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Research Article

Evaluation of Xanthone and Cinnamoylbenzene as Anticancer Agents for Breast Cancer Cell Lines through *In Vitro* and *In Silico* Assays

Yehezkiel Steven Kurniawan, Hanif Amrulloh, Ervan Yudha, Nela Fatmasari, Faris Hermawan, Anggit Fitria, Harno Dwi Pranowo, Eti Nurwening Sholikhah, and Jumina Jumina*

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Abstract
Breast cancer is a severe global disease for women as the number of deaths increases annually. Therefore, attempts to find new anticancer agents are critical and inevitable. In this work, we report the investigation on the anticancer activity of xanthone and cinnamoylbenzene compounds against two breast cancer cell lines, i.e., T47D and MCF-7, through experimental *in vitro* and theoretical *in silico* assays. Xanthone and cinnamoylbenzene exhibit anticancer activity with a half-maximal inhibitory concentration (IC_{50}) of 136.7–194.3 and 235.8–262.4 µg/mL against T47D and MCF-7 cancer cells, respectively. Cinnamoylbenzene generates less cytotoxicity to normal Vero cells with a selectivity index of 1.095–2.102. The molecular docking studies agree with the experimental data in which cinnamoylbenzene is more active against T47D with an IC_{50} of 136.7 µg/mL due to Topoisomerase II inhibition through π-π stacked interactions with Adenine12 and Guanine13 nitrogen bases. Meanwhile, xanthone is more active against MCF-7 with an IC_{50} of 235.8 µg/mL due to EGFR inhibition through van der Waals interaction and hydrogen bond with Glutamic acid767 and Methionine769 amino acid residues, respectively. Additionally, the pharmacokinetic parameters of xanthone and cinnamoylbenzene are predicted through absorption, distribution, metabolism, excretion, and toxicity (ADMET) analysis, and they show better suitability than doxorubicin as the commercial anticancer drug.

Keywords: breast cancer, xanthone, cinnamoylbenzene, cytotoxicity, molecular docking

1. INTRODUCTION

Recently, breast cancer has been receiving world attention in the medical field because one in eight women develops breast cancer in her lifetime [1][2]. Breast cancer is the most frequently diagnosed among women and the second leading cause of cancer-related deaths worldwide. In 2020, the World Health Organization reported that 2.26 million new breast cancer cases were diagnosed, with around 685 thousand deaths in a year [3]. That means breast cancer contributes to 30% of cancer patients. Surprisingly, 85% of breast cancer patients had no family history of breast cancer [4]. Therefore, researchers shall give profound awareness to treat breast cancer diseases to save human lives in the following years [5]-[7].

Breast cancers can be generated by the uncontrolled growth of T47D and MCF-7 cancer cell lines [8][9]. The T47D are epithelial cells from a female with an infiltrating ductal carcinoma on her breasts. This cancer cell stimulates the breast cancer cells' growth and the cancer genesis process. Otherwise, MCF-7 cancer cell lines are isolated from a woman with metastatic adenocarcinoma and they are characterized by rapid protein transcription and apoptosis regulation [10]. Both cancer cell lines have been commonly used for research on anticancer drugs [8]-[10]. The overexpression of specific proteins could cause the rapid growth of breast cancer cells. It is reported that the Topoisomerase II protein was overexpressed in T47D cancer cells [11]. Meanwhile, the epidermal growth factor receptor (EGFR) protein is overexpressed in MCF-7 cancer cells [12]. Because of that, either Topoisomerase II or EGFR protein has been extensively used to investigate the action mechanism of new anticancer drugs.

Hundreds of new anticancer agents have been designed and developed; however, most of them suffer from complicated isolation and synthesis, as well as time-consuming purification processes, expensive raw materials, poor anticancer activity, and high toxicity to normal cells [13]-[16]. Xanthone and cinnamoylbenzene derivatives are

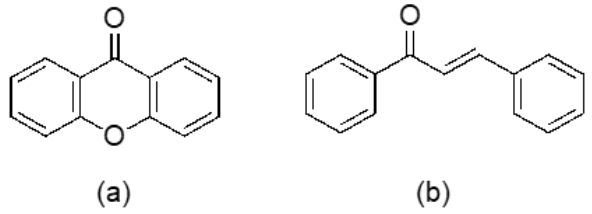

Figure 1. Chemical structures of (a) xanthone and (b) cinnamoylbenzene.

simplest aromatic compounds that have been investigated and proven as efficient anticancer agents [17]-[19]. Xanthone, 9*H*-xanthen-9-one, is a natural oxygenated heterocyclic compound with high biocompatibility and wide biological activities. As the secondary metabolites, xanthones are produced from mixed shikimic acetate and acetate polymalonic acid pathways and they are commonly found in Clusiaceae, Gentianaceae, Hypericaceae, Moraceae, and Polygalaceae [20]. On the other hand, cinnamoylbenzene, mostly known as chalcone, is produced from the shikimate pathway with the help of the chalcone synthase enzyme [21]. Otherwise, xanthone can be synthesized from phenolic and salicylic acid derivatives through cyclo-condensation reaction. Meanwhile, cinnamoylbenzene is prepared through Claisen-Schmidt condensation or Friedel-Crafts acylation reaction. Both compounds comprise two aromatic rings connected by unsaturated ketone and 4-pyrone moieties, respectively (see Figure 1).

Xanthone and cinnamoylbenzene are known for their privileged structure in medicinal chemistry yielding various biological activities as anticancer, anti-inflammatory, antiviral, antioxidant, antidiabetic, antibacterial, antifungal, and antimalarial agents. Some of their modified derivatives are under investigation in clinical trials as new anticancer agents such as gambogic acid and lucanthone [20][21]. The anticancer activity of xanthone and cinnamoylbenzene derivatives depends on the functional groups attached to their chemical structures. For example, hydroxylated xanthone gives remarkable anticancer activity against MCF-7 cancer cells by inhibiting the Topoisomerase II protein. The 1,3,8-trihydroxyxanthone, 1,6-dihydroxyxanthone, and 1,5,6-trihydroxyxanthone gave the half-maximal inhibitory concentration (IC_{50}) value of 184, 450, and 419 µM, respectively, against MCF-7 cancer cells. Their selectivity indexes are in a range of 0.535–18.42 depending on the number and position of the hydroxy group on the xanthone structure [22].

