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Synthesis, Biological Activity, and Molecular Docking Study of Xanthenol and Its Disproportionation Products as Anticancer and Antimalarial Agents

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Abstract

Xanthone and its derivatives are well known for their broad biological activities. This research aims to investigate the anticancer and antimalarial activities of synthesized xanthenol and xanthene compounds and to elucidate their mechanisms of action through molecular docking. The xanthenol compound was obtained through xanthone reduction with sodium triacetoxyborohydride, and xanthene synthesis was studied through three different reaction conditions: uncatalyzed and catalyzed by Lewis acid or Bronsted acid. The reduction reaction produced xanthenol in 30.50% yield, whereas the three reaction conditions produced xanthene and xanthone in 39.35–75.48% yield. An anticancer assay for Vero, WiDr, HeLa, and T47D cell lines was evaluated with a microculture tetrazolium assay, and an antimalarial activity test was examined using the heme polymerization inhibition method. The xanthene compound showed the lowest IC_{50} value (44 µg/mL) among the products in the T47D cell line. Meanwhile, the antimalarial assay showed that the xanthone compound could inhibit heme polymerization, with an IC_{50} value of 114 µg/mL. The molecular docking study revealed that the anticancer activity of xanthene occurred through the inhibition of the cyclooxygenase-2 (COX-2) enzyme, and the antimalarial activity of xanthone occurred through the inhibition of the *Plasmodium falciparum* lactate dehydrogenase enzyme. These results showed that xanthene and xanthone compounds are potential anticancer and antimalarial drugs, respectively.

Keywords: anticancer, antimalarial, xanthene, xanthenol, xanthone

Introduction

Cancer and malaria have the highest mortality rates worldwide. Global Cancer Incidence, Mortality, and Prevalence reported approximately 19.3 million new cancer cases in 2020, and the number of global cancer deaths reached 10 million cases. In Indonesia, 396,000 new cancer cases were reported in 2020, resulting in 234,000 deaths. Breast cancer had the highest number of cases in the country (66,000 new annual cases), followed by cervical cancer (37,000 new cases) and colorectal cancer (35,000 new cases) [1]. Cancer cells can evade chemotherapy effects and develop multidrug resistance (MDR) [2]. MDR mechanism could increase deoxy-ribonucleic acid repair capacity, catalyze xenobiotic metabolism, and enhance growth and genetic factors. Each of these mechanisms contributes to the decreased efficacy of commercial anticancer drugs, thus impeding cancer treatment [3]. Consequently, MDR considerably increases the number of annual deaths. Serious efforts need to be made for cancer prevention and treatment to decrease the number of cancer cases and annual deaths in the world [1].

Malaria cases reached 241 million in 2020, which was higher than those in 2019 with 227 million cases. The World Health Organization stated that the South and Southeast Asia regions contribute to approximately 2% of global malaria cases. Unfortunately, Indonesia ranked second after India, with 785,000 malaria cases in 2020 [4]. Similar to cancer treatment, malaria treatment is subjected to problems related to resistance. Some malaria parasites are resistant to commonly used antimalarial drugs, such as quinine, chloroquine, and primaquine. For example, *Plasmodium falciparum* builds resistance to quinine and chloroquine, preventing them from inhibiting heme polymerization. Resistance to standard antimalarial drugs increases the probability of malaria treatment failure [5].

Anticancer and antimalarial drugs derived from natural compounds have been developed. Garcia-Oliveira *et al.* [6] reported that most phenolic and sulforaphane compounds showed potential activity at the preclinical test stage. In addition, heterocyclic compounds exhibit various biological activities, and thus they have been employed in the development of anticancer and

antimalarial drugs [7]. Xanthone, one of the natural compounds with phenolic and oxygen-based heterocyclic structure, has been widely investigated in the medical field. Natural xanthone derivatives are well known for their chemopreventive and chemotherapeutic activities during carcinogenesis, including initiation, promotion, and progression. Ibraheem *et al*. [8] evaluated the efficacy natural xanthones in vitro and in vivo; they found that xanthone derivatives bound to heme and suppressed hemozoin formation in some red blood cell samples. However, the major drawback of utilizing natural xanthones is that their isolation and purification require complicated steps. Therefore, a synthesis method is an alternative for obtaining xanthone compounds.

