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Computational Model of Trisindoline 1 Conjugate to Protein P53 and P53R2: Targets For Breast Cancer Therapy

Shabrina Syifa Ghaissani[1,*] Awik Puji Dyah Nurhayati[1] Vencka Azzahra Putri[2]

[1] *Postgraduate School of Biology, Department of Biology, Faculty of Science and Data Analytics, Institute Technology of Sepuluh Nopember, Surabaya, East Java, Indonesia, 601112*
[2] *Undergraduate School of Biology, Department of Biology, Faculty of Science and Data Analytics, Institute Technology of Sepuluh Nopember, Surabaya, East Java, Indonesia, 60111*
[*]*Corresponding author. Email: shabrinafassani@gmail.com*

ABSTRACT
In 2020, there were 2.3 million women diagnosed with breast cancer with average, 7 percent to 11 percent of women with early breast cancer experience a local recurrence during this time. Resistance mechanisms in breast cancer include DNA repair mechanisms that protect cancer cells from endogenous or exogenous DNA-damaging agents. p53R2 is a potential target for cancer gene therapy like RRM2 because of its role in dNDP synthesis and DNA repair. Inhibition of p53R2 enhances the sensitivity of cancer cells in vitro. Meanwhile, the P53 protein plays a role in apoptosis and can respond to signals of DNA damage, hypoxia, oxidative stress, and oncogene activation. The common cancer therapy given is chemotherapy or radiotherapy, but it is known that cancer cell has a DNA repair mechanism pathway that resists that treatment. Due to the severity of side effects of current cancer therapy, such as resistance and non-specific target to the cancer cell, natural compound sources were believed to have multiple specific targets with minimally acceptable side-effects are now of interest to many researchers, such as a group of alkaloids, Trisindolina 1 that derived from a marine sponge. Trisindolina is a group of alkaloid compounds that showed the highest cytotoxicity activity in the next cytotoxicity test, Trisindolina 1. The cytotoxicity test was carried out on 7 cell lines, namely HepG2, HELA, T47D, WiDr, RAJI, 4T1 and Vero with IC50 values 0.183; 1,532; 1,293; 1,431; 0.392 μg / ml. Thus, we need to confirm the potency of Trisindoline 1 against the DNA repair-inducing protein, P53R2, and its capability to interact with the apoptotic-inducing protein, P53, by In-silico drug design. So, this study focused mainly on computer-aided drug design processes like structure-based pharmacophore modeling, virtual screening, ADMET, molecular docking, and dynamic simulation approaches to identify the possible natural antagonist against P53r2 protein and enhanced protein p53-apoptotic inducing protein to treat breast cancer.

Keywords: *Breast Cancer, Molecular Docking, P53, P53R2.*

1. INTRODUCTION

Cancer is a disease caused by genetic disorders, characterized by continuous proliferation, damage to growth suppressor genes, absence of cell death processes, uncontrolled cell replication, induction of angiogenesis, cells capable of metastasizing, and invading tissues surrounding the network [1]. Based on the latest Globocan data, in 2020, there were around 2,261,419 new cases of breast cancer, so this cancer is one of the most critical health problems in the world. On average, 7 percent to 11 percent of women with early breast cancer experience a local recurrence during this time [2]. Several patients with breast cancer may relapse; there is an urgent need to have a better understanding of the resistance mechanisms in breast cancer. Several factors that trigger resistance mechanisms in breast cancer include drug absorption, transport, and efflux; enzyme system

