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Molecular Docking Simulation of Trisindolina 1 Compound Against Pi3k Protein in Hepatocellular Carcinoma

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ABSTRACT
The increased cancer burden globally, from 12.7 million new cases in 2008 to a predicted 22.2 million in 2030, makes cancer a critical global problem with high unmet medical needs. *Hepatocellular carcinoma* (HCC) is the fifth most frequently diagnosed cancer worldwide, and HCC is the third leading cause of cancer death globally. One of the pathways that can affect liver cancer is apoptosis. Phosphatidylinositol 3-kinases (PI3Ks) are lipid kinases that play a central role in regulating apoptosis. Researchers worldwide have widely carried out research and development of natural ingredients as cancer therapeutic agents because natural ingredients are safer, cheaper, more effective, and have specific bioactive targets; they also have a few side effects. One example of a natural product is marine animals, namely sponges. Based on several studies, one of the natural ingredients with high potential as an anti-cancer is Trisindolina. Trisindolina 1 showed high cytotoxic activity on HepG2 cells with an IC50 value of 0.183 g/ml by apoptosis. The study aims to evaluate the antiproliferative activity and the mechanism, a PI3K inhibitor, and mechanism of action of a series of Trisindolina 1 as an anti-HCC agent. Then the underlying mechanism of Trisindolina 1 as the promising compound was evaluated using molecular docking. The type of research used is descriptive exploratory which is carried out through several stages, including data mining, ligand preparation, protein preparation, running docking, docking result validation, docking visualization, and data analysis. The result shows that the lowest docking score of the Trisindolina 1 ligand to the PI3K protein was -10.7 Kcal/mol while the Doxorubicin ligand score to the PI3K protein was -8.9 Kcal/mol. Trisindolina 1 can inhibit PI3K on some amino acids such as Met804 (Methionine number 804) and Met953 (Methionine number 953) through pi-sulfur bonds, Trp812 (Tryptophan number 812) through pi-sigma bonds, Pro810 (Proline number 810) via pi-alkyl bonds, Lys833 (Lysine number 883) through hydrogen bonds at the binding site. The biological activity of alkaloid compounds of PI3K protein can be through apoptosis in some pathways, such as PI3K.

Keywords: *Apoptosis, Cancer, Docking, Hepar, PI3K, Trisindolina*

1. INTRODUCTION

Cancer is a disease characterized by uncontrolled growth and the spread of abnormal cells [1]. Liver cancer is the sixth most common cancer in the world, with 854,000 cases in 2015, with an annual mortality rate of 810,000 [2] [3] [4]. One of the pathways that affect liver cancer is apoptosis [5]. The PI3K pathway plays an important role in regulating apoptosis induced by many chemotherapeutic agents. This pathway undergoes hyperactivation of protein kinase B (PKB) in cancer cells. Excessive activity of PKB causes inhibition of activation of pro-apoptotic proteins, activation of anti-apoptotic proteins, and increased metastasis [6].

One of the treatments for liver cancer is chemotherapy. Chemotherapy agents that are often used are doxorubicin. Doxorubicin plays a role in inducing apoptosis, DNA intercalation, inhibition of topoisomerase II, and formation of ROS (Reactive

Oxygen Species) [7]. But, there are side effects of doxorubicin, namely hepatotoxicity and Multi Drug Resistant (MDR) in liver cancer (Hepatocellular Carcinoma) [8, 9]. Many researchers have carried the research and development of natural ingredients as cancer therapeutic agents because they have low toxicity effects and have specific bioactive targets. One of them is marine sponges which are the largest number of natural products compared to other invertebrates [10, 11, 12].

Based on several studies that have been carried out, one of the natural ingredients that have high potential as anti-cancer is Trisindolina. This compound was isolated for the first time from Vibrio sp., which is in symbiosis with the sponge Hyrtios altum in the waters of Okinawa, Japan. Trisindoline has been widely developed in the latest anti-cancer research due to the success of the synthesis method and its high potential for cytotoxicity. Santoso and Mursyidah [13] synthesized trisindoline compounds into four compounds to see their activity against various types of cancer cells, including the 1: 5' compound. -nitro-[3,3':3',3”-terindoline]-2'-one with the addition of a nitro group, compound 2: 1,1”-dimethyl-5'-nitro-[3,3':3' ,3” terindoline]]-2'-one with the addition of a dimethyl group, compound 3: 5,5”,7,7”-tetrabromo-[3,3':3',3”-terindoline]-2' -one with the addition of a bromo group, compound 4: 5’-chloro-1,1”-diethyl-1H,1”H-[3,3’:3’,3”-terindol]-2’(1’H)ne.

