Synthesis of 5-(4-bromobutyl)-2,3-dimethyl-1,4-benzoquinone and the Solubility Test in n-Octanol/Water Partition System

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Abstract. The modification structure of 1,4-benzoquinone is fascinating in the frame of drug design. The solubility properties of a new drug are important, especially for oral drug administration. The 2-isopropyl-5-methyl-1,4-benzoquinone (known as thymoquinone) which is the major constituent in Nigella sativa seed extract is known as an active compound with poor lipophilicity. Here, we reported the synthesis of 5-(4-bromobutyl)-2,3-dimethyl-1,4-benzoquinone which analog with thymoquinone having higher lipophilicity. The synthesis proposed by oxidation reaction of 2,3-dimethyl-1,4-hydroquinone (1) with H₂SO₄ followed by bromoalkylation reaction using bromopentanoic acid in the presence of AgNO₃ and (NH₄)₂S₂O₈. The oxidation product 2,3-dimethyl-1,4-benzoquinone (2) and alkylated-quinone 5-(4-bromobutyl)-2,3-dimethyl-1,4-benzoquinone (3) were obtained in 52.64% and 5.85%, respectively. The FTIR analysis of 3 showed the additional C-Br stretching at 562 cm⁻¹. The solubility test in n-octanol/water system using HPLC gave the log P value of 5-(4-bromobutyl)-2,3-dimethyl-1,4-benzoquinone (3) is 2.99, higher than thymoquinone. By this result, it is suggested that the modification of 1,4-benzoquinone by bromo alkylating agent increased the solubility of the compound.

1. Introduction
The development of herbal medicines in Indonesia is currently increasing since Indonesia has large biodiversity for the medicinal plant. One of the herbal medicines is black cumin or Nigella sativa. Nigella sativa was found in Mediterranean countries and Southeast Asia, including Indonesia. Nigella sativa seed oil was reported to contain with 2-isopropyl-5-methyl-1,4-benzoquinone, trivially known as thymoquinone (18.4-24%). Thymoquinone is an active ingredient that was reported to have hepatoprotective through antioxidant and anti-inflammatory properties [1]–[3].

A new drug design should be considered the bioavailability issue, involved the balance of lipophilicity and hydrophobicity. Drugs should be able to penetrate the membrane cell and then give the biological effects. In previous research, oral administration of thymoquinone in rabbits showed a rapid elimination and slow absorption rate in the body. It also reported that the partition coefficient (log P) of thymoquinone is 2.55 [4]. According to Lipinsky et al., drug candidates should have log P less than 5 [5]. This is the main reason for drug development in the first step.

The bioavailability of a drug candidate can be improved by modifying the chemical structure. Antonenko and the groups (2008), reported the modification of quinone derivatives by the addition of an alkyl group. The results of the modification showed increasing lipophilicity and antioxidant activity in the mitochondrial tissue [6]. In accordance with this report, Ulfa et al. published the bromoalkylation
or alkyl phosphonium substitution into 1,4-benzoquinone. It is reported that the synthesized compound has an increase in its lipophilicity. The in-silico determination suggested the activity of 1,4-benzoquinone derivatives, such as, 3-(10-bromodecyl)-5-isopropyl-2-methyl-1,4-benzoquinone, 5-(7-bromoheptyl)-2,3-dimethyl-1,4-benzoquinone, and 5-(10-bromodecyl)-2,3-dimethyl-1,4-benzoquinone have the ability to work as anticancer drug candidate [7]–[10].

Based on this background, the synthesis of 5-(4-bromobutyl)-2,3-dimethyl-1,4-benzoquinone (3) was carried out. The structure of 3 is the mimic of thymoquinone with the longer alkyl group in order to increase the lipophilicity. The solubility of 3 in n-octanol/water system is determined and compared with thymoquinone.

2. Material and method

2.1. Material and instrumentation

2,3-Dimethyl-1,4-hydroquinone (1), bromopentanoic acid, (NH$_4$)$_2$SO$_4$ by Sigma Aldrich Singapore. KBrO$_3$, Na$_2$SO$_4$, AgNO$_3$ anhydrous by Merck, Singapore. A solution of H$_2$SO$_4$ 2.5 M was prepared by diluted 98% H$_2$SO$_4$ from Smartlab, Indonesia. The solvents chloroform, n-hexane, diethyl ether, ethanol, acetonitrile, and n-octanol by Merck.

The instruments used are Ohaus analytical balance, IKA RV 10 digital rotary evaporator, Orbital Shake Brand Edmund Buhler SM 25, FTIR spectrophotometer Shimadzu 8400S, spectrophotometer UV-Vis double beam Shimadzu 1600, $^1$H-NMR spectrometer Varian 600 MHz, and LC-20AD Prominence HPLC spectrophotometer from Shimadzu.

