Oral anticancer prediction of hexadecenoic acid on p50 target receptor by molecular docking

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Abstract

Ambonese banana stem extract (*Musa paradisiaca var. sapientum*) has been proven to contain the active compound hexadecenoic acid (Hexa) which can suppress the growth of cancer cells through the apoptosis process. The aims to determine HA interaction to nuclear factor-kappa-B p50/ReLA for the development of oral anticancer drugs through molecular docking. In silico study carried out include prediction of activity spectra of substances (PASS), drug-likeness analysis based on the lipinski rule of five principles, absorption, distribution, metabolism, excretion, and toxicity (ADMET) study, molecular docking and HA bond visualization (CID: 985), along with the positive control comparison compound 5-fluorouracil (Fluo) (CID: 3385) and the derivative compound 9-octadecenoic acid (Octa) (CID: 445639) which bind to the proteins target RelA (PDB ID: 6NV2). Results data shows HA compounds have potential as anticancer therapy because of their distribution throughout the body and their activity against cancer. Molecular docking shows that HA is predicted to bind more effectively to the binding pocket of both RelA, while 9-octadecenoic acid has a fairly good interaction with RelA when compared to 5-Fluorouracil. From this study, we can conclude that Hexadecenoic acid compound found in *Musa paradisiaca var. sapientum* (L.) *Kuntze* represents a breakthrough in developing new drugs with potential and effectiveness against RelA/p50.

Keywords: drug development, hexadecenoic acid, molecular docking, oral anticancer, human well-being

1. Introduction

Premalignant lesions are morphologically altered tissue that can lead to cancer. Oral lichen planus, oral submucous fibrosis, and leukoplakia are premalignant mucosal lesions that can develop into malignant tumors in the oral cavity in the form of Oral Squamous Cell Carcinoma (OSCC) [1, 2]. Approximately 90% of oral cancers are OSCC cases. According to GLOBOCAN (Global Cancer Observatory), there were 5,780 cases of oral cancer in Indonesia in 2020, of which 3,087 died [1, 3].

The etiology of precancerous lesions in the oral cavity is currently unknown. Several risk factors such as chewing tobacco, smoking, and alcohol consumption play an important role in the development of oral diseases leads to malignancy [4]. Oral cancer cells or pre-malignant lesions can express Toll-like receptors (TLR) and activate inflammatory, proliferation, and migration signaling pathways [5]. *Rubo Nucleid Acid* (RNA) analysis confirmed that in OSCC cells, stimulation of TLR 2 can activate the nuclear factor-κB (NF-κB) pathway and induce cytokines and chemokines that depend on the NF-κB pathway [6, 7]. NF-κB is a transcription factor in the regulation of the innate immune response which plays an important role in physiological events such as inflammation, apoptosis, growth, and cell differentiation [8]. NF-κB consists of five members, which are p50 (RELA), p50 (RELB), c-REL, NF-κB1, and NF-κB2. RelA has a transcriptional activation domain and is involved in cell survival, invasion, proliferation, metastasis, angiogenesis, and cell chemoresistance. The NF-κB subunit, RelA/p50, can mediate apoptosis through cross-talking with p53. Therefore, NF-κB inhibitors for cancer therapy need to be thoroughly reviewed [9].

Medical plants have proven to be an effective natural source of treatment, and do not replace the function of modern medicine, in other words as alternative therapy. One plant that has many benefits is the banana plant [10, 11]. In previous research, gas chromatography-mass spectrometry (GC-MS) analysis of Ambonese banana stem extract (*Musa paradisiaca var. sapientum (L.) Kuntze*) was carried out and the active compounds obtained included hexadecenoic acid (Hexa) which had the highest percentage (5.40%) for ethanol extract. Meanwhile, octadecadienoic acid had the highest percentage in ethyl acetate extract compound (11.47%) [10]. These two compounds belong to the palmitic acid group which has an antioxidant role and suppresses the growth of cancer cells through the apoptosis process and can modulate protein kinase. That's why we need to study in silico with a specific design to evaluate the role of these two compounds [10, 12].

