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Potential of Bioactive Compounds from Jamblang (*Syzygium Cumini*) And Hanjeli (*Coix Lacryma-Jobi*L.) Essential Oils As SARS-COV-2 Antivirus Targeting NSP5 And ACE2 Receptors

Diky Setya Diningrat[1,*], Ayu Nirmala Sari[2], Kusdianti[3] , Novita Sari Harahap[4]

[1]*Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Negeri Medan (Medan State University), Indonesia;*[2]*Biology Study Program, Faculty of Science and Technology, Ar-Raniry State Islamic University (UIN), Banda Aceh, Indonesia;*[3]*Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Pendidikan Indonesia;*[4]*Department of Sports Science, Faculty of Sports Science, Universitas Negeri Medan (Medan State University), Indonesia.*

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Abstract

This study uses bioactive compounds from jamblang (*Syzygium cumini*) and hanjeli (*Coix lacryma-jobi L.*) essential oils, namely Aziridine-2-carbothioamide and 4-Dibenzofuranamine which are thought to have antiviral activity on MERS-CoV and H3N2 HA. The specific target receptors for current anti-SARS-CoV-2 drugs are NSP5 (nonstructural protein) and ACE2 (angiotensin-converting enzyme 2). The purpose of this study was to determine the antiviral activity of SARS-CoV-2 from compounds of Aziridine-2-carbothioamide and 4-Dibenzofuranamine in silico. The compound was prepared in advance by downloading the PDB ID code, preparing 2D and 3D structures, determining the minimum energy, generating SMILES codes, and predicting physicochemical properties and toxicity. After preparation, the process continued with molecular binding using the PyRx-Vina® application. Docking results were analyzed using PyMOL® software and Discovery Studio Visualizer®. The results of the physicochemical profile showed that the compounds of 4-Dibenzofuranamine and Aziridine-2-carbothioamide had complied with Lipinski's five rules, and each compound had LD50 values of 1350 mg/kg and 340 mg/kg. The docking resulted in interactions of Aziridine-2-carbothioamide at the 2GZ7 receptor and 4-Dibenzofuranamine at the 3D0G, and 1R4L receptors showed significant differences, respectively, to lopinavir and chloroquine with p-value < 0.05 so that these compounds were predicted to have better antiviral potential. This research shows that bioactive compounds from the essential oils of *S. cumini* and *C. lacryma-jobi* can act as SARS-COV-2 antivirals, which have been compared with antivirals used medically in silico. In vivo and in vitro testing needs to be done so that it can be applied medically.

Keywords: COVID-19, essential oil, *Syzygium cumini*, *Coix lacryma-jobi*, Physicochemistry, Toxicity, and In silico.

1. Background

Coronavirus Disease (COVID-19) is caused by the severe acute respiratory syndrome coronavirus-2 (SARSCoV-2). The coronavirus that became the etiology of COVID-19 is included in the betacoronavirus genus (Lai et al, 2020). The results of phylogenetic analysis show that this virus belongs to the same subgenus as the coronavirus that caused the severe acute respiratory illness outbreak (SARS) in 2002-2004, namely Sarbecovirus (Malik et al, 2020; Oglat et al, 2022). The replication process of the COVID-19 virus, namely SARS-CoV-2, binds to the receptors and makes its way into the cell. Then, the glycoprotein contained in the viral spike envelope will bind to the cellular receptor in the form of ACE2 in SARS-CoV-2. SARS-CoV-2 will duplicate genetic material, synthesize the required proteins, and then form new virions that appear on the cell surface (Awaid, 2022; Beyerstedt et al, 2021).

NSP5 plays an indispensable role in SARS-CoV-2 proliferation (Yashvardhini et al, 2022). This research is important to do. This was done to determine the role of bioactive compounds from *Syzygium cumini* essential oil and *Coix lacryma-jobi* essential oil in their activity against the NSP5 protein and ACE2 inhibitors in their role in preventing SARS-CoV-2 infection.

Aziridine-2-carbothioamide is a small molecule biologic as one of the FDA-approved drugs. These small molecules have been selected to ensure that they meet the five criteria of Lipinski's law. One of the criteria is molecular weight, where if the molecular weight is above 500 Da, the drug cannot diffuse through the cell membrane (Oroojalian et al, 2020). Aziridine-2-carbothioamide has shown a very high IC50 concentration of 0.83 g/ml against Middle East respiratory syndrome coronavirus (MERS-CoV) (Ugwuja and Nwankwo, 2022). Aziridine-2-carbothioamide has been shown to decrease the expression of the pro-inflammatory cytokine IL6 in SARS-CoV-2 infection (Ugwuja and Nwankwo, 2022). In previous research, molecular docking of Aziridine-2-carbothioamide against the Mpro (6LU7) receptor was carried out; 3CLpro (1UK4); ACE2 (6M0J); NSP12 (6NUR) (Fadaka et al, 2022), but this has not been done for NSP5 and other ACE2 receptors.

* Corresponding author. e-mail: dikysd@unimed.ac.id.

One of the other small molecule inhibitors is 4-Dibenzofuranamine. 4-Dibenzofuranamine has been shown to inhibit the low-pH conformational change of hemagglutinin (HA) and block the fusion process (da Silva Hage-Melim et al. 2020). Influenza virus (H3N2) HA has a similar sequence or structure to the SARS-CoV-2 spike glycoprotein (Oroojalian et al, 2020). Previous molecular docking studies have shown that 4-Dibenzofuranamine interacts with the ACE2 receptor (6LZG) so that it can inhibit SARS-CoV-2 membrane fusion into host cells (Sharma et al. 2021). Neither 4-Dibenzofuranamine nor Aziridine-2-carbothioamide is registered in Indonesia based on the Indonesian Food and Drug Supervisory Agency. Therefore, based on the literature regarding indications of 4-Dibenzofuranamine and Aziridine-2-carbothioamide, these two compounds have potential as new drugs in Indonesia.

