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Exploring Potential Therapeutic Agents for the Treatment of COVID-19 via in-silico Methods

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Abstract: *Molecular docking was used to assess the binding affinities of different active compounds to the SARS-CoV-2 3CLpro and PLpro proteins. According to the results, the native ligands of the evaluated proteins had the highest binding affinities. The native ligand of 3CLpro is titled WU-02 and has a binding affinity of -7.9 for the protein. The native ligand of Plpro is titled inhibitor 3k and has an affinity of -8.6 towards the protein. Out of the evaluated molecules, shogaol and 6-gingerol had high affinities for 3CLpro. Other molecules that showed a high affinity were 8 and 10 gingerol (-5.4 kcal/mol), and S-allyl-cysteine (-5.5 kcal/mol). In the case of PLpro, shogaol stands out with an affinity of -7.6 kcal/mol followed by 6 and 8 gingerol. The findings imply that these compounds could be good inhibitors of the SARS-CoV-2 PLpro and 3CLpro proteins. However, further research is needed to establish the effectiveness and safety of these drugs in vitro and in vivo.*

Keywords*: SARS-CoV-2, COVID-19, gingerols, allicin, shogaol, alliin, molecular docking*

1. Introduction

Originating primarily from Southeast Asia, Ginger (Zingiber officinale) has gained a reputation as one of the healthiest plants worldwide [1]. Although ginger contains more than 400 different compounds, the terpene and phenolic compounds are responsible for their pharmacological effects. Ginger-derived terpenes have been shown to possess a wide range of pharmacologic properties, such as anticancer, antioxidant, anti-inflammatory, antiviral, antibacterial, antidiabetic, antihyperalgesic, gastroprotective, and neuroprotective effects [2, 3].

Previous studies analyzed the potential of active compounds contained in ginger to target essential SARS-CoV-2 proteins. Molecular docking studies have shown that some active compounds from ginger have a significant affinity towards the spike proteins of SARS-CoV-2 and towards the human angiotensin-converting enzyme 2 (ACE2), an essential protein that allows SARS-CoV-2 to enter the body [4]. Another study analyzed the clinical manifestation in hospitalized patients infected with SARS-CoV-2, where the control group received the standard medication. At the same time, the intervention group received ginger supplements orally (3g of ginger supplements divided into two doses). The intervention group had a significant reduction in the number of hospitalization days [5].

Another plant rich in bioactive compounds, garlic, was thoroughly analyzed for the potential to improve SARS-CoV-2 infection outcomes. The main active constituents of garlic are organosulfur compounds, which are also responsible for the specific odour. The bioactive compounds in garlic belong to the following chemical classes: l-cysteine sulfoxides and γ-glutamyl-l-cysteine peptides. The active compound in the highest concentration is alliin, or S-allyl-l-cysteine sulfoxide. [6,7]. Garlic has been investigated for its potential to treat multiple viral infections. Pre-clinical data showed that the active compounds in garlic could prevent viral entry into the host [8]. In silico studies have been conducted to

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analyze garlic's therapeutic potential in treating SARS-CoV-2 infection. The study concluded that garlic's organosulfur compounds could potentially target some SARS-CoV-2 proteins [9,10]. Another in-silico study focused on organosulfur compounds targeted the ACE2 protein to analyze the potential of these compounds to prevent cell entry [11].

For the following study, the 3CLpro and PLpro proteins from SARS-CoV-2 were selected as targets for 11 active compounds found in ginger and garlic. The binding affinities and the binding method to the protein were evaluated. 3CLpro and PLpro are non-structural proteins that play an essential role in the virus's life cycle, making them viable targets for inhibition [12,13].

2. Materials and methods

2.1. Ligand selection and ligand preparation

The active compounds were selected from garlic (Allium sativum) and ginger (Zingiber officinale). The following compounds were selected for molecular docking studies: allicin, S-allyl-cysteine, alliin, and ajoene from garlic and shogaol, 6-gingerol, 10-gingerol, and 8-gingerol from ginger. The chemical structure of these compounds is presented in Figure 1.

The structures of the selected active compounds were downloaded from PubChem (https://pubchem.ncbi.nlm.nih.gov/) and converted to the appropriate format using OpenBabel version 3.1.1 [14]. The ligands were then imported into AutoDock Vina version 1.5.7 to add rotatable bonds, a process that generates different conformations of the ligands to facilitate docking studies [15].

