Synthesis of 2-hidroxyxanthone from xanthone as a basic material for new antimalarial drugs

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SYNTHESIS OF 2-HIDROXYXANTHONE FROM XANTHONE AS A BASIC MATERIAL FOR NEW ANTIMALARIAL DRUGS

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ABSTRACT

Objective: The purpose of this research is to synthesize 2-hydroxyxanthone from xanthone and to evaluate its antiplasmodial activity.

Methods: The synthesis of 2-hydroxyxanthone followed the sequence of these synthetic stages, namely: 2-nitroxanthone, 2-aminoxanthone, and 2-hydroxyxanthone. The products were separated by chromatography methods including thin layer chromatography and vacuum liquid chromatography. Compound structures of the isolated products were determined based on their infrared and nuclear magnetic resonance spectra. To support these findings, the spectra were also matched to the corresponding data from literatures. The biological properties of the synthetic compound were evaluated toward Plasmodium falciparum 3D7.

Results: 2-nitroxanthone was obtained as a brownish-yellow crystal in 69.00% yield with Madhya Pradesh of 181°C. Reduction of 2-nitroxanthone using SnCl2·2H2O/hydrogen chloride produced 2-aminoxanthone as a pale-yellow solid in 60.60% yield. Finally, the desired 2-hydroxyxanthone was achieved by initially reacting 2-aminoxanthone with sodium nitride to produce diazonium salt. Then, hydrolysis of the salt yielded 2-hydroxyxanthone as a white solid in 69.81% yield. Synthesis of 2-hydroxyxanthone from xanthone had an overall yield of 38.35%. In vitro antimalarial assay against P. falciparum 3D7 showed that the half maximal inhibitory concentration value was 0.44 μg/mL.

Conclusions: An antimalarial compound (2-hydroxyxanthone) was successfully synthesized from xanthone in three steps of synthetic reactions, i.e., the formation of 2-nitroxanthone, 2-aminoxanthone, and 2-hydroxyxanthone.

Keywords: 2-nitroxanthone, 2-aminoxanthone, 2-hydroxyxanthone, in-vitro, antimalarial, Plasmodium falciparum 3D7.

INTRODUCTION

Malaria is a kind of disease, which still threatens residents in both developing and developed countries. In 2010, malaria caused 660,000 deaths, especially in children. According to the World Health Organization (WHO), malaria is actually a preventable and treatable disease. Prevention and control of this disease may decrease malaria cases in various places [1], and the WHO targets a 50% or more reduction in cases and deaths from malaria in 2000 to 2010 and 75% or more between year 2000 and 2015 [2]. Efforts to overcome malaria can be done medically and non-medically. Medical efforts, among others, are done by finding new antimalarial drug through isolation from nature and modifying it, and also synthesizing or derivatizing existing antimalarial compounds [3-5].

The natural product compounds that have anti-plasmodial potencies are lignan [6], quinolone [7], and xanthone derivatives [8]. Xanthone derivatives have potency not only as antimalarial but also as cyclooxygenase-2 inhibitor agents [9]. Tropical plants that have been used as a traditional antimalarial drug, especially in Indonesia are Alectronserratus [10], Artocarpus heterophilius, Artocarpus altius, and Artocarpus camansi [11], and also Garcinia dulcis (Guttaferae). Further study of G. dulcis, potency as antimalarial have been done by in vivo test against Swiss Webser mice [12]. Phytochemical studies show that Garcinia plants commonly produce xanthone compounds. For example, the leaves of G. dulcis produce terpenoids, benzophenone, biflavonoid, and xanthone [13]. 7-O-methylgulinone-E, a new type of xanthone was reported from Garcinia cowa [14,15]. This compound was isolated from the bark of the plant and in fact, this xanthone was found to be active as an antimalarial against Plasmodium falciparum with half maximal inhibitory concentration (IC50) values ranging from 1.50-3.00 μg/mL. Moreover, 1,3,7-trihydroxy-3-xanthoneobtained from Calophytae caleonicum had activity as antimalarial with IC50 value of 1.0 μg/mL [8].