In this study, we studied the anticancer and antimalarial activities of xanthenol and its disproportionation products. First, a xanthenol compound was obtained by reducing the core structure of xanthone, and then xanthene compounds were obtained through disproportionation reactions with three different methods (Figure 1). Given that ether, hydroxyl, and carbonyl groups are critical for the anticancer and antimalarial activities of organic compounds [9], the anticancer activities of xanthenol and xanthene in T47D, WiDr, and HeLa cancer cell lines were evaluated using a microculture tetrazolium (MTT) assay. In addition, the antimalarial activity of xanthenol and xanthene after the inhibition of heme polymerization was investigated, and the interaction between the synthesized compounds in the active sites of protein receptors was explored with a molecular docking study.

Experiment

Materials. Xanthone, glacial acetic acid, tetrahydrofuran, dimethyl sulfoxide, anhydrous sodium sulfate, aluminum chloride, acetic anhydride, sodium bicarbonate, and sulfuric acid, were purchased from Merck®. The chemicals were of pro-analytical quality and employed without any further purification. Other materials, such as chloroform, hydrochloric acid, and sodium borohydride, were obtained in pro-analytical quality from J.T.Baker® and Loba Chemie®. The materials used for the in vitro anticancer test were the breast cancer cell line T47D, colon cancer cell line WiDr, cervical cancer cell lines HeLa, and normal cell line Vero, Dulbecco's modified eagle medium, medium 199, fetal bovine serum, phosphate buffer solution (PBS), penicillin–streptomycin, fungizone, MTT, doxorubicin, cisplatin, 5-fluorouracil, trypsin, and sodium dodecyl sulfate. Meanwhile, quinine hydrochloride, sodium hydroxide, and hematin in pro-analytical quality (Merck®) were used for the antimalarial test.

Instrumentation. The evaporation of solvent during the purification of the synthesized products was performed using a rotary evaporator Buchi type R-114. The melting point of the product was determined using Electrothermal Melting Point 9100 apparatus. Infrared spectra were recorded using Shimadzu Prestige-21 on KBr pellets. ^{1}H-NMR and ^{13}C-NMR spectra were obtained with a JEOL JNM-ECS400 spectrometer (400 MHz for ^{1}H and 100 MHz for ^{13}C). The purity of the target compounds and their mass spectra were determined using GC-MS Shimadzu QP2010S. Mass balance (Sartorius), inverted microscope (Axiovert 25), 96-well plate (NEST), micropipette (VWR), centrifuge (Sorvall), and ELISA reader (BIO-RAD Benchmark) were used for the anticancer and antimalarial tests.

Synthesis of xanthenol. Xanthone (1.96 g, 10 mmol) was dissolved in 50 mL of tetrahydrofuran and stirred for 15 min. Then, sodium borohydride (2.27 g, 60 mmol) and glacial acetic acid (3.60 g, 60 mmol) were added to the solution. The mixture was refluxed for 12 h, and then water was added to precipitate the product. The solid obtained was filtered and dried.

Synthesis of xanthene using lewis acid. Xanthenol (0.33 g, 1.6 mmol) was dissolved in 30 mL of chloroform and stirred until a clear solution was obtained. Then, $AlCl_3$ (0.44 g, 3.0 mol) and acetic anhydride (0.25 g, 4.2 mmol) were added to the mixed solution, which was stirred for 15 min at 27 °C and refluxed for 9 h. Afterward, the mixture was poured in 20 mL of crushed ice and 0.5 mL of concentrated HCl and stirred for 1 h. The mixture was extracted with chloroform (30 mL). The organic layer was washed with $NaHCO_3$ solution and dried with anhydrous Na_2SO_4, and the solvent was removed using a rotary evaporator.

Synthesis of xanthene using bronsted acid. Xanthenol (0.26 g, 1.6 mmol) was dissolved in 30 mL of chloroform, and the mixture was stirred until a clear solution was obtained. Then, acetic anhydride (0.85 g, 8.0 mmol) was added to the mixture, which was stirred for 15 min at 27 °C. The concentrated sulfuric acid (0.1 mL) was added dropwise to the mixture, which was then refluxed for 6 h. After the reaction, the $NaHCO_3$ solution was added to neutralize the mixture. The organic layer was separated and dried over anhydrous Na_2SO_4, and the solvent was removed using a rotary evaporator.

Uncatalyzed synthesis of xanthene. Xanthenol (0.33 g, 1.6 mmol) was dissolved in 30 mL of chloroform, and the mixture was stirred until a clear solution was obtained. Then, acetic anhydride (0.25 g, 4.2 mmol) was added to the mixture, which was stirred for 15 min at 27 °C and refluxed for 14 h. The mixture was poured in 20 mL of crushed ice and stirred for 1 h, and the desired compound was extracted with chloroform (30 mL). The organic layer was washed with water and dried over anhydrous Na_2SO_4, and the solvent was removed using a rotary evaporator.