Another study was conducted on 81 cases (45 cases in the umifenovir/4-Dibenzofuranamine group and 36 cases in the control group) moderate or severe COVID-19 by comparing the results of CT scans after administration, and the results showed that there was no difference in changes in CT values in 1 week, thus indicating that giving umifenovir does not give better results or shorten the duration of treatment in COVID-19 patients. However, because this study is a single center (retrospective study with small sample size), which is biased and with potentially subjective conclusions, further verification in randomized controlled clinical trials is necessary (Sharma et al. 2021).

In this study, two target receptors were used: NSP5 and ACE2. The NSP5 receptor (nonstructural protein) is known as Mpro (Roe et al, 2021) and 3-chymotrypsin-like protease (3CLpro) (Mody et al, 2021). This NSP5 receptor was chosen because of its mechanism in mediating viral protein replication and transcription (Singh et al, 2022). Another receptor is ACE2 (angiotensin-converting enzyme 2) which has three physiological functions, namely as a negative regulator of the renin-angiotensin system, facilitator of amino acid transport, and receptor for the binding site for SARS-CoV and SARS-CoV-2 (Diningrat et al, 2021; Diningrat et al, 2021).

To determine the effectiveness of the tested ligands as new drugs, molecular binding, physicochemical and toxicity tests were carried out in this study. In addition, a comparison of the binding affinity values of the comparator of lopinavir to Aziridine-2-carbothioamide and the comparator of chloroquine to 4-Dibenzofuranamine was carried out. This research is important to do because in the development of COVID-19 drugs, it is necessary to predict properties absorption, distribution, metabolism, excretion (ADME), toxicity, and describe drug interactions with receptors. Physicochemical tests describe the solubility/solubility of a compound, whether it is soluble in water (hydrophobic)/fat (hydrophilic), and this solubility property is required to penetrate cell membranes by the movement of drugs from high to low concentrations (passive diffusion) (Fadaka et al, 2022). Toxicity is the ability of a chemical substance to cause damage to organisms both when used and when in the environment (da Silva Hage-Melim et al, 2020).

2. Materials and Methods

2.1. Materials

The materials used in this study were PyRx 0.8®, Discovery Studio Visualizer®, PyMOL®, and AutoDockTools-1.5.6®.

2.2. Tools

The hardware tools used were a set of laptops with specifications for an Intel® Celeron® CPU N3350 @ 1.10GHz, 2 GB of RAM, and Windows 10 Pro 64-bit operating system software (10.0, Build 19041)(Diningrat et al, 2021).

2.3. Preparation of Ligands and Comparative Compounds

Ligand samples were produced from GCMS analysis of metabolites obtained from essential oils of *S. cumini and C. lacryma-jobi*. The bioactive compounds are Aziridine-2-carbothioamide from *S. cumini* essential oil and 4-Dibenzofuranamine from *C. lacryma-jobi* essential oil. Sample preparation began with downloading the ligands and comparison compounds from the Protein Data Bank website https://www.rcsb.org in *pdb format. For visualization data in the form of two-dimensional structures, compounds were drawn using the ChemDraw 19.0® program. The ligand preparation process to become a ready-to-use file with a 3D chemical structure format is carried out using the AutoDockTools-1.5.6® conversion facility. The ligands used were Aziridine-2-carbothioamide and 4-Dibenzofuranamine, as well as their comparison compounds, lopinavir and chloroquine. Then, the downloaded ligand complex file will be opened in pdb format. The next step was ligand preparation by eliminating solvent (water), all residues, and small molecules, then storing in the form of pdbqt (Protein Data Bank, Partial Charge (Q), & Atom Type (T))(Diningrat et al, 2021).

2.4. Receptor Preparation

A Receptor preparation was initiated by downloading the NSP5 (7BQY and 2GZ7) and ACE2 (3D0G and 1R4L) receptor structures on the Protein Data Bank (PDB) site in pdb format. Receptor protein preparation was carried out using the PyMOL® application. At this stage, the elimination of solvents (water) and small molecules is carried out. After that, the file was saved in the form of pdbqt (Protein Data Bank, Partial Charge (Q), & Atom Type (T)) (Diningrat et al, 2021; Diningrat et al, 2021).

2.5. Determination of Minimum Energy

The determination of the minimum energy was carried out using the Chem3D 19.0® program. It was replicated three times using the Merck Molecular Force Field 94 (MMFF94) method, but first the three-dimensional structure was downloaded from the https://pubchem.ncbi.nlm.nih.gov/ site in *sdf format. After that, the minimum energy calculation is carried out through Chem3D 19.0® by importing file > calculation > MMFF94 > Perform MMFF94 Minimization.

2.6. Determination of Grid Box Center and Sizes

The determination of grid box centers and sizes was done with blind docking tools automatically using the CB-Dock® application. CB-Dock® predicts receptor binding

sites, calculates centers and sizes with a curvature-based cavity detection approach (Mills et al, 2015). Ligands and receptors in pdb format were uploaded to the site http://cao.labshare.cn/cb-dock/ and then calculated. The results obtained were interactions between ligands and receptors in 3D form and a table containing the vina score, cavity size, center, and size. The results were then saved or copied into Microsoft Word.

2.7. Method Validation

The validation process in this in silico test was carried out through re-docking of native ligands that have been downloaded through the Protein Data Bank website https://www.rcsb.org, and there are several native ligands that were prepared using the Discovery Studio Visualizer® application. Receptor validation was performed three times using the PyRx-Vina® application. The parameter observed at this stage is the root mean square deviation (RMSD) value resulting from the re-docking of the native ligand with its protein (Basu et al. 2020). The method is said to be valid and good if the resulting RMSD value is < 2 (Perrella et al, 2021).