Several studies have shown that the Trisindolina compound with the highest cytotoxicity effect is Trisindolina 1, indicated by the lowest IC50 of 2.059 M [14]. One type of cytotoxic activity of the Trisindolina 1 compound in several cell lines showed that liver cancer cells (HepG2) were the most sensitive cancer cells compared to T47D cells (breast cancer cells), HELA cells (cervical cancer cells). The results of other studies also showed that Trisindolina 1 had high cytotoxic activity in HepG2 cells with an IC50 value of 2.837 g/ml. In the latest study, Trisindolina 1 showed high cytotoxic activity in HepG2 cells with an IC50 value of 0.183 M by inducing apoptosis [15, 16].

Therefore, it is necessary to conduct a molecular study to explain the interaction between Trisindolina 1 compounds and proteins. It plays a role in the PI3K pathway to know the potential of these compounds, also can indirectly inhibit apoptosis in human liver cancer and develop new drug candidates. This study examines secondary metabolites from natural ingredients, namely alkaloids from the H. Altum sponge in molecular docking. The molecular docking method can help determine stable molecular bonds with minimum energy on apoptotic pathway targets and Trisinsolina 1 compounds. This method is suitable for liver cancer patients and can add information about the benefits of marine natural ingredients, especially the H. altum sponge.

2. MATERIALS AND METHODS

2.1. Tools and Materials

The tool used in this research was a notebook with a specification AMD Ryzen 3 3250U 2.6 GHz dual-core hp, Windows 10 Home 64-bit RAM 8GB DDR4 2400 MHz. The software used is PyRx, AutoDock Vina [17], ChemDraw, Biovia Discovery Studio 2020. The materials used in this study were the PI3K protein database, the chemical structure of the compound Trisindolina 1, and doxorubicin. Some data sources included: Protein Data Bank (GDP) (http://www.rscb.org/pdb/), NCBI database, and Pubchem (http://pubchem.ncbi.nlm.nih.gov/).

2.2. Data Retrieval

The structural data of the PI3K target protein (code 4FJZ) and Doxorubicin (code 32874) was taken through the Protein Data Bank (PDB) and Pubchem. The Protein Data Bank is the global repository for 3D processing and distributing biomolecular structure data. Protein structures were downloaded from the site with specific keywords [19]. The target protein used is PI3K which would be docked with ligands (target drugs), namely Trisindolina 1 and Doxorubicin compounds.

2.3. Protein Database Preparation

Receptor preparation used Biovia Discovery Studio 2020 software. The software was opened and then the receptor file was entered. "Select Water Molecules" then press the delete key on the keyboard to remove the water molecules. "Select Ligands" to remove contaminant ligands and then press the delete key on the keyboard. Files were saved.

2.4. Ligand Database Preparation

Ligand preparation using PyRx software. click the "Open Babel" menu and then click "Insert new item" to input the ligand. Sdf format ligand file was selected. Click the ligand file then select "Convert Selected to AutoDock Ligand (pdbqt)". Then the file was saved in pdb format.

2.5. Docking with PyRx and Autodock Vina

The PyRx software was opened, the receptor and ligand files were entered by selecting "Load Molecule (MolKit)" then click the receptor file, select "AutoDock" and select "Make Macromolecule". Clicked “AutoDock” then selected “Make ligand”. Click start, forward and select "Maximize" then select "Forward". Then wait until the running was complete. Click on Mode 0 or Mode 1. Next, right-click on the docking ligand file and save it in PDB format.