In any case, developing a drug requires a very long time and costs a lot of money. In the past few decades, research using computational assistance has been developed that is called in silico. The in-silico test was chosen because it can optimize the results of the in vivo test [13]. Physicochemical, pharmacodynamic, pharmacokinetic, and molecular docking analyses can be carried out to predict the activity of a

compound and the binding interactions between the ligand and the target protein. The Hexa ligand and its derivatives, with carbon, hydrogen, and oxygen donor groups, can enhance the diversity of the complex structure and influence the biological activity properties of the complex compound [14]. The hypothesis of this research is the hexadecenoic acid compound contained in *Musa paradisiaca var. Sapientum*, which acts as a ligand, can bind to RelA proteins so it can be a candidate for oral anti-cancer drugs through in silico studies.

2. Material and methods

2.1 Pharmacodynamic analysis Prediction of Activity Spectra of Substances (PASS)

The study compound used was "Hexadecenoic acid" contained in *Musa paradisiaca var. sapientum*. This analysis uses the PubChem database (https://pubchem.ncbi.nlm.nih.gov/), and downloads the structure of the compound in 2-dimensional form in .sdf (SQL Server Compact Database File) format. The Simplified Molecular Input Line Entry System (SMILES) structure is also stored. The results obtained are in the form of PASS test analysis, producing Pa (Potential activity) and Pi (Potential inhibitory) values for each biological activity that the compound may have. A Pa value > 0.7 means the compound is very biologically active, conversely, if a *p* value < 0.5 means the compound is not biologically active [15].

2.2 Pharmacokinetic analysis of ADME and toxicity

Absorption, distribution, metabolism, excretion, and toxicity (ADMET) studies of drug candidates to assess biological activity in the body using "pkCSM online" (https://biosig.lab.uq.edu.au/pkcsm/prediction) by entering SMILES compounds which were obtained previously in the "Provide a SMILES String" column, and predict the pharmacokinetic properties by pressing "ADMET" on that page. It is also possible to compare ADMET results with positive control compounds and derivative compounds. The ADMET test results detect the five aspects above based on the pharmacokinetic parameters of each part. Drug absorption is expressed by water solubility and intestinal absorption. Distribution parameters are expressed by volume of distribution (VDss), metabolism is based on whether the study compound is an inhibitor and substrate of cytochrome: CYP3A4, and excretion is seen from the total clearance value, while toxicity parameters are in the form of AMES toxicity, Max. tolerated dose (human), Oral Rat Acute Toxicity (LD50), and Skin Sensitization [16, 17].

2.3 Physicochemical analysis of drug-likeness

Physicochemical analysis is based on the Lipinski Rule of Five principles on the web (http://www.scfbioitd.res.in/software/drugdesign/lipinski.jsp) by entering compound structure files in 2-dimensional form in .sdf format. The information that will be obtained is in the form of compound characteristics including molecular mass, donor hydrogen bonds, acceptor hydrogen bonds, log P, and molar refractivity. A compound is said to be drug-like if it meets the requirements for each parameter [18, 19]. The terms for Drug-Likeness are:[19] (Molecular weight ≤ 500 daltons, Log P value (partition logarithm) ≤ 5, Donor hydrogen bond value ≤ 5; Acceptor hydrogen bond value ≤ 10; and Molar refractivity value in the range 40-130).

2.4 Molecular docking analysis

2.4.1 Download of study compounds and RelA proteins

The 3D structures of the selected target proteins were obtained from the RSCB PDB database (https://www.rcsb.org/), which are RelA (PDB ID: 6NV2). The 3D structures of the control ligand and study compounds, namely 5-Fluorouracil (CID: 3385), Hexadecenoic Acid (CID: 985), and 9-Octadecenoic acid (CID: 445639) were obtained from the PubChem database (https://www.pubchem. ncbi.nlm.nih.gov).

2.4.2 RelA protein preparation

The ligands were energy-minimized and converted to PDB file format using Open Babel within the PyRx software. The RelA receptors were separated from their ligands and water molecules using BIOVIA Discovery Studio Visualizer 2021, ensuring that only the structures of the RelA proteins were used as the target proteins.