inactivates antitumor drugs by affecting their metabolism; The quantity and affinity of hormone receptors alter; Cancer-related genes; DNA repair; Cancer stemness; and Tumor microenvironment [3]. DNA repair pathways play roles in maintaining genetic stability and integrity when cancer cells interact with endogenous or exogenous DNA-damaging agents [4]. The deregulation of DNA repair pathways is associated with the initiation and progression of cancer [5]. Due to its ability in dNDP synthesis and DNA repair, some studies reported that p53R2 is a potential target for cancer gene therapy. Downregulation of P53R2 expression would enhance sensitivity to cancer cell line in vitro. Several studies have also observed that upregulation of p53R2 expression was positively correlated with anticancer agent resistance of human malignancies [7]. Recently, a study by Devlin et al showed that p53R2 was overexpressed in prostate tumor cell lines; we can assume that downregulating the expression of p53R2 will increase the apoptotic effects of ionizing radiation and doxorubicin. George et al. reported that the roles of p53R2 in the biological characteristics of cancer cells and the underlying mechanisms remain largely unclear even controversial [8].

Meanwhile, the P53 protein plays a role in apoptosis and can respond to signals of DNA damage, hypoxia, oxidative stress, and oncogene activation [9] [10]. Loss of p53 activity has been associated with tumor progression and unfavorable tumor prognosis [11]. However, targeting and overcoming abnormalities in cancer such as P53R2 that suppress apoptosis could generate a potent proapoptotic stimulus, for example, P53, which can drive apoptosis. Such novel therapeutics try to prime the apoptotic machinery to act as promising apoptosis-inducing agents, bearing high hopes for managing cancers resistant to conventional treatments [12] [13].

The most common cancer treatments, including chemo- or radiotherapy, are designed to induce cell death by direct or indirect DNA damage [14]. However, tumor cells will repair their DNA to resist these anticancer agents during chemo- or radiotherapy [15]. Many approaches to cancer management are often ineffective due to adverse reactions, drug resistance, or inadequate target specificity of single anti-cancer agents. Natural compound mixtures that are believed to have multiple specific targets with minimally acceptable side effects are now of interest to many researchers due to their cytotoxic and chemosensitizing activities. As a result, several natural products with unique mechanisms of action have been identified and recently entered into clinical trials [16] [17] [18].

Kobayashi et al. have researched the anticancer activity of marine sponge alkaloid compounds *Cinachyrella anomala* through apoptosis induction in target cancer cells. The next strategy is to synthesize a new compound with a structure similar to its alkaloid. Trisindolina is a group of alkaloid compounds 1,4,9-triazatricyclo [7,3,1,0] trideca-3,5 (13), 10-trien-8-ol. The synthesis of Trisindoline compounds is Trisindoline 1,2,3, and 4. Cytotoxicity tests of Trisindolina 1,2,3 and 4 against MCF (Michigan Cancer Foundation) -7 cell line / breast cancer were IC50, amounting to 2,059; 0; 3,9759 and 15.46 μM. Trisindolina 1 showed the highest cytotoxicity activity, so in the next cytotoxicity test, Trisindolina 1. The cytotoxicity test was carried out on 7 cell lines, namely HepG2, HELA, T47D, WiDr, RAJI, 4T1 and Vero with IC50 values 0.183; 1,532; 1,293; 1,431; 0.392 μg / ml [19].

In-silico drug design approaches like pharmacophore modeling, virtual screening, molecular docking, and dynamic simulation approaches are widely used to discover, develop, and analyze drugs and similar biologically active molecules [20] [21]. In molecular docking, we evaluate the structure, and a ligand-based model can be able to identify similar active molecules against a specific target protein, where the binding affinity of a large scale compound with target macromolecule. A compound's biological activity can be evaluated whenever the compound binds with a targeted macromolecule and triggers a specific response. Trisindoline compound is one of the anti-cancer compounds that have the potential to be developed as a cancer therapy drug because of its cytotoxicity, which can induce the apoptotic pathway, so it does not have a toxic effect on normal cells. p53R2 and p53 in breast cancer cells (T47D) via molecular docking, so further research is needed on this matter. The docking method proved helpful, especially in the early stages of selecting the test compound for further development by describing the interaction between a molecule as a ligand with a receptor or protein [24].