A thoroughly study of the correlation between xanthone derivatives with antimalarial activity showed that the antimalarial activities of hydroxyxanthones were affected by the amount of hydroxyl groups in the xanthone cellonete. In general, the more hydroxyl groups attached to the xanthone framework, the better anti-plasmodial activity of the compound [8]. One of the most potent antimalarial compounds derived from xanthone compounds against P. falciparum is 2-hydroxyxanthone from G. dulcis root barks with IC50 value 0.44 μg/mL [15]. This compound has only one hydroxyl group, so that its anti-malarial activity may be increased by adding hydroxyl groups as substituents. However, the quantity of these compounds that could be isolated from natural products is very low. One way to increase the quantity is through synthesis.

The synthesis of 2-hydroxyxanthone from xanthone and its biological activity evaluation against P. falciparum strain 3D7 has not been reported yet. This article discussed 2-hydroxyxanthone synthesis and its anti-plasmodium activity evaluation. The steps of the synthesis were following this sequential order. First, xanthone was synthesized from 2-phenoxybenzoic acid, followed by preparation of 2-nitroxanthone,
then 2-aminoxanthone and finally 2-hydroxyxanthone. Furthermore, its antiplasmodial potency was evaluated toward in vitro \textit{P. falciparum} strain 3D7.

**MATERIALS AND METHODS**

**General procedures**

Thin layer chromatographic (TLC) analyses were performed on Kiesel gel 60 F254 plates from Merck. Detection was carried out under ultra violet (UV) light. Column chromatography for substance purifications was performed on silica gel 60 N, 40-50 μm. Solvents’ evaporation was performed using Iwaki Rotary Evaporator REN-1000 with reduced pressure. Perkin-Elmer spectrum one Fourier-transform infrared (IR) spectrophotometers was used to record infrared spectra. JEOL nuclear magnetic resonance (NMR) of [NJ MCA 500 MHz was utilized in analysis of \textit{H} and \textit{13C} NMR spectra. The operations of the JEOL spectrophotometer were at 500 MHz for \textit{H NMR} and at 125 MHz for \textit{13C}, using acetone-\textit{d6} as solvent and TMS as internal standard. Anti-plasmodial evaluation used the standard facilities for in vitro antimalarial test.

**Materials**

Glacial acetic acid (Merck), dichloromethane (CH$_2$Cl$_2$) (Merck), ethanol (Merck), diethylether (Merck), hydrochloric acid (Merck), Na$_2$SO$_4$ anhydrous (Merck) SnCl$_2$.2H$_2$O (Merck), sodium nitride (NaNO$_3$) (Merck), NaOH (Merck), hydrazinium mono formate (Merck), and distilled water. The materials used for in vitro plasmodium test including chloroquine diphosphate, 10% serum, Medium roswell park memorial institute 1640 and Giemsa.

**Experiments**

**Synthesis of 2-nitroxanthone**

The slurry mixture of 4.90 g (25 mol) of the xanthone in 10 mL of glacial acetic acid was reacted at 0°C (ice cooling) with 1.039 ml nitric acid (concentrated). To the reaction mixture, 1.31 mL of sulphuric acid (concentrated) was added, and the mixture was stirred for 4 hrs. The progress of the synthetic reaction was monitored with TLC in which after starting to form yellow solution, the product was tested with TLC using CH$_2$Cl$_2$ as an eluent and the RF was compared to the starting material (xanthone) RF. Once the TLC indicating there was a difference in the RF value, then the heating was stopped and let it overnight to cool to room temperature. The resulted yellow solids were filtered, and washed it in a sequential order with 2.5 mL of dichloromethane, 10 mL of water, 10 mL of ethanol, and 10 mL of diethylether. The organic layer was collected and dried with Na$_2$SO$_4$ anhydrous. After filtration, the solvents were removed through evaporation with Buchi rotary evaporator. The obtained solid was recrystallized to remove the reaction residue; finally, the desired product was obtained. Identification of the products was determined by measuring its melting point and assessing its UV-vis and IR spectra.

