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Research article

In silico screening of potential inhibitor from *Andrographis paniculata* constituents against three targets of SARS-CoV-2: Main protease, Spike protein, and Nsp15

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Abstract: The current pandemic of COVID-19 is caused by Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), which has increased the morbidity and mortality rate throughout the world. World Health Organization has declared this COVID-19 outbreak as a health emergency throughout the world. At this time, there are very few drugs against SARS-CoV-2. So, this study aimed to screen 91 *Andrographis paniculata* against three targets of SARS-CoV-2: main protease, spike protein, and Nsp15 by molecular docking. The calculation result revealed that mostly bioactive compounds from *Andrographis paniculata* are a good binding affinity with the main protease than that of Nsp15 and spike protein. The top six compounds and their interactions with the active site were visualized. Among them, 7,8-dimethoxy flavone-5-β-D-glucopyranosyloxy flavone and Stigmasterol compounds from *Andrographis paniculata* had a superior binding affinity of -11.65 and -11.33 kcal/mol toward the main protease. A detailed understanding of ligand-protein interaction could be helpful in further drug design and development for COVID-19 treatment.

Keywords: COVID-19; Molecular docking; Thai herbs; MPro; Nonstructural proteins

1. Introduction

Coronavirus disease 2019 (COVID-19), an infectious disease caused by SARS-CoV-2 (severe acute respiratory syndrome coronavirus-2) has become a pandemic. It has infected more than 285 million people all over the world since it was discovered and reported 5.5 million deaths by end of 2021 (https://www.worldometers.info/coronavirus/). COVID-19 is clinically characterized by fever, slight pain in the neck, headache, dry cough, breathing problem, vomit, and it might lead to death through multi-organ failure [1]. The SARS-CoV-2 re-emergence in Thailand especially in April 2021 has kept the government under high alert and has made a severe situation demanding fast treatment to prevent the infected patients. Currently, there are no efficient antiviral drugs for the prevention of COVID-19 infection. The current prevention

methods are directed on quarantine and containment of infected patients for preventing human-to-human transmission along with vaccinations. The need for rapid time-to-solution in drug discovery has become accentuated by the COVID-19 pandemic. Therefore, structure-based molecular docking for screening anti-SARS-CoV-2 potential drug candidates from natural sources is the main goal of this study.

The coronavirus is a single-strand RNA virus (+ssRNA) that can cause severe respiratory syndrome in humans [2]. SARS-CoV-2 (also called 2019-nCoV) is an enveloped, single-stranded, positive-sense RNA virus with genome sizes ranging from 26 to 32 kb and virion sizes from 50 to 200 nanometers in diameter. Four essential structural proteins, i.e., Spike protein (S), Envelope protein (E), Membrane protein (M), and Nucleocapsid protein (N) are found in SARS-CoV-2 and to generate the major components of the virus particle, polyprotein processing is an essential mechanism [3]. Each of the key viral proteins of the SARS-CoV-2 viral replication cycle steps such as spike protein, nonstructural proteins 15 (Nsp15), and main protease have been used as a target region for screening anti-SARS-CoV-2 potential drugs. The spike (S) glycoprotein homotrimer on the COVID-19 virus surface plays an essential role in receptor binding and virus entry in which it uses the angiotensin-converting enzyme 2 (AEC2) receptor for cell entry. The S protein is a multifunctional molecular machine that plays key role in the early steps of viral infection by interacting with host susceptibility factors, including receptors and proteases [4]. While the nonstructural proteins (Nsps) and the N protein are required for packaging of the viral genome into the newly assembled virion. SARS-CoV-2 Nsp15 is a uridine-specific endoribonuclease with a C-terminal catalytic domain belonging to the EndoU family that is highly conserved in coronaviruses [5]. The polyproteins are cleaved and transformed into mature nonstructural proteins by the main proteases and are responsible for playing a role in the replication/transcription process. Therefore, spike protien, Nsp15 and main protease could be an effective drug targets for anti-SARS-CoV-2.