2.6. Docking Result Validation

Docking of native ligands was carried out to find the 3D conformation of the native ligand to the receptor by taking into account the coordinates of the center of mass of the structure and the grid box size of the binding site pocket in angstroms (Vina) or the number of points (AutoDock). The conformation of the docking results obtained was aligned with the conformation of the native ligand from the crystallographic measurements expressed in the root mean square deviation (RMSD) (Figure 1) [11]

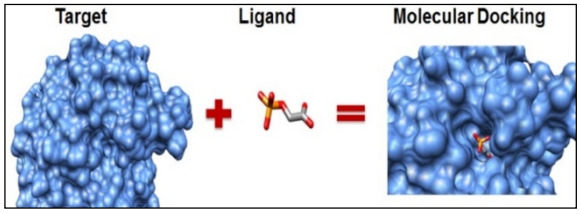

Figure 1. Docking results [11]

2.7. Docking Result Visualization

The docking results visualization can be viewed with the Biovia Discovery Studio 2020 software. The steps taken to visualize the docking results were the docked ligand files and the receptors were accessed in Discovery Studio. Right-click the ligand file and select “Copy”. On the receptor file page, right click then select “Paste”. The ligand file was selected then click "Define Ligand: <undevined>". Then select "Ligand Interaction" to see the interaction between the receptor and the ligand and select "Show 2D Diagram" to see the interaction between the receptor and the ligand displayed in 2D. The results of the docking visualization would be shown through the amino acids that play a role in the binding between the ligand and the target protein. Docking of native ligands was carried out to find the root mean square deviation (RMSD) value.

2.8. Result Interpretation

Manual folder results would generate nine input conformations. The conformation with the lowest score would be selected. Docking results with reference to experimental results were calculated by RMSD (Root Mean Square Distances). RMSD calculations were performed for validation evaluation. RMSD was a measurement of two poses by comparing the position of the docked atom compared to the reference. The RMSD value < 2.0 is usually used as a success criterion for the docking method [17]. If the value is less than 2Å, it can be used as a docking protocol for the next virtual sample screening. The smaller the RMSD value, the better the predicted ligand pose/bond was closer to the native conformation. The RMSD formula is in Equation (1), where N is the atomic number, i is 1, min *j rij* 2 is the distance of two I atoms based on two structures, and unit RMSD is Ǻngstrom.

$$\mathrm{RMSD}_{ab} = \max(\mathrm{RMSD}'_{ab}, \mathrm{RMSD}'_{ba}), \qquad (A.1)$$

where

$$\mathrm{RMSD}'_{ab} = \sqrt{\frac{1}{N}\sum_{i} \min_{j} r_{ij}^2}, \qquad (A.2)$$

(1)

2.9. Data Analysis

This research is a bioinformatics approach using molecular docking analysis of ligands on proteins. This research was descriptive. Data analysis was carried out by scoring values. The molecule with the lowest scoring value indicates that the molecule has a good stability affinity, then visualization was carried out using Biovia Discovery Studio 2020.

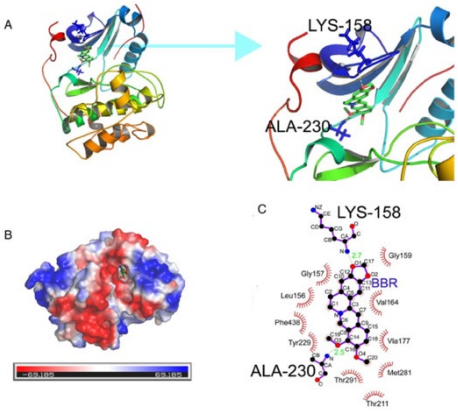

Figure 2. Example of Docking Results Visualization and Molecular Interaction Analysis [12]

3. RESULT AND DISCUSSION

3.1. Structural Data Collection of PI3K, Trisindolina 1, and Doxorubicin

Data retrieval of protein in the PI3K (phosphoinositide3-kinase) pathway, namely PI3K protein with PDB code: 4FJZ through the website www.rcsb.org. The structure of the drug as a positive control in the form of Doxorubicin with code PubChem: 32874 can be accessed at www.pubchem.ncbi.nlm.nih.gov, and the structure of the candidate drug Trisindolina 1 drawn through ChemDraw has been successfully carried out. The following is the structure of the PI3K protein in the PI3K pathway before being prepared using the Biovia Discovery Studio (Figure 3). The candidate ligand structures used were Trisindolina 1 and Doxorubicin (Figure 4).

