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Synthesized of 2,7 dihydroxyxanthone from xanthone and antimalarial activities

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Abstract: The purpose of the research is to synthesize 2,7- di-hydroxyxanthone compounds from xanthone and to evaluate antiplasmodial against activities. The synthesize of 2,7-di-hydroxyxanthone compounds worked with chromatogramphy methods including Thin Layer Chromatography (TLC), Vacuum Liquid Chromatography (VLC). A compound structures were determined based on the spectroscopic evidences including, Infrared (IR), one dimension (1-D) and two dimension (2-D) Nuclear Magnetic Resonance (NMR) spectra and comparison the spectroscopy data with related data from references. The biological properties of compounds are evaluated towards antiplasmodial against activity. The result of the product was obtained as white solid in 63.49% yield. The IR spectrum showed the absorption at 3433 cm^{-1} Which was reinforced with a sharp attack at 1087 cm^{-1} indicating the stretching of OH, while the stretching of aromatic C=C appeared at 1620 cm^{-1} . The ¹H-NMR (500MHz, and DMSO -d₆) spectrum showed that the aryl protons appeared in the region of $\delta_{\text{H}} 12.98$ ppm. In this region, there were 2 singlet at $\delta_{\text{H}} 12.98$ ppm (1H, 2-OH) and (1H,7-OH) and shows the presence of two OH groups. Based on spectroscopy analyses, it could be started that the reaction of 2.7 di-aminoxanthone with NaNO₂/HCl and H₃PO₄ produced 2.7- di-hydroxyxanthone. *In vitro* antiplasmodial assay of the product synthesized 2,7 di-hydroxyxanthones against *Falciparum* strain of 3D7 showed that the IC₅₀ values of 2,7-di-hydroxy xanthone, were 0.31 $\mu\text{g/mL}$, respectively.

1 Introduction

Malaria is global health problem in developing countries. The efforts to eliminate this disease have been doing in many ways. However, the expected results is still not given. Evenmore, malaria becomes one of threatening disease in the world. This is indicated by the increase of malaria incidence especially in the endemic area. There are several problems against malaria. The main problems is the presence of malaria vectors (mosquito) which are resistant to insecticide and the resistant malaria parasites (such as



plasmodium falciparum) to anti-malaria drugs (such as chloroquin). The parasites are widely spread almost in all endemic area at whole over the world. Therefore, such anti-malaria drugs are not effective and sensitive anymore. These reasons lead the researchers to find the new anti-malaria drugs. One of strategies is based on the development of active compounds obtained from medicinal plants, which are traditionally used by people to cure malaria [1]. From 400 of *Garcinia* plants, it was found that xanthone was the major component, beside terpenoid, benzophenone and biflavonoid. 2-hydroxy xanthone, 2,7 dihydroxyxanthone had the potential biological activities. The 2,7-di-hydroxyxanthone as antimalaria agent has not been reported.

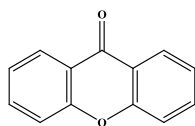


Figure 1. Structure xanthone

The efforts to find the new antimalarial drugs could be done in several ways, such as: a) synthesis of 2-hydroxyxanthone compound which could inhibit the specific metabolism of parasite, b) isolation of active compounds of natural products which are traditionally employed to cure malaria, and c) synthesis of analog compound of anti-malaria drugs, [2]. This research was initialized by isolating and identifying the xanthenes from the root of *G. Dulcis* as well as performing the anti-malaria assay [3]. However, the yield obtained was very low. Several xanthone derivatives were reported to display antimalaria activities [4]. Xanthenes could selectively inhibit the growth of *P. falciparum* in culture. Study on anti-malaria activity of xanthone derivatives showed that there was correlation between the structure and the anti-malaria activity (IC_{50}). Preliminary study on IC_{50} value (the inhibition value of *P.falciparum* growth) with semi empirical method of PM3 showed that the anti-malaria activity was correlated with the electronic properties of the substituents [5]. Xanthone and its derivatives were commonly obtained from isolation of natural products. Isolation of xanthone has been conducted from the leave [6] and bark [7] of *Garcinia dulcis*. [8] has obtained new xanthone derivatives of 7-O-methyl garcinon-E from *G. cowa* with IC_{50} of 1.50-3.00 $\mu\text{g/mL}$. Other xanthone derivatives of 1,3,7-trioxygenated and prenylated xanthone have been isolated from *Calophyllum caledonicum* [9]. The originality of this research could be seen as the synthesized for 2,7-dihydroxy xanthenes have been conducted. The *in vitro* antiplasmodium assay of the product synthesized 2,7-dihydroxyxanthenes to *P. falciparum* strain 3D7 has not been reported. This research was synthesized of 2,7 di-hydroxyxanthone and analyzed of the product synthesized 2,7dihydroxyxanthenes using UV-Vis, IR, $^1\text{H-NMR}$, $^{13}\text{C-NMR}$ spectrometers and Test *in vitro* anti-malaria assay of the synthesized 2,7-dihydroxy xanthenes against *P.falciparum*. This research was conducted with the main aims of synthesizing the 2,7-dihydroxyxanthenes and performing the *in vitro* antimalaria assay of the 2,7 di-hydroxyxanthone. The specific aims were 1) To synthesized the 2,7dihydroxy xanthone from 2,7-diaminoxanthone; 2) To analyzed the product synthesized 2,7dihydroxyxanthenes using spectroscopy method (FTIR, $^1\text{H-NMR}$, $^{13}\text{C-NMR}$, spectrometer); and 3). To conduct new antimalarial activity of the 2,7-di-hydroxyxanthone.