The structural-based virtual screening of small molecules from natural sources with key viral proteins target will provide the potential drug candidates. Many natural products showed inhibitory activity on the COVID-19 virus. Andrographolide and its derivatives derived from *Andrographis paniculata* have been used for the treatment of COVID-19 virus with fever [6-7], sore throat, chronic cough, and other human diseases [8-9]. The herbs such as gallic acid, curcumin, and its derivatives and mushroom [1, 6, 10-11], have attracted increasing attention for COVID-19 virus agent targeting. Though several in vitro studies showed that natural compounds have anti-virus activity , their inhibition mechanism of action is not known and even more difficult to analyze at the molecular level using experimental methods. Therefore, comprehensive studies are required to determine their molecular interactions. Recently, several studies have used the molecular docking method to study the interactions and conformations of ligand against the SARS-CoV-2 virus [3, 10-12] which leads away to in vitro and in vivo studies. In this study, we aimed to explore the lead compound from *Andrographis paniculata* herb for their medicinal potentials as therapeutic agents against the spike protein, Nsp15, and the main protease of SARS-CoV2. So, the 91 bioactive molecules (Figure 1) were used to explore the lead compound for their medicinal potentials as therapeutic agents against influenza. A detailed understanding of ligand-protein interaction could be helpful in further drug design and development for COVID-19 treatment.

2. Materials and Methods

2.1. Compound data set

The 91 structures of *Andrographis paniculata* were structurally drawn using Gauss View [13]. Their three-dimensional (3D) structures were optimized at the Hartree-Fock level of theory with a 6-31G* basis set using the Gaussian 03 program [14]. The optimized structures (Figure 1) were viewed and converted to .pdb format using GaussView [13] for molecular docking study. The three-dimensional (3D) structures of the two targets of SARS-CoV-2*:* main protease and spike protein were retrieved from the Brookhaven Protein Data Bank (PDB) [15]. The PDB ID of main protease, spike protein and Nsp15 were 6XBG (Resolution*:* 1.45 Å, R-Value Free*:* 0.206, R-Value Work*:* 0.181*)* [16], 7BZ5 (Resolution*:* 1.84 Å, R-Value Free*:* 0.191, R-Value Work*:* 0.167) [17] and 6WXC *(*Resolution*:* 1.85 Å, R-Value Free*:* 0.194, R-Value Work*:* 0.171*)* [5], respectively. The water molecules were eliminated while the missing hydrogen atoms were added to this protein.

2.2. Molecular docking

The molecular docking technique was applied to study the binding orientation and affinity of the bioactive compounds from Thai herbs toward the binding site of the main protease of COVID-19 using Autodock 4.2 [18]. Partial atomic charges of protein and ligands were assigned using the Gasteiger–Marsili method [19] implemented in AutoDock Tools [20]. By semiflexible docking protocol, the protein molecule was kept rigid, while the ligand was flexible to attain a degree of freedom torsions bridged by the rotational parameter. The protein and ligands were converted to .pdbqt format after initial addition of hydrogen bonds and charges. These molecules were considered for docking analysis. The cubical grid box of 50 × 50 × 50 sizes with 0.375 Å was fixed around the key residues of the three proteins. UAW246 was re-docked into the main protease binding site for assessing binding affinity. Favipiravir, lopinavir, hydroxychloroquine, and ritonavir were used as control molecules for filtering bioactive compounds from *Andrographis paniculata* herbs. The autogrid4 parameter was used to attain a rigid grid box. Furthermore, autodock4 with Lamarckian genetic algorithms was conducted to obtain the best docking conformation. Note that the AutoDock estimates free energies of binding based on the typical molecular mechanics' energy terms for dispersion/repulsion, hydrogen bonding, and electrostatic interactions [21], torsional entropy upon binding, and the desolvation upon the ligand binding and corresponding hydrophobic effect [22].

The AutoDock estimates the free energy of binding *(*ΔG*)* based on empirical weighting factors*:*

$$\Delta G = \Delta G_{vdw} + \Delta G_{hbond} + \Delta G_{elec} + \Delta G_{tor} + \Delta G_{sol}$$

where ΔG_{vdw}, ΔG_{hbond}, and ΔG_{elec} are the typical molecular mechanics' energy terms for van der Waals, hydrogen bonding, and electrostatic interactions, respectively. While ΔG_{tor} characterizes the loss of torsional entropy upon binding and ΔG_{sol} displays the desolvation upon the ligand binding and corresponding hydrophobic effect.

