Synthesis and Activity Analysis of 3-(10-Bromodecyl)-5-isopropyl-2-methyl-1,4-benzoquinone: In-silico Approach

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Abstract

The 3-(10-bromodecyl)-5-isopropyl-2-methyl-1,4-benzoquinone (1) have been synthesized and structurally characterized by mass spectrometer (LC-MS), FT-IR, ¹H-NMR and ¹³C-NMR spectra. The synthesis was carried out by refluxed Thymoquinone (TQ) with bromoundecanoic acid in the presence of acetonitrile:water (2:1 v/v) for 1.5 h. Purification of the products was conducted by open-column chromatography. Determination of product is mainly observed from C–Br stretching at 562–563 cm⁻¹ by FT-IR and the chemical shift at δ = 3.4 ppm (¹H-NMR) and 33–34 ppm (¹³C-NMR). In addition, δ = 1.38–3.05 ppm (¹H-NMR) and 21–32 ppm (¹³C-NMR) considered as aliphatic alkane which substituted at C–6 on Thymoquinone ring. Analysis using LC-MS showed the molecular weight of 1 is 382.96 g/mol. The yield of 1 is 12.09% as brownish-yellow liquid. Activity test of 1 against the cytochrome protein (CYP) as macromolecule model revealed a good fit with IC₅₀ 1.23 mg/mL lower than TQ (36.11 mg/mL). It is showed that compound 1 exhibit anticancer activity better than TQ.

Keywords: thymoquinone, lipophilicity, bromo alkylation, anticancer, cytochrome protein

1. INTRODUCTION

Thymoquinone (TQ) is a potentially active compound of black cumin seeds (*Nigella sativa L*)¹. TQ have biological activity as an antioxidant, antimicrobial, anticancer and anti-inflammatory effect². Although it has benefits for therapy, its effectiveness and oral bioavailability are limited by poor solubility and poor lipophilicity³. TQ have activity for oral administration in a wide panel of animal disease models. Its revealed to be safe to consume if the doses were kept between 10–100 mg/kg body weight⁴. On otherhand, TQ ability to bind serum proteins affects and reduces its anticancer activity.⁵ By this fact, the bioavailability of TQ over oral administration is limited. Modification structure of TQ is proposed to increase the activity by addition of bromoalkyl-phosphoniumcation (TQBrP). It showed that TQBrP gave antioxidant activity in mitochondria⁶. Here, we reported the synthesis of TQ derivative by substitution reaction using bromoalkyl group and analysis its activity against the cytochrome protein (CYP) as macromolecule model to determine the physicochemical properties and interactions between the receptor ligand and the active side.

2. EXPERIMENTAL

Materials and Methods

Thymoquinone was purchased from Sigma Aldrich with 99% purity and used without further purification. Bromoundecanoic acid and $(\text{NH}_4)_2\text{S}_2\text{O}_8$ were also purchased from Sigma Aldrich. Na_2SO_4 and AgNO_3 were supplied by Merck. The solvents, that is acetonitrile, diethyl ether, chloroform, and n-hexane were obtained from Merck in pro analysis grade.

Synthesis of 3-(10-bromodesil)-5-isopropyl-2-methyl-1,4-benzoquinone

Bromoalkylation reaction of thymoquinone was carried out by reflux. Thymoquinone (4 mmol, 0.55 g), bromoundecanoic acid (4.2 mmol, 1.12 g), AgNO_3 (2 mmol, 0.37 g), and 14 mL $\text{AcCN}:\text{H}_2\text{O}$ (2:1) stirred until 80 °C then added with $(\text{NH}_4)_2\text{S}_2\text{O}_8$ in 3 mL water and continued the reaction for 1.5 hour. The product mixture was extracted with diethyl ether. The organic was dried over Na_2SO_4 and evaporated in vacuum. The crude product of reaction purified by chromatography column using n-hexane:chloroform (9:1) as the eluent. Products were analyzed using TLC, FT-IR, UV-Vis, LC-MS, ^1H - and ^{13}C -NMR.

