal/mol. In this computational study, the top seven compounds are reported to have antagonistic effects against the ACE2 receptor for the first time.

In silico study of physicochemical properties, ADME and toxic properties of the screened compounds was conducted in the next step. Physicochemical properties of all the selected cyanobacterial metabolites were examined in accordance with Lipinski's rule of five, which considers pharmacokinetic properties like hydrogen bond donor, hydrogen bond acceptor and molecular weight. Lipophilicity, oral bioavailability, and membrane permeability of the selected elements can be predicted through the above-mentioned properties [23]. To be a potential drug candidate, the compound needs to satisfy properties such as 1. Molecular weight should be less than 500; 2. LogP value should be less than 5; 3. The number of H-bond donors should be less than five; 4. The number of hydrogen bond acceptors should be less than ten, as Lipinski et al. (2001) suggested. Compounds with all four properties above show a reasonable membrane permeability and gastrointestinal absorption [23]. Mycosporine–glycine–valine obeyed all the rules and can be easily absorb into the cell; however, rest of the chosen compounds violated Lipinski's first and third rules. As all the selected ligands belong to the category of natural products they are cited as an exception to Lipinski's rules. We believe this is because nature has learned to maintain low hydrophobicity and intermolecular H-bond donating potential when it needs to make biologically active compounds with high molecular weight and large numbers of rotatable bonds [30].

Toxicity prediction through the ProTox-II web server reveals that reduced scytonemin and dimethoxyscytonemin belong to class V with LD_{50} values higher than 2000 mg/kg, Tetramethoxyscytonemin and scytonin belong to toxicity class IV having LD_{50} values between 300 mg/kg and 2000 mg/kg. Mycosporine–glycine–valine and shinorine come under Class III toxicity having LD_{50} values between 50 and 300 mg/kg. Despite having a good docking score and remarkable distribution in the human body, toxic effects such as carcinogenicity and mutagenicity of a compound became an important concern. It is crucial to evaluate the novel products for their poisonous impact during the early phase of drug development. Even though our study shows scytonemin and its derivatives as top hits with least binding energy, information from the ProTox-II web server declares them unfit for drug development due to their carcinogenic, mutagenic, cytotoxic and organ toxic activity (Table 3). Thus, we eliminated all the compounds with toxic behavior and left with only mycosporine–glycine–valine and shinorine.

Both compounds' docked structures are further studied to analyze their molecular interaction with the target residue. Visualization study reveals mycosporine–glycine–valine docked and stabilize in the active site of ACE2 by forming eight hydrogen bonds with six amino acid residues (Table 4). Unlike this compound, shinorine interacted and stabilized with ACE2 active site by forming five hydrogen bond with five amino acid residue (Table 4). Comparison of target binding residue of mycosporine–glycine–valine, shinorine and known inhibitor MLN-4760 reveals Arg371, Thr371 as common interacting residue in all three. Thus we expect that binding of screened ligands may cause some conformational changes in the receptor protein (ACE2) to disable its binding with spike glycoprotein, thus restricting viral entry into the cell protoplasm.

The biological activity prediction of both the selected compounds is obtained through PASS online server. In silico's prediction proves that the selected compounds have antiviral activity against previously known viral diseases (Table 5). Thus we anticipate that consumption of drugs derived from photoprotective compounds may show some inhibitory action against SARS-CoV-2. In this in silico study, we have tried to report the antiviral potential of the photoprotective compounds for the first time.

Conclusions

Docking study, ADME and toxicity analyses suggest effective inhibitory potential of mycosporine–glycine–valine and shinorine against SARS-CoV-2, ACE2 receptor protein.Both the compounds may potentially bind to the active sites of ACE2 and inhibit the binding of virus, thereby reducing the spread of the virus inside the human body. So the current study strongly indicates mycosporine–glycine–valine and shinorine can be presented as prototypes for the drug development against SARS-CoV-2. However, this experiment needs in vivo and in vitro

validation to confirm the antiviral properties of studied compounds. We expect that our information will be helpful and supportive for novel drug development against SARS-CoV-2.

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Author's Contributions All authors contributed equally to the concept and design of study. Material preparation, data collection and analysis were performed by N. Sahu, S. Mishra, M. Kesheri, S. Kanchan and R. P. Sinha. The first draft of the MS was written by Niharika Sahu and edited by R. P. Sinha. All authors commented on previous version of the MS and approved the final draft.

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Declarations

Conflict of interest The authors declare no conflict of interest.

Human Participants and/or Animals No human participants and/or animals were used during the experiment.

Informed Consent Not required.

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