Anticancer activity. Cytotoxicity test was carried out on cancer cells with an in vitro assay. The cell lines were

thawed, trypsin was added, and the cells were counted. A cell line (100 µL) was placed in each well on a 96-well plate and incubated for 24 h. The sample (10 mg) was dissolved in 200 µL of DMSO and diluted in a culture media with a concentration of 7.81, 15.6, 31.2, 62.5, 125, 250, or 500 g/mL. The aliquots (100 µL) of each concentration were added to each well. The cell lines were incubated again for 24 h. The culture medium was removed and cleaned with PBS. The MTT solution (100 µL) was added to a 96-well plate and incubated for 4 h, and a stopper solution (100 µL) was added to each well. The plate was incubated overnight at room temperature, and the absorbance of the solution was recorded using an ELISA reader at a wavelength of 595 nm for the calculation of the IC_{50} values.

Antimalarial activity. A heme polymerization inhibition test was carried out using a previously described method [9] with some modifications. Hematin solution in 0.2 M NaOH (100 µL) was placed in an Eppendorf tube. Each sample (50 µL) at a concentration of 2.5, 1.25, 0.63, 0.31, 0.16, or 0.08 mg/mL was added to the Eppendorf tube, which already contained a hematin solution. All samples were replicated three times. Glacial acetic acid solution (50 µL) was added to an Eppendorf tube containing a hematin solution and a sample. The samples were incubated at 37 °C for 24 h. After incubation, the Eppendorf tube was centrifuged at 8,000 rpm for 5 min. The supernatant was removed, and the precipitation was washed twice with DMSO (200 µL). The precipitate was centrifugated again at 8,000 rpm for 5 min. The obtained residue was mixed with 0.2 M NaOH (200 µL). Each well contained 100 µL of NaOH solution, and the absorbance was read with an ELISA reader at a wavelength of 405 nm for the calculation of the IC_{50} values.

Molecular docking. A computer with Intel Core i3-1005G1 CPU @ 1.20GHz, 4.09 GB RAM was used for the molecular docking study. The protein structures of COX-2 and *Plasmodium falciparum* lactate dehydrogenase (P*f*LDH) proteins were downloaded from the protein data bank with PDB ID 1CX2 and PDB ID 1LDG, and their mechanisms of action as anticancer and antimalarial agents were elucidated. The structures of xanthenol and xanthene were optimized using a semi-empirical AM1 method and GaussView 5.0 software, whereas protein and native ligand structures were examined using Discovery Studio 2017 R2 software. The proteins were cleaned from residues (such as H_2O molecules or metal ions), and native ligands were separated from the target proteins. Proteins and native ligands were redocked using Autodock Vina software in a 50 Å × 50 Å × 50 Å grid box. The redocking process was carried out until the RMSD value was less than 2 Å. Molecular docking was carried out between the proteins and synthesized compounds. Afterward, the results were visualized and analyzed using Discovery Studio 2017 R2 software.

Results and Discussion

Synthesis of xanthenol. 9*H*-xanthen-9-ol was obtained as a yellowish-white solid (0.22 g, 30.50%), m.p.: 128 °C. FTIR (cm^{-1}): 3,426 (O–H str.), 2,931 (C_{sp2}–H str.), 1,458 (C=C str.), 1,265 (Ar–O–Ar str.). ^{1}H-NMR (400 MHz, CD_3OD): δ 7.55 (d, *J* = 7.6 Hz, 2H), 7.31 (t, *J* = 8.0 Hz, 2H), 7.10–7.16 (m, 4H), 6.98 (s, OH), 3.97 (s, 1H). ^{13}C-NMR (100 MHz, CD_3OD): δ 151.07, 129.66, 128.88, 123.10, 122.92, 116.02, 62.64. MS (EI): m/z calcd. for $C_{13}H_{10}O_2$ $[M + H]^+$, 198.22; found, 198.00.

Synthesis of xanthene using lewis acid. 9*H*-Xanthene was obtained as a yellowish-white solid (0.22 g, 75.48%), m.p.: 115 °C. FTIR (cm^{-1}): 2,924 (C_{sp2}–H str.), 1,458 (C=C str.), 1,265 (Ar–O–Ar str.). ^{1}H-NMR (400 MHz, CD_3OD): δ 7.73 (t, *J* = 8.0 Hz, 2H), 7.49 (d, *J* = 8.5 Hz, 2H), 7.39 (t, *J* = 7.5 Hz, 2H), 7.05 (d, *J* = 8.0 Hz, 1H), 7.01 (d, *J* = 8.0 Hz, 1H), 4.05 (s, 2H). ^{13}C-NMR (100 MHz, $CDCl_3$): δ 152.05, 151.68, 129.02, 127.74, 124.04, 123.06, 116.56, 27.97. MS (EI): m/z calcd. for $C_{13}H_{10}O$ $[M + H]^+$, 182.22; found, 182.00.