2.8. Binding Process

The binding process was carried out between the ligand and the comparison compound at each receptor through the PyRx-Vina® software. Receptors and compounds that have been prepared were inserted into the PyRx 0.8® application in the form of pdbqt. The receptor was then set to a macromolecule, while the compound is set to a ligand. The ligands and receptors would be saved on the computer automatically and would be listed in the navigator section "AutoDockTools-1.5.6®". Receptors and ligands that have been stored earlier are entered in the control section of the Vina Wizard select molecule selection. Then, the forward button is selected and the grid box center and sizes from the resulting CBDock® application are set.

Then, vina search space information (center and dimension) was recorded, and this data was used for administrative needs, validation, and when analyzing relative positions. Next, Run Vina was selected, and the results were obtained in the form of affinity values and RMSD values for validation need (Diningrat et al, 2021; Diningrat et al, 2021).

2.9. Prediction of Physicochemical Properties and Toxicity

The prediction of the physicochemical properties of the ligands was carried out by checking the simplified molecular input line entry system (SMILE) code of the ligands and the comparison compounds obtained from the ChemDraw 19.0® application. The SMILE code is uploaded to the SwissADME website http://www.swissadme.ch/. The results were analyzed based on Lipinski's five rules. Lipinski contains five rule that must be met by the ligand in order to proceed to the docking simulation stage. Compounds are said to not meet Lipinski's five rules if there are errors of more than one criterion [8], by means of molecular weight analysis (BM), logarithm of partition coefficient (Log P (XLogP3)), hydrogen bond acceptors (HBA), hydrogen bond donors (HBD), and molar refractivity. To predict ligand toxicity based on LD50 values, skin sensitization, AMES toxicity, and hepatotoxicity, the prepared SMILES code was entered through the pkCSM online tools site https://biosig.unimelb.edu.au/pkcsm/prediction and the Protox II online site tools https://toxnew.charite.de/protox_II/ to predict the LD50 value.

3. Results

The chemical structure of Aziridine-2-carbothioamide and 4-Dibenzofuranamine compounds as well as the comparison compounds of lopinavir and chloroquine can be seen in Figure 1. The minimum average results obtained for the compounds Aziridine-2-carbothioamide and 4-Dibenzofuranamine were 12,148 kcal/mol and 72,0405667, respectively.

The results of the docking validation can be seen in Table 1.

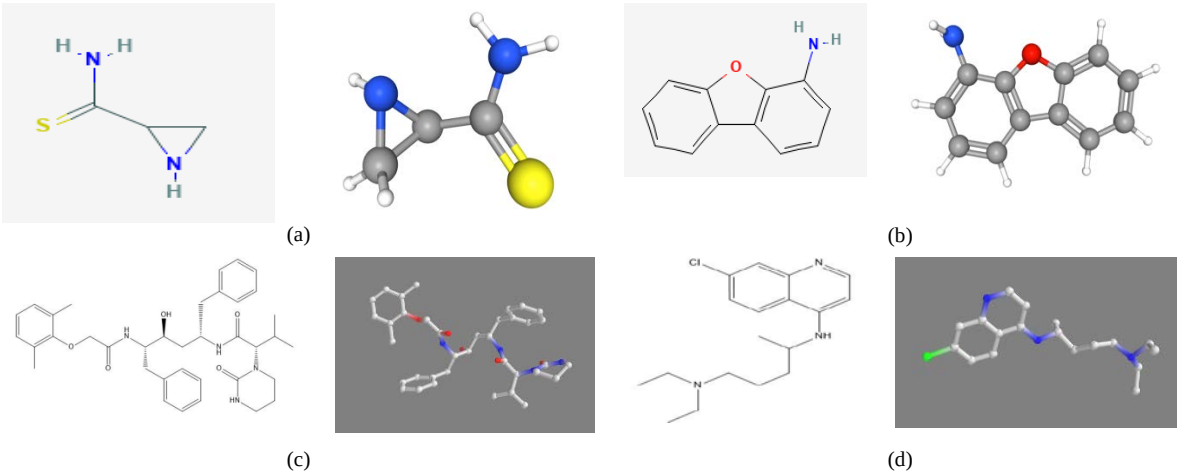

Figure 1. Chemical structure of compounds (a) Aziridine-2-carbothioamide and (b) 4-Dibenzofuranamine, as well as comparison compounds (c) lopinavir and (d) chloroquine

Table 1. The results of the docking validation on RMSD

Receptors	Native Ligand	RMSD Lower (Å)	RMSD Upper (Å)
7BQY	Native ligand 1	1.751±0.060	3.469 ± 0.736
2GZ7	Native ligand 2	0.185 ± 0.009	2.950 ± 0.093
3D0G	Native ligand 3	1.846 ± 0.047	2.202 ± 0.079
	Native ligand 4	1.852 ± 0.029	5.257 ± 0.034
1R4L	Native ligand 5	1.356 ± 0.668	2.279 ± 0.458
	Native ligand 6	1.805 ± 0.157	2.276 ± 0.214

Native ligand 1: n-[(5-methylisoxazol-3-yl)carbonyl]alanyl-l-valyl-n~1~-((1r,2z)-4(benzyloxy)-4-oxo-1-{[(3r)-2oxopyrrolidin-3-yl]methyl}but-2enyl)-l-leucinamide, Nativeligand 2: 2-[(2,4-dichloro-5-methylphenyl)sulfonyl]-1,3-dinitro-5-(trifluoromethyl)Benzene

Native ligand 3: 2-acetamido-2-deoxy-beta-D-glucopyranose,Native ligand 4: 2-acetamido-2-deoxy-alpha-D-glucopyranose , Native ligand 5: (s,s)-2-{1-carboxy-2-[3-(3,5-dichloro-benzyl)-3h-imidazol-4-yl]-ethylamino}-4-methyl-pentanoic acid

The results of docking using the application of PyRx-Vina® on the compound of Aziridine-2-carbothioamide, the comparison compound of lopinavir, and the native ligand against the 7BQY and 2GZ7 receptors, as well as the 4-Dibenzofuranamine compound, the comparison compound of chloroquine, and the native ligand against the 3D0G and 1R4L receptors are shown in Figure and. Table 2.