2.4.3 Analysis

Docking was carried out using Autodock Vina integrated in Pyrx v.1.1 [20]. Docking was also carried out using the targeted docking method with exhausted parameters 100 and mode 9. The size of the gridbox was adjusted to the position of amino acid residues based on predictions using PrankWeb (Table 1). The docking results obtained are in the form of binding affinity or affinity energy resulting from the interaction of the compound with the protein. It is said to have good effectiveness if it has a lower binding affinity value compared to the binding affinity value of the comparison compound. It is said to have the most optimal values in zero mode, zero upper bond, and zero lower bond. Furthermore, the interaction between the compound and the docked protein was visualized using BioVia Discovery Studio 2021 software [21, 22].

Table 1: Molecular docking analysis gridbox

	RelA (6NV2)	
	Center	**Dimensions**
X	20.7633	20
Y	22.0097	203
Z	2.3536	20

3. Results

Physiochemistry and PASS prediction

The results of the physicochemical tests (Table 2) showed that the three compounds met Lipinski's principles, with the Fluo compound having a molecular mass of 130 Da, a hydrogen bond donor of 2, a hydrogen bond acceptor of 2, log P -0.22, and a molar refractivity of 20.70. Further data from the Hexa compound meets Lipinski's requirements because the compound has a molecular mass of 256 Da, hydrogen bond donor 1, hydrogen bond acceptor 1, log P 4.32, and molar refractivity 88.26. The Octa compound meets Lipinski's requirements because it has a molecular mass of 282 Da, hydrogen bond donor 1, hydrogen bond acceptor 2, log P 4.73, and molar refractivity 96.86.

Table 2: Physicochemical study results

Compound	Molecular mass (Da)	Hydrogen bond donor	Hydrogen bond acceptor	Log P	Molar refractivity
Batasan	≤500 Da	≤5	≤10	≤5	40-130
Fluo*	130	2	2	-0.22	20.70
Hexa*	256	1	2	4.32	88.26
Octa*	282	1	2	4.32	96.86

*Fluo: 5-Fluorouracil; Hexa: hexadecenoic acid; Octa: 9 Octadecenoic acid

The potention of the Fluo, Hexa, and Octa compund shown in Table 3. Fluo compound has the potential to act as a RelA inhibitor with an efficacy of 0.915. The potential of being an inactive RelA inhibitor is 0.005. First, ΔP was obtained of 0.91 so it was categorized as an active RelA inhibitor compound. For the Hexa compound, the potential to become an active RelA inhibitor is 0.821 with the potential to become inactive at 0.003. Second, we obtained ΔP of 0.818 so it can be categorized as an active RelA inhibitor compound. Further data regarding the Octa compound has the potential to become an active RelA inhibitor is 0.791 with the potential to become inactive at 0.004. The results obtained ΔP of 0.787 so it was categorized as an active RelA inhibitor compound.

Table 3: PASS rediction results

Compound	Fluo*	Hexa*	Octa*
Pa	0.915	0.821	0.791
Pi	0.005	0.003	0.004
ΔP (Pa-Pi)	0.91	0.818	0.787

*Fluo: 5-Fluorouracil; Hexa: hexadecenoic acid; Octa: 9 Octadecenoic acid

ADME and toxicity

Examination findings of the ADME prediction test (Table 4) demonstrated the parameters of each aspect consisting of administration, distribution, metabolism, and excretion. The Fluo compound has an intestinal absorption value of -3.72, water solubility of -1.55, VDss of -0.23 log L/kg, negative for CYP3A4 substrates and inhibitors, and an excretion rate of 0.639 ml/min. The Hexa compound has an intestinal absorption value of 92,004, water solubility of -5,562, VDss of -0.543 log L/kg, positive for CYP3A4 substrates and negative for CYP3A4 inhibitors, and an excretion rate of 1,763 ml/min. The Octa compound has an intestinal absorption value of 91,823, water solubility of -5,924, VDss of -0.558 log L/kg, positive for CYP3A4 substrates and negative for CYP3A4 inhibitors, and has an excretion rate of 1,884 ml/min.