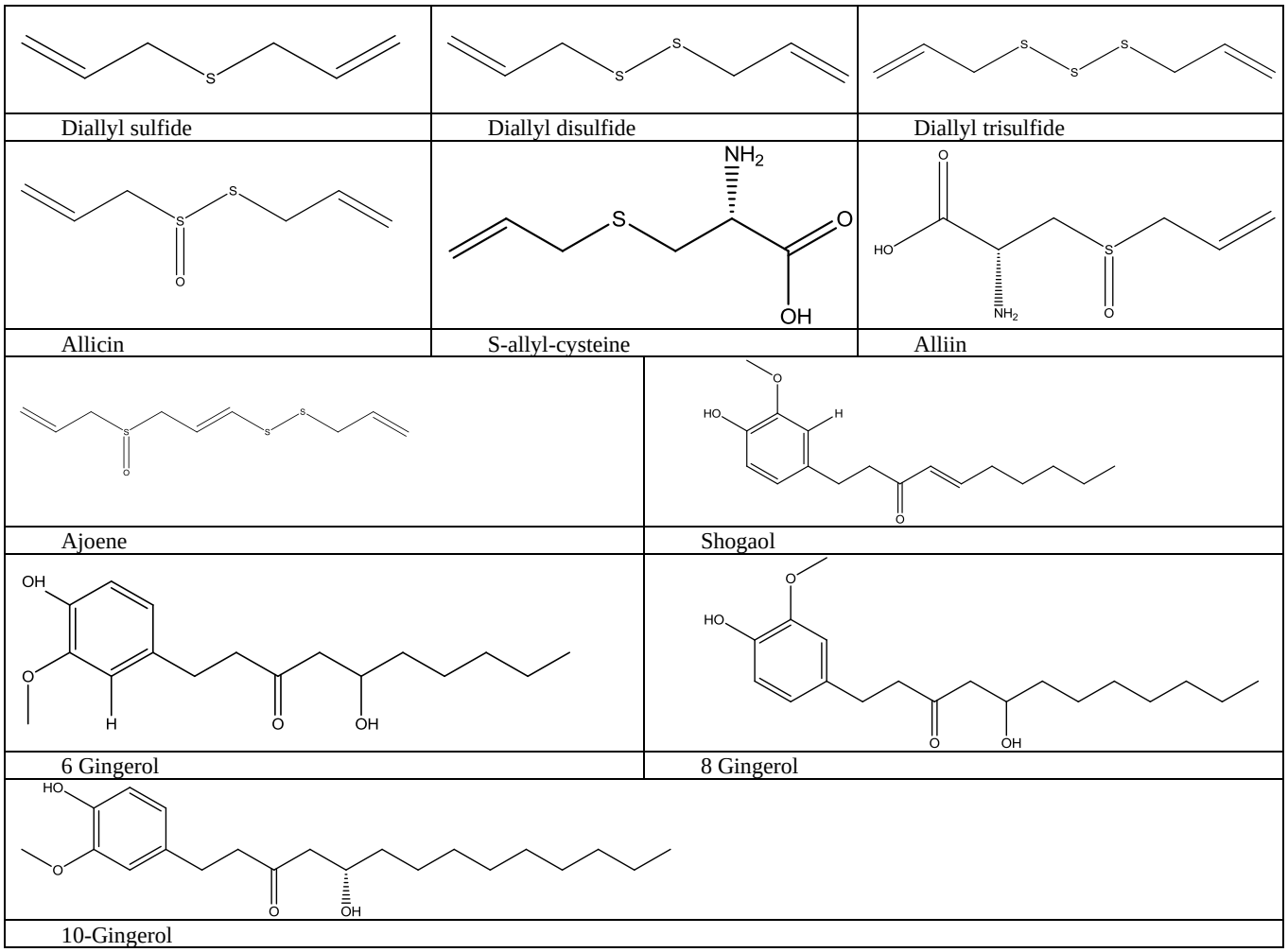


Figure 1. Chemical structure of the docked compounds

2.2. Protein selection and preparation

The crystal structure of the target proteins was downloaded from the Protein Data Bank (PDB, https://www.rcsb.org/) website as .pdb files. The protein was prepared for docking by removing any water molecules and other ligands in the crystal structure using Molegro Molecular Viewer version 2.5 (http://molexus.io/). The protein was then imported into AutoDock Vina, optimizing the structure for molecular docking studies.

2.3. Molecular docking

Molecular docking studies were performed using AutoDock Vina. The previously prepared protein and ligand structures were used as inputs for the docking simulations. The docking grid was selected based on the active site of the protein. The binding affinities and the way the ligands interacted with the protein were analyzed using the Discovery Studio suite [16]. The docking method was validated by determining the RMSD (root-mean-square deviation) between the native ligand and the re-docked version of the native ligand.