The types of interactions in three-dimensional form are shown in Figure 2

Table 2. Results of docking of test compounds, comparison compounds, and native ligands for each receptor

Receptor	Compound	Affinity (kcal/mol)	Center			Size (x*y*z)
			X	Y	Z	
NSP5 (7BQY)	Aziridine-2-carbothioamide*	-5.7 ± 0	-6 ± 0	2 ± 0	9.7 ± 0.929	21*21*21
	Lopinavir (Control Compound)	-5.633 ± 0.058	9 ± 0	3 ± 0	9.2 ± 1.701	26*26*26
	Native ligand 1	-5.467 ± 0.208	9 ± 0	3 ± 0	5.6 ± 3.650	29*29*29
NSP5 (2GZ7)	Aziridine-2-carbothioamide**	-6.667 ± 0.153	-1 ± 0	37 ± 0.058	14.4 ± 2.223	21*21*21
	Lopinavir (Control Compound)	-7.8 ± 0.1	-1 ± 0	-37 ± 0	15 ± 4.114	26*26*26
	Native ligand 2	-5.6 ± 0	-23 ± 0.058	-40 ± 0.058	14.1 ± 0.305	21*21*21
ACE2 (3D0G)	4-Dibenzofuranamine***	-6.567 ± 0.208	19 ± 0.058	39 ± 0.058	68.4 ± 0.889	22*22*22
	Chloroquine (Control Compound)	-4.1 ± 0.1	47 ± 0.058	-11 ± 0.058	77.6 ± 3.980	24*24*24
	Native ligand 3	-3.367 ± 0.058	61 ± 0	17 ± 0	68.2 ± 0.346	18*32*18
ACE2 (1R4L)	4-Dibenzofuranamine***	-6.667 ± 0.115	46 ± 0	5 ± 0	18.7 ± 2.285	35*24*35
	Chloroquine (Control Compound)	-9.167 ± 0.058	46 ± 0	2 ± 0	26.7 ± 1.4	35*23*35
	Native ligand 4	-6,267 ± 0.058	46 ± 0	5 ± 0	27.3 ± 0.153	35*18*35

The significance value between the test compound and the comparison compound at each receptor .

* : 0.114 kcal /mol, * * : 0.000 kcal / mol, ** * : 0.000 kcal /mol, *** * : 0.034 kcal /mol

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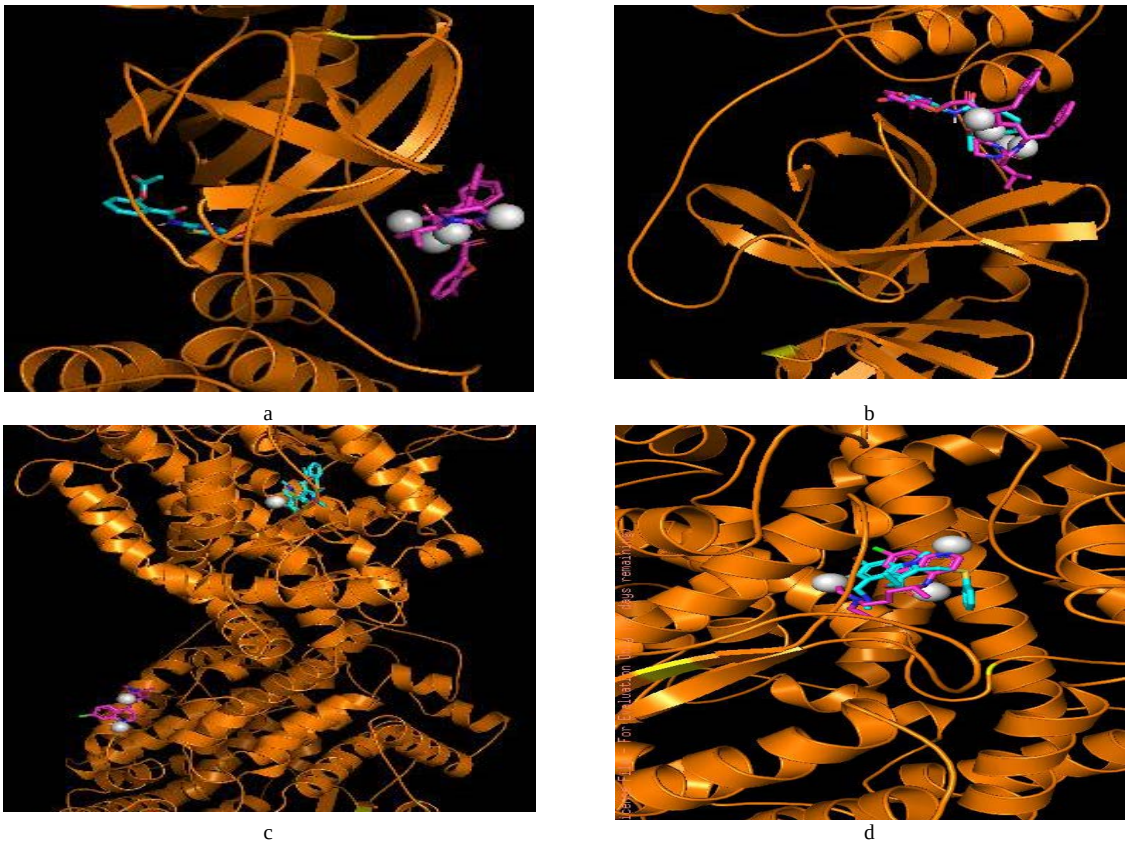

