One-pot four-component synthesis of phenazine derivative using 2-hydroxy-1,4-naphthoquinone

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Abstract. Phenazine is one of the most important organic compounds because of its various biological activities. This study aims to synthesize the phenazine derivatives compound using 2-hydroxy-1,4-naphthoquinone (lawsone) with a one-pot four-component method. Phenazine derivative that had been successfully synthesized was carried out by reacting four reactants at once in one flask with the same mole ratio. The reaction occurred between lawsone, o-phenylenediamine, benzaldehyde, and malononitrile. Characterization of the synthesized products was confirmed using FT-IR, UV-Vis Spectrophotometer, and LC-MS. The results of the analysis using several of these instruments obtained one compound, namely 3-amino-2-cyano-1-phenyl-1H-benzo[a]pyrano[2,3-c]phenazine. The reaction conditions were carried out at 50°C for 6 h in ethanol with unassisted catalysts and obtained a product yield of 45%.

Keywords: Phenazine derivative, 2-hydroxy-1,4-naphthoquinone, one-pot four-component.

1. Introduction

1,4-naphthoquinone are generally found in both natural (occur in plants, fungi, and some animals with chromatic pigments) and synthetic products and represent several biological activities [1-3]. Previous studies have revealed that 1,4-naphthoquinone derivated compounds have classified as molluscsicide, antiproliferative, antifungal, antimalarial, antibacterial, antileishmanial, and antiviral activities [4-10]. The biological activity of a quinone cannot be separated from the uniqueness of its structure which is capable of transferring electrons and protons [11,12]. Its ability to accept one or two electrons will be useful in the formation of suitable radical anions or radical di-ions [13]. It makes easy to modify the structure by adding the substituents [14]. The existence of these structural aspects, derivatives of naphthoquinone compounds can act as a variety of biological activities. One of the derivatives of naphthoquinone that has an impressive structure and bioactivity is lawsone. Lawsone, 2-hydroxy-1,4-naphthoquinone, is the primary coloring agent found in the henna plant (Lawsonia inermis L.) [15]. Lawsone has been reported to have biological activities such as antibacterial, antifungal, and antitumor, and often used as a hair dye and as a sunscreen in ultra-violet (UV) filters [16-20].

In order to increase the wealth of structure and biological activity, the compound 2-hydroxy-1,4-naphthoquinone can be modified into a phenazine derivative compound. Phenazine compound is nitrogen-containing heterocyclic that found mostly as secondary metabolites. It is primarily in Pseudomonas, Streptomycyes, and several genera from the soil and marine habitats [21]. Phenazine has a diversity of biological functions like anti-mycobacterial [22], antimalarial [23, 24], and fungicides [25]. Phenazine derivatives that have been produced from a naphthoquinone and aromatic diketone...
display an activity as antitumor agents [26]. Besides, conjugated heterocyclic pyran has shown an antioxidant activity against DPPH radicals [27]. Moieties of molecules with phenazines and pyrans have gained a significant attention in the discovery of drug.

Various methods have been carried out and reported to synthesize the phenazine-derived compounds. The methods that have been carried out include the one-pot two-step [28] and one-pot four-component methods which have been carried out to synthesize organic compounds with the help of various types of the catalyst [29]. The one-pot two-step method is carried by first reacting two or three precursors. Then, the compound from the synthesis is added to the next precursor to get the targeted compound. The multicomponent domino reaction (MDR) which is carried out by reacting four precursors at once is a useful way for the synthesis of important biological compounds. This method has advantages including environmentally friendly, reduces chemical waste formation, cost-effective, and produces higher yields [30, 31]. Therefore, in this research, the synthesis of phenazine-derived compounds will be carried out with the one-pot four-component method by using lawsone as a precursor.

2. Materials and method

2.1. General

All chemicals were obtained from Merck and Aldrich and applied without further purification. IR spectrum was recorded on a Shimadzu Prestige 21 spectrometer. UV-Vis spectrum was recorded on a Shimadzu 2600 spectrophotometer. The mass spectrum was recorded on an LC-MS (Liquid Chromatography-Mass Spectrometer) Agilent Technologies Type 7890A-5975C. Thin-layer chromatography (TLC) was performed on a GF254 plate.

2.2. General procedure for the synthesis of phenazine derivatives

2-hydroxy-1,4-naphthoquinone (1 mmol), o-phenylenediamine (1 mmol), benzaldehyde (1 mmol), and malononitrile (1 mmol) were mixed in a round-bottomed flask at 50 °C in ethanol (10 mL). In general, the reaction takes place as illustrated in figure 1. The flask was fitted with a condenser. Then, the mixture was heated to reflux under magnetic stirring and monitored by TLC. Once completed, the reaction was cooled and the product was filtered and cleansed two times with water (2 x 5 mL). Then, the product was dried and immediately recrystallized with hot ethanol. The product was evaluated with instruments FT-IR, UV-Vis Spectrophotometer, and LC-MS to determine the vibration of the functional group, the maximum wavelength, and its molecular weight, respectively.