Synthesis of xanthene using bronsted acid. 9*H*-Xanthene was isolated as a yellowish-white solid (0.18 g, 39.35%), m.p.: 120 °C. FTIR (cm^{-1}): 2,924 (C_{sp2}–H str.), 1,458 (C=C str.), 1,265 (Ar–O–Ar str.). ^{1}H-NMR (400 MHz, CD_3OD): δ 7.72 (t, *J* = 8.0 Hz, 2H), 7.50 (dd, *J* = 8.5 Hz, 2H), 7.38 (t, *J* = 7.5 Hz, 2H), 7.04 (d, *J* = 8.0 Hz, 1H), 7.01 (d, *J* = 8.0 Hz, 1H), 4.04 (s, 2H). ^{13}C-NMR (100 MHz, $CDCl_3$): δ 152.03, 129.03, 127.74, 124.04, 123.06, 116.56, 27.96. MS (EI): m/z calcd. for $C_{13}H_{10}O$ $[M + H]^+$, 182.22; found, 182.00.

Uncatalyzed synthesis of xanthene. 9*H*-Xanthene was isolated as a pale-yellow solid (0.29 g, 45.67%), m.p.: 114 °C. FTIR (cm^{-1}): 2,924 (C_{sp2}–H str.), 1,458 (C=C str.), 1,265 (Ar–O–Ar str.). ^{1}H-NMR (400 MHz, CD_3OD): δ 7.73 (t, *J* = 7.8 Hz, 2H), 7.49 (dd, *J* = 8.5 Hz, 2H), 7.38 (t, *J* = 6.5 Hz, 2H), 7.04 (d, *J* = 8.0 Hz, 1H), 7.01 (d, *J* = 8.0 Hz, 1H), 4.04 (s, 2H). ^{13}C-NMR (100 MHz, $CDCl_3$): δ 152.04, 129.03, 127.5, 124.04, 123.06, 116.56, 27.96. MS (EI): m/z calcd. for $C_{13}H_{10}O$ $[M + H]^+$, 182.22; found, 182.00.

Anticancer. The anticancer activity of xanthone, xanthenol, and xanthene on Vero, HeLa, WiDr, and T47D cell lines was tested using the MTT assay. A living cell line produces an oxidoreductase enzyme that catalyzes the reduction of tetrazolium salt to formazan. The process is indicated by the change in the color of the solution from yellow to purple. Thus, the purple color indicates that many living cells are present in a solution. The IC_{50} value is the concentration value that produces 50% of the antiproliferative properties in cells. The results are presented in Table 1.

Antimalaria. The antimalarial activity of xanthone, xanthenol, and xanthene was evaluated according to the inhibition percentage of parasite growth and was indicated by the IC_{50} values obtained through probit analysis. The results are presented in Table 2.

Molecular docking. The interaction was determined with a molecular docking study, and the binding affinity of synthesized compounds on the active site of protein receptors was calculated. The interactions are illustrated in Figures 2 and 3, and the docking results are tabulated in Tables 3 and 4.

In this work, xanthone was reduced using $NaBH_4$-CH_3COOH as a reducing agent for xanthinol production. Sodium borohydride was reacted first with glacial acetic acid to produce the reducing agent sodium triacetoxy-borohydride. Owing to its selective and mild reduction properties, sodium triacetoxyborohydride is a common reducing agent for ketones and aldehydes [10]. The mild nature of sodium triacetoxyborohydride is generated in the presence of acyl as the electron-withdrawing agent and the steric effect of the acetoxy group, which stabilizes the boron–hydrogen bond. This reagent has yet to be applied to xanthenol synthesis, and the commonly used reagents are $NaBH_4$, $NaBH_4$-$NiCl_2$, and Zn-NaOH [11, 12]. Triacetoxyborohydride was used in the synthesis of xanthenol from xanthone with a reflux method. Xanthenol was obtained after 12 h. Sodium triacetoxyborohydride is more effective than other reducing agents, including $NaBH_4$, $NaBH_4$:$NiCl_2$, and Zn-NaOH, and xanthenol is not generated when $NaBH_4$ and $NaBH_4$:$NiCl_2$ are used as reducing agents [11]. Furthermore, reduction reactions using Zn-NaOH require 20 h to generate a xanthenol compound [12].