Table 4: ADME prediction test results

	Administration		Distribution	Metabolism	Excretion
Compo-und	Intestinal Absroption (%)	Water Solubility	VDss (Human) (log L/kg)	CYP3A4 substrat; inhibitor	Total clearance (ml/min)
Fluo*	-3.725	-1.555	-0.23	Negative; Negative	0.639
Hexa*	92.004	-5.562	-0.543	Positive; Negative	1.763
Octa*	91.823	-5.924	-0.558	Positive; Negative	1.884

Based on Table 5 results, the Fluo compound is negative for AMES toxicity so the compound does not cause genetic mutations; has a maximum dose threshold of 1,359 mg/kg, oral acute toxicity of 1,939 mol/kg, and does not cause mucosal / skin irritation because it is negative for skin sensitization. The Hexa compound is negative for AMES toxicity so the compound does not cause genetic mutations; has a maximum dose threshold of -0.708 mg/kg, oral acute toxicity of 1.44 mol/kg, and can cause mucosal/skin irritation due to positive skin sensitization parameters. The Octa compound is negative for AMES toxicity so the compound does not cause genetic mutations, has a maximum dose threshold of -0.81 mg/kg, oral acute toxicity of 1,417mol/kg, and is negative in skin sensitization parameters.

Table 5: Toxicity test results

Compound analysis	AMES toxicity	Human maximum tolerated dose log (mg/Kg BB)	Oral acute toxicity (LD50) (mol/kg)	Skin sensitization
Fluo*	Negative	1.359	1.939	Negative
Hexa*	Negative	-0.708	1.44	Positive
Octa*	Negative	-0.81	1.417	Negative