3. Results and discussions

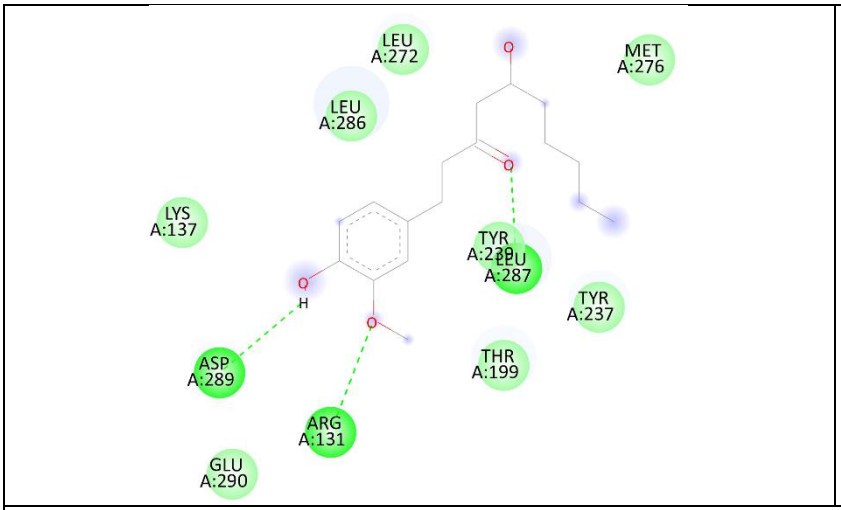
3.1. Targeting SARS-CoV-2 3CLpro

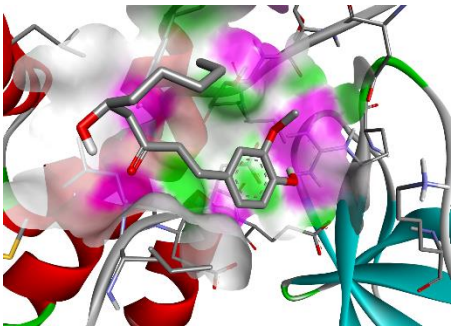
For this study, the protein with the following PDB ID was selected: 7TZJ. The grid box coordinates were set to X= 15.94, Y= 26.70 and Z= -18.19. The size of the grid box was set to 60x60x60. The RMSD value between the native ligand (5-bromanyl-~{N}-methyl-3-nitro-2-[(4~{R},5~{S})-2-(7-oxidanyl-isoquinolin-4-yl)carbonyl-4-phenyl-2,7-diazaspiro[4.4]nonan-7-yl]benzamide, titled WU-02), and the re-docked native ligand was 1.65 Å (angstrom) which falls into the accepted values by the literature [17]. The binding affinities of the studied ligands are presented in Table 1.

Table 1. Binding affinities of the evaluated compounds

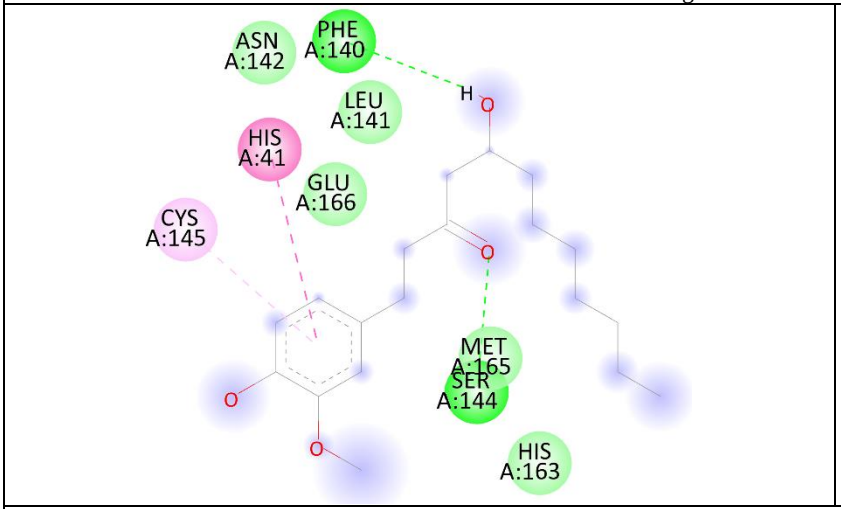
Compound	Binding affinity Kcal/mol
10-Gingerol	-5.4
6-Gingerol	-5.9
8-Gingerol	-5.4
Ajoene	-4.7
Allicin	-4.1
Alliin	-5.3
Diallyl disulfide	-3.8
Diallyl sulfide	-3.9
Diallyl trisulfide	-4.0
S-allyl-cysteine	-5.5
Shogaol	-5.9
WU-02	-7.9