The prepared xanthenol compound was then reacted with acetic anhydride in reaction systems with Lewis acid or Bronsted acid catalysts or without catalysts. Xanthenol compound is an unstable compound under acidic conditions, and the reaction tends to produce xanthene and xanthone compounds [13]. The formed products are xanthene and xanthone compounds. Xanthenol undergoes an elimination reaction and produces a carbocation, which is a xanthylium cation [14]. Xanthylium cation reacts with another xanthenol compound to form xanthene and xanthone compounds as disproportionation products. Xanthene is the product of a reduction reaction, whereas xanthone is the product of an oxidation reaction [15]. The reactions can occur without a catalyst, but the reaction rates are relatively slow, that is, 3.26%/h. Lewis acid and Bronsted acid increased the reaction rates, and thus the yields of the products were increased to 8.39%/h (Lewis acid; 75.48%/9 h) and 6.56%/h (Bronsted acid; 39.35%/6 h). Lewis acid was more effective in inducing the disproportionation of xanthenol than Bronsted acid. Further research is required to elucidate this phenomenon.

Table 1. IC_{50} Value of the Synthesized Products Against Several Cancer Cell Line

Compound	IC_{50} (µg/mL)			
	Vero	**HeLa**	**WiDr**	**T47D**
Xanthone	123	272	>500	>500
Xanthenol	148	>500	290	>500
Xanthene	98	189	>500	44
Doxorubicin	>500	-	-	0.5
Cisplatin	-	7	-	-
5-Fluorouracil	-	-	7	-

Table 2. IC_{50} Value of the Synthesized Products Against Heme Polymerization Assay

Compound	IC_{50} value (µg/mL)
Xanthone	114
Xanthenol	7,122
Xanthene	10,012
Quinine	409

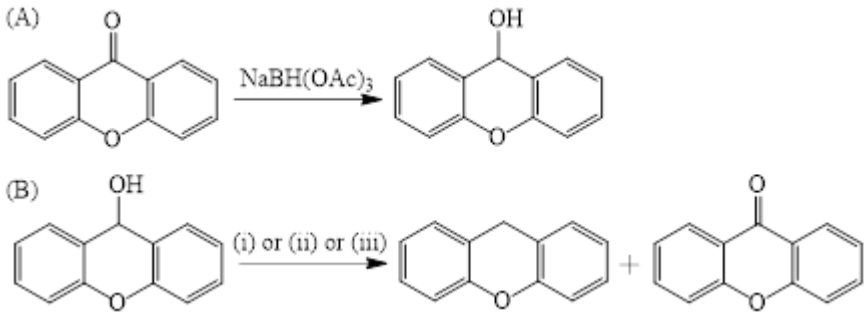

Figure 1. (A) Reduction Reaction of Xanthone; (B) The Disproportionation Reaction of Xanthenol to Form Xanthene and Xanthone Compounds; (i) $AlCl_3$ and Ac_2O; (ii) H_2SO_4 conc. and Ac_2O. (iii) Ac_2O