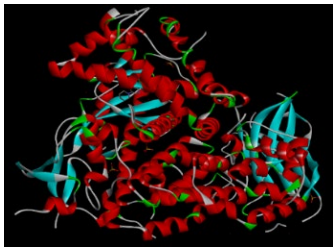

Figure 3. Structure of PI3K PDB ID: 4FJZ

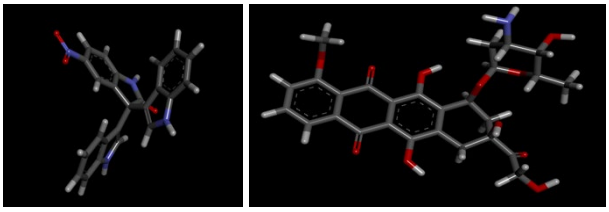

Figure 4. Structure of Trisindoline 1 (left) Structure of Doxorubicin (right)

3.2. Receptor Preparation

Receptor preparation was carried out using the Discovery Studio 2020 by removing water and ligand molecules, and the file was saved in pdb format (Figure 5).

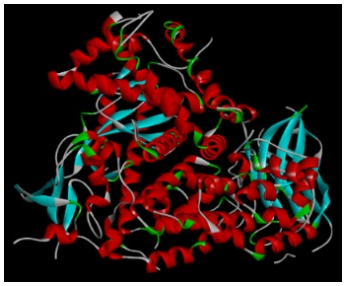

Figure 5. PI3K protein structure in the prepared PI3K pathway (PDB ID: 4FJZ)

3.3. Ligand Preparation

Ligand preparation was carried out using PyRx add version,developer/country and file was saved in pdb format (Figure 6).

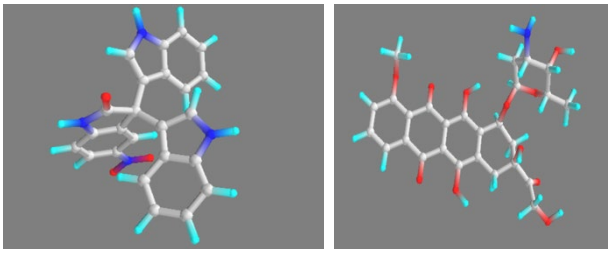

Figure 6. Structure of the prepared ligand (Trisindolina 1 (left), and Doxorubicin (right)).

3.4. Molecular Docking with Autodock Vina

The molecular docking method aims to identify the correct position of the ligand in binding proteins and predict the energy affinity between the ligand and the protein [18]. This study used a molecular docking method using Autodock software [17]. Autodock can also predict the optimal binding conformation of the ligand to the protein [20, 21]. The increased performance of Autodock combined with the availability of high-speed computers can enable much larger experiments [22, 23].

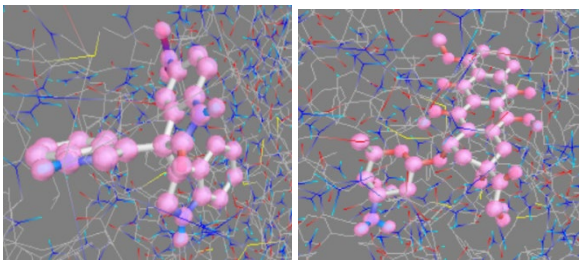

Figure 7. Autodock Vina protein docking results PI3K PDB ID : 4FJZ with Trisindolina 1 (left); Doxorubicin (right)

The results of molecular docking showed a comparison of the scores of a compound with other compounds to explain whether a compound was potent or not. The molecule with the lowest docking score (minus score) showed a good stability affinity [24]. In this study, protein-ligand docking was carried out because it used proteins in the PI3K pathway and Trisindolina 1 ligand as a candidate for liver cancer drugs compared to Doxorubicin. The following table (Table 1 and Table 2) lists the results of the docking scores on the PI3K pathway protein and the drugs used.