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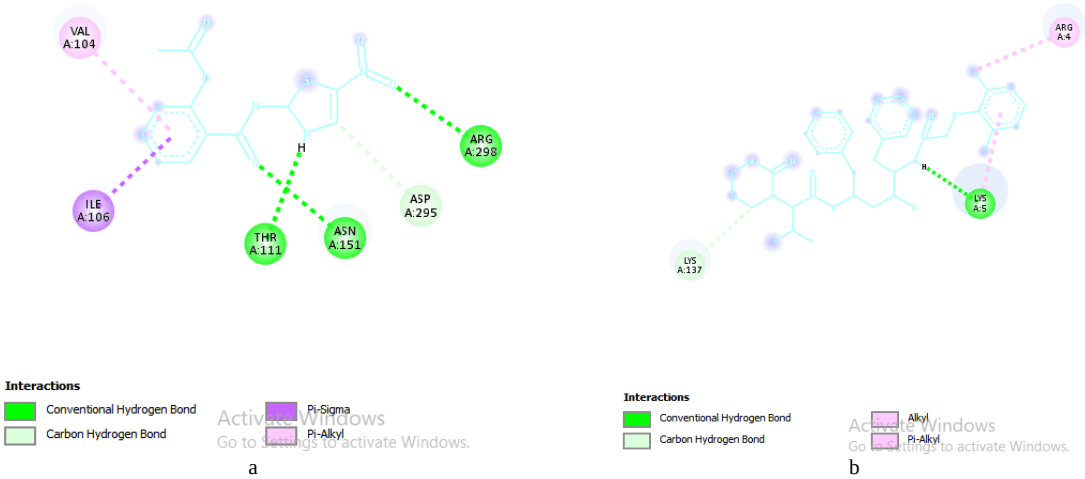
Figure 2. Three-dimensional shape (a) Aziridine-2-carbothioamide (cyan)-lopinavir (purple) against the 7BQY receptor (b) Aziridine-2-carbothioamide (cyan)-lopinavir (purple) against the 2GZ7 receptor (c) 4-Dibenzofuranamine (cyan)-chloroquine (purple) to 3D0G receptors (d) 4-Dibenzofuranamine (cyan)-chloroquine (purple) to 1R4L receptors

The type of interaction in two-dimensional form is shown in Figure 3.



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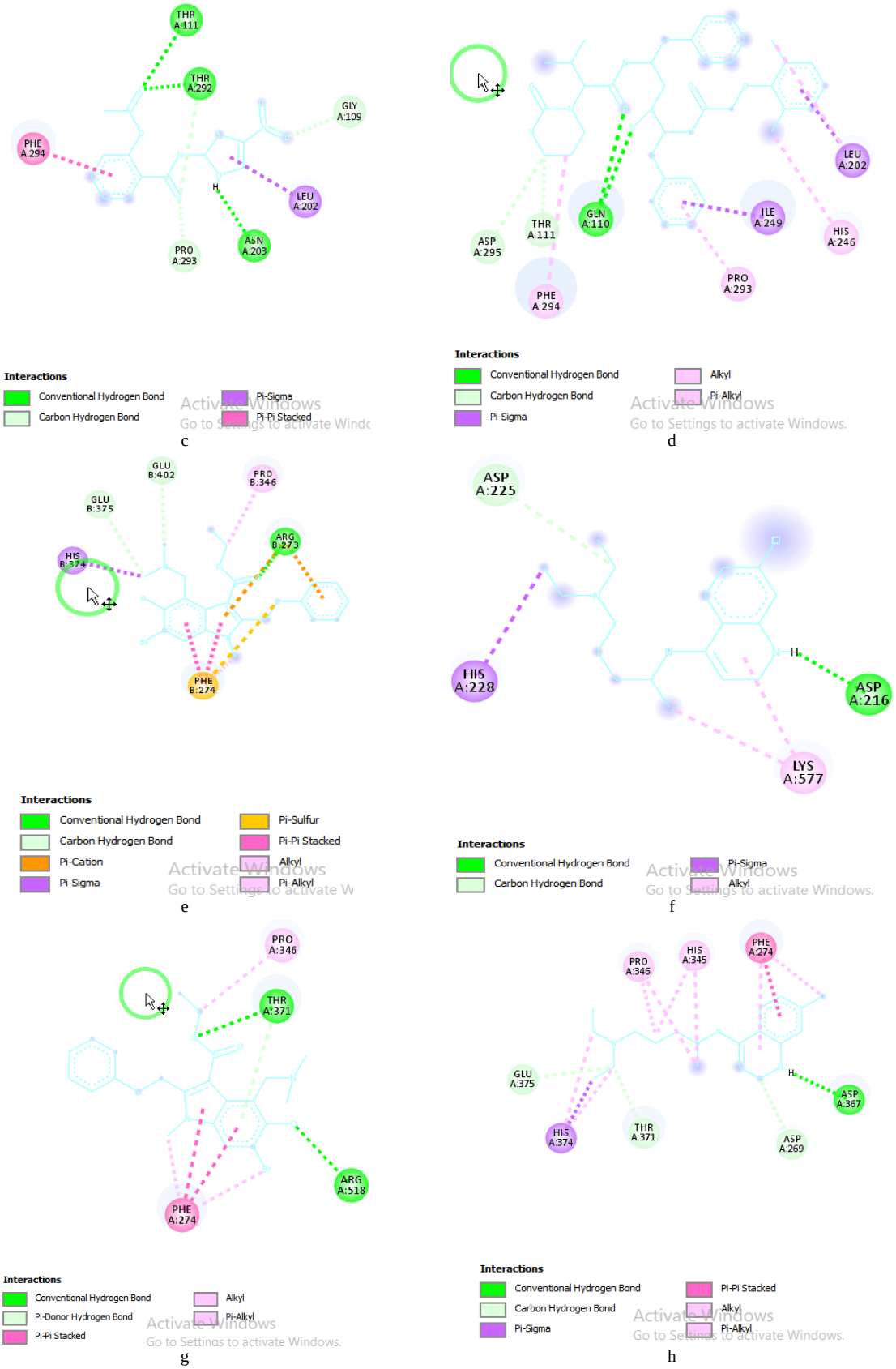