**Synthesis of 2-aminoxanthone**

2-Nitroxanthone (10 mg, 0.0413 mmol) was suspended in 12.5 mL of ethanol, stirred and cooled at 5°C. The cooled suspension was added drop wisely with SnCl$_2$.2H$_2$O (0.6688 g, 0.0413 mmol) in 1.25 mL hydrogen chloride (HCl) (concentrated) at maintained temperature under 100°C. After completion of the addition of SnCl$_2$.2H$_2$O, the reaction mixture was refluxed it at 80°C for 2 hrs and let it to cool to room temperature overnight. After that, it was diluted with distilled water, extracted with 15 mL dichloromethane, filtered, and extracted with 15 mL of ethyl acetate. The organic layer was dried with Na$_2$SO$_4$ anh. and after filtration the solvents were removed by evaporation. The obtained product was dried. The product was characterized using IR spectrophotometer.

**Synthesis of 2-hydroxyxanthone**

Into a three-neck flask containing 2-aminoxanthone (0.01 g, 0.0005 mol) suspended in HCl, 10 mL of NaNO$_3$ 2.8 M was added at 5°C and the solution changed entirely to yellow. The reaction mixture was stirred at 5°C for 30 minutes and added with 15 mL of HCl 1 M and then cooled. The reaction mixture was continually stirred at 5°C for 1 hr, and acidified with phosphoric acid. The product was recrystallized with ethanol-distilled water. Furthermore, the crystal was analyzed using IR and NMR spectrometer.

**In vitro antimalarial activity test**

Testing the effect of in vitro antimalarial activity on \textit{P. falciparum} 3D7 utilizing a procedure outlined by Trager and Jensen and it was modified by Waruyanti [16,17]. To determine the IC$_{50}$ (concentration of test compound which could inhibit parasitic growth by 50%), the obtained data were analyzed using probit analysis method.

**RESULTS**

Rendement of reaction steps in 2-hydroxyxanthone synthesis are displayed in Table 1.

**4HNMRdata of 2-nitroxanthone**

The 4HNMR spectrum of 2-nitroxanthone (500 MHz) $\delta$(ppm) 8.75 (2H, d, J=2.3, H, and H$_d$); 8.36 (1H, d, J=8.3, H$_d$), 8.34 (2H, dd, J=8.3, 2.3, H, and H$_d$); 7.30 (1H, d, J=8.3); 7.10 (1H, d, J=8.3, H$_d$); and 6.86 (1H, d, J=8.3, H$_d$).

**4H and 13C NMR data of 2-hydroxyxanthone**

The 4H and 13C NMR spectra of 2-hydroxyxanthone (500 MHz) $\delta$(ppm): 11.93 (1H, s, O-H); 7.56 (2H, J=8.3 Hz); 7.28 (2H, J=8.3 Hz), 7.20 (1H, s); 6.91 (1H, J=8.3 Hz); and 6.85 (1H, J=8.3 Hz). The spectrum of 13C-NMR (JEOL, JNM ECA 125 MHz showed aromatic carbons at $\delta$9; 106; 110; 117; 137 and 155 ppm. The peak at $\delta$179 came from the carbonyl group while the peak at $\delta$155 ppm was the peak for the carbon next to the hydroxyl group.

**DISCUSSION**

**Synthesis of 2-hydroxyxanthone**

Nitration of xanthone with concentrated nitric acid was conducted at 5°C. This was performed by adapting No K, Noh Y and Firdaus method [18,19]. The nitration product was a brownish-yellow crystal in 69.71% yield, with Madhya Pradesh of 181°C. The product was characterized using UV-Vis and IR spectrometers. From UV-Vis spectra, it was found that there were 2 maximum wavelengths at 215 and 311 nm. The appearance of the two peaks agreed with those of Harborne findings [20], namely, at 202-215; 254-258; 278-284; and 330-358 nm. The IR spectrum of the product showed strong absorption of carbonyl group at 1689 cm$^{-1}$, while aromatic-ether group absorbed at 1288 and 1087 cm$^{-1}$. The presence of strong absorption bands at 1543 and 1350 cm$^{-1}$ was from the vibration of nitro groups.