Based on the molecular docking studies, each docking result has nine conformation scores. The lowest docking score compared with the energy affinity obtained based on the RMSD (root mean square deviation) as validation for the docking process with an RMSD value <2A0 [25]. The lowest docking score of the Trisindolina 1 ligand to the PI3K protein was -10.7 Kcal/mol, while the Doxorubicin ligand score to the PI3K protein was -8.9 Kcal/mol. The lowest docking score of Trisindolina 1 to PI3K was slightly different from the docking score of Doxorubicin to PI3K. Based on the docking score obtained, Trisindolina 1 successfully bound to the target protein (PI3K) in the PI3K pathway with an affinity value less than the Doxorubicin docking score. The lower the docking score, the easier it was for a compound to bind. Thus, based on the in silico approach, the compound Trisindolina 1 could be said to be a potent candidate for inhibiting the PI3K pathway in liver cancer. Experimentally the free bond energy on the docking score was directly related to the inhibition constant. Determination of free bond energy values could predict the ability of compounds to inhibit the work of

proteins or enzymes [26]. This result followed the theory that the lower the score (minus), the more stable the ligand-protein interaction was [27].

Table 1. Results of PI3K Docking and Ligand

Conformation	Binding Afinity (Kcal/mol)	
	Trisindolina 1	Doxorubicin
Data 1	-10.7	-8.9
Data 2	-10.5	-8.6
Data 3	-9.8	-7.7
Data 4	-9.8	-7.4
Data 5	-9.7	-7.4
Data 6	-9.5	-7.3
Data 7	-9.4	-7.3
Data 8	-9.4	-7.3
Data 9	-9.3	-7.3

Table 2. Best Docking Score Selection Results

Ligands	Docking Score to PI3K (Kcal/mol)
Trisindolina 1	-10.7
Doxorubicin	-8.9

As a comparison, here are the results of another study related in silico compounds containing steroids to the PI3K pathway on the same target protein (PI3K). Based on the study conducted by Kattan *et al*. [28], the following was a table of the results of the in silico study (Table 3).

Table 3. Results of PI3K Pathway Protein Docking and Steroid Ligand

Protein target	Docking Score (Kkal/mol)
PI3K	-10.37

The molecular docking results for Trisindolina 1 showed that the Trisindolina 1 ligand had a lower score than Doxorubicin on the two target proteins, PI3K. Based on other studies comparing steroid compounds, Trisindolina 1 compounds have a lower docking score. They indicate that Trisindolina 1 is a potential candidate for inhibiting the PI3K pathway in liver cancer.

3.5. Visualization of Docking Results with Discovery Studio

The visualization of the docking used the Discovery Studio and the binding site obtained through the Biovia Discovery Studio [29]. The visualization results were interactions between ligands (compounds) and amino acids in protein macromolecules. The amino acid residue that interacted with the ligand would determine the bond between the ligand and the protein. The following was a table of visualization results of amino acids that bind to the target ligand and receptor (Table 4). The visualization results showed that the Trisindolina 1 compound was proven to be able to bind through the binding site on the target protein receptor.

Table 4. Visualization of Amino Acid Ligands and Targeted Receptors on the PI3K Pathway Protein

Target Ligands and Receptors	Amino Acid Visualization	*Binding Site*
PI3K and Trisindolina 1	Met804, Lys833, Met953, Pro810, Trp812	Met804, Val803, Lys833, Met953, Pro810, Trp812, Glu880, Ile881, Val882, Thr887
PI3K and Doxorubicin	Val882, Ile881, Trp812, Met953, Thr887, Lys833,	Met804, Val803, Lys833, Met953, Pro810, Trp812, Glu880, Ile881, Val882, Thr887

Trisindoline 1 can inhibit PI3K on the amino acids Met804 (Methionine number 804) and Met953 (Methionine number 953) through pi-sulfur, Trp812 (Tryptophan number 812) through pi-sigma, Pro810 (Proline number 810) via pi-alkyl, Lys833 (Lysine number 883) through hydrogen bonding at the binding site. Binding area (Binding site) was an area of protein binding to ligands that will affect the conformation and function of the protein. Binding sites showed amino acid residues that play an important role in forming interactions between macromolecules and ligands such as hydrogen, hydrophobic, and electrostatic [30].

Doxorubicin can inhibit PI3K through binding to the amino acids Val882 (Valin number 882) through conventional hydrogen bonds, Ile881 (Isoleucine number 881), Met953 (Methionine number 953), Trp812 (Tryptophan number 812) through pi-alkyl, Lys883 (Lysine number 812) 883), Thr887 (Threonine number 887) through hydrogen bonding at the binding site.