2. METHODOLOGY

2.1. Structural Investigation in the PDB

Data retrieval is done by downloading the receptor file and the ligand file. The structure of the target protein (receptor) p53R2 was downloaded via the Protein Data Bank (PDB) with PDB ID, namely 2VUX (Human Ribonucleotide Reductase, M2 B Subunit (Gene name: p53R2)) and p53 with PDB ID 1TUP (Tumor Suppressor p53 Complex with DNA). The file is saved in PDB format with the file name according to the receptor name. The file is then stored in a folder,

namely the download folder. Next, ligan files were downloaded, namely Doxorubicin, through the Pubchem site or the ZINC15 site. The ligand file from the Pubchem site was downloaded in the 3D Conformer 'SDF' format and placed in the same folder as the previous receptor folder with the file name 'ligand'. In addition to doxorubicin, the ligand used is Trisindolina 1, whose chemical structure was made using Chemdraw software, then saved using the 'SDF' file format. Trisindolina 1 was not downloaded through the Pubchem/ZINC15 site because this ligand was not yet available on the site. After all, it was still a new compound. The target protein used is p53R2 which will then be docked with ligands (drug target), namely Trisindolina 1 and doxorubicin compounds.

The structure of the target protein (receptor) p53R2 was downloaded via the Protein Data Bank (PDB) with PDB ID, namely 2VUX (Human Ribonucleotide Reductase, M2 B Subunit (Gene name: p53R2)) and p53 with PDB ID 1TUP (Tumor Suppressor p53 Complex with DNA). Next, the ligand was downloaded, namely Doxorubicin, through the Pubchem site or the ZINC15 site. The ligand file from the Pubchem site was downloaded in the 3D Conformer 'SDF' format. In addition to doxorubicin, the ligand used is Trisindolina 1, whose chemical structure was made using Chemdraw software, then saved using the 'SDF' file format. The molecular structure of the protein that has been downloaded is opened using Biovia Discovery Studio 2020. The remaining protein molecules on the computer's main screen display are receptors that will be used in the docking process.

2.2. Receptor Preparation

The next step is to open the PyRx software. After the main software interface has been opened, the next step is to set the location of the worksheet (the 'Molecular Docking' folder) by clicking Edit Preferences. The selected molecule is a protein file that has been separated from its accompanying components.

2.3. Ligand Preparation

Ligand preparation is the next step after receptor preparation is done. The first step in preparing the ligand is to enter the structure file of the ligand compound into the PyRx software. Click Open Babel on the Controls box toolbar, clicks the icon click Open. After the ligand appears in the PyRx display, right-click with the mouse on the ligand name click Minimize selected to reduce the energy value of the ligand to be docked. When finished, right-click on the ligand name, then click Convert All to AutoDock Ligand (pdbqt) to change the ligand format such as the receptor format (.pdbqt) before running the molecular docking process. The ligand preparation process has been completed.

2.4. Molecular Docking

The docking process was carried out using the PyRx software, which has been integrated with AutoDock Vina. First of all, after receptor and ligand preparation, AutoDock Vina was opened then Vina Wizard was clicking. Next, the receptor and ligand to be docked were selected in the Navigator display box of the AutoDock menu. The determination of receptors and ligands were clicked multiple times by pressing the 'ctrl' key. Next, forward in the Controls box were chosen, then a grid box will appear on the macromolecule view. The size of the grid box was adjusted so that it will cover the entire binding site location of the receptor by moving the white round lever. The last step in docking is clicking Run Vina. The software will automatically run the docking process. The process will take time to finish until the binding affinity and RMSD values appear in the Controls box, which is displayed in tabular form. The docking folder on the computer desktop was opened to view the docking results. There will be three folders formed entitled Etc, Ligand, and Macromolecule Open the macromolecule folder, another folder will appear (example: 2VUX), and the results can be seen in the folder contains three files, namely receptor name.pdbqt, Conf.txt, and Dugname.pdbqt.