On the other side, substituted cinnamoylbenzene is an effective anticancer agent against the T47D cancer cell line caused by inhibiting the EGFR protein's function. The 4-chlorocinnamoylbenzene, 4-chloro-4'methoxycinnamoylbenzene, 4-chloro-3',4'-dimethoxycinnamoylbenzene, 4-chloro-4'-hydroxy-3'methoxycinnamoylbenzene, and 4-chloro-4'dimethylaminocinnamoylbenzene exhibited IC_{50} values of >100, 99.51, 0.800, 7.580, and >100 µg/mL, respectively, against MCF-7 cancer cells. Meanwhile, these compounds yielded IC_{50} values of >100, >100, 0.340, 5.610, and >100 µg/mL, respectively, against T47D cancer cells. It means that the type and position of the functional group on the cinnamoylbenzene structure influence the anticancer activity against breast cancer cells [23]. However, the anticancer activity of unsubstituted xanthone and cinnamoylbenzene against breast cancer cell lines has not been investigated yet to the best of our knowledge. The unsubstituted compound could exhibit unique anticancer activity due to the fact that there is no steric hindrance reaching the cancer cells. Indeed, by knowing the anticancer activity of the unsubstituted structure will give a deeper insight into a broader map of drug design and development in the future. Thus, we herein report the anticancer activity of pristine xanthone and cinnamoylbenzene through *in vitro* and *in silico* assays. The *in vitro* investigation has been conducted through 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay while the *in silico* molecular docking studies have been performed against Topoisomerase II and EGFR proteins.

2. MATERIALS AND METHODS

2.1. Materials

The chemicals are purchased in pro analytical grade from Merck and Sigma-Aldrich and used without further purification. Xanthone ($C_{13}H_8O_2$, CAS: 90-47-1), cinnamoylbenzene ($C_{15}H_{12}O$, CAS: 614-47-1), and doxorubicin in the form of hydrochloride salt ($C_{27}H_{29}NO_{11}\cdot HCl$, CAS: 25316-40-9) were examined as the anticancer agents.

Meanwhile, MTT ($C_{18}H_{17}N_5S$, CAS: 57360-69-7), fetal bovine serum (FBS, lyophilized from 0.5% $(NH_4)_2SO_4$, CAS: 9014-81-7), sodium dodecyl sulfate (SDS, $NaC_{12}H_{25}SO_4$, CAS: 151-21-3), and dimethyl sulfoxide (DMSO, C_2H_6SO, CAS: 67-68-5) are utilized to perform the *in vitro* anticancer assay.

2.2. Methods

2.2.1. In Vitro Anticancer Assay of Xanthone and Cinnamoylbenzene

The anticancer assay of xanthone and cinnamoylbenzene compounds has been examined through MTT assay as the standard method [24]. Briefly, cancer and normal cells are separately cultured in an incubator containing 5% carbon dioxide (CO_2) atmosphere at 310 K. The cells are placed in the FBS medium. Afterward, the cells are transferred in 96-well microplates and added with various concentrations of xanthone and cinnamoylbenzene compounds in DMSO as the solvent. The plates are incubated for 24 h, and then 5% MTT solution is added. The plates are incubated for 4 h and then added by SDS for an additional incubation for 24 h. The optical density of the mixture is recorded using an Elisa reader at a fixed wavelength of 495 nm. The IC_{50} values are estimated using a probit analysis, while the selectivity index is calculated by dividing the IC_{50} value for normal cells by the IC_{50} value for the cancer cells.

2.2.2. In Silico Assay of Xanthone and Cinnamoylbenzene as Anticancer Agents through Molecular Docking Studies

The *in silico* anticancer assay of xanthone and cinnamoylbenzene is evaluated through molecular docking studies. Two protein receptors examined in this work are EGFR (ID: 1M17) and human Topoisomerase II (ID: 4G0V). The water molecules are eliminated from the three-dimensional structure of each protein receptor, and then the structure of the proteins is recovered by adding hydrogen atoms. The molecular docking is studied by adjusting the parameters for the re-docking of native ligands using AutoDockTools 1.5.6 and AutoDock4 softwares to reach a root-mean-square deviation (RMSD) value less than 2.0 Å [25]. Afterward, the formed interactions with the amino acid and/or nitrogen base residues of the protein receptor are visualized using Discovery Studio 2019 software. Additionally, the drug-likeness and predictions for absorption, distribution, metabolism, excretion, and toxicity (ADMET) have been also performed in the pkCSM software (https://biosig.lab.uq.edu.au/pkcsm/prediction). Meanwhile, the toxicity profiles of the evaluated compounds are estimated in ProTox II software (https://tox-new.charite.de).

3. RESULTS AND DISCUSSIONS

3.1. In Vitro Anticancer Assay of Xanthone and Cinnamoylbenzene

At first, the anticancer activity of xanthone and cinnamoylbenzene has been evaluated through an *in vitro* MTT assay. The MTT assay is a well-established method to screen anticancer activity [24][25]. In this assay, the MTT chemical is reduced by the mitochondrial reductase enzyme in living cells to form formazan. This reduction reaction changes the color of the solution from yellow to purple. Since the reaction could be monitored using the colorimetric method, spectrophotometric measurement at 495 nm can calculate the percentage of remaining cancer cells in the 96-well microplate [26]. The IC_{50} values represent the minimum required concentration of synthetic compound to cause the death of half the population of examined cancer cells. A higher IC_{50}

Table 1. *In vitro* anticancer activity of xanthone and cinnamoylbenzene.