* Fluo: 5-Fluorouracil; Hexa: hexadecenoic acid; Octa: 9 Octadecenoic acid

Molecular docking

Hexadecenoic acid compound against RelA protein

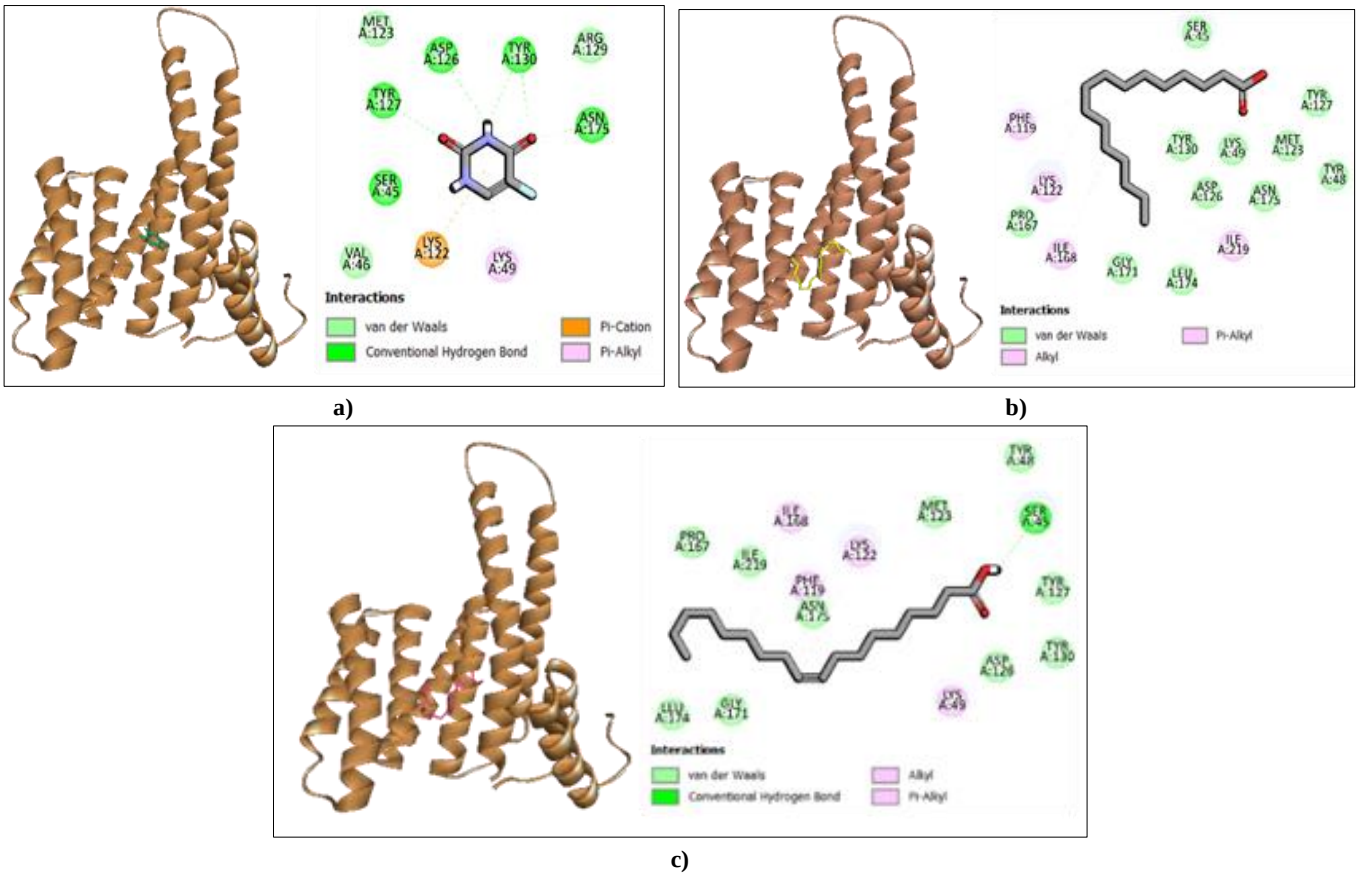
Results of the molecular docking test (Table 6) determined the ability and potential of the hexadecenoic acid compound from Ambon banana stem extract as a RelA inhibitor compared to the comparison group. The Hexa compound in mode and RMSD 0 has a bond affinity of -4.9 kcal / mol, where this value is higher than the positive control compound, which is Fluorouracil, and lower than the Octadecenoic acid derivative compound which is the comparison group.

Table 6: Molecular docking results of the active compound hexadecenoic acid from Ambon banana stem extract and the reference compound against RelA

Target	Compounds	Binding affinity (kkal/mol)	Mode	RMSD	
				Lower bound	Upper bound
RelA PDB ID 6NV2	5-Fluorouracil	-4.6	0	0	0
	Hexadecanoid Acid	-4.9	0	0	0
	9-Octadecenoic acid	-5.2	0	0	0

Visualization of RelA docking results

The visualization of the molecular docking test (Figure 1) between the study compound and the compared compound to the target protein show identical binding locations. The types of bonds formed are hydrogen interactions, Van der Waals interactions, hydrophobic interactions, and also electrostatic. The types and locations of ties are described in detail in the table below (Table 7). Where the visualization results showed that the peptide bond location of Fluo, Hexa, and Octa has the same location, namely SER45.



a) b)

c)

Fig 1: Visualization of docking results, a) RelA/5-Fluorouracil protein, b) RelA/Hexadecenoic Acid protein, and (c) RelA/9-Octadecenoic acid protein. The left part showed a 3D visualization, and the right part showed the type of bond produced between the ligand-protein.