2.5. Docking Visualization

The docking results were visualized using the Biovia Discovery Studio 2020 software. The first step is to double-click on the protein file in the Docking folder. Next, the docked ligand file is inserted by double-clicking on the ligand file in the same Docking folder. Then, on the software's main screen, a 3D structure of the protein with docked ligands will appear. Visualizing the docking results in 2D is done by clicking on the receptor name in the hierarchy box.

3. RESULT AND DISCUSSION

3.1. Protein Preparation

The protein structure downloaded from PDB generally has a protein structure that still contains solvent (water). Other residues in the preparation process will obtain a protein structure without the original ligand and the original ligand structure. The preparation process is carried out using the Biovia Discovery software.

Table 1. Grid Box Values of Target Proteins and Ligands

	Center X	Center Y	Center Z	Size X	Size Y	Size Z
P53R2 + Trisindolina 1	24.92	-23.74	-8.43	108.78	95.72	113.98
P53R2 + Doxorubicin	26.65	-19.65	-14.2	95.58	101.29	119.01
P53 + Trisindolina 1	55.36	15.93	71.78	113.00	124.79	110.73
P53 + Doxorubicin	61.36	14.32	65.75	117.31	100.25	116.30

The protein's structure obtained is a protein that has lost its original ligand, other molecules such as water, and other single atoms. In the docking process, only the test compound interacts with the protein. These ligands and water molecules must be removed from protein macromolecules because they can prolong the duration of the docking simulation. The separation of native ligands from the protein structure aims to provide a pocket that will be used as a space for binding the test compound to the target protein [24].

3.2. Protein Preparation

Ligand preparation is carried out using PyRx software, namely by minimizing the energy of the ligands to avoid clashes between atoms when the process of adding and breaking hydrogen bonds occurs [25] so that the most stable structure is obtained, from this result obtained a ligand that has a sturdy structure with the lowest energy [26]. This process is done by right-clicking on the compound name, then clicking "Minimize Selected". After that, the power of the ligand will be minimized. Local energy minimization plays a role in optimizing all atoms can move freely and then rely on the minimization of the energy function to stabilize the conformation of the atom due to covalent and non-covalent interactions [28].

3.3. Molecular Docking

The docking simulation in this study was carried out using the blind docking method, which is a docking process carried out without knowing the exact location of the receptor's active site. This docking process uses software called PyRx, which has been integrated with Autodock Vina. A grid box arrangement is made in the docking stage, which will become a space for the native ligand to form a conformation when docked with the target protein [31]. In the docking stage, a grid box arrangement is made, becoming a space for the native ligand to form a conformation when docked with the target protein, as shown in Figure 3 below.

A grid box is a place where the ligand will interact with amino acid residues on the target protein in the form of a cube. Determination of the grid box is done to determine the coordinates of the binding site of a protein. The grid box settings carried out are setting the coordinates of the grid center and setting the grid size. This study uses a large grid box so that the ligands can rotate freely to find the most stable place on the receptor [29] [30].

The range of grid point spacing varies from 0.2Å to 1.0Å; the default is 0.375Å. Each point within the grid map stores the potential energy of an atom or functional group due to all the atoms in the macromolecule. We need to specify an *even* number of grid points in each dimension, n_x, n_y and n_z to add a central point also an odd number of grid points [32]. The grid box values from this research are as Table 1.

Based on the docking results between the ligand doxorubicin and Trisindolina 1 and the target protein p53R2 (PDB ID: 2VUX) obtained nine conformation results or the best value poses. The docking results show that the Trisindolina 1 compound has a docking or binding affinity score of -8.9 kcal/mol in the 1st conformation, which is more stable than the doxorubicin score of -7.6 kcal/mol in the 1st conformation. Trisindolina 1 and Doxorubicin ligands with p53 target protein (PDB ID: 1TUP) showed that Trisindolina 1 had a docking or binding affinity score of -9.0 kcal/mol in the 1st conformation, which was more stable than the doxorubicin score of -7.9. kcal/mol in the 1st conformation.