The function of each bond is in the interaction of the ligand and the protein involved. Pi-sigma bonds to assist drug intercalation at the receptor-binding site [31]. Pi-alkyl bonds donate the drug's dipole moment by electron charge transfer. Dipole moments are important in the orientation of molecules when interacting with bonding sites [32, 33]. The hydrogen bond is the interaction of the H atom as a link between 2 electronegative atoms to stabilize the interaction in the bond. Each hydrogen bond contributes an average of 1.3 kcal/mol to stabilization [34]. Conventional hydrogen bonds are important in forming protein-ligand complexes because they provide stability to the complex [35]. Pi-sulfur bond results from several nitrogen atoms in a hetero-aromatic ring, and this

bond involves charge transfer, which helps in drug intercalation at the binding site [36].

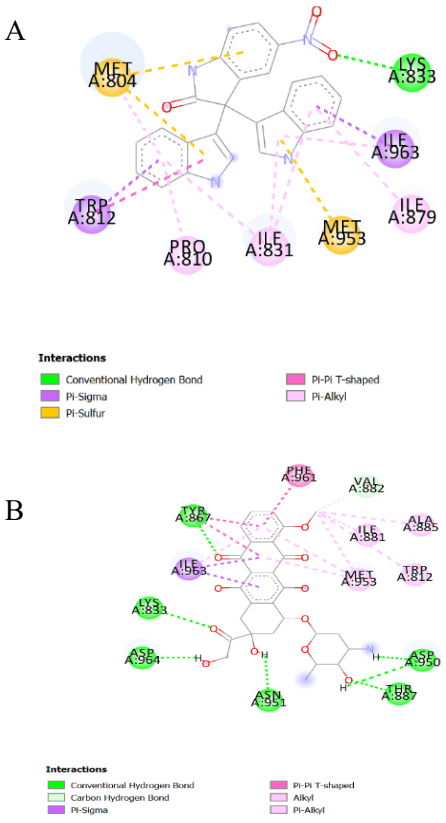

Figure 8. Results of 2D Visualization of Trisindolina 1 (A) and Doxorubicin (B).

The following were amino acid residues in the interaction of ligands and proteins with several functions. The amino acid lysine (code: Lys883) plays a role in cell division and growth [37]. The presence of a positive charge associated with lysine in non-epithelial cells is expressed as a mediation of facilitated diffusion [38]. Amino acids Valine (code: Val882) and isoleucine (code: Ile881) play a role in increasing protein synthesis [37]. The amino acid proline (code: Pro810) played a role in maintaining cell proliferation [37, 39]. The amino acid methionine (code: Met804, Met953) contained elemental sulfur, essential for healthy cartilage and liver. The presence of metabolic disorders can prevent the body from using methionine, which can cause liver damage in the long term. The amino acid tryptophan (code: Trp812) is a precursor to niacin (vitamin B3), melatonin, and serotonin. The tryptophan codon was polypeptide chains and proteins. The amino acid threonine (code: Thr887) can increase the absorption of other amino acids such as phenylalanine. Besides that, it also played a role in fat metabolism stored in the liver [37].

The R groups on the amino acids methionine, proline, valine, and isoleucine were hydrophobic because they are dominated by hydrocarbon chains and have low polarity. Methionine has many alkyl groups and undergoes hydrophobic interactions and functions to stabilize the protein structure. Methionine belongs to the group of non-polar amino acids because it has a non-polar thioether group in the side chain. Proline was the only amino acid with an amino group bound in a circular structure. These secondary amino groups were bound in a rigid conformation. The presence of proline in a polypeptide chain reduces the flexibility of the structure in that area. Tryptophan was an amino acid with an aromatic group that is non-polar and can interact hydrophobically. Tryptophan was polar because it has an N atom in the indole ring. The amino acid threonine was more soluble in water and is hydrophilic. The polarity of amino acids is influenced by the polarity of the functional group and depends on the number of carbon-hydrogen atoms in the alkane chain. The longer the alkane chain, the lower the polarity of the amino acid [40].

