

Original Article

Oral anticancer promising of hexadecanoic acid through molecular interaction to nuclear factor-kappa-B p65/RELA and tumor suppressor-p53

Anticâncer oral promissor do ácido hexadecanoico por meio da interação molecular com o fator nuclear kappa-B p65/RELA e supressor tumoral p53

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Abstract

Ambonese banana stem extract (*Musa paradisiaca* var. *sapientum* (L.) Kuntze) has been proven to contain the active compound Hexadecanoic 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 p65/RELA and tumor suppressor-p53 for the development of oral anticancer drugs through molecular docking. In silico molecular docking 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 Hexa bond visualization (CID: 985), along with the positive control comparison 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) and p53 (PDB ID: 2OCJ). The Hexa, Fluo and Octa compounds' tests were negative for AMES toxicity, indicating that these compounds do not cause genetic mutations. The acute oral toxicity tests yielded values of 1.44 mol/kg for Hexa, 1.939 mol/kg for Fluo and 1.417 mol/kg for Octa. Molecular docking results and bond visualization indicate that the affinity of 9-octadecenoic acid interacts better with RELA and p53 compared to the positive control, i.e. 5-fluorouracil. Hexa compound exhibits a superior binding pocket compared to Fluo and Octa, particularly against the p53 target protein. Hexadecanoic acid compound in *Musa paradisiaca* var. *sapientum* (L.) Kuntze represents a breakthrough in developing a new anticancer potential and effectiveness against RELA and p53.

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

Resumo

O extrato do caule da banana ambonesa (*Musa paradisiaca* var. *sapientum* (L.) Kuntze) provou conter o composto ativo ácido hexadecanoico (Hexa), que pode suprimir o crescimento de células cancerosas por meio do processo de apoptose. O objetivo é determinar a interação do HA com o fator nuclear kappa-B p65/RELA e o supressor tumoral p53 para o desenvolvimento de medicamentos anticâncer orais por meio do encaixe molecular. O estudo de encaixe molecular *in silico* realizado inclui a predição de espectros de atividade de substâncias (PASS), a análise de similaridade a fármacos com base na regra de Lipinski dos cinco princípios, o estudo de absorção, distribuição, metabolismo, excreção e toxicidade (ADMET), o encaixe molecular e a visualização de ligação Hexa (CID: 985), juntamente com a comparação de controle positivo 5-fluorouracil (Fluo) (CID: 3385) e o composto derivado ácido 9-octadecenoico (Octa) (CID: 445639), que se ligam às proteínas alvo RELA (PDB ID: 6NV2) e p53 (PDB ID: 2OCJ). Os testes dos compostos Hexa, Fluo e Octa foram negativos para toxicidade AMES, indicando que esses compostos não causam mutações genéticas. Os testes de toxicidade oral aguda produziram valores de 1,44 mol/kg para Hexa, 1,939 mol/kg para Fluo e 1,417 mol/kg para Octa. Os resultados do acoplamento molecular e a visualização da ligação indicam que a afinidade do ácido 9-octadecenoico interage melhor com RELA e p53, em comparação com o controle positivo, ou seja, 5-fluorouracil. O composto Hexa exibe uma bolsa de ligação superior em comparação com Fluo e Octa, particularmente contra a proteína alvo p53. O composto de ácido hexadecanoico em *Musa paradisiaca* var. *sapientum* (L.) Kuntze representa um avanço no desenvolvimento de um novo potencial anticancerígeno e eficácia contra RELA e p53.

Palavras-chave: desenvolvimento de medicamentos, ácido hexadecanoico, docking molecular, anticâncer oral, bem-estar humano.

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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) (Dohude and Ramaliah, 2022; NCI, 2023). Approximately 90% of oral cancers are OSCC cases. According to global cancer observatory (GLOBOCAN), there were 5,780 cases of oral cancer in Indonesia in 2020, of which 3,087 died (Dohude and Ramaliah, 2022).