The nitration mechanism was displayed in Fig.1. The concentrated nitric acid acted as a direct nitrating agent. The formed nitro nium agent would be attacked by the aromatic ring of xanthone to give 2-nitroxanthone.

| Source of compound | Yielded (%) |
|-------------------|-------------|
| Xanthone          | 69.71 from xanthone |
| 2-nitroxanthone   | 78.81 from 2-nitroxanthone |
| 2-aminoxanthone   | 69.81 from 2-aminoxanthone |

**Table 1: Reaction products (%) of each reaction step**
However, the formation of nitronium ion was relatively slow, thus the yield was not optimal. For this reason a mixture of sulfuric acid and nitric acid was required the reaction rate-stopping-agent.

Conversion of aliphatic nitro group into aliphatic amino group could be carried out using boron as a reductor in THF, while aromatic nitro group was usually performed using Zn as a reductor in neutral condition [17,21] or Zn-NH$_4$Cl/ H$_2$O at 50-55°C. The reduction of nitro group could also be done using SnCl$_2$2H$_2$O/HCl [13,14]. In this research, the reduction of 2-nitroxanthone was employed 3 equivalent of SnCl$_2$.2H$_2$O in HCl per one nitro group. 2-Aminoxanthone was obtained as a pale-yellow solid in 60.60% yield. The solid product was slightly soluble in ethyl acetate. The low yield was probably due to the less solubility of product in extractor solvent and the competition with the formation of Sn(OH)$_2$. Absorption band in IR spectrum at 3093 cm$^{-1}$ confirmed the existence of the amino group of the 2-aminoxanthone. The presence of this group and the disappearance of a band at 1543 cm$^{-1}$ from nitro group, indicated that the nitro group has been completely reduced into amino group. The vibrations of C-N and C-O bonds were shown by the presence of the absorption band at 1288 and 1234 cm$^{-1}$. Based on the IR spectrum, it seemed that the desired product of 2-aminoxanthone was successfully achieved from the reduction of 2-nitroxanthone using SnCl$_2$.2H$_2$O/HCl, with 78.81% yield. The scheme of the formation of 2-aminoxanthone was presented in Fig. 2.

2-Aminoxanthone as the product of 2-nitroxanthone reduction was then reacted with NaNO$_2$, HCl, and H$_3$PO$_4$ to yield 2-hydroxyxanthone, as presented in Fig. 3.

2-Hydroxyxanthone could be synthesized from aminoxanthones with NaNO$_2$ and hydrochloric acid through diazonium salt. This compound was obtained by reacting 2-aminoxanthone with NaNO$_2$ to first produce its diazonium salt. Then, hydrolysis of the salt gave 2-hydroxyxanthone as the desired product. The product was a white solid in 69.81% yield. The IR spectrum showed that the absorption at 3433 cm$^{-1}$ indicated the stretch of OH, while the stretching of aromatic C=C appeared at 1620 cm$^{-1}$.

The spectrum of $^1$H-NMR (JEOL, JNM ECA 500 MHz), showed that the aryl protons appeared in the region of δ 6.85-7.56 ppm. In this region, there were 4 doublet at δ 6.85 (2H, $J=8.3$ Hz), 6.91 (2H, $J=8.3$ Hz), 7.28 (2H, $J=8.3$ Hz), and 7.56 (2H, $J=8.3$ Hz) ppm as well as one triplet peak at δ 7.20 ppm. One singlet peak from hydroxyl proton appeared at δ 11.93 ppm. Identification of the product using $^{13}$C-NMR (JOEL, JNM ECA 500 MHz) showed aryl carbons at δ 96, 106, 110,117, 137, and 155 ppm. The peak at δ 179 came from the carbonyl group while the peak at δ 155 ppm was the peak for the carbon next to hydroxyl group.

Based on spectroscopy analyses, it could be stated that the reaction of 2-aminoxanthone with NaNO$_2$/HCl and H$_3$PO$_4$ had successfully produced 2-hydroxyxanthone. The reaction mechanism was presented in Fig. 4.
Calculation of 2-hydroxyxanthone rendement

Their action products or rendements starting from nitration of xanthone to 2-hydroxyxanthone are shown in Table 1. The data in this Table are stoichiometric calculation results for each step of the reactions.