Activity of 3-(10-bromodesil)-5-isopropyl-2-methyl-1,4-benzoquinone

Activity of 3-(10-bromodesil)-5-isopropyl-2-methyl-1,4-benzoquinone (**1**) was calculated by molecular docking using Auto Dock 4. The structure of ligand (**1**) was optimized by HyperChem save in .pdb format. The macromolecule cytochrome protein (CYP) were downloaded by www.pdb.org and saved in .pdb format. The structure of macromolecule should be free from water and ligand external. Docking process consists of two main step, running Auto grid (modified format changed to .gpf) and running Auto dock (format changed to .dpf). Activity analysis Auto dock include binding interaction (K_i) with minimum energy, amino acid interaction, and ligand interaction.

3. RESULT AND DISCUSSION

Synthesis of 3-(10-bromodesil)-5-isopropyl-2-methyl-1,4-benzoquinone

Synthesis of 3-(10-bromodesil)-5-isopropyl-2-methyl-1,4-benzoquinone (**1**) were summarized in **Scheme 1**. The addition of AgNO_3 initiated the decarboxylation reaction by the release of CO_2 from the carboxyl group to form a nucleophile. Then, the addition of $(\text{NH}_4)_2\text{S}_2\text{O}_8$ in solution initiated nucleophilic substitution of alkyl bromide on the quinone ring at C-6. Substitution at C-6 is preferred over C-3 because of steric hindrance of isopropyl at C-2 is greater than methyl at C-5. It is predicted that the addition of bromoalkyl increased the lipophilicity of **1** by the addition of non-polar substituent.

Figure 1. Synthesis of 3-(10-bromodesil)-5-isopropyl-2-methyl-1,4-benzoquinone (**1**)

The obtained product is brownish-yellow liquid in 12.09% yield. Analysis using TLC showed that compound **1** have retardation factor $R_f = 0.40$ (n-hexane: CHCl_3 (9:1 v/v)). The R_f of **1** is greater than TQ ($R_f = 0.30$) in n-hexane: CHCl_3 (9:1 v/v). It showed that compound **1** has a lower polarity than TQ. Analysis using UV-Vis in 1-octanol gave $\lambda_{\text{maks}} 259.6$ nm. The addition of bromo alkyl group in quinone ring increased the wavenumber of TQ⁷. From FT-IR analysis, compound **1** showed the existence of a new peak at 562.01 cm^{-1} from the vibration of C–Br group. In concomitant, the decreasing of the intensity peak at 3040.38 cm^{-1} ($\text{sp}^2\text{C–H}$ stretching) is evident of the alkyl substitution at quinone ring. Increasing intensity of $\text{sp}^3\text{C–H}$ stretching at 2963.22 cm^{-1} indicates substitution of alkyl group at C6. The double bond C=C quinone at 1612.38 cm^{-1} and the revealed of C=O stretching at 1649.02 cm^{-1} showed the skeleton of quinone.

Elucidation structure of product reaction was conducted by ^1H - and ^{13}C -NMR in CDCl_3 . The successful of synthesis was easily identified from hydrogen splitting in aliphatic alkane at chemical shifts $\delta = 1.38\text{--}2.48$ ppm. Methylene bonded with bromine ($-\text{CH}_2\text{--Br}$) at 3.41 ppm is due electronegativity effect. The hydrogen attached to quinone ring at $\delta = 6.48$ ppm gave the singlet splitting. It showed that alkylation only replaces the hydrogen atom at C-6 in quinone ring. Analysis with ^{13}C -NMR showed that aliphatic alkane substituted in quinone ring at $\delta = 21\text{--}32$ ppm, C–Br at $\delta = 34.18$ ppm, C=C in quinone at $\delta = 130\text{--}154$ ppm, and C=O at $\delta = 187\text{--}188$ ppm. The chemical shift from ^1H - and ^{13}C -NMR was tabulated in **Table 1** and **Table 2**. Correlation of proton and carbon is depicted in **Figure 1**.

