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Effect of methyl substituent on the solubility of 1,4-benzoquinone derivatives in n-octanol/water system

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Abstract

The solubility of the compound is a crucial task for new drug design. Quinone is a promising candidate to develop as a new drug. In this research, the synthesis of 1,4-benzoquinone derivatives, that is, 2-(5-bromoamyl)-3,5-dimethyl-1,4-benzoquinone (**2a**) and 2-(5-bromoamyl)-5-methyl-1,4-benzoquinone (**2b**) were carried out by decarboxylation and insertion reaction of alkyl bromides. The product **2a** and **2b** are purified using SiO_2 gel column chromatography and analyzed by UV-Visible, FT-IR, and NMR. The yield of **2a** is 13.75%, and **2b** is 4.04%. The solubility of **2a** and **2b**, expressed by log P, is measured in the n-octanol/water (3:7 (v/v)) system by the shake flask method. The log P of **2a** and **2b** are 2.99 and 1.36, respectively. It is showed that the log P of **2a** is higher compared to **2b**. The presence of two methyl substituents on the quinone ring of **2a** supports the increase of hydrophobicity of the compound in the n-octanol/water system.

1. Introduction

Quinone is ubiquitous natural compounds which have fascinating chemical and biological properties. One of their derivatives is 2-isopropyl-5-methyl-1,4-benzoquinone (known as thymoquinone/TQ), which is the major constituent in *Nigella sativa* seed essential oil. The structure of the TQ is depicted in **Figure 1**. There are many reports about the activity of TQ, such as anti-inflammatory, antidiabetic, and anticancer [1, 2, 3, 4]. The problem arising from TQ for drug candidates is the rapid elimination and relatively slower absorption when consumed by oral [5]. These properties are related to the structure-activity relationship (SAR) of TQ and the receptor in the body [6]. The presence of the alkyl group is responsible for increasing the hydrophobicity or lipophilicity. By modifying hydrophobicity, the penetration of the compound to the cell membrane is easier. Then, the binding of the compound with the receptor is increased. The addition of alkyl halide, methoxy, and the ionic group is reported to increase the lipophilicity of quinone derivatives based on in-silico approach [7, 8, 9].

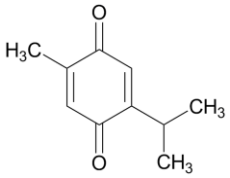


Figure 1. Structure of thymoquinone

Modification of structure should follow Lipinski rules, i.e., the molecular weight is under 500, hydrogen donor less than 5, and hydrogen acceptor not more than 10 [10]. Several structural modifications have been reported. Antonenko *et al.* [11] reported the addition of an alkyl group with an ionic counterpart. In this report, alkyl substituent increased the lipophilicity, and the phosphonic ion connected with terminal carbon increased the ion channel. The insertion of cationic phosphonyl derivatives into 1,4-benzoquinone was smoothly penetrated the mitochondrial cell wall and increased the antioxidant effect [11]. Following that result, our groups reported the addition of alkyl bromine into 1,4-benzoquinone framework and predicted the activity for anticancer, antidiabetic, and antioxidant using macromolecules model protein by in-silico approach [7, 8]. The result showed that the presence of

alkyl bromide or methoxy in the quinone ring increases the activity against the macromolecules model. The in-vitro design by the shake flask method to evaluate the solubility of 1,4-benzoquinone was also reported [9, 12]. The longer alkyl carbon insertion in the quinone skeleton has also increased the hydrophobicity and promote the solubility.

The solubility of the drug candidate expressed by Log P should not exceed 5 points [10]. The Log P-value can be determined by the shake flask method. This method involves the mixing of aqueous solutions of the compounds with the organic phase, such as, n-octanol and allowing the system to reach equilibrium. When equilibrium was formed, the concentration remaining in the water phase or the organic phase was measured by high-performance liquid chromatography (HPLC). Log P is determined by equation 1 as follows [13].

$$\text{Log P} = \log \frac{C\ octanol}{C\ water} = \log \frac{Ao/Vo}{Aw/Vw} \quad (1)$$

In this work, the synthesis of 2-(5-bromoamyl)-3,5-dimethyl-1,4-benzoquinone (**2a**) and 2-(5-bromoamyl)-5-methyl-1,4-benzoquinone (**2a**) is proposed. Compound **2a** and **2b** have different methyl substituents, either in the position or in the amount of methyl group. It is expected that the different methyl substituent position influences the solubility of the compounds.