Compound	IC_{50} (µg/mL)		
	T47D	MCF-7	Vero
Xanthone	194.3	235.8	247.5
Cinnamoylbenzene	136.7	262.4	287.4
Doxorubicin	32.80	38.49	478.6

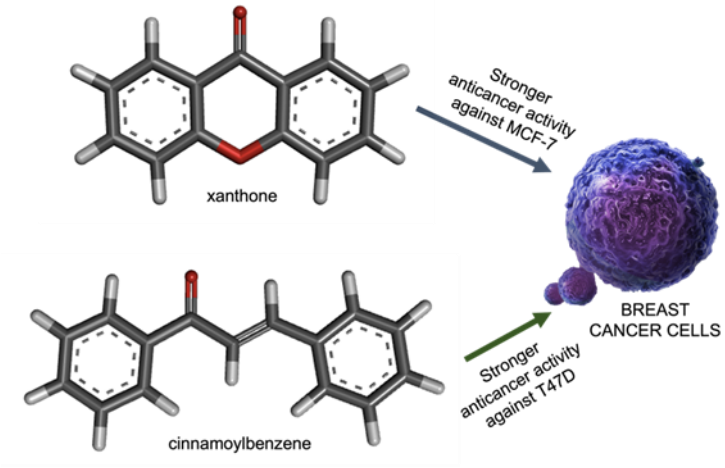

Figure 2. Comparison of the anticancer activity of xanthone and cinnamoylbenzene against breast cancer cell lines.

value reflects a weaker anticancer activity [27]. The IC_{50} values of xanthone and cinnamoylbenzene are listed in Table 1.

Xanthone yields weak anticancer activity against T47D and MCF-7 cancer cell lines with IC_{50} values of 194.3 and 235.8 µg/mL, respectively. Meanwhile, cinnamoylbenzene also exhibits weak anticancer activity with IC_{50} values of 136.7 and 262.4 µg/mL against T47D and MCF-7 cancer cell lines. Despite that, doxorubicin, as the commercial anticancer drug, generates much lower IC_{50} values against T47D and MCF-7 cancer cell lines. This phenomenon could be caused by more functional groups on the doxorubicin structure, i.e., carbonyl, hydroxyl, amino, and methoxy substituents, compared to the bare xanthone and cinnamoylbenzene compounds. These functional groups let the doxorubicin bind with protein receptors on the cancer cell line [28]. This phenomenon will be discussed later through molecular docking studies.

Table 1 shows that either xanthone or cinnamoylbenzene yielded a stronger anticancer activity against MCF-7 than T47D cancer cells due to different metabolic receptors. In contrast, doxorubicin gave a stronger anticancer activity against T47D than MCF-7 cancer cells because it is well known that T47D cells are more sensitive to doxorubicin as reported earlier [28]. The *in vitro* results demonstrate that cinnamoylbenzene is more active than xanthone against the T47D cancer cell line. Besides, xanthone is more active than cinnamoylbenzene against the MCF-7 cancer cell line. Compared to cinnamoylbenzene, xanthone gives 1.42 times weaker anticancer activity against the T47D cancer cell line; however, xanthone exhibits 1.11 times stronger anticancer activity against the MCF-7 cancer cell line (Figure 2).

The anticancer drug should selectively target the cancer cells and be non-toxic against normal cells [29]. The cytotoxicity assay of xanthone and cinnamoylbenzene against the normal Vero cell line has been conducted, and the results are displayed in Table 2. The selectivity index parameter reflects the selectivity properties of xanthone and cinnamoylbenzene as breast anticancer agents. The selectivity index value is obtained by dividing the IC_{50} value for the normal Vero cell line by the IC_{50} value for either the T47D or MCF-7 cancer cell line. Table 2 shows that xanthone yielded a selectivity index of 1.274 and 1.050 against T47D and MCF-7 cancer cell lines, respectively. On the other hand, cinnamoylbenzene exhibits much higher selectivity index values of 2.102 and 1.095 against T47D and MCF-7, respectively. We observe that cinnamoylbenzene is less toxic than xanthone against both breast cancer cell lines.

3.2. In Silico Anticancer Assay of Xanthone and Cinnamoylbenzene

To better understand the anticancer activity of xanthone and cinnamoylbenzene compounds, *in silico* investigations, including molecular docking studies and ADMET predictions, have been performed. EGFR and Topoisomerase II proteins are selected in this study as breast cancer cell lines

overexpressed them, and these proteins stimulate rapid cell division [30][31]. The three-dimensional structures of EGFR and Topoisomerase II proteins are retrieved from the protein databank with protein IDs of 1M17 and 4G0V, respectively. The molecular docking was initiated by re-docking of the native ligand, named *N*-(3-ethynylphenyl)-6,7-bis(2-methoxyethoxy)quinazolin-4-amine (erlotinib) and 1,4-dihydroxy-5,8-bis[2-(2-hydroxyethylamino)ethylamino]-anthracene-9,10-dione (mitoxantrone) on the active site of EGFR and Topoisomerase II proteins. The re-docking is a docking process of the native ligand on the active site of a particular protein to know the validity of the employed docking parameters [25].

As the three-dimensional structures of the protein target and native ligand are crystallized from the reported experimental work, the native ligand should not be significantly different after the re-docking process from the initial conformation as retrieved from the protein databank. The change in conformation is measured by the RMSD value, which must not exceed 2.00 Å [25]. The superimposed structures of the native ligand for each protein receptor are shown in Figure 3. We observe that the structure of each native ligand after the re-docking process is not significantly different from their X-ray crystallographic structure. Additionally, the obtained RMSD value from the re-docking of erlotinib and mitoxantrone ligands on the active site of EGFR and Topoisomerase II proteins is 1.64 and 1.22 Å, respectively. These values fulfil the re-docking requirement as suggested by the previous study [25]; therefore, the parameters could be used for further molecular docking studies.