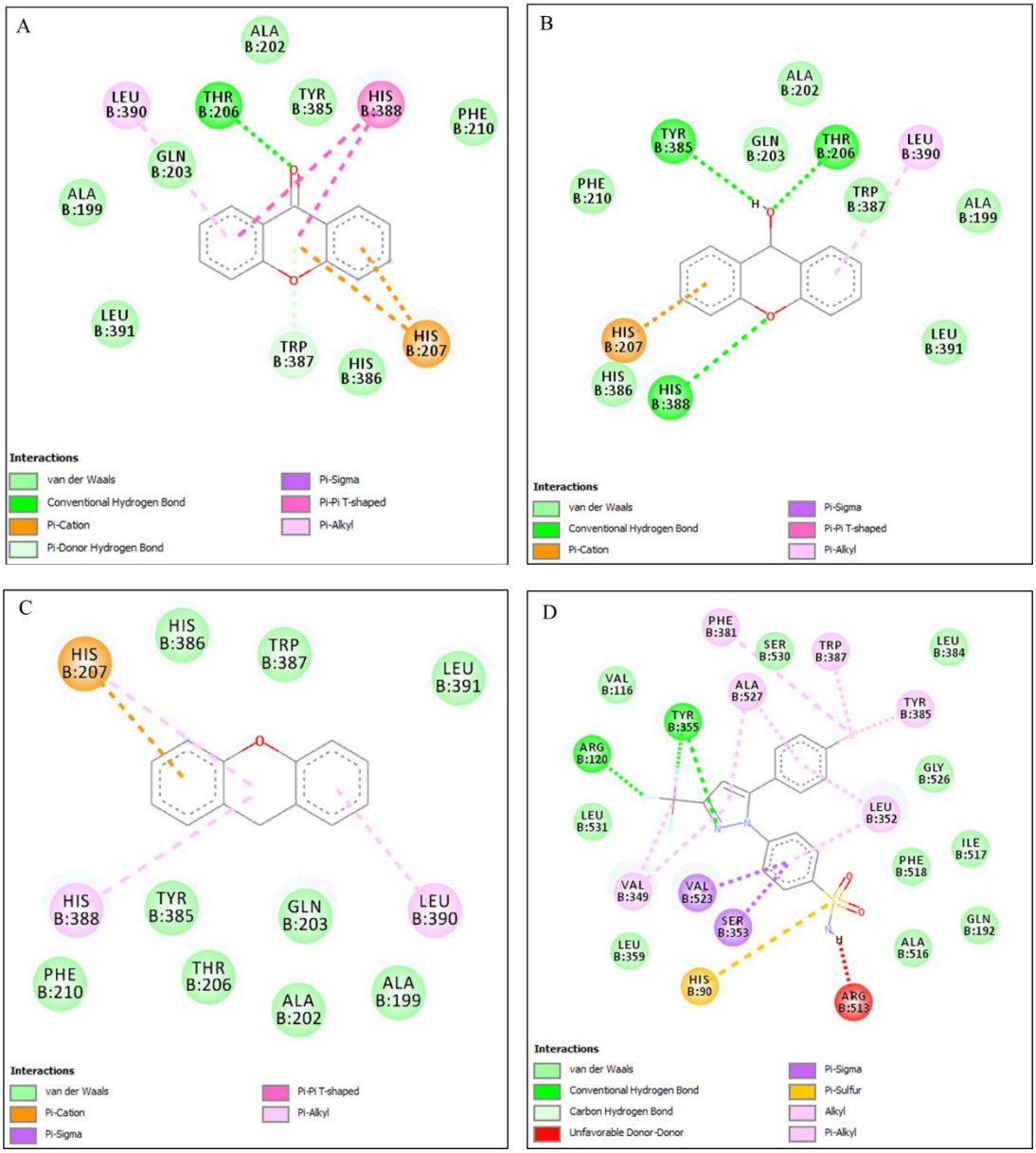

Figure 2. Interaction of the (A) Xanthone, (B) Xanthenol, (C) Xanthene, and (D) Celecoxib as Native Ligands in the Active Site of the COX-2 Enzyme

The experimental results (Table 1) show that xanthenol had a higher level of anticancer activity (IC_{50} = 290 µg/mL) than xanthone and xanthene against the WiDr cancer cell line. The xanthene compound exhibited more potent anticancer activity against the T47D (IC_{50} = 44 µg/mL) and HeLa (IC_{50} = 189 µg/mL) cell lines than xanthone and xanthenol. However, xanthene was the most toxic compound to the normal Vero cell line (IC_{50} = 98 µg/mL). Good anticancer compounds must be selective, i.e., they should have low toxicity to Vero normal cells but are highly toxic to cancer cells. This result shows that either the carbonyl or alcohol group is more effective as a safe anticancer agent than the methylene group.

The antimalarial activity assay was performed using the heme polymerization inhibition method. The heme polymerization method replicates the conditions and principles when *Plasmodium* infection occurs in living red blood cells. According to Baelmans *et al*. [16], a compound with a smaller IC_{50} value than chloroquine diphosphate (7,650 µg/mL) inhibits heme polymerization. Thus, either xanthone or xanthenol compound inhibits heme polymerization. Furthermore, the xanthone compound has a smaller IC_{50} value than xanthenol, xanthene, and positive control (Table 3). This result shows that the carbonyl group is more active in antimalarial activity than the methylene and alcohol groups.

Antimalarial compounds disrupt hydrogen bonds on the propionic acid residue of hematin. In addition to hydrogen bonding, antimalarial compounds with heteroatoms can allow coordination bonds with Fe(III) as the center metal ion, thereby inhibiting polymerization. The hydrogen bond between hematin through the propionic acid residue with Fe(III) ion causes the polymerization of hematin, which will form hemozoin. Xanthone with a C=O functional group could coordinate with the Fe(III) ion of hematin through hard–soft acid–base interaction. This phenomenon leads to high antimalarial activity comparable to that of xanthenol and xanthene compounds with similar molecular structures [17].