The binding energy (ΔGbind), or it can also be called the Gibbs free energy, is a parameter of the strength of the binding affinity between the ligand and the receptor. The more stable the interaction of the ligand with the protein is seen with the lower (minus or negative) the value of the bond energy. The docking simulation results show that the Trisindolina 1 compound has a higher Gbind value than the doxorubicin compound, as shown in Table 2 and Table 3. This indicates that the Trisindolina 1 compound can bind more stably to the 2VUX and 1TUP receptors compared to the active compound of the anticancer drug that is always used for chemotherapy, namely doxorubicin, and has the potential to become a new natural anticancer drug.

A lower binding affinity indicates that a compound requires less energy to bind or interact with the receptor. In other words, lower binding affinity values have a more significant potential to interact with target proteins [33]. The greater the value of Gbind, the lower the affinity between the receptors, and vice versa, the lower the bond energy produced, the more stable the interaction formed between the amino acids of the receptor protein and the ligand, so it is predicted that the activity will be more significant so that cancer becomes unreactive. And its development can be inhibited in the human body [33] [34].

Table 3. Docking Score

Ligan	Docking Conformation Score
P53R2 + Trisindolina 1	-8.9
P53 + Trisindolina 1	-9.0
P53R2 + Doxorubicin	-7.6
P53 + Doxorubicin	-7.9

3.4. Docking Result Validation

The molecular docking method was validated using the PyRx software integrated with Autodock Vina. The docking method validation parameter is the RMSD value. RMSD is a measurement of two poses by comparing the position of the docked atom compared to the reference. The smaller the RMSD value indicates that the predicted ligand position is getting better because it is getting closer to the original confirmation. The bond becomes stronger and more stable [35]. The RMSD value < 2 indicates that the smaller the error from the calculation, it can be said that the calculation is more accurate. However, if the RMSD value > 2 indicates that the deviation from the results of the docking algorithm is more significant [36] [37] so that the results of in silico ligand and receptor interactions cannot be used as a reference [38].

The RMSD value obtained in this study was 0.0 both on the docking results with p53R2 and p53 proteins, which were tested with Trisindolina 1 and Doxorubicin compounds, which means that the molecular docking method used has been validated. From the nine resulting conformational ranks, the top rank has a Gbind value with an rmsd of 0 because it is the best conformation of ligand binding.

3.5. Docking Result Visualization

Receptor interactions with ligands formed after docking results were analyzed through a visualization process using Biovia Discovery Studio software. Observation of residue interactions (amino acids) aims to identify interactions between ligands and receptors. To perform biological functions, proteins fold into specific spatial conformations, driven by several interactions. These interactions are hydrogen bonds, electrostatic interactions, and hydrophobic interactions

Table 2. Results of Docking Ligand and Target Protein Scores

	Docking Energy / Binding Affinity (kcal/mol)			
Conformation	p53R2 + Trisindolina 1	p53R2 + Doxorubicin	P53 + Trisindolina 1	P53 + Doxorubicin
entry_001_conf_1	-8.9	-7.6	- 9.0	- 7.9
entry_002_conf_1	-8.6	-7.2	- 8.9	-7.8
entry_003_conf_1	-8.4	-7.1	-8.8	-7.7
entry_004_conf_1	-8.3	-7.0	-8.8	-7.6
entry_005_conf_1	-8.2	-6.9	- 8.7	-7.3
entry_006_conf_1	-8.1	-6.9	-8.7	-6.9
entry_007_conf_1	-7.9	-6.7	-8.5	-6.7
entry_008_conf_1	-7.7	-6.7	- 8.5	-6.7
entry_009_conf_1	-7.6	-6.6	- 8.3	-6.7