The representations of molecular docking results of xanthone, cinnamoylbenzene, and doxorubicin in the active site of EGFR are shown in Figure 4. Meanwhile, the molecular docking data for EGFR protein are listed in Table 3. Xanthone gives a binding energy of -26.15 kJ/mol with a binding constant of 2.63×10^{-5} mol/L and an RMSD value of 1.02 Å. These parameters come from the interactions of xanthone with Met769 through a hydrogen bond; Val702, Thr766, Gln767, Leu768, Pro770, and Gly772 through van der Waals interactions; and Leu694, Ala719, and Leu820 through π-alkyl interactions.

On the other hand, cinnamoylbenzene yields a binding energy of -24.89 kJ/mol with a binding constant of 4.34×10^{-5} mol/L and an RMSD value of 1.79 Å. These parameters come from the interactions of cinnamoylbenzene with Met769 through a hydrogen bond; Leu768 through a carbon-hydrogen bond; Leu694 through π-σ interaction; and Ala719, Cys751, and Leu820 through π-alkyl interactions as shown in Figure 4. Compared to cinnamoylbenzene, xanthone generates lower binding energy and lower binding constant due to more interactions with ten amino acid residues on the active site of EGFR.

The *in silico* result is in agreement with the *in vitro* data, demonstrating that xanthone (IC_{50} = 235.8 µg/mL) has more potent anticancer activity than cinnamoylbenzene (IC_{50} = 262.4 µg/mL) against the MCF-7 cancer cell line. It means that the stronger anticancer activity of xanthone could be generated by the interaction of xanthone with EGFR protein. It is reported that the interactions with Gln767, Met769, Asn818, and Asp831 residues play a pivotal role in the inhibitory effect of EGFR's function [32]. The interactions with Gln767 and Met769 are observed on the molecular docking data of xanthone (Figure 4). These interactions suppress the proliferation signal, thus killing the MCF-7 cancer cells.

Compared to doxorubicin, both xanthone and cinnamoylbenzene give weaker binding energy, higher binding constant, and fewer amino acid

Table 2. Selectivity index of xanthone and cinnamoylbenzene.

Compound	Selectivity index	
	T47D	MCF-7
Xanthone	1.274	1.050
Cinnamoylbenzene	2.102	1.095
Doxorubicin	14.59	12.43

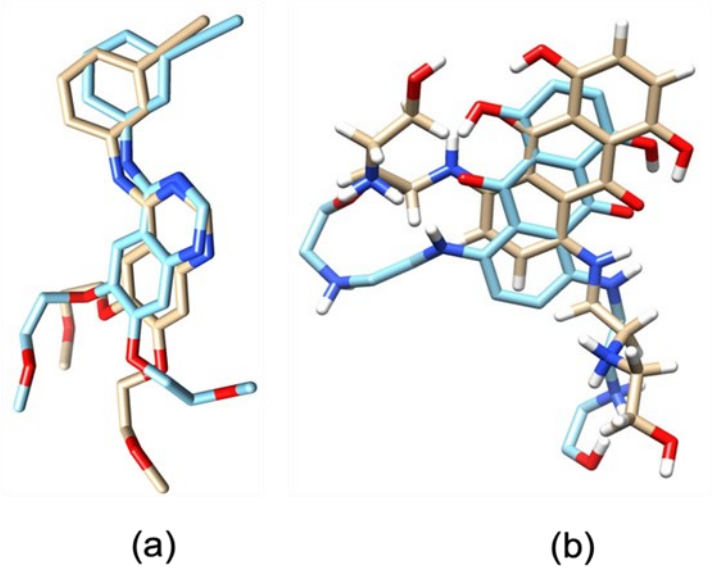

Figure 3. Comparison of the chemical structures of (a) erlotinib in the active site of EGFR and (b) mitoxantrone in the active site of Topoisomerase II from computational re-docking process (blue color) and experimental X-ray crystallographic data (brown color). Carbon atom is indicated by either blue or brown color. Oxygen atom is indicated by red color while hydrogen atom is indicated by white color.

interactions. Figure 4 reveals that doxorubicin yielded binding energy, binding constant, and RMSD value of -40.25 kJ/mol, 8.82×10^{-8} mol/L, and 0.59 Å, respectively. Furthermore, doxorubicin generates hydrogen bonds with Met769, Arg817, Asn818, and Thr830 amino acid residues, as well as van der Waals interactions with Lys721, Glu738, Met742, Thr766, Gln767, Leu768, Pro770, Gly772, Cys773, Asp831, and Phe832. Furthermore, doxorubicin generated π-σ interactions with Leu694, Phe699, and Val702 and formed π-alkyl interactions with Ala719 and Leu820 residues. It means doxorubicin formed interactions with 20 amino acid residues on the active site of EGFR protein, which is more than xanthone (10 interactions) and cinnamoylbenzene (6 interactions). Furthermore, doxorubicin could interact with all vital amino acids, i.e., Gln767, Met769, Asn818, and Asp831. This finding agrees with the lowest IC_{50} value of doxorubicin (IC_{50} = 38.49 µg/mL) compared to xanthone (IC_{50} = 235.8 µg/mL) and cinnamoylbenzene (IC_{50} = 262.4 µg/mL) as revealed in the experimental data.