Xanthone derivative compounds interact with COX-2 receptors with a PDB ID of 1CX2 [18]. The final product of COX-2 can induce sialyltransferase α-2,3-sialyltransferase-3 (ST3Gal-I) expression, thereby increasing the activity of truncated sialylated glycans. Changes in glycan composition affect glycosylation. Abnormal glycosylation is one of the characteristic features of breast, colorectal, and cervical cancers. The presence of a COX-2 inhibitor is required to inhibit this activity, thus disrupting its function in these cancer cells [19].

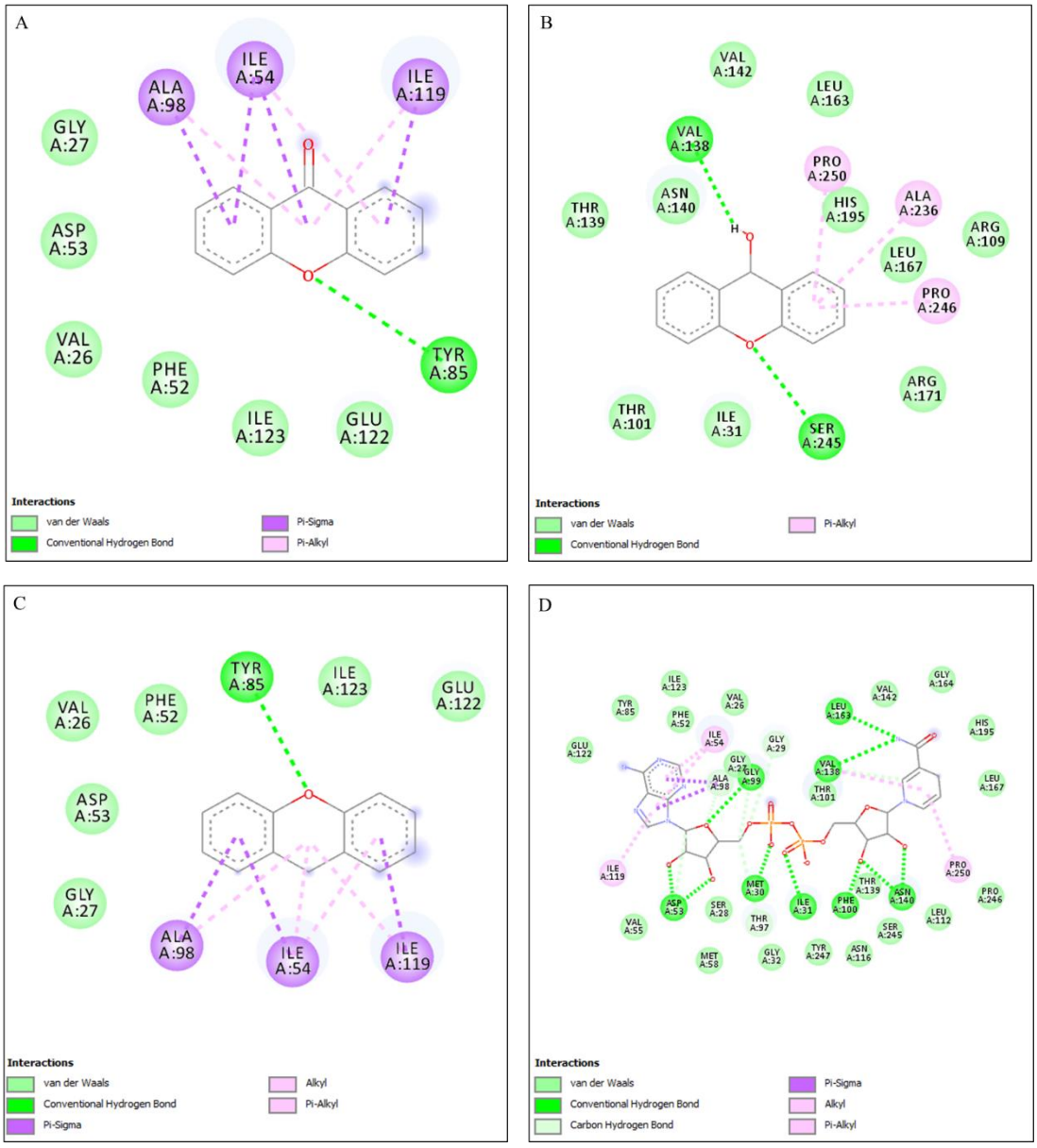

Figure 3. Interaction of the (A) Xanthone, (B) Xanthenol, (C) Xanthene, and (D) NADH as Native Ligand in the Active Site of P*f*LDH Enzyme

Table 3. Docking Results of Xanthene and Native Ligand on the Active Site of COX-2 Enzyme