2.2. Method

2.2.1. Synthesis of 2,3-dimethyl-1,4-benzoquinone (2). The synthesis of 2,3-dimethyl-1,4-benzoquinone was carried out according to the previously published method [7], by reacting 9 mmol of 2,3-dimethyl-1,4-hydroquinone with 3 mmol of KBrO$_3$, 9 mL H$_2$O and 0.45 mL H$_2$SO$_4$ 2.5 M was added while stirring at temperatures 40-50°C for 60 minutes. After completion, the reaction mixture was extracted using diethyl ether. The organic layer is separated and dried using anhydrous Na$_2$SO$_4$ then vacuum evaporated. The crude product was purified by column chromatography using SiO$_2$ column with eluent n-hexane: CHCl$_3$ (7:3 (v/v)). The product was analyzed using TLC, UV-Vis spectrophotometer, FTIR spectrophotometer, and $^1$H-NMR.

2.2.2. Synthesis of 5-(4-bromobutyl)-2,3-dimethyl-1,4-benzoquinone (3). The synthesis of 3 is carried out by firstly reacting (2.1 mmol) of bromopentanoic acid and AgNO$_3$ (1 mmol) in 7 ml of the mixture of acetonitrile and water (2:1 v/v), then stirred until 90°C. After that, added with the solution of 2,3-dimethyl-1,4-benzoquinone (2) (2 mmol) and (NH$_4$)$_2$SO$_4$ (2 mmol) in 3 mL H$_2$O added dropwise to the mixture while stirring followed by heating at 80-90°C for 3 hours. The reaction mixture then extracted with diethyl ether, dried the organic layer using Na$_2$SO$_4$ anhydrous. The solvent was evaporated with a rotary evaporator. The product was purified by SiO$_2$ column chromatography using n-hexane: CHCl$_3$ (7:3, v/v). The product was analyzed using TLC, UV-Vis spectrophotometer, FTIR spectrophotometer.

2.2.3. Solubility test in n-octanol/water system. The solubility test is perform followed Andres methods by some modification [11]. Prior to the solubility test, n-octanol and water system (phosphate buffer pH 7.4) were prepared by saturation. Phosphate buffer solution pH 7.4 made by mixing 67 mL of 0.1 M Na$_2$HPO$_4$, 0.4 mL mixed with 330 mL Na$_2$HPO$_4$, 0.73 H$_2$O 0.1 M, adjusted to pH of solution 7.4. The water system was saturated with n-octanol. The saturation is carried by taking a 20 mL water phase and added dropwise with n-octanol, shaking in separating funnel, and lay aside until complete separation then separated the water phase from an n-octanol layer. The n-octanol was also saturated with water with a similar procedure. The saturated n-octanol and water phase are then put in a separating funnel, shaken and then allowed to stand for at least 24 hours.
The synthesized compound 3 (10 mM) and thymoquinone are dissolved in saturated n-octanol, then the saturated aqueous phase is added with the volume ratio 3:7 (n-octanol: water). This solution is shaken with an orbital shaker for 1 hour at room temperature (200 rpm). The n-octanol phase and water phase are separated. The concentration in each solution is measured using HPLC methods.

2.2.4. Measurement of solubility test using HPLC. The HPLC analysis was carried out by injecting 2 µL of the sample into the HPLC instrument with a flow rate of 0.5 mL/min. The detector is set 254 nm for thymoquinone and 259 nm for compound 3. The column temperature is set at 37°C using isocratic phase acetonitrile:water (70:30 (v/v)) and 0.5% of acetic acid. The log P was calculated by equation (1), where \( C_{\text{octanol}} \) and \( C_{\text{water}} \) represent the total drug concentration in octanol and water phase, respectively. Thus, total drug concentration is derived by peak areas of compound 3 in octanol (\( A_{\text{o}} \)) and in water (\( A_{\text{w}} \)) and the volumes of octanol (\( V_{\text{o}} \)) and in water (\( V_{\text{w}} \)) partition [11].

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\log P = \log \frac{C_{\text{octanol}}}{C_{\text{water}}} = \log \frac{A_{\text{o}}/V_{\text{o}}}{A_{\text{w}}/V_{\text{w}}}
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3. Result and discussion

3.1. Synthesis of 2,3-dimethyl-1,4-benzoquinone (2)

![Scheme 1. The synthesis of 1,4-benzoquinone derivatives.](image)

| Compound                                      | Rf   | Wavelength (nm) | Yield (%) |
|-----------------------------------------------|------|-----------------|-----------|
| 2,3-dimethyl-1,4-hydroquinone (1)             | 0.01 | 204             | -         |
| 2,3-dimethyl-1,4-benzoquinone (2)             | 0.18 | 245             | 52.64     |
| 5-(4-bromobutyl)-2,3-dimethyl-1,4-benzoquinone (3) | 0.23 | 259             | 5.85      |

a) TLC analysis, mobile phase n-hexane: chloroform (7:3)  
b) UV-Vis analysis, ethanol solvent