Table 4. Amino Acids Visualization

Ligand and Receptor	Amino Acid Visualization	Active Site
P53R2 dan Trisindolina 1	Ile128, Ser112, Glu105, Glu131, Glu109, Cys122, Ser135, Asn104, Asn132, Gln116, Arg121, Pro118, Ile44	Ile128, Ser112, Glu109, Cys122, Ser135, Arg121, Ile44
P53R2 dan Doxorubicin	Glu32, Leu35, Asn30, Lys37, Gln116, Arg121, Gln113, Arg179, Glu114, Arg186, Arg110, Thr182, Phe183	Arg121, Gln113
P53 dan Trisindolina 1	Val172, Gly199, His168, Glu171, Lys139, Thr140, Thr170, Glu198, Gln167, Asn235, Ala138, Arg196, Met237, Ser185	Val172, Gly199, His168, Glu171, Thr140, Thr170, Glu198, Gln167, Ala138
P53 dan Doxorubicin	Phe212, Arg174, Val172, Thr140, Thr170, Gly199, Glu198, Arg196, Glu171, Arg249, Asn235, Lys139, His168, Thr123, Asn247	Phe212, Val172, Thr140, Thr170, Gly199, Glu198, Glu171, His168

[39].

Hydrogen bonding contributes to the affinity of a molecule for the target protein that forms electrostatic interactions (hydrogen donor and acceptor) [40]. Hydrogen bonds consist of 2 types: conventional hydrogen bonds and carbon-hydrogen bonds, where conventional hydrogen bonds are much stronger than carbon-hydrogen bonds [41]. Hydrophobic interactions play a role in determining the stability of the ligand to the receptor. This interaction can increase the binding affinity between the ligand-receptor and increase the biological activity of the ligand. Electrostatic interactions play a role in the stability of the ligand to the receptor. These interactions include weak and non-covalent interactions so that they are easily separated. Still, because of the large number of electrostatic interactions, they have a significant contribution to the formation of protein conformations [42] [43] [44]. The Table 4. is visualization results of amino acids that bind to the target ligand and receptor along with amino acid residues that attach to the protein's active site.

Based on the results of docking visualization between Trisindoline 1 and p53r2 protein (Fig.1), it shows the interaction of conventional hydrogen bonds with Arg121 amino acids. This bond increases the bond

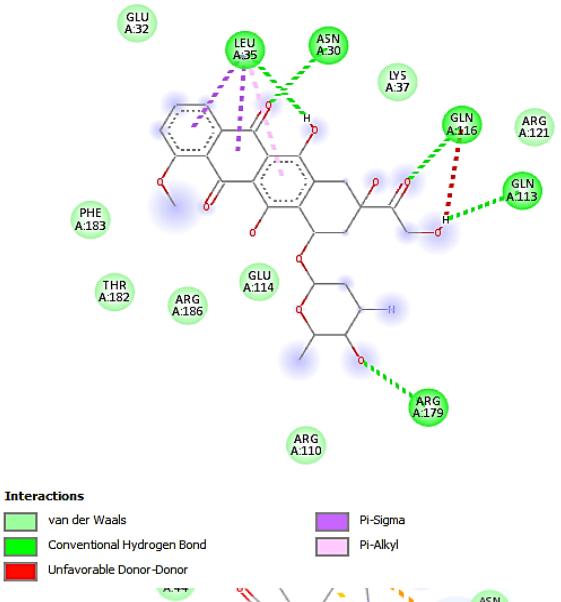

Figure 2. Visualization of Doxorubicin Compound Docking with p53R2 protein (PDB ID: 2VUX)

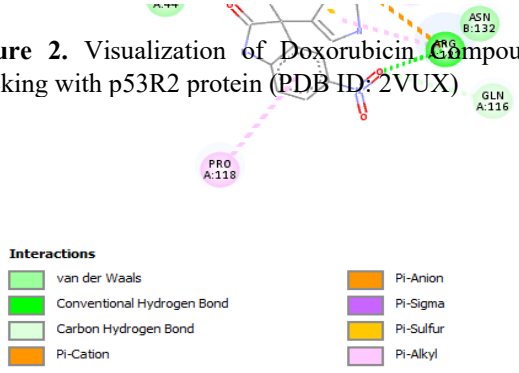

Figure 1. Visualization of Docking for Trisindoline 1 Compound with p53R2 protein (PDB ID: 2VUX)