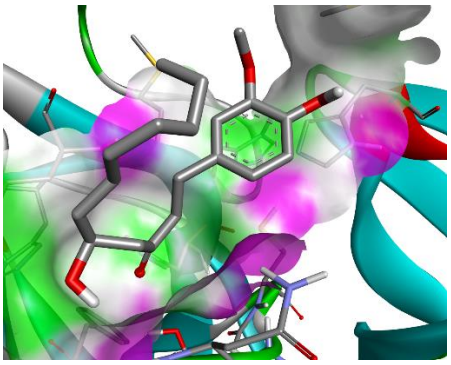
The results show that the native ligand (WU-02) had the highest binding affinity of -7.9 Kcal/mol, which indicates a strong affinity for the protein. 10-Gingerol, 6-gingerol, 8-gingerol, shogaol, and S-allyl-cysteine also exhibited strong binding affinity to the protein. In contrast, allicin and diallyl disulfide had lower binding affinities of -4.1 and -3.8 Kcal/mol, respectively. Figure 2 presents the 2D and 3D diagrams of the compounds with a high affinity towards the protein.



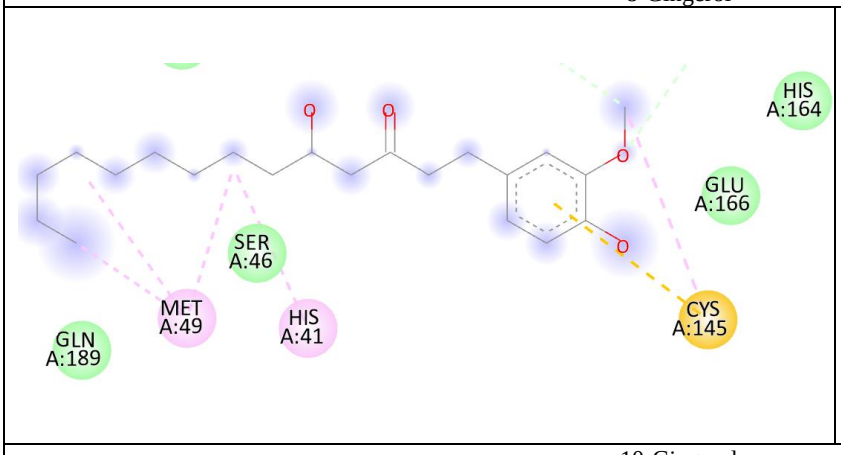


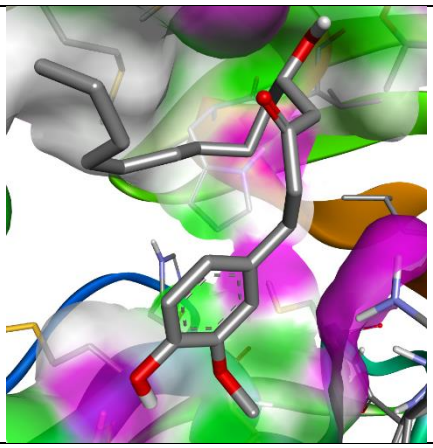
6-Gingerol



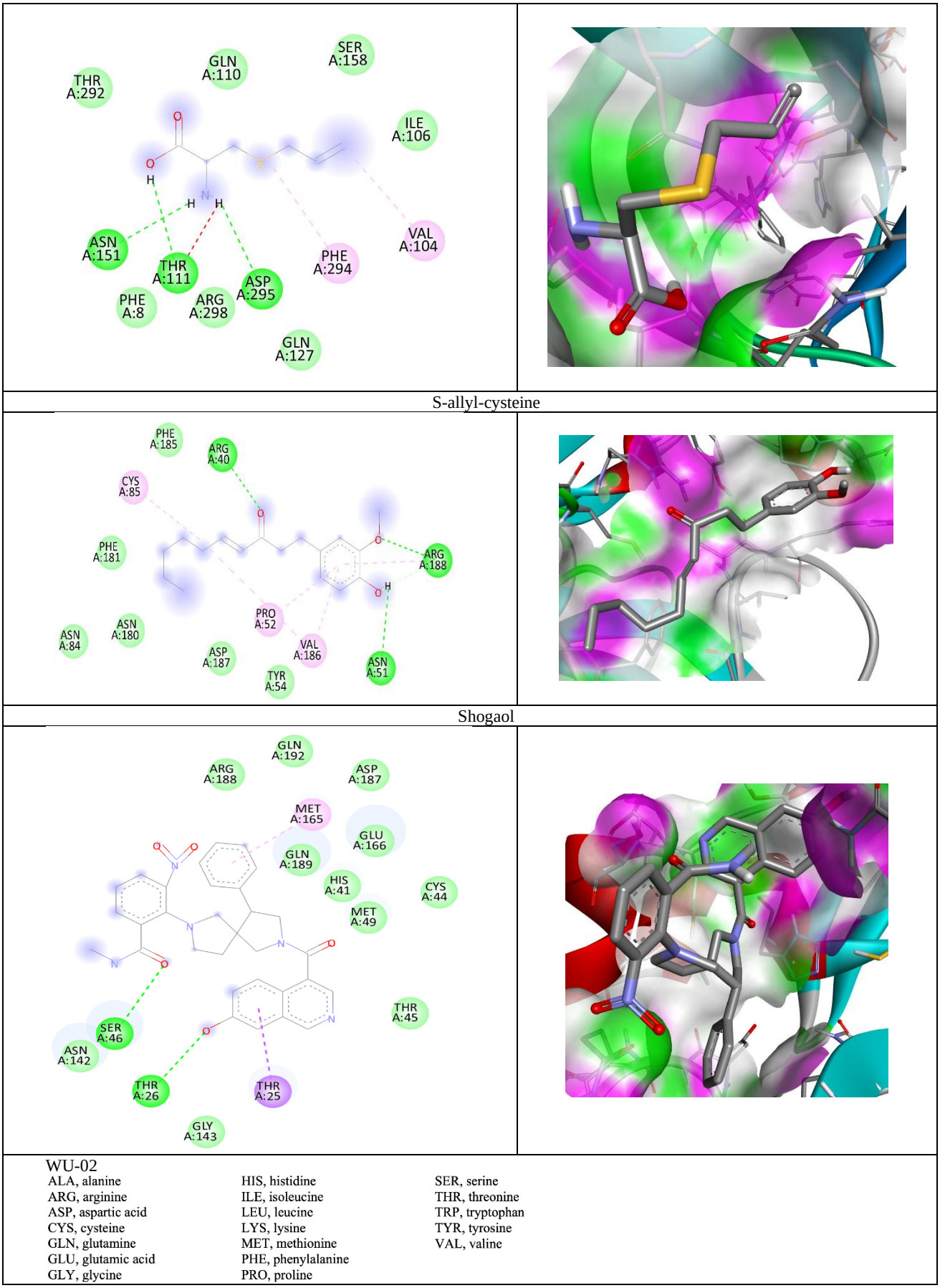


8-Gingerol





10-Gingerol



ALA, alanine	HIS, histidine	SER, serine
ARG, arginine	ILE, isoleucine	THR, threonine
ASP, aspartic acid	LEU, leucine	TRP, tryptophan
CYS, cysteine	LYS, lysine	TYR, tyrosine
GLN, glutamine	MET, methionine	VAL, valine
GLU, glutamic acid	PHE, phenylalanine	
GLY, glycine	PRO, proline	

Figure 2. The compounds that had the highest affinity towards 3CLpro. 2D-left. Middle, amino acid abbreviation and name. 3D-right

6-Gingerol binds to the following amino acids: LEU 287, ARG131 and ASP289. 8-Gingerol interacts with PHE140, HIS41, CYS145, and MET144. 10-Gingerol interacts with CYS145, HIS41, and MET49. S-allyl-cysteine binds to ASN151, THR111, ASP295, PHE294, VAL104. Shogaol binds to ARG188, ASN51, VAL186, PRO52, CYS85, and ARG40. The native ligand interacts with MET165, SER46, THR26 and THR25.