The molecular docking results of xanthone, cinnamoylbenzene, and doxorubicin in the active site of Topoisomerase II are shown in Table 3 and Figure 5. Xanthone gives a binding energy of -25.52 kJ/mol with a binding constant of 3.39×10^{-5} mol/L and an RMSD value of 0.43 Å. Xanthone generates interactions with Arg503 and Gly504 through van der Waals interactions, and Cytosine8, Adenine12, and Guanine13 through π-π stacked interactions (see Table 3). On the other hand, cinnamoylbenzene yields a binding energy of -30.17 kJ/mol with a binding constant of 5.19×10^{-6} mol/L and an RMSD value of 0.39 Å. Cinnamoylbenzene interacts with Cytosine14, Arg503, Gly504, Ile506, Leu507, and Asn520 through van der Waals interactions. Moreover, cinnamoylbenzene interacts with Glu522 through π-anion interaction; with Lys505 through π-lone pair interaction; and with Adenine12 and Guanine13 through π-π stacked interactions as depicted in Figure 5. Compared to xanthone, cinnamoylbenzene produces a lower binding energy and lower binding constant due to more interactions with seven amino acids and three nitrogen base residues on the active site of Topoisomerase II.

These data are linear to the higher anticancer activity of cinnamoylbenzene (IC_{50} = 136.7 µg/mL) than xanthone (IC_{50} = 194.3 µg/mL) against the T47D cancer cell line. It means that the stronger anticancer activity of cinnamoylbenzene could be caused by the interaction with the Topoisomerase II protein, which is overexpressed in the T47D cancer cells, as reported earlier [10]. The interactions with

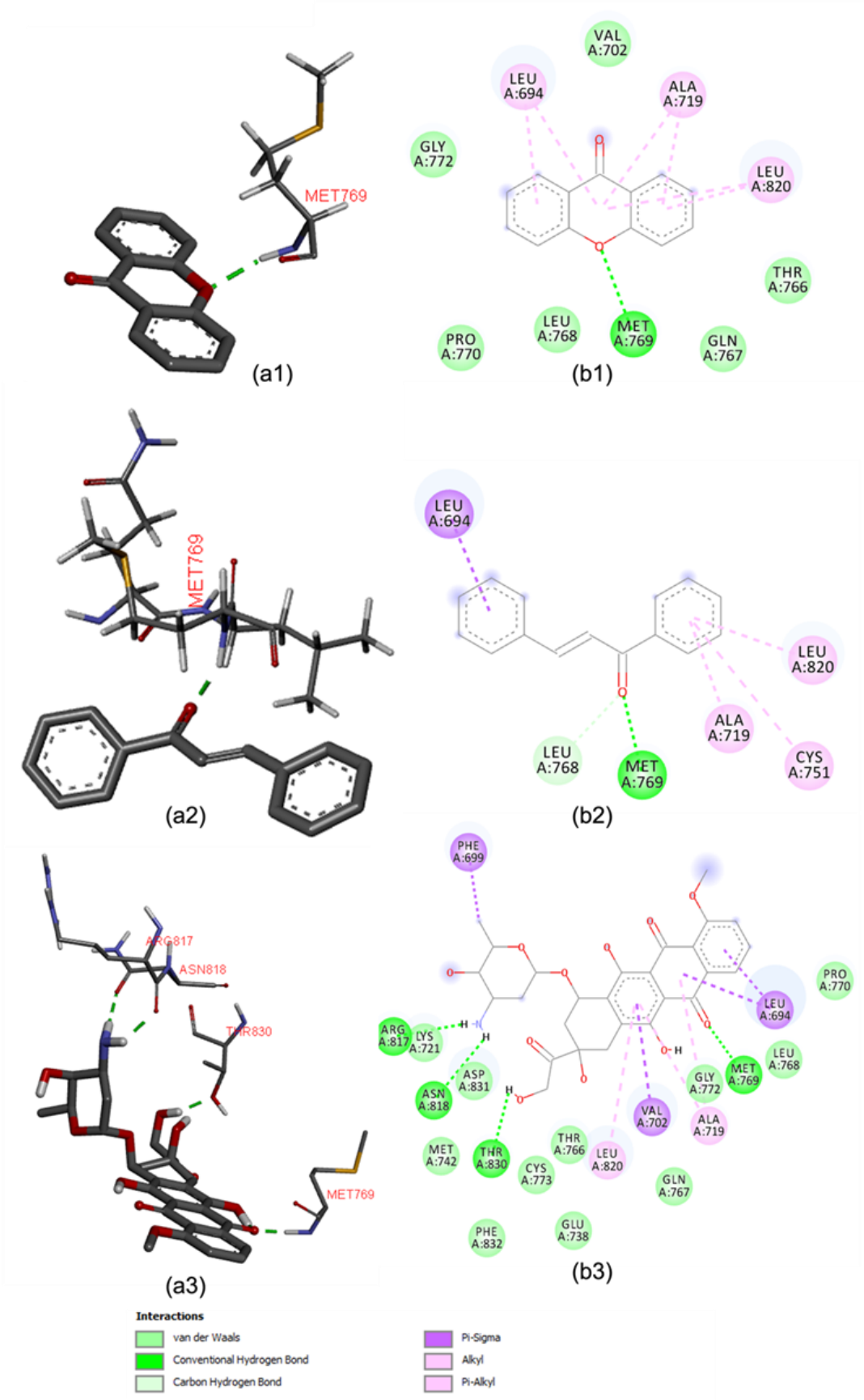

Figure 4. Representation of molecular docking results of (1) xanthone, (2) cinnamoylbenzene, and (3) doxorubicin on the active site of EGFR through (a) three-dimensional and (b) two-dimensional visualizations. Carbon, oxygen, nitrogen, sulfur, and hydrogen atoms are indicated by black, red, blue, gold, and white color, respectively.