Figure 3. Two-dimensional shape (a) Aziridine-2-carbothioamide-7BQY (b) Lopinavir-7BQY (c) Aziridine-2-carbothioamide-2GZ7 (d) Lopinavir-2GZ7 (e) 4-Dibenzofuranamine-3D0G (f) Chloroquine -3D0G (g) 4-Dibenzofuranamine-1R4L (h) Chloroquine-1R4L

By using the tools, the pharmacokinetic profile of compounds such as absorption, distribution, and ligand metabolism could be evaluated. The results of screening the physicochemical properties of Aziridine-2-carbothioamide and 4-Dibenzofuranamine compounds, as well as the comparison compounds of lopinavir and chloroquine can be seen in table 3.

Table 3. The results of reading the physicochemical properties by applying Lipinski's five rule to receptor protein compounds

Compound	Lipinski Five Rules					Result
	MW (Dalton)	Log P	NBD	HBA	MR (cm3 mol-1 K- 1)	
Aziridine-2-carbothioamide	307.28	2.04	1	6	76.65	Yes
4-Dibenzofuranamine	477.41	4.43	1	4	122.69	Yes
Lopinavir	628.80	5.92	4	5	187.92	No
Chloroquine	319.87	4.63	1	2	97.41	Yes

MW: Molecule Weight < 500 Dalton ,LogP: Coefficient Partition < 5, HBD: Hydrogen Bond Donor < 5 ,HBA: Hydrogen Bond Acceptor < 10, MR: Molar Refractivity (40 < MR < 130)

Many kinds of toxicity assessment were carried out in this test, such as LD50, skin sensitization, Ames toxicity (Ames test devised by a scientist "Bruce Ames" is used to assess the potential carcinogenic effect of chemicals (Pan, 2021)), and hepatotoxicity. The Globally Harmonized System classifies LD50 into 6 classes: class I (fatal if swallowed): LD50 5mg/kg; class II (fatal if swallowed): 5 < LD50 50 mg/kg; class III (toxic if swallowed): 50 < LD50 300 mg/kg; class IV (harmful if swallowed): 300 < LD50 2000 mg/kg); class V (may be harmful if swallowed): 2000 < LD50 5000 mg/kg; and class VI (non-toxic): LD50 > 5000 mg/kg [43]. The results of the predicted toxicity are shown in Table 4.

Table 4. The predicted results of the toxicity properties of the protein compounds of Aziridine-2-carbothioamide and 4-Dibenzofuranamine, as well as the comparison compounds of lopinavir and chloroquine by using pkCSM Online Tools and Protox II Online Tools receptor protein compounds.

Compounds	Toxicity Category				Results (GHS)
	LD50 (mg/ kg)*	Skin sensitization**	Ames toxicity**	Hepatotoxicity **	
Aziridine-2-carbothioamide	1350	No	Yes	Yes	4
4-Dibenzofuranamine	340	No	No	No	4
Lopinavir	5000	No	No	Yes	5
Chloroquine	750	No	Yes	Yes	4

*Protox II Online Tools ,** pkCSM Online Tools

4. Discussion

The determination of the minimum energy in the compounds of Aziridine-2-carbothioamide and 4-Dibenzofuranamine was carried out with the aim of obtaining a more accurate calculation of the molecule and a more stable final conformation (Joshi et al. 2020). This energy is the most possibly minimum energy in the stereochemical form and the most stable form for docking. The validation process was carried out with the aim of ensuring the method used was validated and a good method was obtained so that it can be continued in the next research stage. The parameter observed at this stage was the RMSD value resulting from the redocking of native ligand with its protein (Yanagisawa et al. 2022). RMSD is the process of measuring two poses by comparing the atomic positions between the experimental structure and the docked protein structure (Sarathi & Padhi, 2021). The method is said to be valid and good if the resulting RMSD value is < 2 (Yanagisawa et al. 2022).

PyRx-Vina® produces two types of RMSD: RMSD lower and RMSD upper. However, only the value of the lower RMSD was analyzed because the lower RMSD was obtained by searching for all possible atoms in a symmetrical molecule (Shi et al. 2022). That is, the lower RMSD is calculated from the conformational approach of the native ligand in all symmetries. Atoms that cannot be distinguished will give correct results by correcting the symmetry so that the lower RMSD value can be more precise (Prateeksha et al. 2021).

Based on the validation process, the RMSD value was obtained. These values were different, possibly because the molecules analyzed are symmetrical molecules so that the potential substructures correspond to positions in the molecule (not all with the same position) (Laksmiani et al. 2020). The six native ligands were known to meet the validation standards with RMSD values < 2 so that docking of the test compounds on the respective receptors can be carried out.

The results of molecular docking of molecules in this study include the value of binding affinity and its RMSD. Binding affinity is the strength of the interaction between two or more reversibly bound molecules (Aljahdali et al. 2021). The score is a parameter of the strength of the binding affinity of the test ligand to the receptor (Takaya et al. 2020). The more stable ligand-protein interaction will be reflected by the lower score (minus). If the ligand binding to the receptor is more stable, it can be predicted that its activity will also increase (Morris & Corte, 2021).

Table 2 shows that there were compounds with native ligands that have different binding affinity replication data, and this difference was predicted due to differences in ligand binding to amino acids at the receptor (Aljahdali et al. 2021). At the 7BQY receptor, Aziridine-2-carbothioamide obtained a binding affinity value of -5.7 kcal/mol, lopinavir of -5,633 kcal/mol, and native ligand of -5,467 kcal/mol. From this value, it was known that Aziridine-2-carbothioamide had the best binding affinity value so that the compound was more effective (as a drug) and was able to inhibit the replication process of the SARS-CoV-2 virus better. Meanwhile, at the 2GZ7 receptor, Aziridine-2-carbothioamide obtained a binding affinity value of -6,667 kcal/mol, lopinavir of -7.8 kcal/mol, and a native ligand of 5.6 kcal/mol. From these values, it is known that lopinavir had the bestbinding

affinity value so that the comparison compound is more effective (as a drug) and was able to better inhibit the replication process of the SARS-CoV-2 virus. However, Aziridine-2-carbothioamide had a better binding affinity value than the native ligand.