Analysis using LC-MS showed that molecular weight of product reaction is 382.87 g/mol. From all the analysis, we success to synthesized 3-(10-bromodesil)-5-isopropyl-2-methyl-1,4-benzoquinone (**1**) in one-step reaction.

TABLE 1. ^1H -NMR of 3-(10-bromodesil)-5-isopropyl-2-methyl-1,4-benzoquinone in CDCl_3 at 600 MHz

Chemical Shift (δ , ppm)								Coupling constant ($J_{\text{H,H}}$, Hz)			
H-a	H-b	H-c	H-d	H-e	H-f	H-g	H-h	$J_{\text{b,f}}$	$J_{\text{c,h}}$	$J_{\text{f,g}}$	$J_{\text{d,g}}$
6,48	3,41	3,05	2,48	2,02	1,86	1,38	1,11	6,95	6,85	7,13	6,82

TABLE 2. ^{13}C -NMR of 3-(10-bromodesil)-5-isopropyl-2-methyl-1,4-benzoquinone in CDCl_3 at 125 MHz

Number of Carbon	Chemical Shift (δ , ppm)	Number of Carbon	Chemical Shift (δ , ppm)
C-1	188.64	C-11	29.54
C-2	187.15	C-12	29.51
C-3	154.68	C-13	29.47
C-4	145.51	C-14	28.87
C-5	139.98	C-15	28.29
C-6	130.09	C-16	26.85
C-7	34.18	C-17	26.89
C-8	32.96	C-18	21.23
C-9	30.03	C-19	21.58
C-10	29.92	C-20	11.87

Figure 2. Elucidation of **C1** based on ^1H -NMR and ^{13}C -NMR

Activity of 3-(10-bromodesil)-5-isopropyl-2-methyl-1,4-benzoquinone

The activity of **1** was determined by molecular docking using cytochrome protein (CYP). Evaluated the activity of compound **1** is resume in **Table 3**. The physicochemical properties of compounds were subjected for the coefficient partition ($\log P$), Gibbs energy (ΔG°), and inhibition constant (K_i). Visualization interaction of compound **1** with CYP macromolecule showed in **Figure 2**.

TABLE 3. Molecular docking result of ligand C1 with CYP macromolecule

Ligan	Ki (μM)	ΔG° (kcal/mol)	IC ₅₀ (mg/mL)	Log P*
TQ	109.95	-5.4	36.11	2.80
C1	1.61	-7.9	1.23	6,71

*calculated log P using HyperChem

Figure 3. Visualization interaction of compound **1** with CYP

The activity of compound **1** has evaluated by molecular docking, and the calculation of log P carried out by HyperChem. Based on HyperChem, compound **1** has a log P value greater than TQ. Log P value has a correlation with lipophilicity of determined compound. It showed that addition bromoalkyl group in TQ is increasing the absorption rate and affinity of compound **1** to penetrate lipophilic membrane of macromolecule. The Gibbs energy (ΔG°) is energy needed by the ligand and amino acid to binding macromolecule. Lower ΔG° means that ligand can easily to bind the macromolecule. By this calculation, compound **1** has $\Delta G^\circ = -7.9$ kcal/mol lower than TQ. On the other hand, Ki of compound **1** is smaller than TQ. Activity test of **1** against the cytochrome protein (CYP) as macromolecule model revealed a good fit with IC₅₀ 1.23 mg/mL lower than TQ (36.11 mg/mL). It is showed that compound **1** exhibit anticancer activity better than TQ.

4. CONCLUSION AND OUTLOOKS

Synthesis of 3-(10-bromodecyl)-5-isopropyl-2-methyl-1,4-benzoquinone (**1**) was performed in one-step reaction by alkylation of Thymoquinone with bromoundecanoic acid in mild condition. The substitution of the bromoalkyl into Thymoquinone ring gave the increasing activity against CYP macromolecule model compared with the parent compound. Future works will focus on antimicrobial analysis of compound **1** by In-vitro and In-vivo approach.

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