Ligand	Binding Affinity (kcal/mol)	Hydrogen Bonds	Van der Waals
Xanthone	−8.5	Thr206	Ala199, Ala202, Gln203, Phe210, Tyr385, His386, Leu391
Xanthenol	−8.7	Thr206, Tyr385, His388	Ala199, Ala202, Gln203, Phe210, His386, Trp387, Leu391
Xanthene	−8.5	-	Ala199, Ala202, Gln203, Thr206, Phe210, Tyr385, His386, Trp387, Leu391
Doxorubicin	−8.3	Leu82, Lys83, Leu93, Arg120,	Pro84, Ile92, Trp100, Val116, Ser119, Leu123, Ser471
5-Fluorouracil	−6.0	Ala202	Gln203, Phe210, Tyr385, Leu390
Celecoxib	−11.0	Arg120, Tyr355	Val116, Gln192, Leu359, Leu384, Ala516, Ile517, Phe518, Gly526, Ser530, Leu531

Table 4. Docking Results of Xanthene and Native Ligand on the Active Site of P*f*LDH Enzyme

Ligand	Binding affinity (kcal/mol)	H-bond
Xanthone	−7.5	Tyr85
Xanthenol	−7.4	Val138, Ser245
Xanthene	−7.4	Tyr85
Quinine	−6.9	Thr97
NADH	−11.5	Met30, Ile31, Asp53, Gly99, Phe100, Val138, Asn140, Leu163

The interaction between protein and synthesized compounds can be predicted using a molecular docking in the same binding site as the native ligand in the three-dimensional structure of a protein receptor. After the redocking of the COX-2 enzyme, the RMSD value was 1.46 Å, demonstrating that the molecular docking method was valid (RMSD < 2.00 Å). The value of the binding affinity of xanthene was higher than the native ligand (see Table 3 and Figure 2(A)). In addition, no hydrogen bonds formed between xanthene and the active site of COX-2 enzyme. However, the van der Waals interactions with Ala202, Tyr385, and Trp387 amino acid residues may contribute to the moderate anticancer activity of xanthene. Therefore, xanthene can still be considered an anticancer agent because of its mechanism that inhibits COX-2 enzyme activity particularly in T47D cancer cells. When the function of COX-2 enzyme is disrupted, proliferation is discontinued and leads to apoptosis of cancer cells.

The antimalarial activity of xanthone was also studied using molecular docking. P*f*LDH complex with NADH and oxamate substrates has been widely used as targets for antimalarial compounds. Antimalarial drugs, such as chloroquine, interact with P*f*LDH in the NADH binding pocket. Chloroquine can prevent the formation of hemozoin by forming a complex with hematin dimers. Hematin (ferriprotoporphyrin IX) competes with NADH and interact with the P*f*LDH site and enables hemozoin formation. The formation of hemozoin allows parasites to survive [20].

After redocking, the obtained RMSD value was 1.11 Å. Table 4 and Figure 2(B) show that the binding affinity of xanthone was higher than that of the native ligand. Xanthone interacted through a hydrogen bond with Tyr85, which is a key amino acid residue in the P*f*LDH enzyme inhibition mechanism. Therefore, xanthone is an antimalarial candidate because it inhibits P*f*LDH enzyme activity and has a low IC_{50} value. The disruption of P*f*LDH enzyme activity inhibited glycolysis, leading to the death of *Plasmodium*.

Conclusion

Xanthenol and its disproportionation products (xanthene and xanthones) were successfully synthesized in this work. Although the synthesis of xanthenol disproportionation products was successfully carried out using three different reaction conditions, Lewis acid was more effective in inducing the disproportionation of xanthenol than Bronsted acid. Further, research is needed

to explain the difference between the effects of the catalysts in the reaction. Xanthenol exhibited a stronger anticancer activity (IC_{50} = 290 µg/mL) against the WiDr cancer cell line than xanthone and xanthene compounds. The xanthene compound had a higher level of anticancer activity than xanthone and xanthenol compounds against the T47D (IC_{50} = 44 µg/mL) and HeLa (IC_{50} = 189 µg/mL) cell lines. The molecular docking study reveals that xanthene could inhibit COX-2 enzyme through van der Waals interactions; thus, xanthene showed moderate anticancer activity against the T47D cell line. However, xanthene was the most toxic compound to the normal Vero cell line (IC_{50} = 98 µg/mL), showing that the methylene group as an anticancer agent has low selectivity. Xanthone gave a very strong inhibitory activity for heme polymerization with an IC_{50} value of 114 µg/mL. Meanwhile, the molecular docking results show that xanthone could disrupt the function of the P*f*LDH enzyme through a hydrogen bond with Tyr85, which may be responsible for its extremely strong antimalarial activity.

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