At the 3D0G receptor, 4-Dibenzofuranamine obtained a binding affinity value of -6.567 kcal/mol, chloroquine of -4.1 kcal/mol, the first native ligand of -3.367 kcal/mol, and the second native ligand of -5.3 kcal/mol. From this value, it is known that 4-Dibenzofuranamine has the best binding affinity value, so that the compound is predicted to be more effective as a drug and able to inhibit the attachment of the virus to the ACE2 receptor.

Meanwhile, the second native ligand was known to have a better binding affinity value than the first native ligand. Furthermore, at the 1R4L receptor, 4-Dibenzofuranamine obtained a binding affinity value of -8.1 kcal/mol, chloroquine of -6,667 kcal/mol, the first native ligand of -9,167 kcal/mol, and the second native ligand of -6,267 kcal/mol. From this value, it is known that the first native ligand with the name (s,s)-2-{1-carboxy-2-[3-(3,5-dichloro-benzyl)-3himidazole-4-yl]-ethylamino}-4- methyl-pentanoic acid has the best binding affinity value so that the compound is more effective (as a drug) and is able to inhibit the attachment of the virus to the ACE2 receptor better.

However, 4-Dibenzofuranamine had a better binding affinity value than the comparison compound, namely chloroquine. A hydrogen bond is one that occurs between a hydrogen atom (H) in one molecule and one atomic element (F, O, N) in another molecule, which is the strongest dipole-dipole force (Meyer-Almes, 2020). In biological systems, nitrogen or oxygen atoms are donors and acceptors, especially atoms in the amine (-NH2) and hydroxyl (-OH) groups. Due to the polar nature of the N-H and O-H bonds, the H atoms can hydrogen bond with acceptor atoms (Diningrat et al, 2021). Hydrogen bonds will be stable and have strong bonds if they have a bond length of < 2.7 (Domínguez-Villa et al. 2021; Diningrat et al. 2021).

The smaller the hydrogen bond distance between the ligand and the acid group is, the greater the affinity value is. The smaller the bond distance is, the stronger the bond is and not easily separated or the other way round (Domínguez-Villa et al, 2021). Hydrophobic bonds are nonpolar molecules that do not contain hydrated ions or have a dipole moment. This happens because in water, these molecules are insoluble or almost insoluble (Meyer-Almes et al. 2020). This binding is important in the process of combining the nonpolar region of the ligand with the nonpolar region of the receptor. The nonpolar region of the water-insoluble molecule and the surrounding water molecules will combine through hydrogen bonds to form a quasi-crystalline structure (icebergs) (Zhang et al. 2020). Hydrophobic binding is a parameter of the strong amino acid interaction between the ligand and the receptor which is useful in helping to maintain the binding conformation (Aljahdali et al. 2021) (Figure 2).

Electrostatic bonds describe the forces between polar atoms and are usually represented by the Coulomb potential. In general, there were two grading function approaches for hydrogen bond interactions: (i) using specific force field-based parameters related to van der Waals and electrostatic energy potentials; (ii) using a directional term, where the hydrogen bond contribution was a function of the deviation of the geometric parameter from the ideal hydrogen bond (Zhang et al. 2020). Hydrophobic interactions and electrostatic interactions can increase conformational stability (Zhang et al. 2020).

The 3D visualization results showed that Aziridine-2-carbothioamide-lopinavir at the 2GZ7 receptor and 4-Dibenzofuranamine-chloroquine at the 1R4L receptor have the same binding position, so it could be predicted that the test compound has inhibitory activity (2GZ7) or attachment (1R4L) of the SARS-CoV virus. -2. Meanwhile, Aziridine-2-carbothioamide-lopinavir at the 7BQY receptor and 4-Dibenzofuranamine-chloroquine at the 3D0G receptor did not have the same position and angle of each atom. The similarity of the ligand pose with the comparison compound could be influenced by the RMSD value, where an RMSD value that was close to zero would cause the pose similarity between the two (Aljahdali et al. 2021).

Figure 3 shows the dotted lines. They are the bond distance (Aljahdali et al. 2021). Hydrogen bonds are indicated by dotted lines in lime green and salted egg green; Electrostatic interactions are indicated by the orange dotted line and hydrophobic interactions are indicated by the purple, neon pink, and pink dotted lines.

In Figures 3(a) and 3(b), it can be seen that the Aziridine-2-carbothioamide compound was more stable, supported by the presence of hydrogen bonds with the amino acids Asn 151, Arg 298, Thr 111, and Asp 295; and hydrophobic interactions with amino acids Ile 106 and Val 104. Meanwhile, the comparison compound lopinavir only had two hydrogen bonds with the amino acids Lys 5 and Lys 137; and hydrophobic interaction with amino acid Arg 4. Meanwhile, in Figures 3(c) and 3(d), the comparison compound of lopinavir was more stable, supported by the presence of hydrogen bonds with amino acids Gln 110 (2), Thr 111, and Asp 295; and hydrophobic interactions with the amino acids Leu 202 (2), Ile 249, His 246, Phe 294, and Pro 293. Aziridine-2-carbothioamide had hydrogen bonds with the amino acids Thr 111, Thr 292, Asn 203, Gly 109, and Pro 293; and had only two hydrophobic interactions with the amino acids Leu 202 and Phe 294.