Table 3. Molecular docking results of xanthone and cinnamoylbenzene as anticancer agents

Compound	Binding energy (kJ/mol)	Binding constant (mol/L)	RMSD (Å)	Interactions
EGFR receptor				
Xanthone	-26.15	2.63×10^{-5}	1.02	Hydrogen bond: Met769 π-alkyl: Leu694, Ala719, Leu820 van der Waals: Val702, Thr766, Gln767, Leu768, Pro770, Gly772
Cinnamoylbenzene	-24.89	4.34×10^{-5}	1.79	Hydrogen bond: Met769 Carbon hydrogen bond: Leu768 π-σ: Leu694 π-alkyl: Ala719, Cys751, Leu820
Doxorubicin	-40.25	8.82×10^{-8}	0.59	Hydrogen bond: Met769, Arg817, Asn818, Thr830 π-σ: Leu694, Phe699, Val702 π-alkyl: Ala719, Leu820 van der Waals: Lys721, Glu738, Met742, Thr766, Gln767, Leu768, Pro770, Gly772, Cys773, Asp831, Phe832
Topoisomerase II receptor				
Xanthone	-25.52	3.39×10^{-5}	0.43	π-π stacked: Cytosine8, Adenine12, Guanine13 van der Waals: Arg503, Gly504
Cinnamoylbenzene	-30.17	5.19×10^{-6}	0.39	π-π stacked: Adenine12, Guanine13 π-anion: Glu522 π-lone pair: Lys505 van der Waals: Cytosine14, Arg503, Gly504, Ile506, Leu507, Asn520
Doxorubicin	-47.15	5.45×10^{-9}	0.19	Hydrogen bond: Guanine13, Glu522 Carbon hydrogen bond: Adenine12 van der Waals: Cytosine8, Arg503, Gly504, Ala521

Adenine12 and Guanine13 nitrogen bases play a pivotal role in stopping protein synthesis in the cancer cells, thus leading to apoptosis of the T47D cancer cells. Compared to doxorubicin, xanthone and cinnamoylbenzene give lower binding energy and binding constant. Figure 5 reveals that doxorubicin leads to binding energy, binding constant, and RMSD value of -47.15 kJ/mol, 5.45×10^{-9}, and 0.19 Å, respectively. Furthermore, doxorubicin generated hydrogen bonds with Guanine13 and Glu522, and van der Waals interactions with Cytosine8, Arg503, Gly504, and Ala521 residues. Moreover, doxorubicin creates a carbon-hydrogen bond with Adenine12 and forms π -π stacked interactions with Adenine12 and Guanine13. It denotes that doxorubicin formed interactions with four amino acids and three nitrogen base residues on the active site of the Topoisomerase II protein.

Even though these interactions are more than xanthone (2 amino acids and 2 nitrogen bases), the formed interactions of doxorubicin are fewer than

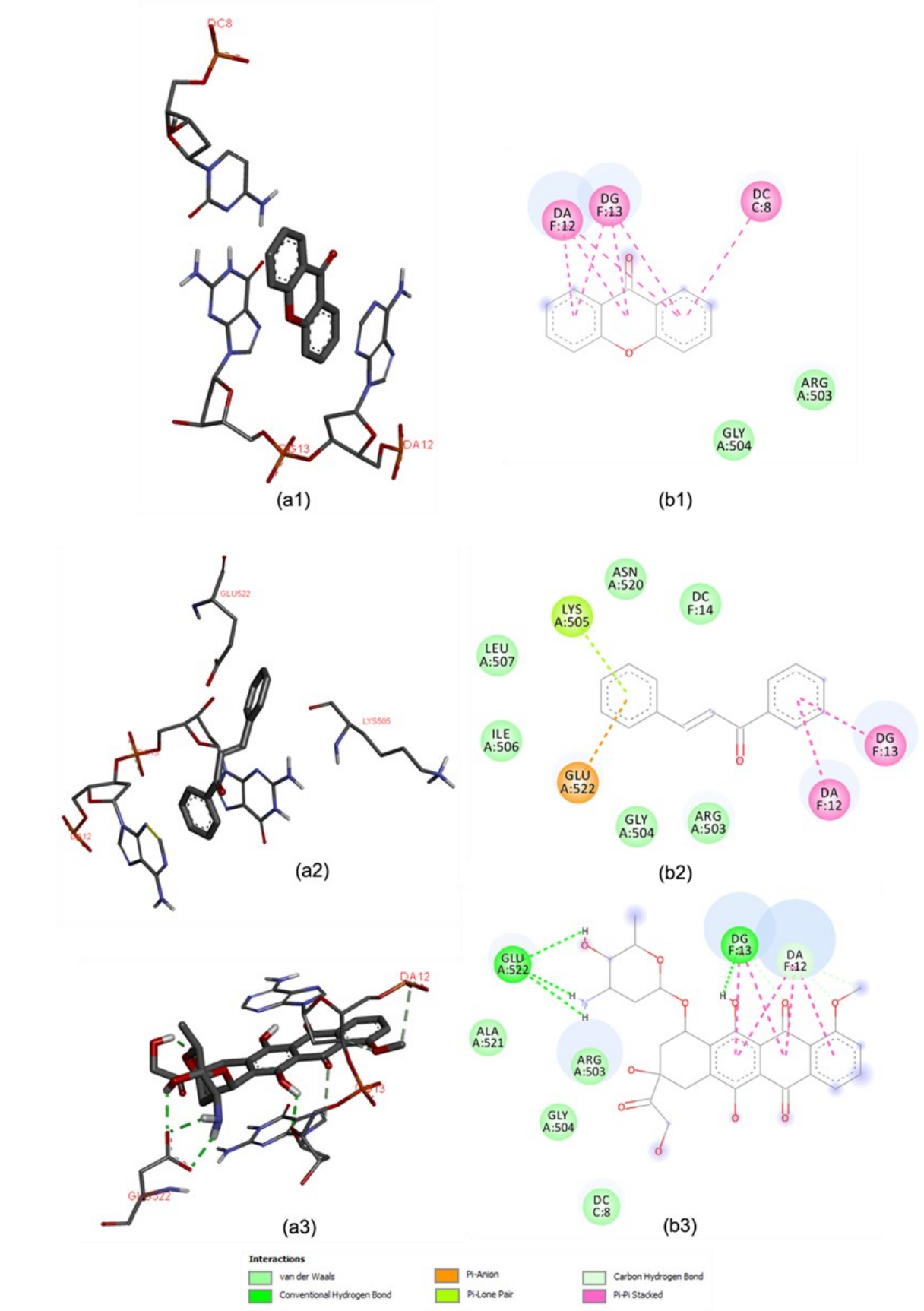

Figure 5. Representation of molecular docking results of (1) xanthone, (2) cinnamoylbenzene, and (3) doxorubicin on the active site of Topoisomerase II through (a) three-dimensional and (b) two-dimensional visualizations. Carbon, oxygen, nitrogen, sulfur, and hydrogen atoms are indicated by black, red, blue, gold, and white color, respectively.