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 (Yardimci et al., 2014). Oral cancer cells or pre-malignant lesions can express *toll-like receptors* (TLR) and activate inflammatory, proliferation, and migration signaling pathways (Rusanen et al., 2017). *Rubo nucleid acid* (RNA) analysis confirmed that in OSCC cells, stimulation of TLR-2 can activate the *nuclear factor- κ B* (NF- κ B) pathway and induce cytokines and chemokines that depend on the NF- κ B pathway (Pisani et al., 2017; Palani et al., 2017). NF- κ B consists of five members, which are p65 (RELA), p50 (RELB), c-REL, NF- κ B1, and NF- κ B2. RELA has a transcriptional activation domain and is involved in cell survival, invasion, proliferation, metastasis, angiogenesis, and cell chemoresistance. The NF- κ B subunit, p65/RELA, can mediate apoptosis through cross-talking with p53. Therefore, NF- κ B inhibitors for cancer therapy need to be thoroughly reviewed (Bu et al., 2016).

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 Hexadecanoic acid (Hexa) which had the highest percentage (5.40%) for ethanol extract. Meanwhile, octadecenoic acid (Octa) had the highest percentage in ethyl acetate extract compound (11.47%) (Budi et al., 2022). 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. Hence, it is imperative to engage in silico studies with a tailored framework to assess the impact of both compounds (Budi and Farhood, 2023; Agamah et al., 2020; Parrales and Iwakuma, 2016).

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 (Madden et al., 2020). 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 (Meng et al., 2021). This study was conducted to analyze the hexadecenoic acid compound

contained in *Musa paradisiaca* var. *sapientum* (L.) Kuntze for oral anticancer drugs, which can bind to p65/RELA and p53 proteins through in silico studies.

2. Materials and Methods

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

Structure molecule of Hexadecanoic acid contained in *Musa paradisiaca* var. *sapientum* (L.) Kuntze is provided from 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 potential activity (Pa) and potential inhibitory (Pi) 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 Pa value < 0.5 means the compound is not biologically active (Guy et al., 2005).

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 (Ferreira and Andricopulo, 2019). 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, maximum tolerated dose (human), oral rat acute toxicity (LD50), and skin sensitization (Abdjan et al., 2020; Ibrahim et al., 2021; Izzaturrahmi et al., 2023).

2.3. Physicochemical analysis of drug-likeness

Physicochemical analysis is based on the *lipinski rule of five* principles on the web (Jayaram et al., 2024) 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 (Karami et al., 2022; Khaled et al., 2022). The terms for *drug-likeness* are (Khaled et al., 2022): (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. Structural molecule of Hexadecanoic acid, 5-Fluorouracil and 9-Octadecenoic acid

The three dimensional (3D) structures of the selected target proteins were obtained from the *research collaboratory for structural bioinformatics protein data bank* (RSCB PDB) database (RCSB, 2024), which are RELA (PDB ID: 6NV2) and p53 (PDB ID: 2OCJ). The 3D structures of the control ligand and study compounds, namely Hexadecanoic acid (CID: 985), 5-Fluorouracil (CID: 3385) and 9-Octadecenoic acid (CID: 445639) were obtained from the PubChem database (NLM, 2024).

2.5. RELA and p53 protein preparation

The ligands were energy-minimized and converted to PDB file format using *open babel* within the *PyRx* software. The RELA and p53 receptors were separated from their ligands and water molecules using *BIOVIA discovery studio visualizer 2021*, ensuring that only the structures of the RELA and p53 proteins were used as the target proteins.

2.6. Docking analysis and visualization

Docking was carried out using *autodock vina* integrated in *PyRx v.1.1* (Kumar et al., 2023). 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* (Lipinski, 2004).

3. Results

3.1. Physicochemistry and PASS prediction

The results of the physicochemical tests (Table 2) showed that the three compounds met *lipinski's* principles, with Hexa 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 data from Fluo 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. 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.

The potentiation of the Hexa, Fluo and Octa shown in Table 3. The Hexa compound has potential to become an

Table 1. Molecular docking analysis gridbox.