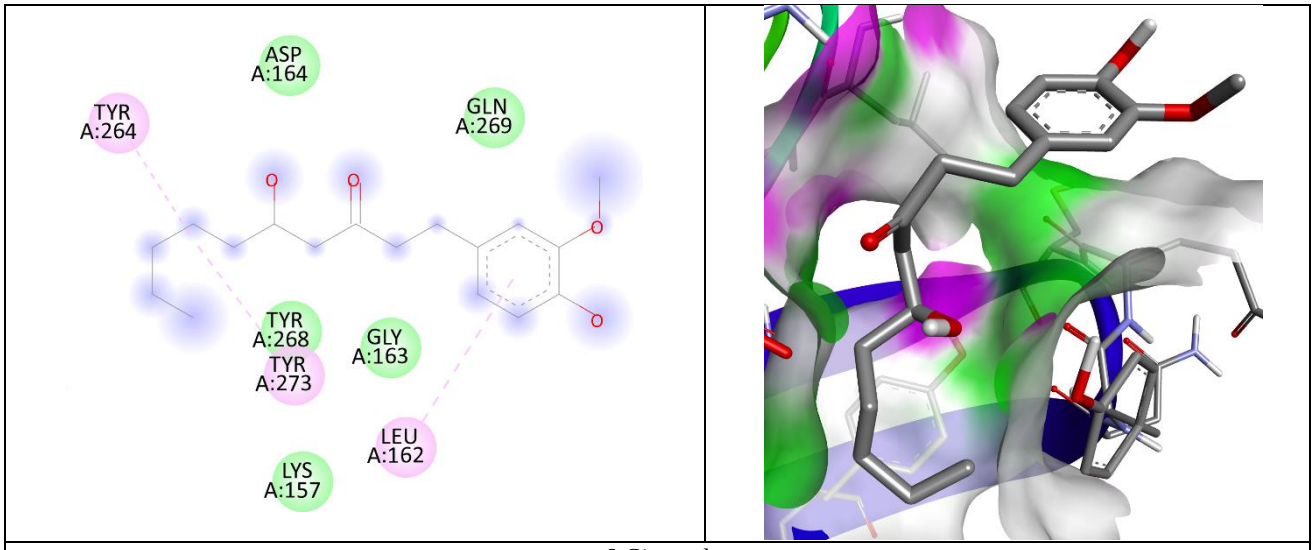
3.2. Targeting SARS-CoV-2 PLpro

The selected PLpro protein has the PDB ID: 7TZJ. The grid box coordinates were set to X= -2.69, Y= 4.74 and Z= -35.21. The size of the grid box was set to 60x60x60. The RMSD value between the native ligand N-[(3-fluorophenyl)methyl]-1-[(1R)-1-naphthalen-1-ylethyl]piperidine-4-carboxamide (inhibitor 3k) and the re-docked native ligand was 1.74 Å (angstrom) which falls into the accepted values by the literature. The binding affinities of the studied ligands are presented in Table 2.

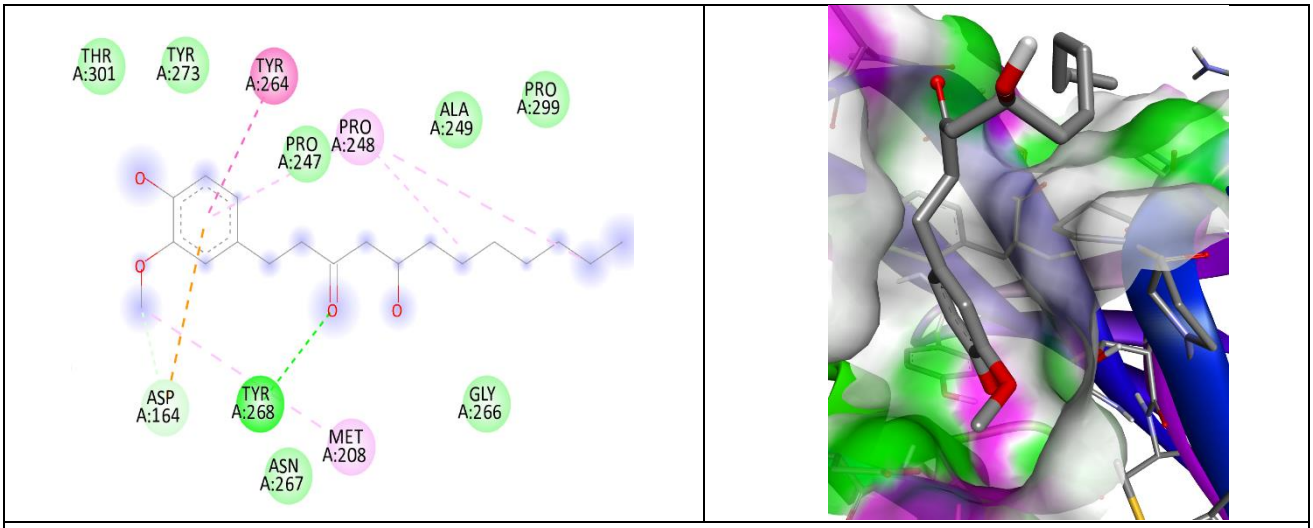
Table 2. Binding affinities of the evaluated compounds

Compound	Binding affinity Kcal/mol
10-Gingerol	-6
6-Gingerol	-6.7
8-Gingerol	-6.6
Ajoene	-4.8
Allicin	-5.2
Alliin	-6.1
Diallyl disulfide	-4.9
Diallyl sulfide	-4.7
Diallyl trisulfide	-4.6
S-allyl-cysteine	-5.6
Shogaol	-7.6
Inhibitor 3k	-8.6