3. Results and Discussion

The molecular docking was performed using the AutoDock program to screen the binding affinity of 482 bioactive compounds against the three targets of SARS-CoV-2*:* main protease, spike protein, and Nsp15. The 3D structure of these bioactive compounds from 91 *Andrographis paniculata* (A1-A91) compounds is shown in Figure 1. The key residues of main protease, spike protein, and Nsp15 are considered as active residues to bind with bioactive compounds. The binding energy (BE, kcal/mol) and the percentage of possible conformation or dock score (% DS) of 91 bioactive compounds were varying from -3.89 to -11.65 kcal/mol and 13 to 100%, respectively. The BE and %DS of bioactive compounds from *Andrographis paniculata* are summarized in Table 1.

The docking program was verified by re-docking of Tipiracil back into Nsp15 [5] binding site together with the re-docking of UAW246 [16] back into the main protease binding site. Tipiracil, an FDA-approved drug that is used in the treatment of colorectal cancer, as a potential anti-COVID-19 drug by interacting with the uridine binding pocket in the enzyme's active site of SARS-CoV-2 Nsp15. The docking model of Tipiracil and UAW246 were well-posed in the Nsp15 and main protease binding site, respectively *(*Figure not show*)*. The BE and % DS of Tipiracil and UAW246 re-docking were -5.09 and -6.58 kcal/mol and 45% and 7%, respectively, suggesting that the setting parameters were suitable for this study. Darunavir, favipiravir, hydroxychloroquine, lopinavir, remdesivir, and ritonavir were also docked into its binding site of the three targets of SARS-CoV-2*:* main protease, spike protein, and Nsp15 (Table 1). The result shows that all the well-known drugs are a good binding affinity in the cavity of the main protease than that of the spike protein. Lopinavir had the highest binding energy (-8.91 kcal/mol) but the %DS was quite low (4%) while favipiravir had the highest %DS (48%) but the binding energy is lower (-4.88 kcal/mol) than that of lopinavir in the binding pocket of the main protease. Most of the *Andrographis paniculata* compounds have lower BE than the known drugs. This finding result can be suggested that *Andrographis paniculata* is more effective in treating Covid-19 than the drug Fapiravir, which is commonly used in Thailand. However, to confirm this suggestion, the result from the experimental laboratory is necessary. The docking result revealed that mostly bioactive compounds are a good binding affinity with the main protease than that of the spike protein. On comparing the binding energy values of all compounds, the top twenty-four bioactive compounds and well-known drugs are shown

in Table 1. The 3,19-isopropylidene andrographolide, 7,8-dimethoxy flavone-5-ß-D-glucopyranosyloxy flavone, 14-acetyl-3,19-isopropylidene andrographolide, alpha1-Sitosterol, indosterol, oleanolic acid, and stigmasterol from *Andrographis paniculata* were possessed a higher binding affinity with the binding site of the COVID-19 main protease and Nsp15.