	RELA (6NV2)		P53 (2OCJ)	
	Center	Dimensions	Center	Dimensions
X	20.7633	20	36.9863	40
Y	22.0097	20	43.8929	20
Z	2.3536	20	33.4365	30

Table 2. Physicochemical analysis of structural molecule.

Compounds	Molecular Mass (Da)	Hydrogen Bond Donor	Hydrogen Bond Acceptor	Log P	Molar Refractivity
	≤500 Da	≤5	≤10	≤5	40-130
Hexa	256	1	2	4.32	88.26
Fluo	130	2	2	-0.22	20.70
Octa	282	1	2	4.32	96.86

Noted: Fluo: 5-Fluorouracil; Hexa: Hexadecanoic acid; Octa: 9 Octadecenoic acid.

Table 3. PASS prediction.

Compounds	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

Noted: Pa: probability "to be active"; Pi: probability "to be inactive"; Fluo: 5-Fluorouracil; Hexa: Hexadecanoic acid; Octa: 9 Octadecenoic acid.

active RELA inhibitor and active p53 activator 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 and an active p53 activator compound. The Fluo compound has the potential to act as a RELA inhibitor and p53 activator with an efficacy of 0.915. The potential of being an inactive RELA inhibitor and an inactive p53 activator is 0.005. First, ΔP was obtained of 0.91 so it was categorized as an active RELA inhibitor compound and an active p53 activator compound. Further data regarding the Octa compound has the potential to become an active RELA inhibitor and the active p53 activator 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 and an active p53 activator.

3.2. ADME and toxicity effect

Examination findings of the ADME prediction test (Table 4) demonstrated the parameters of each aspect consisting of administration, distribution, metabolism, and excretion. 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 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 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.

Based on Table 5 results, the Hexa 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 Fluo 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 Octa 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.

3.3. Molecular docking analysis of Hexa to RELA

Results of the molecular docking test (Table 6) determined the ability and potential of the Hexadecanoic acid compound from Ambonese 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

Table 4. The prediction of administration, distribution, metabolism and excretion.

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

Noted: Fluo: 5-Fluorouracil; Hexa: Hexadecanoic acid; Octa: 9 Octadecenoic acid.

Table 5. The prediction of toxicity.

Compounds	AMES Toxicity	Human Maximum Tolerated Dose	Oral Acute Toxicity (LD ₅₀)	Skin Sensitization
		Log (mg/Kg BB)	(mol/kg)	
Hexa	Negative	-0.708	1.44	Positive
Fluo	Negative	1.359	1.939	Negative
Octa	Negative	-0.81	1.417	Negative

Noted: Fluo: 5-Fluorouracil; Hexa: Hexadecanoic acid; Octa: 9 Octadecenoic acid.

Table 6. Molecular docking analysis on RELA.

Protein Target	Compounds	Binding affinity (kcal/mol)	Mode	RMSD	
				Lower bound	Upper bound
RelA	Hexa	-4.9	0	0	0
PDB ID 6NV2	Fluo	-4.6	0	0	0
	Octa	-5.2	0	0	0

Noted: Fluo: 5-Fluorouracil; Hexa: Hexadecanoic acid; Octa: 9 Octadecenoic acid.

control compound, which is Fluorouracil, and lower than the Octadecenoic acid derivative compound which is the comparison group.

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 Table 7. Where the visualization results showed that the peptide bond location of Hexa, Fluo and Octa has the same location, namely **SER45**.

3.4. Molecular docking analysis of Hexa to p53

The results of the molecular docking determined the ability and potential of the Hexadecanoic acid compound