In Figures 3(e) and 3(f), the 4-Dibenzofuranamine compound was more stable, supported by the presence of hydrogen bonds with amino acids Arg 273, Glu 375, and Glu 402; hydrophobic interactions with the amino acids His 374, Phe 274 (3), and Pro 346; and electrostatic interactions with the amino acid Arg 273 (2). Meanwhile, chloroquine has only two hydrogen bonds with the amino acids Asp 216 and Asp 225 and has only three hydrophobic interactions with the amino acids His 228 and Lys 577 (2).

Furthermore, in Figures 3(g) and 3(h), chloroquine compounds were more stable, supported by the presence of hydrogen bonds with amino acids Asp 367, Asp 269, Thr 371, and Glu 375; and hydrophobic interactions with the amino acids His 374, Phe 274 (3), and Pro 346 (2). Meanwhile, 4-Dibenzofuranamine has only three hydrogen bonds with the amino acids Thr 371 (2) and Arg 518; and has only five hydrophobic interactions with the amino acids Phe 274 (4) and Pro 346.

The prediction of physicochemical properties was carried out by checking the ligands of Aziridine-2-

carbothioamide and 4-Dibenzofuranamine, as well as comparison compounds lopinavir and chloroquine. Compounds are said to not meet if there are errors of more than one criterion, by means of the analysis of Molecular Weight (BM), logarithm of partition coefficient (Log P (XLogP3)), hydrogen bond acceptors (HBA), hydrogen bond donors (HBD), and molar refractivity. SwissADME was chosen because this platform does not incur costs and functions in calculating the molecular properties of ligands based on Lipinski's five rules (Ferdausi et al, 2022).

From Table 3, it can be seen that the compounds of Aziridine-2-carbothioamide, 4-Dibenzofuranamine, and chloroquine had a molecular weight of less than 500 Daltons, a LogP value less than 5, a hydrogen bond donor value less than 5, a hydrogen bond acceptor value less than 10. , and the value of the molar refractivity was between 40 and 130. Thus, the compound of 4-Dibenzofuranamine and chloroquine complies with Lipinski's five rules and can be said to be easy to absorb and have high permeability. Therefore, the above compounds can be administered orally (Basu et al. 2020).

Meanwhile, lopinavir did not meet Lipinski's five laws because there were three criteria that were not met that are the molecular weight value of 628.80 Da (BM < 500 Da), the LogP value of 5.92, and the molar refractivity value of 187.92 (40 < MR < 130). Compounds can be said to not meet if there is more than one criterion that deviates. The condition for the value of LogP (XLogP3) is -0.4-5. The larger or the more positive the log P value is, the more hydrophobic is the molecule. If it is too hydrophobic, the level of toxicity will also be high because it will be retained longer in the lipid bilayer or the base of the cell membrane structure and distributed more widely in the body so that the selectivity of binding to the target enzyme is reduced (Missioui et al. 2022). Molar refractivity that does not meet the requirements would cause nonpolar compounds to be unable to form momentum so that they cannot bind to receptors, and their polar nature cannot excrete residues from compound metabolism (Cheng et al. 2021). Therefore, lopinavir compounds will be difficult to absorb and have low permeability.

Toxicity is the ability of a chemical substance to cause damage to organisms either when used or when in the environment (Upreti et al. 2021). A toxicity test is carried out if it is known that the compound has a better predictive activity than the comparison compound based on the equation of the Quantitative Structure and Activity Relationship (HKSA) (Cheng et al. 2021).

Based on Table 4, the results obtained for all compounds were proven to have no toxicity on skin sensitization analysis so all compounds did not cause skin sensitization. For the results of the Ames toxicity test, there were two positive compounds, Aziridine-2-carbothioamide and chloroquine, which means that both compounds are mutagenic and therefore can act as carcinogens (Ahmad et al. 2021). Then, the hepatotoxicity test showed positive results on the protein compound Aziridine-2-carbothioamide and the comparison compound of lopinavir and chloroquine, so it could be predicted that the three compounds were toxic to the liver. In addition, for oral toxicity in experimental animals (LD50), testing and classification of toxicity are carried out on the Protox II Online Tools website. Lethal dose 50 (LD50) is statistical data of a quantity to express a single dose of a compound that is estimated to cause death or toxic effects in 50% of experimental animals after treatment. The smaller the toxic value is, the more toxic is the compound, or the other way round (Khaerunnisa et al. 2020).

From these tests, it can be predicted that the compound of Aziridine-2-carbothioamide has an LD50 value of 1350 mg/kg in experimental animals, while the compound of 4-Dibenzofuranamine is 340 mg/kg and the comparison compound of chloroquine is 750 mg/kg so that the three compounds are classified in class 4 GHS (300 < LD50 2000 mg/kg), which means the compound has a relatively low toxicity effect (Ahmad et al. 2021). Meanwhile, the comparison compound of lopinavir is predicted to have a value of 5000 mg/kg so that the compound is classified in class 5 GHS (2000 < LD50 5000 mg/kg), which means it has a low acute toxicity effect. The greater the toxic value, the less toxic is a compound or the other way round (Khaerunnisa et al. 2020; Ahmad et al. 2021).

5. Conclusion

The Aziridine-2-carbothioamide compound at the NSP5 receptor (7BQY) did not have a significant difference in binding affinity values with the comparison compound of lopinavir, while Aziridine-2-carbothioamide at the NSP5 receptor (2GZ7); and 4-Dibenzofuranamine at the ACE2 receptors (3D0G and 1R4L) had a significant difference in binding affinity respectively to the comparator lopinavir and chloroquine. However, the binding affinity of 4-Dibenzofuranamine is less effective than the native ligand of the 1R4L receptor, so further research is recommended to test 4-Dibenzofuranamine at the ACE2 receptor with a different code. To develop the antiviral potential of SARS-CoV-2, the ligand needs to be observed through molecular dynamics analysis at a later stage.

6. Conflict of Interest

The authors declare no financial or commercial conflict of interest.

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