Synthesis of 3-(7-triphenylphosphonioheptyl)-2,6-dimethyl-1,4-benzoquinone) and The Activity Test Toward Glycogen Phosphorylase Enzyme: \textit{In silico} Approach

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Abstract. The synthesis of 3-(7-triphenylphosphonioheptyl)-2,6-dimethyl-1,4-benzoquinone) (TFH) is carried out by bromoalkylation and insertion of triphenylphosphonium moiety by refluxed of 2,3-dimethyl-1,4-benzoquinone using bromooctanoic acid triphenylphosphine. The alkylation gave 17.14% yield of 2-(7-bromoheptyl)-3,5-dimethyl-1,4-benzoquinone (C7) and 3-(7-triphenylphosphonioheptyl)-2,6-dimethyl-1,4-benzoquinone) (TFH) is obtained at 40.35% yield. Structure characterization of the synthesized product was performed mainly using UV-Vis, FT-IR, and \textit{1}H-NMR. UV-Vis characterization showed maximum wavelength of TFH at 198 nm, 226 nm, and 260 nm. FT-IR characterization showed \textit{sp}\textsuperscript{3} character at 2930.43 cm\textsuperscript{-1} and the disappearance of C-Br peak at 687.37 cm\textsuperscript{-1}, indicates the presence of long chain alkyl group and the replacement of Br with triphenylphosphonium. In \textit{in silico} investigation by using Glycogen Phosphorylase enzyme as molecular target estimated IC50 value of thymoquinone, C7, and TFH, that is, 16.14 ppm, 162.2 ppm, and 1.26 ppm, respectively. Thus, TFH has the smallest IC50 value suggested that most effective drug candidate compared with thymoquinone and C7.

Keyword: thymoquinone, bromoalkylation, triphenylphosphonium moiety, Glycogen Phosphorylase (GPA), anticancer

1. Introduction

\textit{Nigella sativa} L. (Black Cumin) is herbal plant originally from Asia and also cultivated in Europe and North Africa that widely used as traditional medicine. \textit{Nigella sativa} volatile oil contain saturated fatty acid, i.e, thymoquinone (TQ), thymohydroquinone (THQ), nigellonene, limonene, citronellol, etc [1]. Thymoquinone (2-isopropyl-5methyl-1,4-benzoquinone, TQ) is a major compound (28-57\%) founded in \textit{Nigella} sativa oil. It is reported that thymoquinone is an active compound for anticancer, antioxidant, and anti-inflammatory [2-4]. Among the broad therapeutic benefits, thymoquinone has a poor bioavailability resulting to the rapid elimination and relatively slow absorption in the biological system. The lipophilicity and/or hydrophilicity balance is one of the factors that caused bioavailability problems [5-6].

Modification of thymoquinone to improve the bioavailability were reported by Antonenko et al. (2008) and Saverina et al. (2013). The substitution by bromoalkyl group and triphenylphosphonio moieties to the quinoid ring can improve the lipophilicity of the compound. The addition of a
functional group with a rigid and bulky structure such as triphenylphosphonio that can delocalize its atom charge can create an ion permeability to the membrane [7]. Later, Ravindran et al. (2010) reported the insertion of thymoquinone into poly-nanoparticle (lactide-co-glycoside) by nano formulation to improve the bioavailability of thymoquinone. Compared to physical modification, the structural modification by the addition of alkyl [8] and triphenylphosphonio moieties is the promising approach to deal with stability and solubility problems.

Activity test to evaluate the pharmacological effect of a drug candidate was mainly conducted by in vitro and in vivo approach. Currently, in silico design were increased interest because of its less costly and time consuming compared to conventional method. In silico approach using molecular docking is an interaction modelling of two or more molecules in the most stable condition [9]. This approach was aimed to predict the effectiveness of a drug candidate based on the interaction between ligand and macromolecule as the drug target. The output of molecular docking is partition coefficient (log P), Gibbs energy ($\Delta G^o$), inhibition constant ($K_i$), inhibition concentration ($IC_{50}$), and amino acids residue from macromolecule which interact with specific ligand [10].

Glycogen phosphorylase protein (GPA) is an important role in catalyse the breakdown of glycogen into glucose-1-phosphat inside the liver. Pharmocology inhibitor of GPA has been developed and learned as potential therapy for diabetic type 2 [11]. By this reason, macromolecule target for thymoquinone and the derivatives is chosen.

In the present study, the synthesis of benzoquinone derivative by bromoalkylation using bromooctanoic acid (C7) followed by addition of triphenylphosphonio moieties was carried out to increase the bioavailability of the compounds. An in-silico investigation to evaluate the activity of ligand over the GPA macromolecule model for antidiabetic agent was also reported.