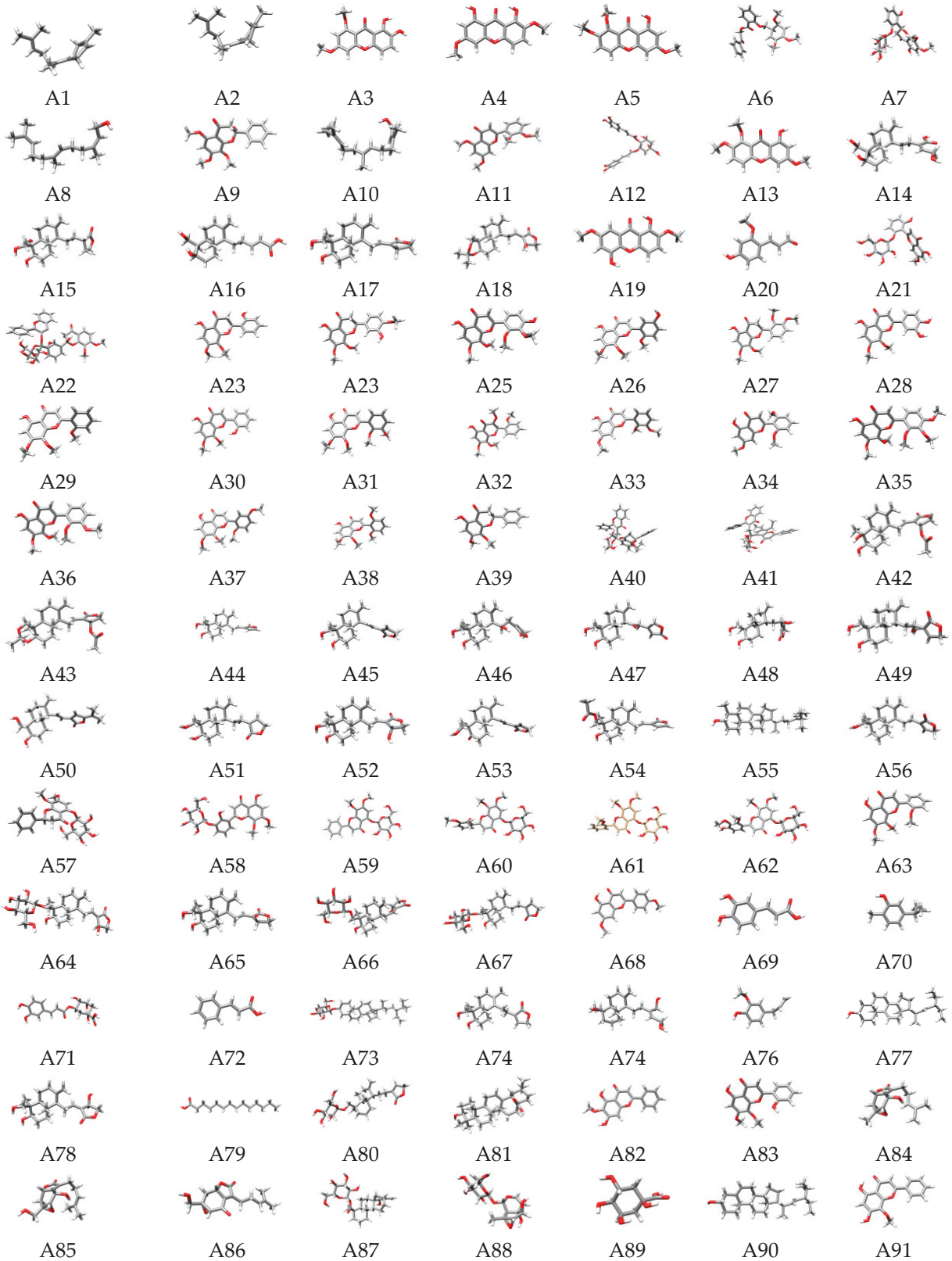

Figure 1. Structures of optimized geometry of 91 *Andrographis paniculata* compounds in HF/6-31 G* level of theory.

Table 1. The BE and %DS of commercial drugs and selected most favorable *Andrographis paniculata* compounds in the binding pocket of spike protein, Nsp15, and main protease.