cinnamoylbenzene (7 amino acids and 4 nitrogen bases). However, the binding energy of doxorubicin is the highest (-47.15 kJ/mol), as well as its IC_{50} value is the lowest (IC_{50} = 32.80 µg/mL). It shall be noted that the binding energy and IC_{50} value are not always correlated with the number of generated non -covalent interactions between the ligand and the active site of the protein receptor. The stronger interactions with key amino acids and/or nitrogen base residues are also important factors to be counted on Kurniawan et al (2024) [33].

The ligand binding to Adenine12 and Guanine13 stimulates the Topoisomerase II protein to generate toxic oligopeptides that kill the T47D cancer cells [34]-[36]. The molecular docking studies reveal that doxorubicin interacted with Adenine12 through a carbon-hydrogen bond and π-π stacked interaction. The interaction with Guanine13 is also observed through a hydrogen bond and π-π stacked interaction. This chelation seems to significantly inhibit the function of Topoisomerase II on the proliferation of T47D cancer cells; thus, it could explain why doxorubicin exhibited the strongest anticancer activity against T47D cancer cells with an IC_{50} value of 32.80 µg/mL.

Further examination has been performed using the pkCSM program for both xanthone and cinnamoylbenzene compounds to predict the pharmacokinetic parameters during the absorption, distribution, metabolism, and excretion processes [37]. Table 4 shows the ADMET analysis of xanthone and cinnamoylbenzene as the anticancer agents. As the initial screening, Lipinski's rule of five is applied to evaluate drug-likeness properties. Lipinski stated that a drug candidate should not have a molecular weight of more than 500 g/mol, generate less than 5 H-bond donors and 10 H-bond acceptors, and have an octanol-water partition coefficient (log P) less than 5 [38]. From these criteria, xanthone and cinnamoylbenzene do not violate all of Lipinski's rules. In contrast, doxorubicin does not fulfil three of four of Lipinski's rules, showing its unsuitability for the ADMET processes in the human body.

From the absorption prediction, cinnamoylbenzene (-4.46 log mol/L) is less soluble in water than xanthone (-3.63 log mol/L) and doxorubicin (-2.92 log mol/L). The high solubility of doxorubicin is generated from 6 H-bond donors and 12 H-bond acceptors. Unfortunately, a very soluble drug is not highly recommended due to its poor penetration on the membrane of cancer cells, as well as its rapid excretion process in the digestive system without reaching the cancer cells [37]. Nevertheless, either xanthone or cinnamoylbenzene shows a moderate solubility in the water, thus contributing to its slow excretion and preferable oral absorption from the human intestinal absorption (HIA) and Caco-2 permeability point of view. Compared to doxorubicin, the HIA percentages of xanthone (98.51%) and cinnamoylbenzene (94.98%) are higher. Furthermore, the Caco-2 permeability values of xanthone (1.22×10^{-6} cm/s) and cinnamoylbenzene (1.34×10^{-6} cm/s) are also higher than doxorubicin indicating their favorable absorption processes because Caco-2 permeability related to *in vitro* drug absorption in the human intestinal mucosa.

On the other hand, pharmacokinetic parameters during the distribution process, i.e., blood-brain barrier (BBB) permeability, steady-state volume distribution (VDss) for human, and central nervous system (CNS) permeability, were also investigated. The log BBB permeability reflects the ease of crossing the blood-brain barrier. A higher log BBB than 0.3 is considered effective in crossing the blood-brain barrier [37]. Meanwhile, VDss represents the dispersion of drugs between tissues and plasma proteins in which high (>0.45) and low (<0.15) VDss values represent a strong bind in tissues and plasma proteins, respectively [39]. The CNS parameters reflect the permeability to penetrate the central nervous system. The drug could penetrate the central nervous system if the CNS value is higher than -2; however, the drug cannot penetrate the central nervous system if the CNS value is lower than -3. Table 4 shows that only cinnamoylbenzene is effective in crossing the blood -brain barrier. Meanwhile, both xanthone and cinnamoylbenzene have moderate VDss values, indicating that they are not firmly bound to tissues or plasma proteins either. Both xanthone and cinnamoylbenzene show a CNS value higher than -2, demonstrating their ability to penetrate the central nervous system. These superior distribution pharmacokinetic parameters are not found in doxorubicin as it has a log BBB lower than 0.3, a

Table 4. ADMET analysis of xanthone and cinnamoylbenzene as anticancer agents.

Pharmacokinetic parameter	Xanthone	Cinnamoylbenzene	Doxorubicin
Lipinski rule			
Molecular weight (g/mol)	196.21	208.26	543.53
Log P	2.95	3.58	1.30×10^{-3}
H-bond donor	0	0	6
H-bond acceptor	2	1	12
Absorption			
Water solubility (log mol/L)	-3.63	-4.46	-2.92
HIA (%)	98.51	94.98	63.37
Caco-2 permeability (cm/s)	1.22×10^{-6}	1.34×10^{-6}	4.57×10^{-7}
Distribution			
log BBB permeability	0.15	0.56	-1.38
VDss for human	0.26	0.37	1.65
CNS permeability	-1.39	-1.24	-4.31
Metabolism			
CYP2D6 inhibitor	No	No	No
CYP3A4 inhibitor	No	No	No
CYP2C9 inhibitor	No	Yes	No
Excretion			
Total clearance (mL/min/kg)	0.23	0.22	0.99
Renal OCT2 substrate	No	No	No
Toxicity			
Hepatotoxicity	No	No	Yes
Immunotoxicity	No	No	Yes
Mutagenicity	No	No	Yes
Predicted LD_{50} (mg/kg)	1680	1048	205

very high VDss (1.65), and a very low CNS value (-4.31).