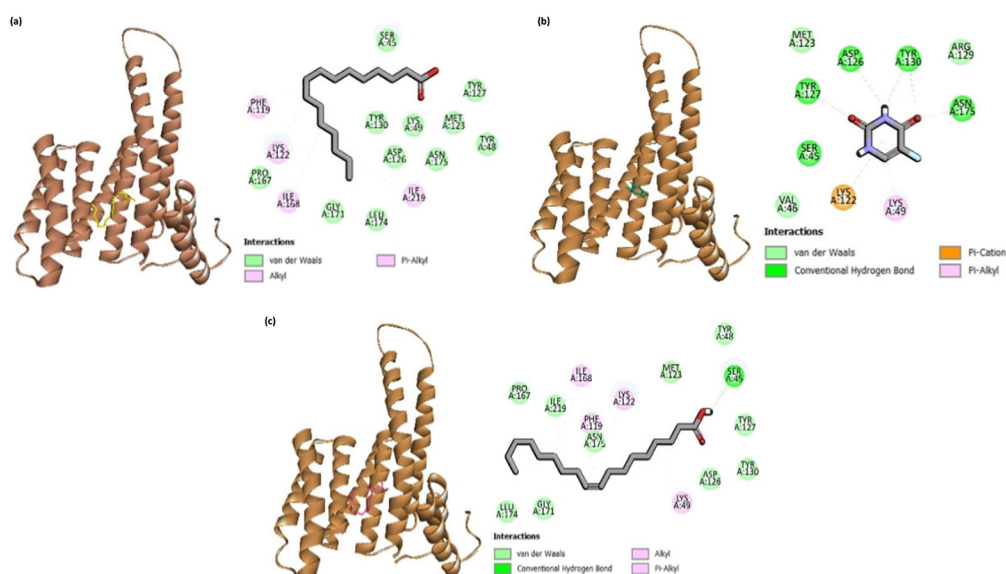


Figure 1. Visualization of docking results, (a) RELA/Hexadecanoic Acid, (b), RELA/5-Fluorouracil, and (c) RELA/9-Octadecenoic acid. The left part showed a 3D visualization, and the right part showed the type of bond produced between the ligand-protein.

Table 7. Interaction of amino acid residues produced in the RELA.

Protein Target	Compounds	Types and Locations of Bonding
RELA	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: -
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
	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: -

Noted: Fluo: 5-Fluorouracil: Hexa: Hexadecanoic acid: Octa: 9 Octadecenoic acid.

from Ambonese banana stem extract as a p53 activator compared to the comparison group (Table 8). The Hexa compound in mode and RMSD 0 has a bond affinity of -4.5 kcal / mol, where this value is higher than the compound, Fluorouracil and Octadecenoic acid which are the comparison group. The visualization test results (Figure 2) of the molecular docking test between the

study compound and the comparison compound for the target protein showed identical binding locations. This is indicated by the location of the compound binding to p53 in the active site. The types of bonds formed are hydrogen interactions, Van der Waals interactions, hydrophobic interactions, electrostatic bonds, and halogen bonds. The types and locations of ties are described in Table 9.

Table 8. Molecular docking analysis on p53.

Compounds	Binding Affinity	Mode	RMSD	
	(kcal/mol)		Lower bound	Upper bound
Hexa	-4.5	0	0	0
Fluo	-4.8	0	0	0
Octa	-4.7	0	0	0

Noted: Fluo: 5-Fluorouracil; Hexa: Hexadecanoic acid; Octa: 9 Octadecenoic acid.

Table 9. Interaction of amino acid residues produced in the p53.

Protein Target	Compounds	Types and Locations of Bonding
p53 PDB ID 2OCJ	Hexa	- Hydrogen Bonding: B:ARG273 - Van der waals bonding: B:ARG280, B:CYS275, B:SER240, B:ASN239, B:VAL274, B:ARG248, D:THR102, D:ASP148, D:GLY108, D:SER106, D:GLY105, D:GLN104 - Hidrofobik: B:ALA276, D:TYR103
	Fluo	- Hydrogen Bonding: B:VAL274, B:ARG273, B:SER240, B:ALA276 - Van der waals bonding: B:ARG280, B:ASN239, B:SER241, D:ASP148 - Hidrofobik: B:CYS275, B:ALA276 - Elektrostatik: - - Halogen: D:SER106
	Octa	- Hydrogen Bonding: - - Van der Waals Bonding: B:ALA276, B:ASN247, B:ARG248, B:ARG273, B:SER241, D:TYR107, D:GLY108, D:ASP148, D:GLN104, D:GLY105, D:SER106 - Hidrofobik: B:MET243, D:TYR103

Noted: Fluo: 5-Fluorouracil; Hexa: Hexadecanoic acid; Octa: 9 Octadecenoic acid.