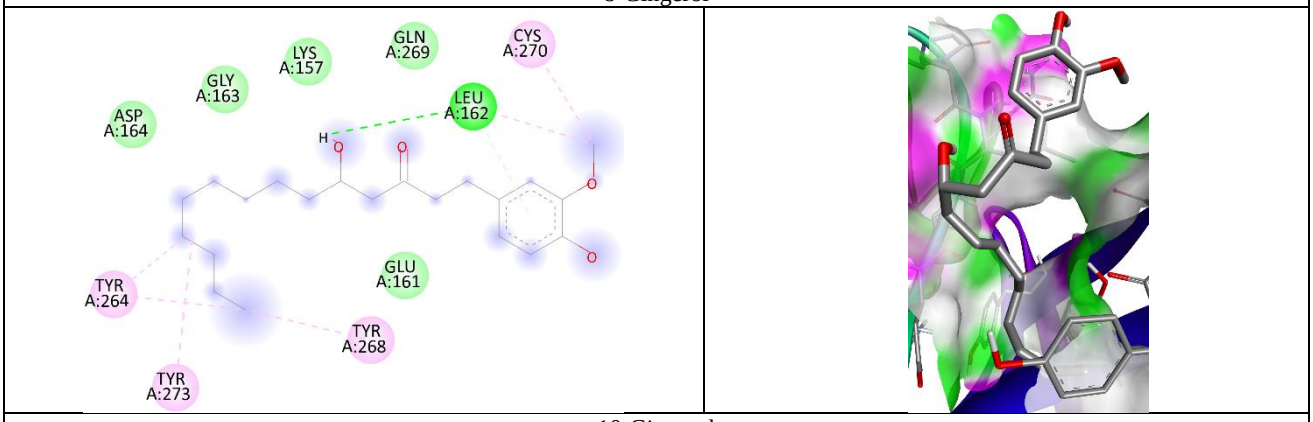
Out of the analyzed compounds, Shogaol had the highest affinity for the protein (-7.6 kcal/mol). 10, 6 and 8 gingerol and alliin also showed a high affinity towards the proteins. The native compound had an affinity of -8.6 kcal/mol. Figure 3 presents the 2D and 3D diagrams of the compounds with a high affinity towards the protein.