Synthesis of 2-nitroxanthone

Xanthone = 4.9 g = \( \frac{4.9}{196} \) = 0.025 mol

2-nitroxanthone product (theory) = 0.025 mol = 0.025 \( \times \) 241 = 6.025 g

Rendement = \( \frac{\text{Weight of product}}{\text{Weight of product (theory)}} \) \( \times \) 100% = \( \frac{6.025 g}{4.2 g} \) \( \times \) 100% = 69.71%

Synthesis of 2-aminoxanthone

2-nitroxanthone = 10 g = \( \frac{10}{241} \) = 0.0414 mmol

2-aminoxanthone product (theory) = 0.0414 mmol = 0.0414 \( \times \) 211 mg = 8.7354 mg

Product (2-aminoxanthone) from synthesis = 6.884 mg

Rendement = \( \frac{\text{Weight of product}}{\text{Weight of product (theory)}} \) \( \times \) 100% = \( \frac{6.884 g}{8.7354 g} \) \( \times \) 100% = 78.81%

Synthesis of 2-hydroxyxanthone

2-aminoxanthone = 10 mg = \( \frac{10}{211} \) = 0.0473 mmol

Rendement = \( \frac{\text{Weight of product}}{\text{Weight of product (theory)}} \) \( \times \) 100% = \( \frac{10.0276 g}{7.0 mg} \) \( \times \) 100% = 69.81%

In general, synthesis of 2-hydroxyxanthone from xanthone can produce 38.35% of 2-hydroxyxanthone which came from 69.71% of 2-nitroxanthone, 78.81% of 2-aminoxanthone, and 69.81% of 2-hydroxyxanthone.

Evaluation of antimalarial activity

The in vitro antimalarial assay of 2-hydroxyxanthone was conducted using microscopic method with the incubation time of 48 hrs. The assay was carried out against *P. falciparum* strain of 3D7. The parasite was sensitive to chloroquine diphosphate. The parasite inhibitory activity was reported as IC \(_{50}\). The antimalarial assay against *P. falciparum* was performed using candle jar methods [15,21]. The parasite culture was cultivated and then employed in the assay. From the *in vitro* assay, the IC \(_{50}\) value could be obtained. This value was the parameter indicating the potential of the 2-hydroxyxanthone as new antimalarial. In the antimalarial assay, the synthesized 2-hydroxyxanthone was dissolved in dimethyl sulfoxide (DMSO) to give concentrations of 10, 1, 0.1, 0.01, and 0.001 µg/mL. The concentration of DMSO used was less than 0.5% as such concentration would not affect the parasite growth [19]. Distilled water was used as the negative control and used as the comparison in the calculation of parasite inhibitory activity of xanthone derivatives. In addition, chloroquine diphosphate was used as the positive control [16,22] since *P. falciparum* strain of 3D7 was sensitive to chloroquine diphosphate. Moreover, the additional reason is that the drug is still applied as standard antimalarial drug in Indonesia.

The assay was performed in 48 hrs as the asexual cycle of parasite in blood. In this phase, clinical symptoms of malaria such as fever and anemia were observed. Synchronization was carried out to make the parasite stadium to be the same. The inhibitory activity of each concentration was determined by calculating the inhibitory percentage of the test compound to the growth of *P. falciparum*. Parasitemia percentage was the amount of infected eritrosit compared to the total
eritrosit. The total eritrosit was the amount of eritrosit in the field of view. The calculation of the average parasitemia percentage was required to calculate the parasite inhibitory percentage to the negative control using statistical method of probit analysis and finally to give IC₅₀ value. Based on the results obtained from in vitro antimalarial assay, it showed that the IC₅₀ value of 2-hydroxyxanthone was 0.44 μg/mL, which is better than IC₅₀ value of xanthone (0.68 μg/mL) as shown in Table 2.

**CONCLUSION**

According to the results and discussion, it could be concluded that: (1) Synthesis of 2-hydroxyxanthone from xanthone was successfully done in three steps of synthetic reactions: 2-nitroxanthone, 2-aminoxanthone, and 2-hydroxyxanthone. These reactions had an overall yield of 38.35%. In vitro antiplasmodial assay of 2-hydroxyxanthones against *P. falciparum* strain of 3D7 showed that the IC₅₀ values of 2-hydroxyxanthone is 0.44 μg/mL.

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