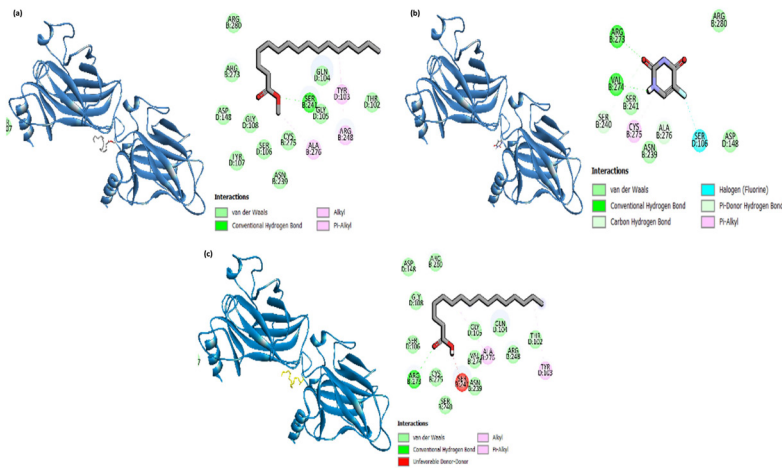


Figure 2. Visualization of docking results. (a) p53/Hexadecanoic acid; (b) p53/5-Fluorouracil; and (c) Protein p53/9-Octadecenoic acid. The left part showed a 3D visualization, and the right part showed the type of bond produced between the ligand-protein.

The visualization of Hexa, Fluo and Octa have binding pocket locations that match the *web prank analysis* data that has been carried out previously. From *prankweb*, it stated that the bonds between p53 and the three ligands are: B:240, B:241, B:248, B:273, B:274, B:275, B:276, B:277, B:280, B:281, D:103, D:104, D:105, D:106, D:108, D:148. In all three bonds, there are amino acid residues that match the *prankweb* prediction results, while the Hexa and Fluo have the same peptide bond location, which is at ARG273.

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. Ambonese banana stem extract (*Musa paradisiaca* var. *sapientum* (L.) Kuntze) has active compounds including Hexa which has an antioxidant role and suppresses the growth of cancer cells through the apoptosis process. To determine the potential of the Hexa compound to be an anticancer drug preparation, as well as its ability to act as a ligand and bind to the RELA and p53 proteins so it can become a candidate for oral anti-cancer drugs.

4.1. Physicochemistry of drug likeness of Hexadecanoic acid

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 (Agamah et al., 2020; NCI, 2023). Additionally, analysis of the log P parameter indicates favorable polarity for distribution through blood plasma (Ferrari, 2023). The molar refractivity parameter further confirms Hexa and Octa compound's ability for intestinal and oral absorption, contrasting with Fluo's weaker absorption capacity (Jayaraman et al., 2022; Ma'arif et al., 2021; Madden et al., 2020).

4.2. Pharmacokinetics (ADME) and pharmacodynamics prediction of Hexadecanoic acid

The prediction outlined in Table 4 sheds light on various aspects of Hexa compound distribution and its comparison with Fluo and Octa. 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 (Mazrouei et al., 2019; Meng et al., 2021). Hexa and Octa exhibit high human intestinal absorption rates, surpassing 90%, while Fluo shows poor absorption which has a negative value (Palani et al., 2017; Parrales and Iwakuma., 2016). Additionally, the volume distribution in steady state (VDss) parameters suggests a high affinity, considered when $\log 0.45 < \text{VDss} < -0.15$, for plasma binding in all compounds (Perumalsamy et al., 2018; Pinzi and Rastelli 2019). Moreover, Hexa and Octa act as substrates for CYP3A4, leading to increased metabolisme. Unlike

Fluo, it is not an inducer or inhibitor of CYP3A4, so it is safe when given together with other drugs that affect the activity of this enzyme. Lastly, the total clearance values indicate a longer duration of action for Hexa and Octa compared to Fluo due to their slower elimination rates (Perumalsamy et al., 2018; Pisani et al., 2017).