2. Experimentals

2.1. Material chemical compounds:

2-Hydroxy xanthone, Hydrogen Chloride (HCl), Natrium Nitride (NaNO_2), phosphoric acid, ethanol-aquades. HEPES buffer, *P. falciparum*, Gentamicin sulfate, NaHCO_3 , serum and red blood cells, Giemsa dyes.

2.2. General procedure:

Spektra of Infra Red (IR): Perkin-Elmer Spectrum One FT-IR spectrophotometers. Spektra of ^1H and ^{13}C NMR: spektrofotometer JEOL LTD ECP400, operated at 400 MHz (^1H) and 100.53 MHz (^{13}C), use acetone- d_6 as solvent and TMS as internal standard. Separation and purification used Thin Layer Chromatography (TLC), Vacuum Liquid Chromatography (VLC).

2.3. Prosedure of the synthesized of 2,7di-hydroxyxanthone

The 2,7diaminoxantone compound of 0.01 gram (0.00005 mol) was introduced into the 3-neck flask, suspended in Hydrogen Chloride (HCl) and added 10 mL of Natrium Nitride (NaNO_2) 2.8 M at 5°C until the solution changed entirely to yellow. The mixture was stirred at 5°C for 30 minutes and then added 15 ml of 1 M Hydrogen Chloride (HCl) solution and be cooled. The resulting mixture was stirred at 5°C for 5 hours, acidified with phosphoric acid. The product is recrystallized with ethanol-aquades. The obtained product was analyzed by Infrared (IR) spectrometer, one dimension (1-D) and two dimension (2-D) Nuclear Magnetic Resonance (NMR) spectra.

2.4. Prosedure Testing the effect of antiplasmodium in vitro

Testing the effect of antiplasmodium in vitro is required *P. falciparum* culture. The cultures used were 3D7, bred by the Trager and Jensen method modified by Waruyanti, [10], [11] by candle jar with RPMI 1640, HEPES buffer, Gentamicin sulfate, NaHCO_3 , serum and red blood cells. The breeding is carried out in a sticked glass candle and incubated glass exchanger in the incubator at 37°C . The medium is replaced periodically every 24 hours. The *P. falciparum* stage required for this test was a ring shaped young trophozoite obtained by synchronization in a 5% w/v sorbitol solution. The anti-plasmodium effect test of the 2,7-di-hydroxyxanton compound is performed in a microbial well. Into the micro well plate which has been given the test compound with various concentrations, given 50 μl of *P. falciparum* suspension. Incubate in incubator at 37°C for 24 hours. The results were evaluated by making the dosage form with Giemsa dyes. The number of living schizonts accounted for 200 asexual parasites, used as a criterion for the effects of antiplasmodium. From the observation results obtained IC_{50} value indicates that the test compound has activity inhibiting the growth of *P. falciparum* in vitro. Then continued data analysis with probit analysis.

3 Result and Discussion

3.1 The strategy is via Fries rearrangement of diaryl ester derivatives (Figure 2) [12].

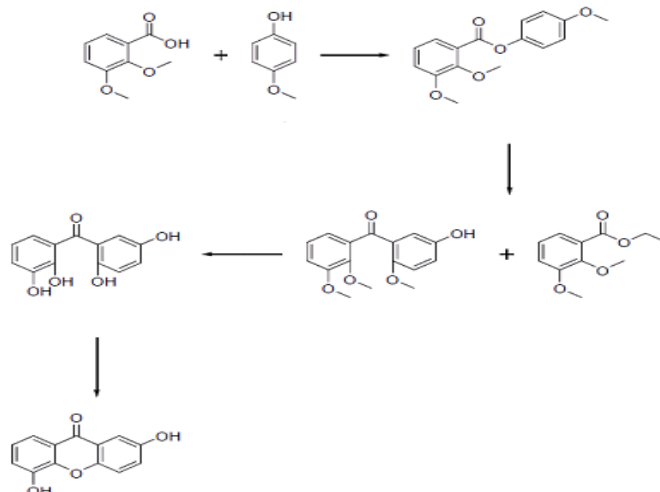


Figure 2. Synthesized of xanthone via benzophenone route

3.2 Synthesized of 2-hydroxyxanthone

The synthesized 2-hydroxyxanthone from monoaminoxanthone was the reacted with NaNO_2 , HCl , and H_3PO_4 (Figure 3).