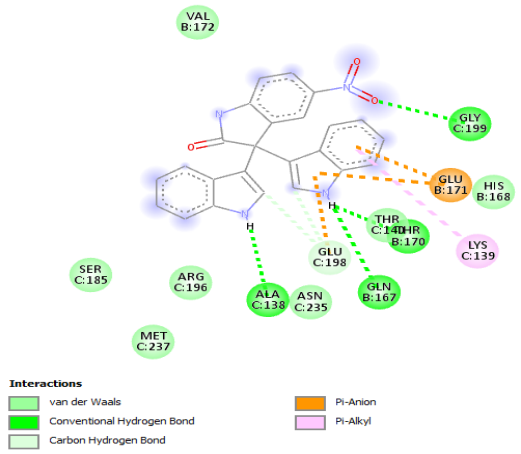

Figure 3. Visualization of Docking Compound Trisindoline 1 with p53 Protein (PDB ID: 1TUP)

strength and maintains the stability of the ligand and protein bonds [45]. There is an interaction of Carbon Hydrogen Bond with the residue of Gln 116; besides, there is also the interaction of Pi-Cation with the residue of Glu105, the interaction of Pi-Alkyl with the residue Pro118. The Pi-alkyl interaction is a non-bond interaction categorized as a form of hydrophobic interaction, while the Pi-Cation and Pi-Anion interactions are electrostatic interactions [47]. The Pi-Sulfur interaction was found with one amino acid residue, Cys112, where Pi-Sulfur belongs to the hydrophobic interaction. In addition to Pi-Cation, Pi-sulfur and Pi-Alkyl interactions, Van Der Waals interactions which are also electrostatic interactions, are also seen with nine amino acid residues, namely Ile44, Ser135, Ile128, Ser112, Glu109, Glu131, Asn104, and Asn132, which give strength addition that affects the bond energy formed [46].

The interactions produced by Doxorubicin and p53R2 protein (Fig.2) include five conventional hydrogen bonds with amino acid residues Leu35, Asn30, Gln116, Gln113, and Arg179. In addition, there were 8 Van Der Waals interactions with residues of Glu32, Phe183, Thr182, Arg186, Glu114, Arg110, Lys37, and Arg121. The interaction by Doxorubicin and p53R2 protein also resulted in Unfavorable Donor-

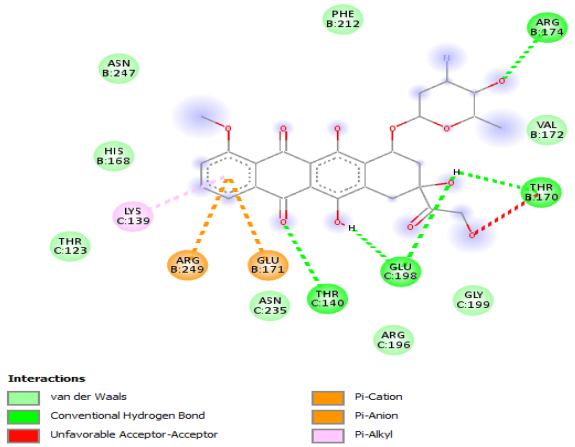

Figure 4. Visualization of Doxorubicin Compound Docking with p53 Protein (PDB ID: 1TUP)

Donor interaction (unfavorable donor-donor bond) with Gln116 residues, which affected the stability of compound activity. All unfavorable protein-ligand interactions will decrease the stability of the complexes because they show repulsion or repulsion between 2 molecules or atoms [40]. Based on the results of the docking visualization between the Trisindolina 1 compound and p53 protein (Fig.3), it shows that there are interactions, namely four conventional hydrogen bonds with amino acid residues, namely Ala138, Gln167, Thr170, and Gly199; carbon-hydrogen bonds as much as 2 with amino acid residues Asn235 and Glu198; 6 van der Waals bonds involved in electrostatic interactions with amino acid residues Val172, Ser185, Arg196, Met237, Thr140, and His168; interaction of 1 alkyl Pi with residue Lys139; and the interaction of Pi Anion as much as 1 with Glu171.