The synthesis of 2,3-dimethyl-1,4-benzoquinone (2) was conducted by oxidation 2,3-dimethyl-1,4-hydroquinone (1) using \( \text{H}_2\text{SO}_4 \) and \( \text{KBrO}_3 \). Compound 2 was obtained as yellow solid in 52.64% by comparing the experimentally obtain product with the theoretically calculated product. The thin layer chromatography analysis showed a greater \( R_f \) value of 2 compared to starting material 1. It is suggested that compound 2 has low polarity compared to 1. Analysis using UV-Vis spectrophotometer gave a longer wavelength for compound 2. The existence of electronic excitation from \( n\rightarrow\pi^* \) and \( \pi\rightarrow\pi^* \) should influence the bathochromic shift for compound 2. The analysis using the FTIR spectrophotometer (Figure 1) showed the formation of carbonyl groups (C=O) at 1657 cm\(^{-1}\). The disappearance of the hydroxyl group at 3262-3225 cm\(^{-1}\) showed that the oxidation of hydroquinone 1 into benzoquinone 2 is successful [7].
Figure 1. FTIR spectrum of 1 and 2.

Analysis using $^1$H-NMR gave two proton character. The peak at $\delta = 2.04$ ppm (s, 6H) designated for methyl group and $\delta = 6.72$ ppm (s, 2H) should be the hydrogen attached in the quinone ring. The $^1$H-NMR spectrum of the oxidation product 2 is shown in Figure 2.

Figure 2. $^1$H NMR of 2 in CDCl$_3$ (400 MHz).

3.2. Synthesis of 5-(4-bromobutyl)-2,3-dimethyl-1,4-benzoquinone (3)
The 5-(4-bromobutyl)-2,3-dimethyl-1,4-benzoquinone (3) was synthesized from previously prepared 2,3-dimethyl-1,4-benzoquinone (2) by bromoalkylation reaction using bromopentanoic acid. The obtained compound 3 is a yellow liquid in a 5.85% yield. The product was analyzed using TLC, UV-Vis, and FTIR spectrophotometer. TLC analysis showed that compound 3 has a greater $Rf$ value than 2, showed the product 3 has more non-polar properties. The UV-Vis analysis of 3 gave a greater
The addition of alkyl in the beta position of the quinone ring is equal to 12 nm [11]. The additional wavenumber at 562 cm$^{-1}$ showed the absorption of the halogen functional group (C-Br) (Figure 3). Based on the overall analysis, it can be suggested that the substitution of bromoalkyl is successful [7].

Figure 3. FTIR spectrum of 2 and 3.

3.3. Solubility test in n-octanol/water partition system

The solubility test was carried out using the HPLC method. The HPLC profile of compound 3 in n-octanol and in water showed in Figure 4. The area in n-octanol and water was noted in Table 2.

Figure 4. HPLC profile of compound 3 (left) and thymoquinone (right) in n-octanol/water phase.

The HPLC profile showed that there was a partition of compound 3 from the n-octanol system (blue) into the water system (red). Compared to the partition between compound 3 and thymoquinone (TQ), it showed that the partition of 3 is larger than TQ. Quantitative analysis using log P calculation gave the log P value of compound 3 is 2.99 slightly higher than TQ that is 2.21. According to computational calculation using Hyperchem software, it showed a similar trend, that is, log P of compound 3 is higher compared with TQ. This calculation prediction supports the in vitro result, suggested that the addition of bromo alkylating agent into 1,4-benzoquinone skeleton supported the increasing of lipophilicity [7].
Table 2. Solubility test analysis.

| Compound                  | The area in octanol (A_o)a | The area in water (A_w)a | log P  | In vitroa | Predictionb |
|---------------------------|-----------------------------|--------------------------|--------|-----------|-------------|
| 3                         | 36350375                    | 87531                    | 2.99   | 2.21      | 2.80        |
| Thymoquinone (TQ)         | 63970392                    | 920323                   |        |           |             |

a) HPLC analysis
b) Predicted using Hyperchem software

4. Conclusion
The synthesis of 2,3-dimethyl-1,4-benzoquinone (2) and 5-(4-bromobutyl)-2,3-dimethyl-1,4-benzoquinone (3) was successful to carried out using oxidation followed by bromoalkylation reaction. The synthesized compound then analyzed using the HPLC method to determine the solubility in n-octanol and water system. The log P value for compound 3 is 2.99. This result is higher than thymoquinone, that is, 2.21. Based on this result, it is suggested that compound 3 which has an additional bromo-aliphatic chain compared to thymoquinone have an increasing lipophilicity which might contribute to the ability of the compound 3 to penetrate in the lipid bilayer, then it can be promoted to be a drug candidate.

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