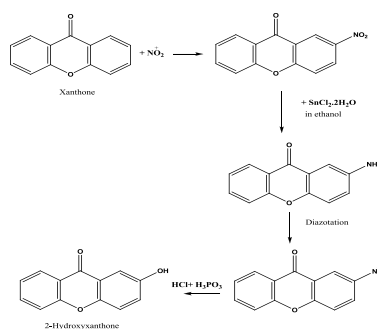


Figure 3. Synthesized of 2-hydroxyxanthone

3.3. Analyzed of 2-hydroxyxanthone

2-Hydroxy xanthone was obtained by reacting 2-aminoxanthone with NaNO_2 to produce diazonium salt. Then, hydrolyzed of the salt produce Produced 2-hydroxyxanthone. The product was obtained as white solid in 69.80% yield. The IR spectrum showed the absorption at 3433 cm^{-1} indicating the stretching of OH, while the stretching of aromatic $\text{C}=\text{C}$ appeared at 1620 cm^{-1} . The $^1\text{H-NMR}$ spectrum showed that the aryl protons appeared in the region of δ 6,97 -7,78 ppm. In this region, there were 4 doublet at δ_{H} 6.97 (H, $J = 8.3 \text{ Hz}$), 7.26 (H, $J=8.3 \text{ Hz}$), 7.37 (H, $J=8.3 \text{ Hz}$) and 7.78 (2H, $J=23\text{Hz}$) ppm as well as one doublet of doublet peak at δ 7.50 ppm (2H, dd, J and 8.3 Hz). One singlet peak from hydroxyl proton appeared at δ_{H} 12.53 ppm. Identification of the product using $^{13}\text{C-NMR}$ showed aryl carbons at δ 106, 110, 117, 137 and

155ppm. The peak at δ 113, 116, 118, 135, 136, 161 dan δ 179,2 from the carbonyl group while the peak at δ 175 ppm was the peak for the carbon (C_2) next to hydroxyl group. Based on spectroscopy analyses, it could be stated that the reaction of 2-aminoxanthone with NaNO_2/HCl and H_3PO_4 produced 2-hydroxyxanthone. The reaction mechanism was presented on Figure 4.

3.4. Synthesized 2.7- di-hydroxyxanthone

The product was obtained as white solid in 63.49% yield. The IR spectrum showed the absorption at 3433 cm^{-1} which was reinforced with a sharp attack at 1087 cm^{-1} indicating the stretching of OH, while the stretching of aromatic $\text{C}=\text{C}$ appeared at 1620 cm^{-1} . The $^1\text{H-NMR}$ (500MHz, and $\text{DMSO}-d_6$) spectrum showed that the aryl protons appeared in the region of δ 12.98 ppm. In this region, there were 2 singlet at δ_{H} 12.98 ppm (1H, 2-OH) and (1H, 7-OH), shows the presence of two OH groups. An aromatic proton appears in the intermediate region of δ 7.30-7.69 ppm i.e. 7.69 for H_1 and H_8 (2H, d, $J = 2.4\text{ Hz}$), 7.43 for H_3 and H_6 (2H, dd, $J = 2.4$ and 8.2 Hz) and 7.30 ppm for H_4 and H_5 (2H, d, $J = 8.2\text{ Hz}$).