The presence of an aromatic ring also reduces the polarity of the amino acid. The polarity of threonine is due to the hydroxyl group (-OH) in its R chain. Functional groups in polar amino acids played an important role in enzymatic reactions. The alcohol (-OH) group on threonine causes these amino acids to act as nucleophiles during enzymatic reactions. *Lysine* is an amino acid that is positively charged at neutral pH. The amino acid lysine had an additional amino group, so the acid group did not neutralize it. The positive charge of lysine comes from the ionization of the -amino group in its aliphatic chain [40].

The structure of trisindoline was part of the indole alkaloids. It was predicted to have the potential to inhibit liver cancer activity because it had a benzene-like structure that binds to N consisting of a heterocyclic ring that can increase activity in the cell line on HepG2. The N atom was important for electron donors. Adding nitrogen to the compound can improve the anticancer activity of HepG2, K562, and HT-29 tumor cell lines. The presence of an aromatic was also important for hydrophobic interactions and provided better lipophilicity. The N-H and carbonyl bonds played an important role in the activity and provided hydrogen bonding [41].

3.6. Docking Result Validation

Based on the molecular docking studies that have been carried out, each docking result had 9 conformation scores. From the scoring value, the lowest docking score was taken and compared with the energy affinity obtained based on the RMSD value as validation for the docking process with an RMSD value <2A0. Based on the study results, the RMSD value used was in accordance with the validation results (value < 2) [42].

RMSD calculations were performed for validation evaluation. RMSD was a measurement of two poses by comparing the position of the docked atom compared to

the reference. Based on the results of the study, the RMSD value used was following with the validation results (value < 2)[17, 42]. If the value is less than 2Å, it can be used as a docking protocol for the next virtual sample screening. The smaller the RMSD value indicates that the predicted ligand pose/binding was getting better because it was getting closer to the native conformation.

Based on the results obtained after molecular docking, mode 1 is the best mode of the nine modes produced [43]. In Table 1 of the Trisindolina 1 compound in mode 1, the docking energy value was -10.7 Kcal/mol, the lowest energy of all modes generated, and the RMSD value is 0.000. Meanwhile, in doxorubicin in mode 1, the docking energy value was -8.9 Kcal/mol, the lowest energy of all the resulting modes, and the RMSD value is 0.000. The lower the docking score, the easier it is for a compound to bind. Thus, based on the in silico approach, the compound Trisindolina 1 can be said to be a potent candidate for PI3K inhibitors in liver cancer.

The free bond energy on the docking score was directly related to the inhibition constant. Thus, the determination of the value of free bond energy can predict the ability of compounds to inhibit the work of proteins or enzymes [26]. This was following the theory, which states that the more stable the ligand-protein interaction is, the lower the score (minus) [27]. The purpose of the scoring function was to describe the true and false poses or binders of the inactive compound during computation. The score will be selected based on the best energy affinity based on the lowest docking score [44].

3.7. Mechanism of Alkaloids on the PI3K pathway

One signal transduction pathway that plays a role in cancer growth is the PI3K pathway. This pathway regulated cellular processes, such as proliferation, growth, cell cycle, autophagy, and apoptosis [45]. Several studies revealed that PI3K is a protein involved in signaling the mTOR pathway and is a significant key in regulating signaling pathways in HCC [28]. Over-activation of the PI3K pathway leads to increased phosphorylation of phosphatidylinositol-4,5-biphosphate (PIP2) to phosphatidylinositol-3,4,5-triphosphate (PIP3). The formation of PIP3 caused phosphorylation of phosphatidylinositol-dependent kinase-1 (PDK1) and caused phosphorylation of PKB so that PKB becomes active. This protein was also a central mediator in the signal transduction of the PI3K pathway. This protein would phosphorylate other intracellular proteins that play a role in cell cycle regulation, cell proliferation, DNA repair systems, and apoptosis [6].