2. Methodology

2.1. Material and instrumentation

The reagent is an analytical grade, otherwise stated. The 2,6-dimethyl-1,4-benzoquinone (**1a**), 2-methyl-1,4-benzoquinone (**1b**), and bromohexanoic acid were purchased from Sigma Aldrich, Singapore. The silver(I) nitrate ($AgNO_3$), ammonium persulfate ($(NH_4)_2S_2O_8$), acetonitrile, Na_2HPO_4, NaH_2PO_4, n-octanol, and chloroform were purchased from Merck, Singapore. The solvent n-hexane and ethyl acetate were purchased from a local vendor and used after distillation.

The instrumentation used is pH meter Schott Gerate/CG 820, UV-Visible Shimadzu 1600, FT-IR Shimadzu 8400S, HPLC Shimadzu LC-20AD Prominence connected with C18 Shim-Pack CLC-ODS column (4.6 mm x 250 mm, I.D 5 µm) and UV Shimadzu SPDM20A detector. The NMR used is JEOL ECS-400, using $CDCl_3$ as a solvent.

2.2. Experiment

2.2.1. Synthesis of 2-(5-bromoamyl)-3,5-dimethyl-1,4-benzoquinone (2a) and 2-(5-bromoamyl)-5-methyl-1,4-benzoquinone (2b)

Synthesis of **2a** and **2b** is according to the previous report [11, 12]. The starting material 3,5-dimethyl-1,4-benzoquinone (**1a**) (2 mmol, 0.544 g) was mixed with bromohexanoic acid (2.1 mmol, 0.82 g), $AgNO_3$ (1 mmol, 0.54 g) and added with 14 mL of acetonitrile: water (2:1 v/v), heated and stirred until 90°C. A solution of $(NH_4)_2S_2O_8$ (2 mmol) in 6 mL water added dropwise to the solution and continue to stir for 2 hours at the subjected temperature. The obtained crude product was purified using a silica gel column with n-hexane: chloroform (7:3 (v/v)). The obtained product **2a** was characterized using Shimadzu 8400S Fourier Transform-Infrared (FT-IR), UV-Visible Shimadzu 1600, and ^{1}H-NMR JEOL ECS-400. A similar procedure for the synthesis of 2-(5-bromoamyl)-5-methyl-1,4-benzoquinone (**2b**) was performed by replacing the starting material into 2-methyl-1,4-benzoquinone (**1b**) in the same mol ratio.

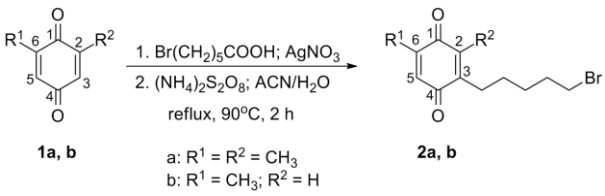
2.2.2. Solubility test of 2-(5-bromoamyl)-3,5-dimethyl-1,4-benzoquinone (2a) and 2-(5-bromoamyl)-5-methyl-1,4-benzoquinone (2b)

A phosphate buffer solution was prepared by mixing $NaH_2PO_4.H_2O$ (0.1 M) and Na_2HPO_4 (0.1 M) until the pH is 7.4 measured by pH meter Schott Gerate/CG 820. The buffer is subjected as the water phase, then saturated with n-octanol to obtain hydrophilic phase (water phase). The hydrophobic phase was obtained by saturating the n-octanol solution with pH 7.4 aqueous buffer (octanol phase). Each partition was taken with a ratio volume of n-octanol:water (3:7 (v/v)). Both solutions were standing for at least 24 hours for complete saturation [13]. Each compound, **2a**, and **2b** were dissolved in the n-octanol phase to prepare a 10 mM solution. The partition was conducted by mixed 7 mL of water phase with 3 mL of n-octanol phase then shaken for one hour at room temperature. After equilibration, n-octanol and water phase were separated, and each of them was injected for analysis using HPLC. The HPLC was performed by injected 2 µL of each partition (flow rate of 0.5 mL/min at 37°C).