3. Results and discussion

The synthesis of phenazine-derived compounds using lawsone as a precursor has been performed through the one-pot four-component method. The proposed mechanism of reaction that occurs in the synthesis of this compound is starting from the reaction between 2-hydroxy-1,4-naphthoquinone with o-phenylenediamine. The results of this reaction will form benzo[a]phenazines. On the other hand, an amination reaction also occurs between benzaldehyde and malononitrile simultaneously and will form the aroyl isothiocyanate compound. In the final stage, the reaction occurs between the benzo[a]phenazine compound and aroyl isothiocyanate to form 3-amino-2-cyano-1-phenyl-1H-benzo[a]pyrano [2,3-c] phenazine. Referring to previous studies, the reaction conditions were carried out at 50 °C for 6 h in ethanol with unassisted catalysts and obtained a product yield of 45 %.

Structure evaluation and determination of the compound obtained was conducted by measuring the functional groups, the maximum wavelength, and the determination of the molecular weight with instruments FT-IR, UV-Vis Spectrophotometer, and LC-MS, respectively. Vibration function measurement results with FTIR obtained several specific peaks that identify the functional group of the target compound (figure 2a). Two specific peaks at wave numbers 3415 cm⁻¹ and 3321 cm⁻¹ are the stretching vibrations of primary amine (NH₂) that will always appear in two absorption peaks.
Figure 1. Reaction scheme for the synthesis of 3-amino-2-cyano-1-phenyl-1H-benzo[a]pyrano[2,3-c]phenazine.

Figure 2. FTIR (a) and Uv-Vis (b) spectrum of phenazine derivative compound.

In addition, there is also an N-H bending vibration at the wavenumber 1403 cm$^{-1}$ with moderate intensity. The stretching vibrations of the C-N, C=N, and C≡N groups are present in the absorption of 1253 cm$^{-1}$, 1625 cm$^{-1}$, and 2187 cm$^{-1}$, respectively. Besides, a peak in 3081 cm$^{-1}$ is a stretching vibration of C-H aromatic (sp$^2$) contained in the benzene ring, while the peak at 3007 cm$^{-1}$ is a stretching vibration of C-H (sp$^3$). C = C stretching vibrations from aromatics are found in 1648 cm$^{-1}$ and 1560 cm$^{-1}$. The peak at 1148 cm$^{-1}$ is the stretching vibration of the C-O-C group. The absorption of functional groups at the peaks, as mentioned earlier, supports that the target compound has all of these groups.

UV-Vis spectrophotometer analysis was performed to detect the chromophore groups from the structure of the obtained compounds. Energy absorbed in the UV region produces a transition of valence electrons in the molecule. This transition occurs from molecular orbitals (usually p and π orbitals) to the higher energy levels (antibonding p* and π*). Measurement of the $\lambda_{\text{max}}$ of the target compound shows the maximum absorption at 295 nm and 412 nm (figure 2b). The peak shifting to the right of the maximum wavelength is called the bathochromic shift or redshift. This shift occurs due to the addition of functional groups that are formed in phenazine-derived compounds. In this case, an electron transition occurs from n orbitals to π* giving an absorption area of $\lambda_{\text{max}}$ more than 300 nm. As a result of this shift occurring in the visible area, the color of the compound can be observed as yellow.

Determination of the molecular weight of the target compound was analyzed using LC-MS by the positive ion method, methanol eluent, and gradient elution system. Figure 3 shows the LC-MS spectrum of the target compound along with the fragments formed from the compound. Based on the analysis results, there is a peak with an m/z value of 401 which is the molecular weight of the synthesized compound. In the LC-MS analysis, the Electrospray Ionization (ESI) setting is used so that the mass that appears is [M+H]$^+$. The m/z value of 401 as [M+H]$^+$ ion corresponds to the compound expected to have
the molecular formula $C_{26}H_{16}N_4O$ with the IUPAC name 3-amino-2-cyano-1-phenyl-1H-benzo [a] pyrano [2,3-c] phenazine. Fragments formed from the results of the analysis using LC-MS can be proposed as seen in figure 4. Several important fragments have a high intensity like $m/z = 281$, $m/z = 247$, and $m/z = 120$.

4. Conclusion
The phenazine derivative was successfully synthesized using lawsone as a precursor through the one-pot four-component method. The synthesized compound was evaluated with some instruments and showed that the analysis of FTIR, Uv-Vis, and LC-MS spectra revealed the characteristics of the target compound. The results of the analysis have identified that the compound formed was a phenazine derivative named 3-amino-2-cyano-1-phenyl-1H-benzo [a] pyrano [2,3-c] phenazine. The target
compound was synthesized with a yield of 45% and the conditions of reaction were conducted at 50 °C for 6 h in ethanol with unassisted catalysts.

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