The interactions produced by Doxorubicin and p53 protein (Fig.4) include the presence of 4 conventional hydrogen bonds with amino acid residues Thr140, Glu198, Thr170, and Arg174; 8 Van der Waals bonds with amino acid residues, namely Phe212, Asn247, His168, Thr123, Asn235, Arg196, Gly199, and Val172; interaction of Pi Alkyl as much as 1 with Lys139; the interaction of Pi Cation and Pi Anion with amino acid residues, namely Glu171 and Arg249; and the Unfavorable Acceptor-Acceptor bond with the Thr170 residue which affects the stability of the compound's activity.

All unfavorable protein-ligand interactions will decrease the stability of the complexes because they show repulsion or repulsion between 2 molecules or atoms [40]. The p53 (p53) protein acts as a tumor suppressor encoded by the p53 (p53) gene. The p53 gene is located on human chromosome 17, and it delays the cell cycle when there is DNA damage. If there is a mutation in p53, the cell cycle will continue to run uncontrollably and reproduce the damaged DNA,

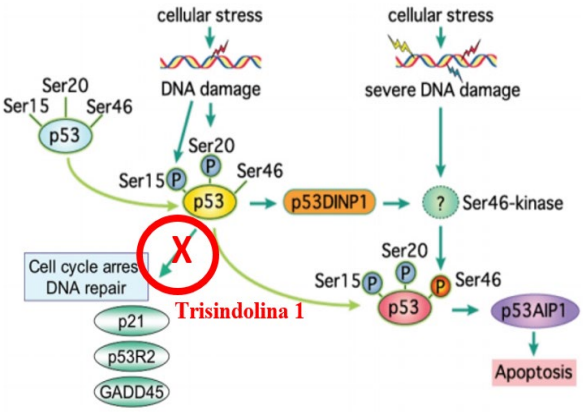

Figure 5. Relationship of p53 and p53R2 pathways on DNA damage [49]

causing uncontrolled cell proliferation and malignancy. The p53 protein is a transcription factor, and its vital role in maintaining the integrity of the genome has earned it the nickname "guardian of the genome".

P53 plays a crucial role in cell cycle regulation, apoptosis, DNA repair, senescence, and angiogenesis [48]. P53 phosphorylation will occur at specific sites (e.g., Ser15 and Ser20 residues), and modified p53 activates genes associated with cell cycle arrest and DNA repair (e.g., p21, GADD45, and p53R2) then DNA damage is limited and repairable. DNA damage in cells becomes severe, p53 has additional phosphorylation at the 46th serine residue by Ser46-kinase in conjunction with p53DINP1, and subsequently activates genes that induce apoptosis (e.g., p53AIP1) [20].

The purpose of administering the Trisindolina 1 compound in this study was to suppress the expression of p53R2 so that active p53 would directly induce apoptosis in cells damaged by DNA damage. The p53R2 gene, which is responsible for DNA repair in cancer cells, will produce primary tumor cells that are genetically stable so that they can attack and cause further metastases [21], so it is hoped that Trisindolina 1 can suppress p53R2 expression by encouraging p53 expression.

Based on the docking results that have been done, the compound Trisindolina 1 has a Gibbs free energy value (ΔGbind), which is more negative than the anticancer drug compound doxorubicin. This indicates that the candidate compound Trisindolina 1 can bind more stably to proteins. However, this research still needs to be continued computationally. It can be continued for molecular dynamic studies and protein-protein interactions. In addition, research validation through in vitro and in vivo is also needed to fulfill and confirm this theory.

AUTHORS CONTRIBUTIONS

SSG and APDN designed research, conducted experiments, performed data analysis, and wrote the manuscript. VAP conducted experiments, performed data analysis, and wrote the manuscript.

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