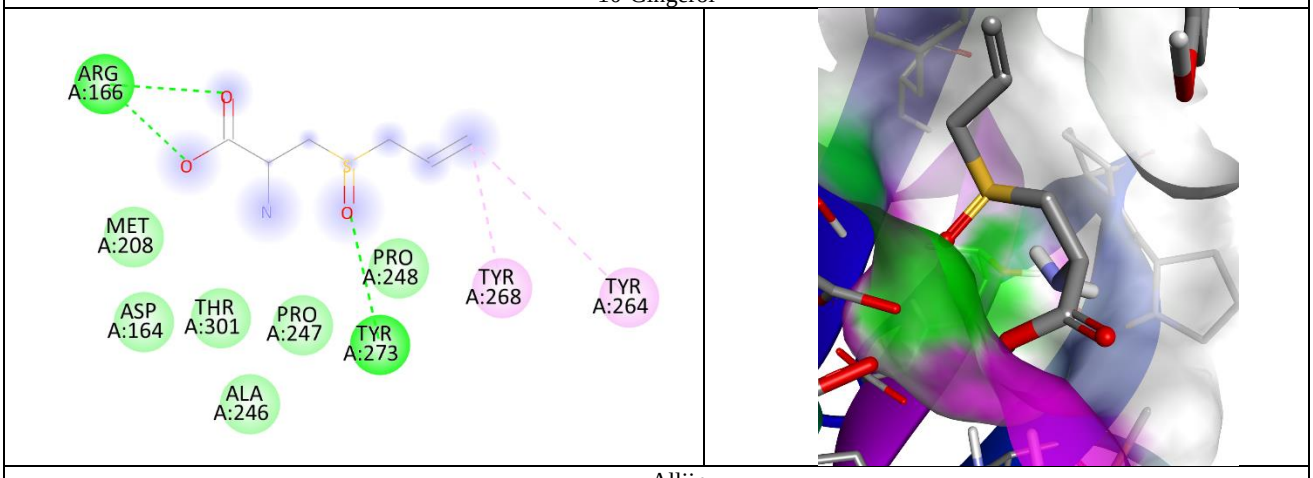
6-Gingerol



8-Gingerol



10-Gingerol



Alliin

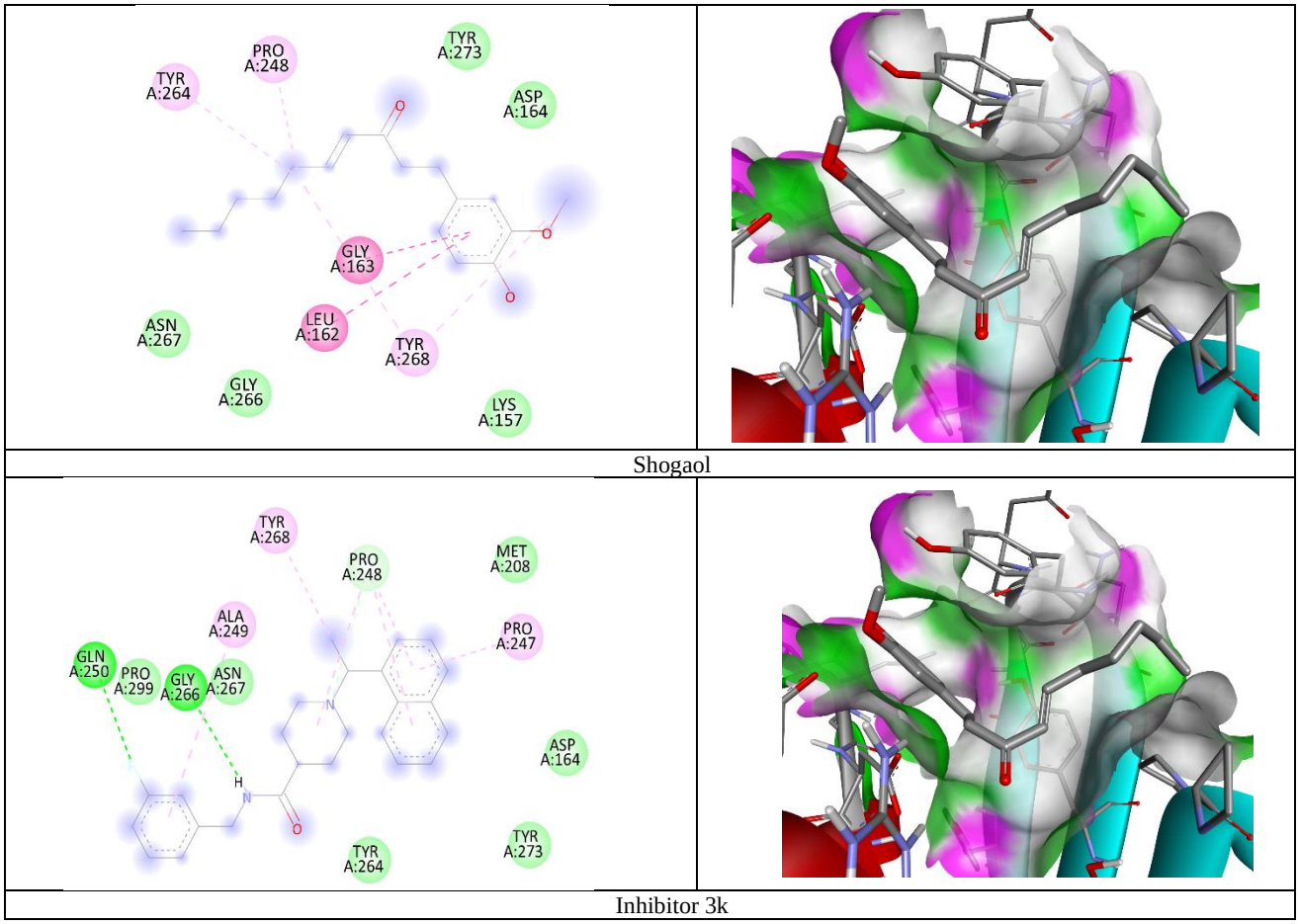


Figure 3. The compounds that had the highest affinity towards PLpro. 2D-left 3D-right. 2D-left. Middle, amino acid abbreviation and name. 3D-right

6-Gingerol bind to TYR264, TYR273, and LEU162. 8-Gingerol binds to TYR264, PRO248, MET208, TYR268, and ASP164, 10-gingerol binds to LEU162, CYS270, TYR268, TYR273, and TYR264. Alliin binds to ARG166, TYR264, TYR268 and TYR273. Shogaol, the ligand that showed the highest affinity for the protein, interacts with PRO248, TYR264, GLY163, LEU162, and TYR268 while the native ligand binds to PRO247, PRO248, TYR268, ALA249, GLY266, and GLN250.

4. Conclusions

Our paper evaluated the binding affinities of various compounds to the SARS-CoV-2 3CLpro, and PLpro proteins using molecular docking. In the case of 3CLpro the results showed that the native ligand (WU-02) had the strongest binding affinity of -7.9 Kcal/mol. Additionally, 6-gingerol, 8-gingerol, shogaol, and S-allyl-cysteine exhibited strong binding affinity to the target protein. In contrast, allicin and diallyl di-sulfide had weaker binding affinities. The results were similar in the case of PLpro, where the native ligand also had the highest affinity towards the protein (-8.6), followed by shogaol with an affinity of -7.6. The gingerols and alliin also showed a significant affinity for PLpro. The binding modes of the compounds with high affinities were determined, revealing specific amino acid interactions. These findings suggest that these compounds could potentially have an effect on SARS-CoV-2, but further studies, including in vitro and in vivo experiments, are necessary to confirm the efficacy and safety of these compounds.

Overall, the results of this study provide insights into the potential use of natural compounds as therapeutic agents against COVID-19, explicitly targeting the SARS-CoV-2 PLpro and CLpro proteins. However, further studies are needed to validate the findings and assess these compounds' safety and efficacy in vivo.

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