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. Pa values >0.7 suggest high likelihood of experimental pharmacological action, while $0.5 < Pa < 0.7$ indicates lower probability, and Pa < 0.5 suggests potential for discovering new compounds. Results for Hexa, Fluo and Octa show Pa values $> Pi$, indicating potential activity as anticancer agents, with ΔP values of 0.91, 0.818, and 0.787, highlighting their specificity in anticancer activity (Shah et al., 2022; Shehzadi et al., 2016).

The pkCSM analysis on toxicity in Table 5 reveals the toxicity profiles of three active compounds: 5-Fluorouracil (Fluo), Hexadecanoic acid (Hexa), and 9-Octadecenoic acid (Octa). All three compounds exhibit negative results for AMES toxicity, indicating a lack of mutagenic potential (Benouchenne et al., 2022). However, Fluo demonstrates a high maximum tolerated dose, while Hexa and Octa have lower values. In terms of oral acute toxicity (LD_{50}), all compounds fall within safe limits for oral consumption (Rusanen et al., 2017). Lastly, only Hexa shows activity in skin sensitization, indicating potential hypersensitivity reactions upon skin contact (Savitri et al., 2023). 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.

4.3. Molecular docking of Hexa to p65/RELA target receptor

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 amino acid residues TYR127, A:TYR130, A:ASP126, A:GLY171, A:LEU174, A:PRO167, A:ILE219, A:ASN175, A:MET123, A:TYR48. 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, dan A: VAL46. Hexa, and Octa are also identified in hydrophobic interactions, namely A: LYS122, A: ILE168, dan A:PHE119. 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 (Aruleba et al., 2018; Coimbra et al., 2021; Trott and Olson, 2010; Ugbe et al., 2022; Venkatachalam and Nadumane, 2018).

4.4. Molecular docking of hexa to p53 target receptor

The molecular docking test (Table 8) indicates that Hexa acts competitively as an anticancer agent, like Fluo and Octa. Hexa shows energy of -4.5 kcal/mol, lower than Fluo (-4.8 kcal/mol), and Octa shows energy of -4.7 kcal/mol. Fluo compound shows 4 hydrogen bonds at residues B: VAL274, B: ARG273, B:SER240, B:ALA276, all binding hydrogen atoms. Additionally, Hexa compound binds with twelve Van der Waals interactions at amino acid residues B:ARG280, B:CYS275, B:SER240, B:ASN239, B:VAL274, B:ARG248, D:THR102, D:ASP148, D:GLY108, D:SER106, D:GLY105, D:GLN104 and Octa binds with eleven Van der Waals interactions at residues B:ALA276, B:ASN247, B:ARG248, B:ARG273, B:SER241, D:TYR107, D:GLY108, D:ASP148, D:GLN104, D:GLY105, D:SER106. Several Fluo residues are also identified to bind with Hexa through hydrogen bonds, namely B: ARG273 (Figure 2). Fluo, Hexa, and Octa are also identified in Van der Waals interactions, namely B: ARG280, B: ARG248, D:ASP148, D: GLY105, D:GLN104. Hexa, Fluo, and Octa are also identified in hydrophobic interactions, namely ALA276, D: TYR103.

In our study, the binding energy consists of four hydrogen bonds, two hydrophobic interactions, twelve van der Waals interactions, and one halogen interaction. Strong hydrogen bonds and Van der Waals interactions are found in Hexa, thus Hexa can influence p53 to induce apoptosis. Octa and Fluo form Van der Waals bonds at the same location on affect p53 regulation. Additionally, they reduce Bcl-2 and increase bax regulation (Yasmin et al., 2022; Zinatizadeh et al., 2020).

5. Conclusion

The Hexadecanoic acid compound contained in *Musa paradisiaca* var. *Sapientum* (L.) Kuntze has potential as an

oral anticancer candidate through a molecular docking approach on RELA and p53. Further in vivo research is needed to specifically determine the anticancer ability of Hexadecanoic acid with RELA and p53 as protein target.

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