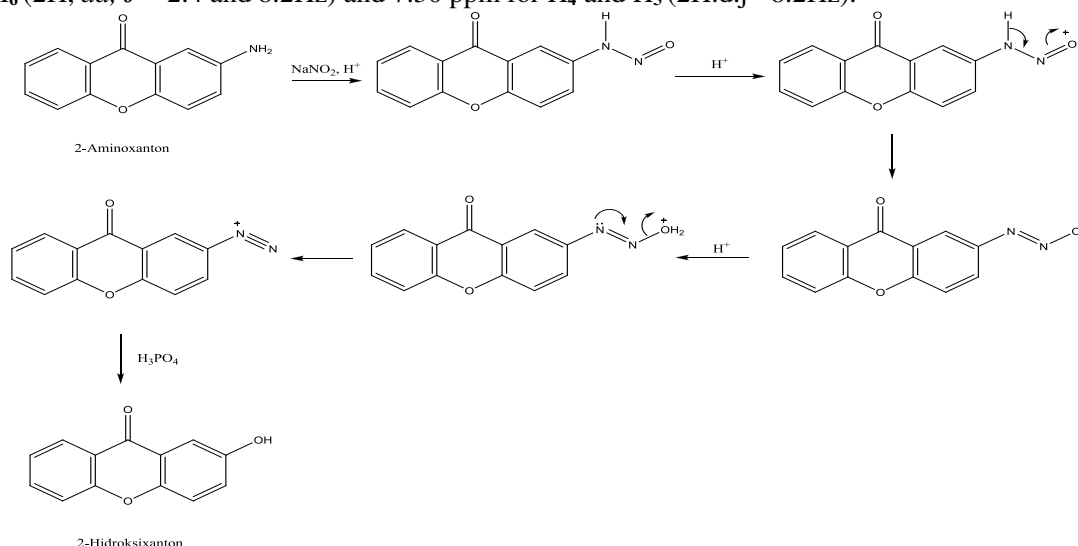


Figure 4. Mechanism of synthesized of 2-hydroxyxanthone

Identification of the product using $^{13}\text{C-NMR}$ (125MHz, CDCl_3) Shows the presence of three peaks of the CH group aryl carbons at 119.56 (2C, C_4 and C_5), 118.74 (2C, C_3 and C_6), and 117.41 ppm (2C, C_1 and C_8). 3 peaks of quaternary carbon groups appear on the region 151.44 (2C, C_9 and C_{12}), 148.58 (2C, C_2 and C_7) and 122.45 ppm (2C, C_{10} and C_{11}), 1 peak of carbonyl group $\text{C}=\text{O}$ at 179.22 ppm at C_{13}

3.5. Anti-malarial Activity Test

Malaria is a disease caused by the infection of protozoa from the genus *Plasmodium*. There are 4 species of *Plasmodium* which could cause malaria. They are *P. falciparum*, *P. vivax*, *P. malariae*, *P. ovale* ([13], [14], [15], [16], [17]). Among them, *P. Falciparum* is the most responsible to the malaria caused death. Moreover, patients could be infected by more than one species of *Plasmodium*, for example *P. falciparum* and *P.vivax* in subtropical area, *P. falciparum* and *P. malariae* in tropical country in Africa. Results of survey showed that there are 15 million case of malaria, where 70 million citizens live in the malaria endemic area. In Jawa and Bali, the case of malaria increased from 0.12 per 1,000 citizens to 0.81 per 1,000 citizens [18]. 90% in Africa, 4% in South East Asia, 4% in Mediterania and 2% in other

area)[19]. Resistance of malaria parasite to standard anti-malaria drugs of chloroquin was found in South America (Columbia and Venezuela) in 1960 [20], and followed in Thailand in 1961. the resistance of *P. falciparum* to chloroquin was reported in Kalimantan in 1974 and spread in all provinces in 1996 [21]. The situation is getting worse due to the case of resistance of *P. falciparum* to drugs of sulfadoxin-pirimetamine in 10 provinces and kina in 5 provinces in Indonesia [22]. Another resistance to mefloquin has also been reported. In fact the drug has not been utilized in Indonesia [23]. Chloroquin as the first line anti-malaria drug in the world has important role in controlling and medication of malaria. The other important factors are pharmacological and transmission factors[21]. In addition, the genetic recombination on sexual stadium between gametocyte and different strain in the mosquitoes could lead the genetic mutation [24]. Chloroquin has been considered to have activity in inhibiting the production of hemozoin on the vacuole of malaria parasites. However, the resistant mechanism of *P. falciparum* to chloroquine has not been known yet [25]. There were several hypotheses regarding the resistant mechanism, such as the change in metabolism pathway, thus the anti-malaria drug which enter the *Plasmodium* could not be metabolized properly. Characteristic of resistant parasites to chloroquin is the fast efflux, while that of sensitive parasites is it could survive against radioisotope-label-drug for longer period [26]. The resistance has forced the researchers to find new anti-malarial drugs to substitute the non sensitive anti-malaria drugs against *P. falciparum*.

The study give to synthesized 2,7-dihydroxyxanthone and biological activity as well as new antimalarial activity with $IC_{50}=0.31\mu\text{g/mL}$

4. Conclutions

According to results and discussion, it could be concluded that: 1) 2,7-di-Hydroxy xanthone which theoretically displayed antimalarial activity. 2) Reaction of 2,7-di-aminoxanthone with NaNO_2 , HCl and H_3PO_4 produced 2,7- di-hydroxyxanthone in 63.49 % yield. 3) *In vitro* antiplasmodial assay of the product synthesized 2,7-dihydroxyxanthone against *P.falciparum* strain of 3D7 showed that the IC_{50} values were 0 31 $\mu\text{g/mL}$, respectively.

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