2. Experimental procedure

2.1. Materials and methods

Starting material 2,6-dimethyl-1,4-benzoquinone with 99% purity, bromooctanoic acid, and triphenylphosphine were purchased from Sigma Aldrich pro analysis. (NH$_4$)$_2$S$_2$O$_8$, AgNO$_3$, and Na$_2$SO$_4$ were supplied by Merck pro analysis. The solvents used in this research are acetonitrile, n-hexane, chloroform, diethyl ether, ethanol, methanol, and H$_2$O which were also supplied by Merck pro analysis.

2.2. Bromoalkylation reaction of 2,6-dimethyl-1,4-benzoquinone

Bromoalkylation reaction of 2,6-dimethyl-1,4-benzoquinone was carried out according to Antonenko et al. (2008) procedure with modification [8]. 2 mmol of 2,6-dimethyl-1,4-benzoquinone (0.272 g), 2.1 mmol of bromooctanoic acid (0.468 g), and 1 mmol AgNO$_3$ (0.170 g) were dissolved in 7 mL solution of AcCN:H$_2$O (2:1) and heated until 90 °C. The solution of 2 mmol NH$_4$(S$_2$O$_4$) in 3 mL H$_2$O were added drop-wise in to the mixture and stirred constantly for 2 h at 85-90 °C. The mixture obtained from the reaction was extracted using diethyl ether and the organic phase obtained was dried using Na$_2$SO$_4$ and evaporated under vacuum. Purification was carried out using silica gel column chromatography with n-hexane:CHCl$_3$ (7:3 v/v) as the eluent. The obtained product, 2-(7-bromoheptyl)-3,5-dimethyl-1,4-benzoquinone (2) was characterized using spectrophotometer FT-IR, spectrophotometer UV-Vis, and spectrometer $^1$H-NMR.

2.3. Reaction of 2-(7-bromoheptyl)-3,5-dimethyl-1,4-benzoquinone with triphenylphosphine

0.1 mmol of 2-(7-bromoheptyl)-3,5-dimethyl-1,4-benzoquinone (0.135 g) and 0.1 mmol triphenylphosphine (0.156 g) were dissolved in 10 mL ethanol and heated inside the stainless steel autoclave at 100 °C using oil bath for 8 h. After the reaction finished, the solvent was evaporated under vacuum and the residue was precipitated by added with diethyl ether which dissolved in CH$_2$Cl$_2$. The crude product was purified using silica gel column with CHCl$_3$:CH$_3$OH (7:1). The obtained product was characterized using spectrophotometer FT-IR, and spectrophotometer UV-Vis.
2.4. Activity test of synthesized product by In-silico Approach

*In silico* analysis using Autodock Vina software was carried out to evaluate the activity of the compound. The 2D structure of synthesized product, i.e thymoquinone, C7, and TFH as ligands was optimized using Hyperchem and converted into (.pdb) format. The macromolecule used was Glycogen Phosphorylase enzyme (GPA) which plays important roles as antidiabetic drug target. The 3D structure of GPA enzyme was downloaded from [www.rcsb.org](http://www.rcsb.org) in (.pdb) format and optimized using Discovery Studio Visualizer to remove water and original ligand structure. Analysis of ligand and macromolecule interaction using molecular docking consists of four main steps. First is preparation of ligand and macromolecule coordinate by converting file format from (.pdb) to (.pdbqt). Second is grid box preparation for determining docking parameter including size and position of grid box. In this research, grid box dimension used was \( x=19.945; y=-60.246; z=18.132 \). The result of grid box preparation was saved in (.gpf) format. Third is docking process using Autodock 4 to get the most stable conformation from ligand and macromolecule interaction. The final result of docking process was saved in (.dpf) format. Analysis of docking parameter including inhibition constant (Ki), Gibbs energy (E), and amino acid interaction with ligand.

3. Result and Discussion

3.1. Synthesis of 3-(7-triphenylphosphonioheptyl)-2,6-dimethyl-1,4-benzoquinone

The synthetic route to the benzoquinone derivative 2 and 3 is shown in **Scheme 1**. The yield of bromoalkylation and triphenylphosphonio reaction is summarized in **Table 1**.