Table 4 also shows that xanthone and doxorubicin are not metabolized during the metabolism process as they do not inhibit CYP2D6, CYP3A4, and CYP2C9 cytochromes [40]. On the other hand, cinnamoylbenzene inhibits the function of CYP2C9 but does not inhibit the CYP2D6 and CYP3A4 cytochromes. Either xanthone or cinnamoylbenzene is not a renal OCT2 substrate; thus, they are not excreted through the kidneys but through liver and biliary clearances. Doxorubicin shows the highest total clearance because its high solubility in water. It means that doxorubicin is not distributed well in the human body (as indicated by its BBB, VDss, and CNS values), but doxorubicin is rapidly eliminated from the human body. Moreover, doxorubicin shows a lower LD_{50} value (205 mg/kg) than xanthone (1680 mg/kg) and cinnamoylbenzene (1048 mg/kg). Furthermore, doxorubicin exhibits hepatotoxicity, immunotoxicity, and mutagenicity but these toxicity issues are not found for both xanthone and cinnamoylbenzene. These toxicity parameters are related to the severe side effects of doxorubicin, i.e., liver failure, heart failure, hypertension, edema, oral sores, nausea, and fatigue, as reported previously [41]. Even though doxorubicin shows very high anticancer activities against T47D and MCF-7 cancer cell lines as presented in the *in vitro* results, the ADMET results disclose that doxorubicin is not a recommended anticancer drug due to its poor pharmacokinetic properties and its toxicity in the human body. The toxic effects of doxorubicin have been reported in some recent medical reports [42] [43].

4. CONCLUSIONS

We report the anticancer activity of xanthone and cinnamoylbenzene through *in vitro* MTT and *in silico* molecular docking studies. The *in vitro* assay reveals that cinnamoylbenzene exhibits a higher activity than xanthone against the T47D cancer cell line, while xanthone is more active against the MCF -7 cancer cell line. Cytotoxicity results show that cinnamoylbenzene is less toxic than xanthone, as shown by its higher selectivity index values (1.095–2.102). Xanthone gives a higher binding energy against EGFR protein with the key interactions to Gln767 and Met769 amino acid residues. On the other hand, cinnamoylbenzene produces a stronger binding energy in the active site of Topoisomerase II due to π-π stacked interactions with key Adenine12 and Guanine13 nitrogen bases. These computational data agree with the experimental *in vitro* assay against MCF-7 and T47D cancer cell lines, demonstrating that both compounds act as the breast anticancer agent through an inhibitory mechanism against both protein receptors. Furthermore, ADMET analysis shows that either xanthone or cinnamoylbenzene has better pharmacokinetic parameters than doxorubicin. Therefore, further research for xanthone and cinnamoylbenzene is opened to design highly active breast anticancer agents based on their structures in the future.

AUTHOR INFORMATION

Corresponding Author

Jumina Jumina — Department of Chemistry, Universitas Gadjah Mada, Yogyakarta-55281 (Indonesia);
orcid.org/0000-0003-2604-7838
Email: jumina@ugm.ac.id

Authors

Yehezkiel Steven Kurniawan — Department of Chemistry, Universitas Gadjah Mada, Yogyakarta-55281 (Indonesia);
orcid.org/0000-0002-4547-239X

Hanif Amrulloh — Department of Islamic Primary School Teacher Education, Universitas Ma'arif Lampung, Metro-34114 (Indonesia);
orcid.org/0000-0001-7458-9258

Ervan Yudha — Department of Chemistry, Universitas Gadjah Mada, Yogyakarta-55281 (Indonesia);
orcid.org/0009-0004-6955-4885

Nela Fatmasari — Department of Chemistry, Universitas Gadjah Mada, Yogyakarta-55281 (Indonesia);
orcid.org/0000-0003-0376-8923

Faris Hermawan — Research Center for Pharmaceutical Ingredient and Traditional Medicine, National Research and Innovation Agency (BRIN), Tangerang Selatan-15314 (Indonesia);
orcid.org/0009-0006-6923-4050

Anggit Fitria — Department of Chemistry, Universitas Gadjah Mada, Yogyakarta-55281 (Indonesia);
orcid.org/0009-0006-6923-4050

Harno Dwi Pranowo — Department of Chemistry, Universitas Gadjah Mada, Yogyakarta-55281 (Indonesia);
orcid.org/0000-0002-0223-5036

Eti Nurwening Sholikhah — Department of Pharmacology and Therapy, Universitas Gadjah Mada, Yogyakarta-55281 (Indonesia);
orcid.org/0000-0002-6545-8691

Author Contributions

Conceptualization, J. J., H. D. P. and E. N. S.; Methodology, H. D. P., E. N. S., F. H. and Y. S. K.; Software, H. D. P.; Validation, H. D. P. and E. N. S.; Formal Analysis, Y. S. K., H. A., N. F., E.Y., F. H. and A. F.; Investigation, J. J., H. D. P., H. A. and Y. S. K.; Resources, J. J.; Data Curation, Y. S. K., H. A., and N. F.; Writing – Original Draft Preparation, Y. S. K.; Writing – Review & Editing, J. J., H. D. P., E. N. S., Y. S. K., H. A., N. F., E. Y., F. H. and A. F.; Supervision, J. J., H. D. P. and E. N. S.; Project Administration, J. J.; Funding Acquisition, J. J.

Conflicts of Interest

The authors declare no conflict of interest.

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