Code	Name	Spike protein		Nsp15		Main protease	
		BE (kcal/mol)	%DS	**BE (kcal/mol)**	%DS	**BE (kcal/mol)**	%DS
	Redocking	-	-	-5.09	45	-6.58	7
a	Darunavir	-5.14	4	-7.17	4	-8.00	4
b	Favipiravir	-3.51	82	-3.44	29	-4.88	48
c	Hydroxychloroquine	-4.17	9	-5.35	21	-8.28	22
d	Lopinavir	-4.75	3	-5.85	3	-8.91	4
f	Remdesivir	-3.3	2	-5.51	3	-7.62	3
g	Ritonavir	-4.03	2	-3.71	2	-6.31	2
A3	*(Z)*-gamma-Bisabolene	-5.02	87	-6.03	89	-7.60	96
A10	2-hydroxy-5,7,8-trimethoxy flavone	-4.60	35	-6.17	42	-7.81	72
A12	3',2,5,7-tetramethoxy flavone	-4.96	54	-6.27	53	-8.29	61
A14	3,7,8-trimethoxy 1-hydroxyxanthone	-4.54	46	-6.10	89	-7.65	99
A19	**3,19-isopropylidene andrographolide**	**-6.41**	**44**	**-8.25**	**94**	**-10.08**	**91**
A23	5,2'-dihydroxy-7,8,-dimethoxy flavone-3'-ß-D-glucopyranosyloxy	-5.06	5	-7.43	13	-11.83	14
A33	5-hydroxy-3,7,8,2'-tetramethoxy flavanone	-4.43	23	-6.24	52	-8.49	66
A40	5-hydroxy-7,8-dimethoxy flavanone	-4.82	74	-5.93	60	-7.53	55
A41	7,8,2'-trimethoxy flavone-5-ß-D-glucopyranosyloxy flavone	-4.71	6	-6.77	16	-10.39	37
A42	**7,8-dimethoxy flavone-5-ß-D-glucopyranosyloxy flavone**	**-5.49**	**15**	**-8.23**	**14**	**-11.65**	**50**
A43	14-acetyl andrographolide	-5.48	23	-7.14	73	-9.03	30
A44	**14-acetyl-3,19-isopropylideneandrographolide**	**-6.54**	**94**	**-7.91**	**93**	**-10.36**	**54**
A51	14-deoxy-15-isopropylidene-11,12-didehydroandrographolide	-5.88	24	-7.61	45	-8.69	53
A55	19-o-acetyl anhydroandrographolide	-5.78	45	-7.20	31	-8.49	41
A56	alpha1-Sitosterol	-6.56	47	-9.11	97	-10.97	44
A57	Andrograpanin	-5.56	48	-7.05	51	-8.22	43
A66	Andrographolide	-5.71	48	-6.55	69	-8.62	53
A67	Andrographoside	-5.30	23	-6.79	11	-9.94	19
A68	Andropanoside	-5.54	32	-6.92	13	-9.32	12
A74	Daucosterol	-7.24	27	-8.75	44	-10.53	23
A78	**Indosterol**	**-7.18**	**87**	**-9.83**	**60**	**-10.56**	**48**
A82	**Oleanolic acid**	**-6.77**	**34**	**-10.07**	**56**	**-9.43**	**100**
A88	Paniculoside I	-5.62	23	-7.11	33	-11.35	44
A91	**Stigmasterol**	**-6.89**	**64**	**-9.10**	**90**	**-11.33**	**65**