**Scheme 1.** Synthetic route of 2 and 3

| Entry | (1) Bromooctanoic acid | (2) Triphenylphosphonium | (3) |
|-------|------------------------|--------------------------|-----|
| 1     | 2 mmol (0.272 g)       | 2.1 mmol (0.468 g)       | 0.22 g (17.6%) |
| 2     | -                      | -                        | 0.1 mmol (0.135 g) | 0.1 mmol (0.156 g) | 0.12 g (40.3%) |

Entry 1 in **Table 1** showed that bromoalkylation reaction gave compound 2 in 17.6% yield and addition of triphenylphosphonio moieties gave 3 in 40.3% yield. The analysis of compound 2 using FT-IR showed the increasing intensity of the peak at 2930 cm\(^{-1}\) that indicates the C-H sp\(^3\) functionality from bromoalkylation. The appearance of a new peak at 687 cm\(^{-1}\) indicates the formation of C-Br bond. All the data is in accordance with Antonenko et al. (2008) report. Both FT-IR spectrum showed an intense peak at 2930 cm\(^{-1}\) due to the addition of long chain alkyl moieties [7].

Further analysis using \(^1\)H-NMR was carried out to confirm the chemical structure of bromoalkylation compound 2. \(^1\)H-NMR spectra gave the information about the number and position of proton from synthesized product. \(^1\)H-NMR spectra of product 2 is shown in **Figure 1** with the interpretation data shown in **Table 2**.
Figure 1. Structure identification of 2-(7-bromoheptyl)-3,5-dimethyl-1,4-benzoquinone (2)

Table 2. Interpretation data of $^1$H-NMR spectra of product 2

| Proton type | Number of proton | Splitting | $\delta$ (ppm) | J-Coupling (Hz) |
|-------------|------------------|-----------|----------------|-----------------|
| 1           | R-CH$_2$-R       | 10        | multiplet      | 1.35            | 6.92 ; 7.68     |
| 2           | R-CH$_2$-R       | 2         | quintet        | 1.85            | 6.92 ; 7.68     |
| 3           | Ar-CH$_3$        | 7         | singlet        | 2.03;2.04       | -               |
| 4           | Ar-CH$_2$-R      | 2         | triplet        | 2.45            | 7.68            |
| 5           | R-CH$_2$-Br      | 3         | triplet        | 3.39            | 7.68            |
| 6           | Ar-H             | 1         | singlet        | 6.54            | -               |

$^1$H-NMR spectra showed chemical shift at 6.54 ppm with singlet peak, indicates that there is one proton attached at the quinoid ring and one of the proton from quinoid ring has been substituted with alkyl group. Chemical shift at 3.39 ppm with triplet peak is suitable with the environment of the proton which attached at carbon atom of C-Br. According to the previous research by Shoimatus et al. (2017), the addition of bromoalkyl functionality to the quinoid ring gave similar pattern of $^1$H-NMR spectra to the present work, both formed a singlet peak at 6.49 ppm suggested that the bromoalkylation replaced one specific proton in quinoid ring and a triplet peak at 3.41 ppm from methylene (-CH$_2$-) bonded with bromide atom [15].

Figure 2. FT-IR spectrum of 2 (orange) and 3 (blue)
Addition of triphenylphosphonio moieties was carried out to facilitate the compound to passing through mitochondria by the presence of positive ion charge from triphenylphosphonio functionality. Ionized atom should be surrounded by bulky hydrophobic residue such as phenyl group that can stabilize the positive charge by electron delocalization, thus the ion will be permeable to the membrane [12].

Characterization of compound 3 using spectrophotometer UV-Vis showed three intense peak with specific wavelength that are 208 nm; 226.5 nm; and 261 nm. The peak at 226.5 nm was from triphenylphosphonio functionality [13], while the peak at 260 nm was from the quinoid ring which substituted by alkyl group. Further characterization using FT-IR showed the disappearance of C-Br peak at 687.37 nm, indicates that the C-Br bond was replaced by triphenylphosphonium. FT-IR spectrum of compound 3 is shown in Figure 2.

3.2. Activity test of 3-(7-triphenylphosphonioheptyl)-2,6-dimethyl-1,4-benzoquinone

Activity test using in silico approach was carried out using molecular docking to show the interaction between ligand and macromolecule model in the most stable condition [9]. The ligand used are compound 2, 3, and thymoquinone (TQ) as standard. The macromolecule used is glycogen phosphorylase (GPA) as antidiabetic drug target. The data obtained from in silico analysis are partition coefficient (Log P), Gibbs energy ($\Delta G^o$), inhibition constant (Ki), inhibition concentration (IC$_{50}$), and the residue of amino acids from macromolecule which showed interaction with specific ligand [14].