Table 7: Interaction of amino acid residues produced in the RelA target protein with control ligands and study compounds

Protein target	Compounds	Types and locations of bonding
RelA PDB ID 6NV2	Fluo*	- Hydrogen Bonding: A:ASP126, A:SER45, A:TYR127, A:TYR130, A:ASN175 - Van der waals bonding: A:MET123, A:ARG129, A:VAL46 - Hidrofobik: A:LYS49 - Elektrostatik: A:LYS122
	Hexa*	- Hydrogen Bonding - Van der waals bonding: A:PRO167, A: GLY171, A: LEU174, A:ASP126, A:TYR130, A:LYS49, A:MET123, A:ASN175, A:TYR48, A:TYR127, A:SER45 - Hidrofobik: A:LYS122, A:ILE168, A:ILE219, A:PHE119 - Elektrostatik
	Octa*	- Hydrogen Bonding: A:SER45 - Van der Waals Bonding: A:TYR127, A:TYR130, A:ASP126, A:GLY171, A:LEU174, A:PRO167, A:ILE219, A:ASN175, A:MET123, A:TYR48 - Hidrofobik: A:LYS49, A:LYS122, A:ILE168, A:PHE119 - Elektrostatik

4. Discussion

The first line of cancer therapy, which is chemotherapy and radiotherapy, has several serious side effects due to its non-specific action on normal cells which can proliferate highly. Ambon banana stem extract (*Musa paradisiaca var. sapientum*) has active compounds including hexadecenoic acid which has an antioxidant role and suppresses the growth of cancer cells through the apoptosis process [10, 12]. To determine the potential of the hexadecenoic acid compound, it is necessary to analyse the ability of the hexadecenoic acid compound to be an anticancer drug preparation, as well as its ability to act as a ligand and bind to the RelA proteins so it can become a candidate for oral anti-cancer drugs.

Physiochemistry and pharmacokinetics of hexadecenoic acid and comparative compounds

Physiochemistry of drug likeness

Physicochemical tests assess compound's drug-likeness, with results indicating that the Hexa compound meets Lipinski's Rule of Five parameters, suggesting its potential as an oral anticancer candidate. An increase in molecular weight has been correlated with a decrease in the degree of permeability in the lipid bilayer. The compound's molecular weight and hydrogen bond properties align with optimal absorption characteristics, enhancing its bioavailability [23-25]. Additionally, analysis of the log P parameter indicates favourable polarity for distribution through blood plasma [26]. The molar refractivity parameter further confirms Hexa and Octa compound's ability for intestinal and oral absorption, contrasting with Fluo's weaker absorption capacity [27-29].

Pharmacokinetics prediction of ADME

The prediction outlined in Table 4 sheds light on various aspects of hexadecenoic acid compound distribution and its comparison with 5-Fluorouracil and 9-Octadecenoic acid. Firstly, their water solubility values indicate their ability to dissolve in water, facilitating systemic distribution. A water solubility value lower than -2 indicates that the compound can dissolve in water solvent [30, 31]. Hexadecenoic acid and 9-Octadecenoic acid exhibit high human intestinal absorption rates, surpassing 90%, while 5-Fluorouracil shows poor absorption which has a negative value [32, 33]. Additionally, the volume distribution in steady state (VDss) parameters suggests a high affinity, considered when log 0.45 <VDss< -0.15, for plasma binding in all compounds [34-36]. Moreover, hexadecenoic and octadecenoic acids act as substrates for CYP3A4 leading to increased metabolism, unlike 5-Fluorouracil, not an inducer or inhibitor of CYP3A4. Lastly, the total clearance values indicate a longer duration of action for hexadecenoic and octadecenoic acids compared to 5-Fluorouracil due to their slower elimination rates [35, 37].

Compound toxicity (pkCSM)

The pkCSM analysis on toxicity in Table 5 reveals the toxicity profiles of three active compounds: 5-Fluorouracil, hexadecenoic acid, and 9-Octadecenoic acid. All three compounds exhibit negative results for AMES toxicity, indicating a lack of mutagenic potential [38]. Hexa compound has a lower maximum tolerance limit compared to Fluo, suggesting potential cytotoxic activity, a trait also observed in its derivative [39]. In terms of oral acute toxicity (LD50), all compounds fall within safe limits for oral consumption [40, 41]. Lastly, only Hexa shows activity in skin sensitization, indicating potential hypersensitivity reactions upon skin contact [42].

This information can be used as a basis for determining the therapeutic dose, drug use dose, and lethal dose before carrying out in vitro and in vivo analysis.