3. Results and Discussion

3.1. Synthesis of 2-(5-bromoamyl)-3,5-dimethyl-1,4-benzoquinone (2a) and 2-(5-bromoamyl)-5-methyl-1,4-benzoquinone (2b)

The synthesis of **2a** and **2b** is described in **Scheme 1**. Bromoalkylation reaction is proposed by decarboxylation and substitution reaction. The reaction is to proceed at pH 1-3. Synthesis of **2a** is easily performed and obtained in higher yield compared to **2b** (**Table 1**). This is probably by the presence of two methyl substituents at C-2 and C-6 in 1,4-benzoquinone derivatives **1a**. The electronic induction effect from methyl substituent into π aromatic conjugated system, such as in benzoquinone, is suppressed by the acidity of the reaction solution then the nucleophilic addition is preferable. A similar result was reported by Mbiya *et al.* [14] for the reaction of quinone with thiol, which was conducted at pH below 5.5. Furthermore, the presence of methyl on C-2 and C-6 on **1a** accidentally promotes the substitution of bromoalkyl on o-/p- position. The presence of carbonyl in C-1 and C-4, either in **1a** or **1b** may not influence in the product selectivity.



Scheme 1. Bromoalkylation reaction of 1,4-benzoquinone

The physical properties of **2a** and **2b** are depicted in **Table 1**. Compound **2a** and **2b** have lower polarity compared to **1a** and **1b**, which analyzed from the retention factor (Rf) elucidated using hexane/chloroform solvent on SiO_2 plate. This is showed that the addition of bromoamyl group on the benzoquinone ring (C-3) reduces the polarity of the compound. Further analysis using FTIR showed the increasing intensity of the CH-sp^3 stretching in 2925-2927 cm^{-1}, and an additional medium intensity peak is detected in 686 cm^{-1} accounted for C-Br functional group (supplementary 1: S1a, S1b) [15]. Analysis of the ultraviolet spectrum of **2a** and **2b** showed the bathochromic shift. All the data is similar to the previous report for 1,4-benzoquinone derivative [12].

Table 1. Physical properties and spectral data of compound **2a** and **2b**

Compound	Shape and color	Yield (%)	Retention factor (Rf) [a]	Wavenumber (cm-1) [b]	Wavelength (nm) [c]
2a	Yellow oil	13.75	0.78	2927 (sharp-intense; CH-sp3) 686 (medium; C-Br)	257
2b	Yellow oil	4.04	0.75	2925 (sharp-intense; CH-sp3) 686 (medium; C-Br)	250

a. Thin Layer Chromatography (TLC) analysis using hexane-chloroform (6:4 (v/v))
b. Fourier Transform Infra-Red (FTIR) analysis using NaCl plate
c. Ultraviolet-Visible (UV-Vis) analysis in octanol

The analysis of proton NMR was carried out based on the chemical shift and the coupling constant of each proton (**Figure 2**, **Table 2,** the NMR spectrum is depicted in Supplementary 2: S2a, S2b). The characteristics of compound **2a** can be detected by the appearance of the peaks at chemical shift 3.40 ppm (t, 2H, *J* = 6.8 Hz) which is identified as methylene protons bounded to 5-bromoamyl carbon atoms. The presence of bromine as an electronegative atom causes the protons to be less protected and shifted to the greater chemical shifts. Six methylene protons bound to the quinone ring with the sequence –CH_2–CH_2–CH_2– are shown with a multiplex spectrum at a chemical shift of 1.45 (m, 4H, *J* = 7.4; 7.8 Hz) and 1.87 ppm (q, 2H, *J* = 6.8; 7.4 Hz). The methylene proton, which is bound directly to the quinone, is measured at 2.46 ppm (t, 2H, *J* = 7.8 Hz). Two methyl groups bound to the quinone appear as a singlet at δ = 2.04 ppm (s, 6H) with integration for six protons. The analysis for compound **2b** is similar to **2a**, except the appearance of two singlets at δ = 6.56 ppm (s, 1H) and 6.51 ppm (s, 1H) for the proton bound to quinone ring.

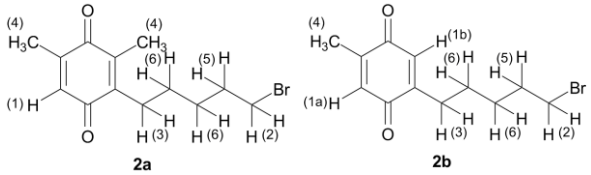


Figure 2. Structure of compound **2a** and **2b**

Table 2. ^{1}H NMR spectral data for **2a** and **2b**

Proton	δ (ppm)	
	2a	**2b**
1a	6.54 (s, 1H)	6.56 (s, 1H)
1b	-	6.51 (s, 1H)
2	3.40 (t, 2H, *J* = 6.8 Hz)	3.41 (t, 2H, *J* = 6.8 Hz)
3	2.46 (t, 2H, *J* = 7.8 Hz)	2.45 (t, 2H, *J* = 7.7 Hz)
4	2.04 (s, 6H)	2.05 (s, 3H)
5	1.87 (q, 2H, *J* = 6.8; 7.4 Hz)	1.89 (q, 2H, *J* = 6.8; 7.7 Hz)
6	1.45 (m, 4H, *J* = 7.4; 7.8 Hz)	1.50 (m, 4H, *J* = 7.7; 6.8 Hz)