According to Liu *et al.* [45], the biological activity of alkaloid compounds on PI3K protein can be through apoptosis and autophagy through a series of pathways such as PI3K, AKT pathway, JNK, and c-Jun. Indole alkaloids can exert an anti-hepatocarcinogenesis effect by influencing cell apoptosis, proliferation, cell cycle, metastasis, and angiogenesis. In addition, inhibition of PI3K significantly increased the number of apoptotic cells induced by alkaloid compounds. Hyperactivation of the PI3K/Akt pathway has been confirmed as essential in initiating and maintaining human tumors. Several studies have shown that the PI3K pathway plays a key role in cell cycle regulation and apoptosis. Hyperactivated PI3K/Akt signaling can prevent apoptosis. The mechanism of apoptosis in PI3K is that PI3K increases Mdm2 protein levels and causes a decrease in p53. The Bcl-2 family also plays an essential role in the regulation of apoptosis. The PI3K/Akt pathway mediates cell viability and apoptosis through Bcl-2 family proteins, including Bad and Bcl-2, which are essential regulators of the mitochondrial apoptotic pathway. Therefore, inhibition of PI3K will cause cell apoptosis through the p53 pathway and mitochondrial apoptosis [46].

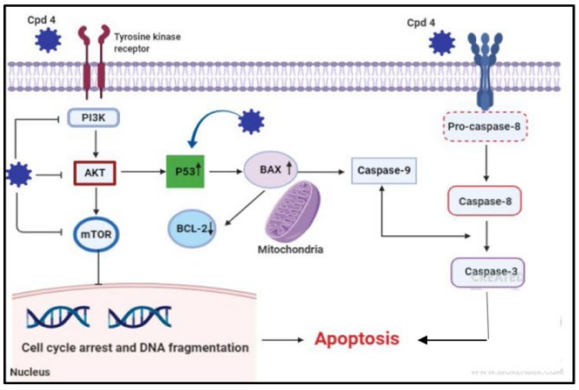

Figure 9. Cytotoxicity potential, and significant regulation of the PI3K/AKT/mTOR pathway, by induction of apoptosis through extrinsic and intrinsic pathways [28].

Previous studies have shown that steroid compounds were able to bind to the active site of the PI3K protein, so it was thought to inhibit the activity of PI3K. The potential of Trisindolina 1 compound, which had a lower binding affinity than steroid compounds, was suspected that Trisindolina 1 compound was able to bind to PI3K protein, inhibit PI3K pathway, and induce apoptosis. Trisindolina 1 compound was thought to induce apoptosis in both intrinsic and extrinsic pathways. The intrinsic pathway involves a mitochondrial-mediated cascade, which includes activating the P53/MDM2 pathway, the pro-apoptotic gene PUMA, Bax, and the caspase 3, 9, with inhibition of the anti-apoptotic Bcl-2 family and the PI3K/AKT/mTOR gene. Whereas the extrinsic pathway included death receptors and activated the caspase 8 cascade, It was suspected that Trisindolina 1 could induce pro-apoptotic genes, such as BAX and the apoptotic genes Caspase-3, Caspase-8, and Caspase-9. In addition, the P53 tumor suppressor gene was also

induced by the activity of this compound. Trisindolina 1 compound was thought to downregulate the anti-apoptotic genes BCL2, PI3K, AKT, and mTOR. Thus, up-regulation of BAX, P53, Caspases-3, -8, -9 and down-regulation of BCL2, Il6, PI3K, AKT, and mTOR genes induced by the compound Trisindolina 1 induced intrinsic and extrinsic apoptotic pathways in liver cancer cells [28].

The Trisindolina 1 compound, which is included in the indole alkaloid compound, can inhibit the activity of the PI3K pathway in liver cancer. This was in accordance with the theory that indole alkaloids could play a role in the anticancer pathway and the proteins involved. Indole alkaloids acted as anti-hepatocarcinogenesis through cell apoptosis and autophagy. One of the roles of indole alkaloids in fighting liver cancer could be achieved by regulating pathways associated with tumor development, such as the PI3K pathway [45].

The compound Trisindolina 1 has a docking score of -10.7, while the docking score of doxorubicin is -8.9. The docking results showed that Trisindolina 1 was a potent compound as an inhibitor of liver cancer in the PI3K pathway. Types of amino acids that were close to the interaction of Trisindolina 1 compounds were methionine, lysine, proline, and tryptophan, which were anticancer compounds against liver cancer.

AUTHORS' CONTRIBUTIONS

The author contributes to making research designs, conducting research, conducting analysis, and making reports.

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