Pharmacodynamics of hexadecenoic acid and comparative compounds

The PASS web server predicts biological activity of compounds based on active probability (Pa) and inactive probability (Pi) scores, with $\Delta P = Pa - Pi$ indicating the most likely activity (Perumalsamy *et al*., 2018). Pa values >0.7 suggest high likelihood of experimental pharmacological action, and Pa <0.5 indicates lower probability also suggests potential for discovering new compounds. Results for Fluo, Hexa, and Octa show Pa values > Pi, indicating potential activity as anticancer agents, with ΔP values of 0.91, 0.818, and 0.787, highlighting Hexa compound exhibits a value closest to that of the positive control for anticancer drugs indicates high specificity in anticancer activity [43, 44].

Molecular docking of RelA/p50 protein subunits and hexadecenoic acid and comparative compounds

The molecular docking test (Table 6) assesses the affinity and binding location between the target protein and the test compound. Lower binding energy and inhibition constant values indicate higher ligand affinity due to stable non-covalent interactions. Hexa shows energy of -4.9 kcal/mol, lower than Fluo (-4.6 kcal/mol), indicating its potential as a RelA inhibitor. The Octa derivative exhibits energy of -5.2 kcal/mol. Fluo compound shows 5 hydrogen bonds at residues A: SP126, A:ASP126, A: SER45, A: TYR127, A: TYR130, A:ASN175, all binding hydrogen. Additionally, Hexa compound binds with eleven Van der Waals interactions at amino acid residues PRO167, A: GLY171, A: LEU174, A:ASP126, A:TYR130, A:LYS49, A:MET123, A:ASN175, A:TYR48, A:TYR127, A:SER45, and Octa binds with ten Van der Waals bonds at residues A: TYR127, A:TYR130, A:ASP126, A:GLY171, A:LEU174, A:PRO167, A:ILE219, A:ASN175, A:MET123, A:TYR48 (Table 7). Several Fluo residues are also identified to bind with Octa through hydrogen bonds, namely A: SER45 (Figure 1). Fluo, Hexa, and Octa are also identified in Van der Waals interactions, namely A: MET123 and A: VAL46. Hexa, and Octa are also identified in hydrophobic interactions, namely A: LYS122, A: ILE168, and A: PHE119 (Table 7). Low binding energy indicates that the compound has a stronger bond with the protein, contributing to one hydrogen bond, 4 hydrophobic interactions, and one electrostatic interaction. Additionally, there are 11 van der Waals forces contributing to energy formation. The higher the binding energy in the ligand-protein complex, the weaker the interactions. This binding energy is influenced by several factors, including the number of bonds, types of bonds, and the structure of the ligand or protein. An increase in the number of bonds and high variation in bond types will decrease binding energy. Similarly, as the structure of the ligand/protein becomes more complex, the energy decreases. Strong bonds between the ligand and the target protein result in the compound not easily dissociating from the compound-protein complex. Molecular docking

visualization shows similarity between Hexa and the reference compound, indicating anticancer potential by inhibiting RelA. This is evidenced by the presence of interactions with eleven amino acid residues binding to RelA. Both compounds bind at SER 45, a competitive inhibitor site for RelA, crucial for inhibiting tumorigenesis. If excessive RelA expression occurs, it can inhibit the process of cell apoptosis, making RelA inhibition causing down-regulation one of the therapeutic targets for cancer treatment [9, 45-47].

5. Conclusion

The *hexadecenoic acid* compound contained in *Musa paradisiaca var. Sapientum* (L.) Kutnze has potential as an oral anticancer candidate through a molecular docking approach on ReLA/p50. Further in vivo research is needed to specifically determine the anticancer ability of *hexadecenoic acid* with RelA/p50 as protein target.

Compliance with ethical standards (WJS-I-Heading no numbering)

Conflict of interest

All authors declare that they have no conflicts of interest.

Statement of ethical approval

This research received approval from The Health Research Ethics Committees Dr. Moewardi General Hospital, School of Medicine Sebelas Maret University, Surakarta.

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