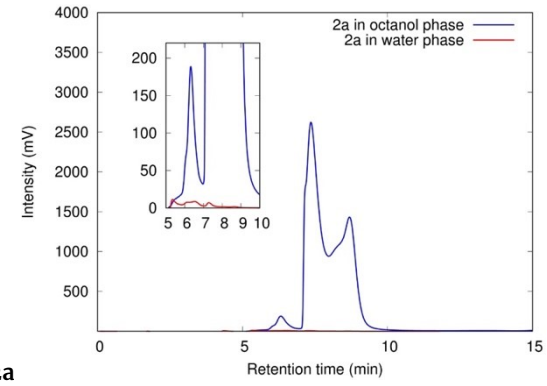
3.2. Solubility test of 2-(5-bromoamyl)-3,5-dimethyl-1,4-benzoquinone (2a) and 2-(5-bromoamyl)-5-methyl-1,4-benzoquinone (2b)

Solubility test of **2a-b** was carried out using the HPLC method [13] and expressed by log P. The log P value can also be used to give recommendations for how the drug is administered in the body. If the synthesized compounds are relatively hydrophobic, the plasma membrane may be permeable, and the compound easily penetrates the membrane. The shake flask method was chosen to measure the solubility of compounds using n-octanol and water (phosphate buffer pH 7.4) for the in vitro approach. At the same time, the marker of lipophilicity (hydrophobicity) refers to solubility compounds is also predicted using ALOGPS 2.1 program for the comparison [16]. In this study, the *n*-octanol/water partition tested in a 3:7 (v/v) ratio. The concentration of the compound in each phase was measured using HPLC, and the ratio area was used to calculate the log P value (**Tables 3**).

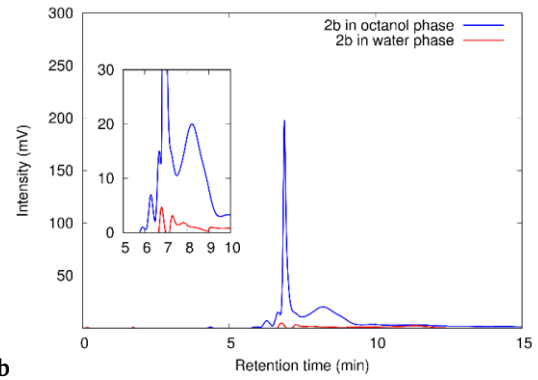
Table 3. Calculated partition coefficient (log P) of compound **2a** and **2b** by HPLC analysis

Compound	The area in octanol partition (Ao) [a]	The area in water partition (Aw) [a]	log P	
			In vitro [a]	Prediction [b]
2a	914422497	217331	2.99	3.21
2b	1583466	210841	1.36	2.66
TQ	93552748	927468	2.37	2.00

[a] HPLC analysis
[b] Predicted using ALOGPS 2.1



2a



2b

Figure 3. HPLC profile of compound **2a**, **2b** in n-octanol/water phase

In the chromatogram (**Figure 3**), it can be observed that the solubility of **2a** and **2b** in the octanol phase is greater than in the aqueous phase. It is showed that the hydrophobicity of compounds tends to be greater than the hydrophilicity. Furthermore, the log P value suggested that the hydrophobicity sequence is as follows, **2a** > **TQ** > **2b**. Compound **2a** gives log P value 2.99, which is greater than **TQ** and **2b**. Based on the recommendations from Comer [17] and Triggle and Taylor [18], the log P value of **2a** is suitable for oral administration drugs. This compound can also penetrate the cell membrane through the intestinal tissue and distributed into the body by the central nervous system or other. However, the log P value of compound **2a** is at a low threshold. Based on this analysis, further effort should be attempted to modify the 1,4-benzoquinone by adjusting the number of alkyl substituents.

4. Conclusion

Compound **2a** and **2b** were synthesized in 13.75% and 4.04%, respectively. The addition of alkyl substituent is analyzed by NMR. The presence of the bromoalkyl group in **2a** and **2b** was also detected using FTIR analysis. The presence of methyl substituent greatly influences the solubility of the compound almost 2-fold. The solubility test by in-vitro analysis using the HPLC approach was in accordance with calculation prediction.