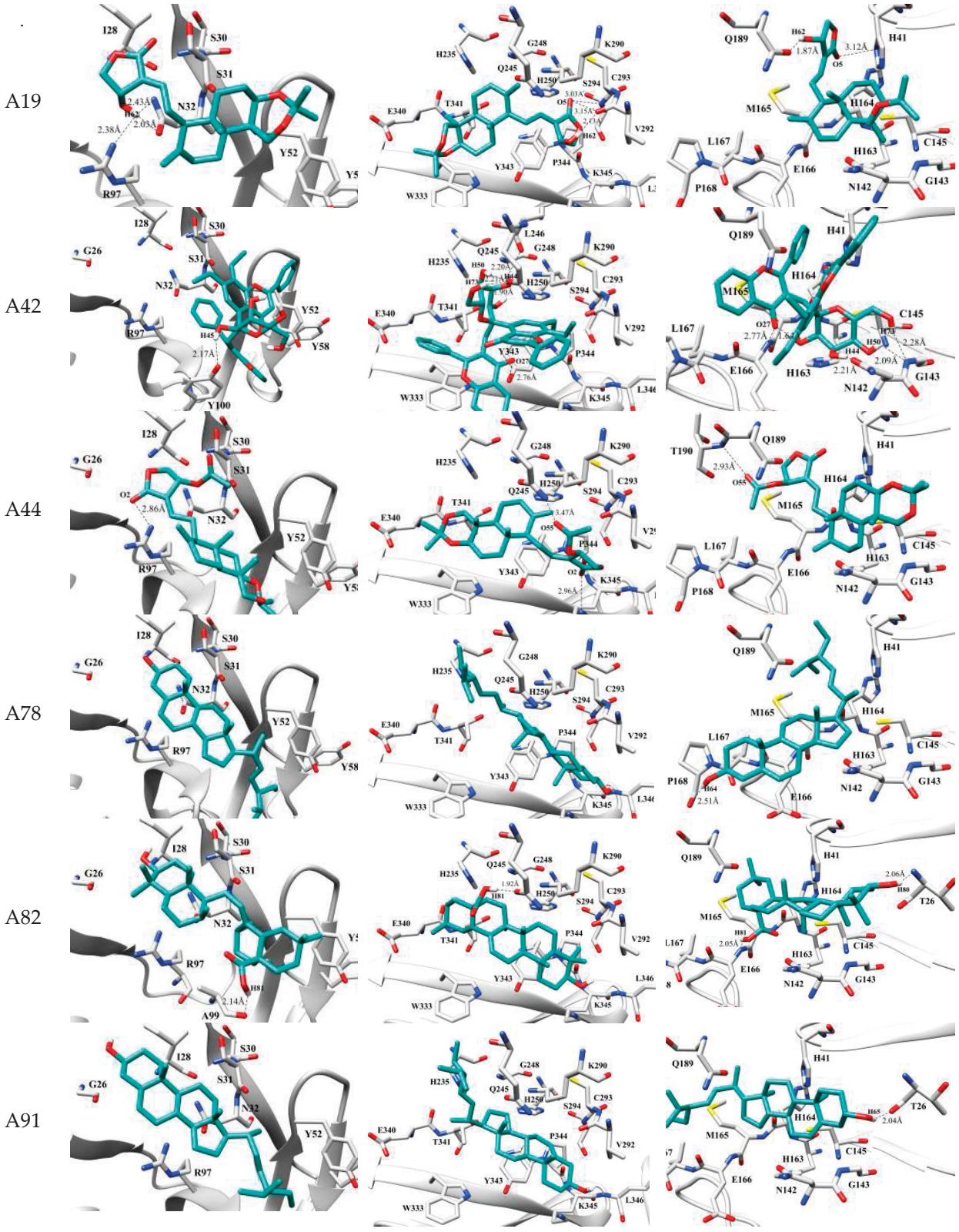

Figure 2. The hydrogen bond interaction and bond distances (Å) of six potential bioactive compounds from *Andrographis paniculata* (A19, A42, A44, A78, A82, and A91) with key amino acid in the binding pocket of the spike protein, Nsp15, and main protease of SARS-CoV-2.

However, the important criteria of strong interaction with key residues in the receptor-binding domain were also considered for finding potential inhibitors. The H-bond interaction of the top six bioactive compounds were shown in Figure 2. Considering all the hydrogen bond data given above, the hydrogen bonds of the 3,19-isopropylidene andrographolide, 7,8-dimethoxy flavone-5-ß-D-glucopyranosyloxy flavone, and stigmasterol ligand with key residues in the binding pocket of the main protease were found more than that of the other ligands. The important amino acid residues in the binding pocket of the main protease are H41, M49, F140, N142, G143, S144, C145, H164, M165, E166, P168, and Q189 [16]. While the important amino acid residues in the binding pocket of spike protein are G26, I28, S30, S31, N32, Y52, Y58, and R97, and the important amino acid residues in the binding pocket of Nsp15 are Q245, G248, H250, K290, C293, S294, E340, T341, Y343, P344, and K345. These hydrogen bond patterns seem to correspond with the binding affinities. From the computational findings, it can be suggested that the 3,19-isopropylidene andrographolide, 7,8-dimethoxy flavone-5-ß-D-glucopyranosyloxy flavone, and Stigmasterol compounds from *Andrographis paniculata* had a superior binding affinity of -11.08, -11.65, and -11.33 kcal/mol toward the main protease. However, the hydrogen bond of many compounds has counts in the active site of nsp15 > spike > the main protease, as opposed to BE that the main protease > nsp15 > spike. This is due to the molecular docking result could provide more interaction types besides hydrogen bonds such as van der Waals and electrostatic interaction.

4. Conclusions

In the present study, a set of commercial drugs and 91 structures of *Andrographis paniculata* (A1-A91) against three targets of SARS-CoV-2*:* spike protein, Nsp15, and main protease were screened by computational chemistry techniques. The docking results showed that mostly bioactive compounds are a good binding affinity with the main protease than that of spike protein and Nsp15 of SARS-CoV-2. Among them, 3,19-isopropylidene andrographolide, 7,8-dimethoxy flavone-5-ß-D-glucopyranosyloxy flavone, and Stigmasterol compounds from *Andrographis paniculata* had a superior binding affinity of -11.08, -11.65, and -11.33 kcal/mol toward the main protease. A detailed understanding of ligand-protein interaction could be helpful for further drug design and development for COVID-19 treatment.

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Conflicts of Interest*:* The authors declare no conflict of interest.

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