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·H2O/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 antiplasmodial 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 activity, 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 antiplasmodium through isolation from nature and modifying it, and also synthesizing or derivatizing existing antiplasmodium 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 antiplasmodium but also as cyclooxygenase-2 inhibitor agents [9]. Tropical plants that have been used as a traditional antimalarial drug, especially in Indonesia are Alstoniaserratus [10], Artocarpus heterophyllus, Artocarpus altulis, and Artocarpus camansi [11], and also Garcinia dulcis (Guttiferae). Further study of G. dulcis, potency as antimalarial have been done by in vivo test against Swiss Webster 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-methylgulonin-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-4-prenyl-xanthone obtained from Calophytae caledonicum had activity as antimalarial 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 antimalarial potency was evaluated toward *in vitro* *P. falciparum* strain 3D7.

**MATERIALS AND METHODS**

**General procedures**

Thin layer chromatographic (TLC) analyses were performed on Kiesel gel 60 F$_254$ 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 JNM ECA 500 MHz was utilized in analysis of $^1$H and $^{13}$C NMR spectra. The operations of the JEOL spectrophotometer were at 500 MHz for $^1$H NMR and at 125 MHz for $^{13}$C, using acetone-$d_6$ as solvent and TMS as internal standard. Anti-malarial evaluation used the standard facilities for *in vitro* antiplasmodium test.

**Materials**

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

**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 reacted at 0°C (ice cooling) with 1.039 ml nitric acid (concentrated). The nitration mechanism was displayed in Equation 1. The concentrated nitric acid acted as a direct nitrating agent. The formed nitronium agent would find its way to the xanthone ring.

The IR spectrum of the product showed strong absorption of carbonyl group at 1689 cm$^{-1}$, while aromatic-ether group absorbed at 1288 cm$^{-1}$. The presence of strong absorption bands at 1543 and 1530 cm$^{-1}$ were characteristic of nitro group.

<table>
<thead>
<tr>
<th>Source of compound</th>
<th>Yiled (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Xanthone</td>
<td>69.71 from xanthone</td>
</tr>
</tbody>
</table>
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 to increase 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-NH₄Cl/ H₂O at 50-55°C. The reduction of nitro group could also be done using SnCl₂·2H₂O/HCl [13,14]. In this research, the reduction of 2-nitro-xanthone was employed using 3 equivalent of SnCl₂·2H₂O 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 absence 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 bands at 1288 and 1234 cm⁻¹, respectively. Based on the IR spectrum, it seemed that the desired product of 2-aminoxanthone was successfully achieved from the reduction of 2-nitroxanthone using SnCl₂·2H₂O/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 NaNO₂, HCl, and H₃PO₄ to yield 2-hydroxyxanthone, as presented in Fig. 3.

2-Hydroxyxanthone could be synthesized from aminoxanthenes with NaNO₂ and hydrochloric acid through diazonium salt. This compound was obtained by reacting 2-aminoxanthone with NaNO₂ 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 ¹H-NMR (JEOL, JNM ECA 500 MHz), showed that the aryl protons appeared in the region of δ 6.65-7.56 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 ¹³C-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 NaNO₂/HCl and H₃PO₄ 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**

\[
\text{Xanthone} = 4.9 \text{ g} = \frac{4.9}{196} = 0.025 \text{ mol}
\]

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

Product (2-nitroxanthone) from xanthone (product of synthesis) = 4.2 g

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

**Synthesis of 2-aminoxanthone**

\[2\text{-nitroxanthone} = 10 \text{ g} = \frac{10}{241} = 0.0414 \text{ mmol}\]

2-aminoxanthone product (theory) = 0.0414 mmol × 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 \text{ g}}{8.7354 \text{ g}} \times 100\% = 78.81\%

**Synthesis of 2-hydroxyxanthone**

\[2\text{-aminoxanthone} = 10 \text{ mg} = \frac{10}{211} = 0.0473 \text{ mmol}\]

Product (2-hydroxyxanthone) from synthesized (theory) = 0.0473 mmol × 212 = 10.0276 mg

Product 2-hydroxyxanthone from 2-aminoxanthone = 7.0 mg

Rendement = \(\frac{\text{Weight of product}}{\text{Weight of product (theory)}} \times 100\% = \frac{7.0 \text{ mg}}{10.0276 \text{ 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 erythrocyte compared to the total.
Heme Polymerization Inhibitory Activity (HPIA) of Asian J Garcinia dulcis Planas. Plasmodium falciparum inhibited by 2-hydroxyxanthone is 0.44 μg/mL. In vitro antiplasmodial assay of 2-hydroxyxanthones against P. falciparum strain 3D7 showed that the IC\textsubscript{50} values of 2-hydroxyxanthone is 0.44 μg/mL. 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%. (2) In vitro antiplasmodial assay of 2-hydroxyxanthones against P. falciparum showed that the IC\textsubscript{50} value of xanthone (0.688 μg/mL) as shown in Table 2. 

**Table 2: IC\textsubscript{50} value of some compounds against P. falciparum**

<table>
<thead>
<tr>
<th>Compound</th>
<th>% Inhibition of test dose (μg/mL)</th>
<th>IC\textsubscript{50} (μg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10</td>
<td>1</td>
</tr>
<tr>
<td>Xanthone</td>
<td>63.01</td>
<td>55.17</td>
</tr>
<tr>
<td>2-hydroxyxanthone</td>
<td>69.35</td>
<td>20.98</td>
</tr>
<tr>
<td>Chloroquine diphosphate</td>
<td>88.55</td>
<td>75.32</td>
</tr>
</tbody>
</table>

IC\textsubscript{50}: Half maximal inhibitory concentration, P. falciparum: Plasmodium falciparum

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%. (2) In vitro antiplasmodial assay of 2-hydroxyxanthones against P. falciparum strain 3D7 showed that the IC\textsubscript{50} values of 2-hydroxyxanthone is 0.44 μg/mL.

ACKNOWLEDGMENTS

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