Acknowledgment

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Supplementary

Supplementary 1 (S1a)

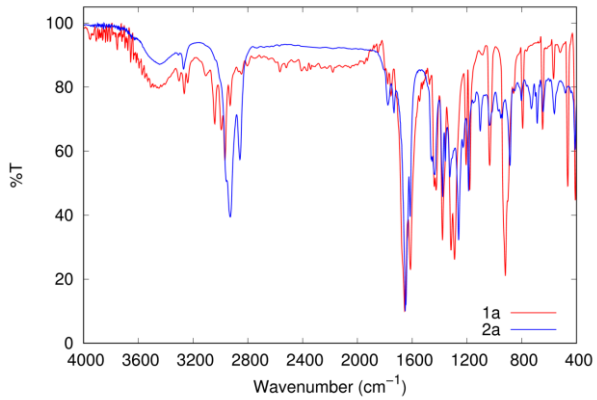

Figure S1a. FTIR spectra of starting material 2,6-dimethyl-1,4-benzoquinone (**1a**) dan synthesized product 2-(5-bromoamyl)-3,5-dimethyl-1,4-benzoquinone (**2a**)

Supplementary 1 (S1b)

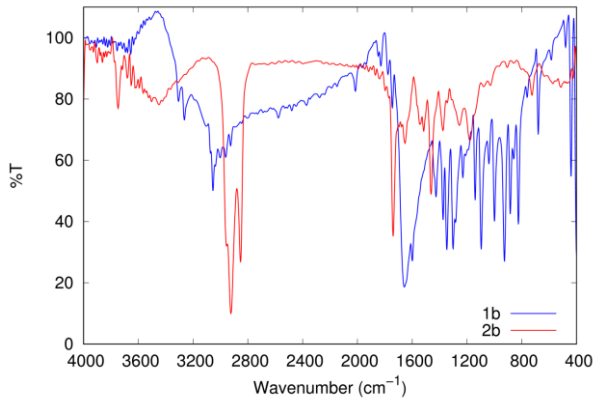

Figure S1b. FTIR spectra of starting material 2-methyl-1,4-benzoquinone (**1b**) dan synthesized product 2-(5-bromoamyl)-5-methyl-1,4-benzoquinone (**2b**)

Supplementary 2 (S2a)

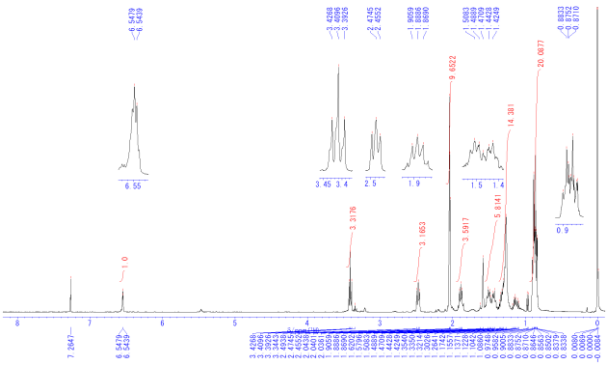

Figure S2a. ^{1}H-NMR spectra of the synthesized product 2-(5-bromoamyl)-3,5-dimethyl-1,4-benzoquinone (**2a**) in $CDCl_3$ recorded at JEOL ECS 400 MHz

Supplementary 2 (S2b)

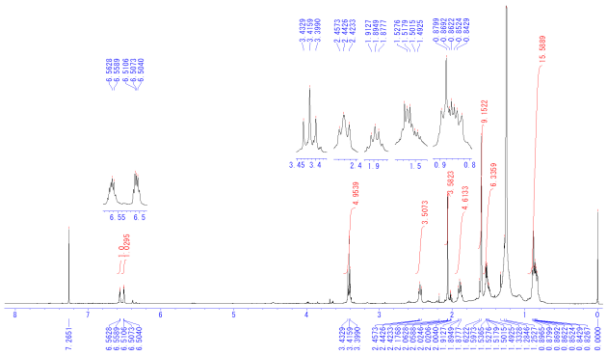

Figure S2b. ^{1}H-NMR spectra of the synthesized product 2-(5-bromoamyl)-5-methyl-1,4-benzoquinone (**2b**) in $CDCl_3$ recorded at JEOL ECS 400 MHz.