The lipophilicity of ligand 2 is higher than that of TQ, indicates that the addition of long alkyl chain group increased the lipophilicity of the compound. While, the lipophilicity value of ligand 3 is slightly different from that of ligand 2, shows that the addition of triphenylphosphonio moieties was not contribute to improve the lipophilicity of the compound (Table 3).

| Ligand | Log P | Ligand structure |
|--------|-------|-----------------|
| TQ     | 2.80  | ![TQ structure](image1) |
| 2      | 4.79  | ![2 structure](image2) |
| 3      | 4.68  | ![3 structure](image3) |

Inhibition constant (Ki) related with IC$_{50}$ value predicted the quantity of ligand as a drug required to inhibited biological process in the body [10]. The data obtained from the interaction of ligand 2, 3, and TQ with GPA macromolecule is shown in Table 4. Ki and IC$_{50}$ value of product 2 are higher than that of TQ, shows that the addition of long chain alkyl group is not contribute to the inhibition of GPA macromolecule. In contrast, Ki and IC$_{50}$ value of ligand 3 are significantly lower than TQ and 2, indicates that the addition of triphenylphosphonio moieties contribute to the improving ligand activity to inhibit GPA macromolecule.

The binding energy ($\Delta G^o$) value is related with the energy required to bind with GPA macromolecule. Based on Table 4, product 3 has the highest $\Delta G^o$ value, means the energy required by product 3 to bind with GPA macromolecule is lower compared with the other ligands, thus it will be
easier for product 3 to interact and inhibit the activity of GPA macromolecule. Visualization of interaction between amino acids of GPA macromolecule and ligand (2 and 3) is shown in Figure 3 with the list of amino acids provided in Table 4.

Table 4. Molecular docking analysis of ligand 2 and 3 with GPA macromolecule

| Ligand | Ki (µm) | ΔG° (kcal/mol) | IC₅₀ (ppm) | Amino acid |
|--------|---------|---------------|------------|------------|
| TQ     | 49.15   | -5.69         | 16.132     | LYS128     |
|        |         |               |            | GLY129     |
|        |         |               |            | LYS125     |
|        |         |               |            | GLY127     |
|        |         |               |            | ALA126     |
|        |         |               |            | TYR131     |
|        |         |               |            | ARG130     |
|        |         |               |            | ASP92      |
|        |         |               |            | CYS124     |
|        |         |               |            | LYS128     |
|        |         |               |            | GLY129     |
|        |         |               |            | LYS125     |
|        |         |               |            | GLY127     |
|        |         |               |            | ALA126     |
|        |         |               |            | TYR131     |
|        |         |               |            | ARG130     |
|        |         |               |            | ASP92      |
|        |         |               |            | CYS124     |
| 2      | 72.21   | -5.65         | 45.231     | ASP324     |
|        |         |               |            | TYR177     |
|        |         |               |            | LEU318     |
|        |         |               |            | TYR176     |
|        |         |               |            | PHE279     |
|        |         |               |            | TYR180     |
|        |         |               |            | ARG172     |
|        |         |               |            | PRO169     |
|        |         |               |            | ARG173     |
|        |         |               |            | GLY282     |
| 3      | 1,28    | -8.04         | 3.315      | ASP324     |
|        |         |               |            | TYR176     |
|        |         |               |            | ASN323     |
|        |         |               |            | TYR177     |
|        |         |               |            | PHE279     |
|        |         |               |            | TYR180     |
|        |         |               |            | ARG172     |
|        |         |               |            | PRO169     |
|        |         |               |            | ARG173     |
|        |         |               |            | PRO281     |

Figure 3. Molecular visualization interaction (a) compound 2 (b) compound 3 with GPA

According to the molecular visualization of ligand (2 and 3) and GPA receptor, ligand 2 only has hydrophobic interaction with GPA amino acids, i.e, tyrosine, leucine, and arginine. These hydrophobic interaction involved aromatic and alkyl functionalities which formed alkyl-alkyl and π-π interaction. While ligand 3 formed three type of interaction that are electrostatic interaction with aspartic acid, hydrogen bonding with arginine, and hydrophobic interaction with arginine, proline, and tyrosine. These interactions involved between ligand 3 and GPA receptor contribute to strengthen the inhibition activity of ligand 3 toward GPA enzyme. From the overall in silico analysis, it estimated that ligand 3 has the best activity to inhibit GPA enzyme.
4. Conclusion
The synthesis of 2 and 3 was obtained in 17.6% and 40.3%, respectively. In silico test using molecular docking with glycogen phosphorylase (GPA) as antidiabetic enzyme model showed that compound 3 has significantly lower IC50 compared to TQ which mean the addition of triphenylphosphonio moieties improve the antidiabetic activity of the compound. The addition of long chain alkyl group is influence the increasing of lipophilicity of the compound shown from higher Log P value of 2 compared to TQ.

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