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Synthesis and antioxidant activity of benzo[a]pyrano[2,3-c] phenazine derivative compound via one-pot multicomponent reaction

Agus Rimus Liandi [1] *, Antonius Herry Cahyana[2]*

[1] Department of Chemistry, Faculty of Science and Technology, Syarif Hidayatullah State Islamic University Jakarta, Tangerang Selatan, 15412, Indonesia
[2] Department of Chemistry, Faculty of Mathematics and Natural Sciences, Universitas Indonesia, Depok, 16424, Indonesia
* Corresponding Author. E-mail: agusrimus@uinjkt.ac.id (A.R.L); herrykim@sci.ui.ac.id (A.H.C).

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ABSTRACT: Phenazine derivative compounds are of concern because of their extraordinary activities in medicine. The aim of this study is to synthesize the derivative of benzo[a]pyrano[2,3-c] phenazine compound based on lawsone with caffeine catalyst using the one-pot multicomponent reaction method and test its antioxidant activity. The formation of the target compound was evaluated for functional group vibration, maximum wavelength, and molecular weight by FTIR, UV-Vis Spectrophotometer, and LCMS, which showed the characteristics of the benzo[a]pyrano[2,3-c] phenazine compound. The synthesized compound was 3-amino-2-cyano-1-(2-hydroxyl-phenyl)-1H-benzo[a]pyrano[2,3-c] phenazine with the molecular formula $C_{26}H_{16}N_4O_2$. At the optimum condition of the reaction, the use of caffeine catalyst was able to give a product yield of 67.2%. The potential of the synthesized compound as an antioxidant could be classified as a good antioxidant with an IC_{50} value of 14.26 ppm.

KEYWORDS: Benzo[a]pyrano[2,3-c] phenazine; lawsone; one-pot multicomponent reaction; antioxidant; caffeine catalyst.

1. INTRODUCTION

Heterocyclic compounds containing nitrogen atoms are an important concern because of their characteristics and bioactivity in medicine [1]. Various approaches to the synthesis of nitrogen-based cyclic compounds have been carried out to enrich their derivatives [2]. One of the nitrogen-containing heterocyclic compounds that have many pharmaceutical benefits is phenazine [3]. Both synthetically and naturally, phenazine compounds are unique and attractive because of their structural diversity and biological abilities [4]. Some of the bioactivity of phenazine compounds and their derivatives that have been investigated have activity as antiphrastic, antimicrobial, fungicidal, antiplatelet, trypanocidal, antimalarial, and antifungal [5–10].

Phenazine-derived compounds in nature are found in several bacterial genera found in marine and terrestrial environments (such as Streptomyces and Pseudomonas) [4], [11]. Synthetically, phenazine compounds are continuously researched and developed using various methods, types of constituent reactants, and the use of catalysts [12–16]. One of them is through the formation of benzo[a]pyrano[2,3-c] phenazine derivatives based on lawsone compounds.

A simple method commonly used in the synthesis of organic compounds is the one-pot multicomponent reaction (MCR) [17,18]. MCR is a green strategy used to produce a product in one flask at a time from a reaction consisting of three or more reactants [19,20]. So in this study, the four reactants (lawsone, o-phenylenediamine, malononitrile, and 2-hydroxybenzaldehyde) were mixed in one flask for the reaction to proceed.

The use of a catalyst in the reaction of organic compounds is needed to shorten the time and energy [21]. Various catalysts that have been used for the synthesis of benzo[a]pyrano[2,3-c] phenazine derivatives were ionic liquids, theophylline, and nano CuO catalysts [5,22,23]. In addition, benzo[a]pyrano[2,3-c]phenazine derivatives have also been synthesized using composite catalysts such as $FeAl_2O_4$ and Ce/PDA/CPTS@$CoFe_2O_4$ [24,25]. Considering the importance of its bioactivities, we report the synthesis of a

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benzo[a]pyrano[2,3-c] phenazine derivative from the reactants of lawsone, o-phenylenediamine, malononitrile, and 2-hydroxybenzaldehyde, with the help of a caffeine (1,3,7-Trimethylpurine-2,6-dione) catalyst using the one-pot multi-component reaction method and tested its bioactivity as an antioxidant (Figure 1).

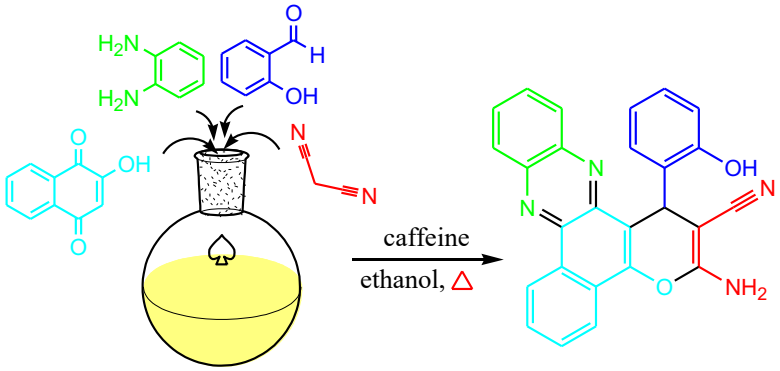

Figure 1. The general design of benzo[a]pyrano[2,3-c] phenazine derivative compound synthesis

2. RESULTS AND DISCUSSION

The synthesis of benzo[a]pyrano[2,3-c] phenazine derivative compound has been conducted with assisted caffeine as a catalyst through the one-pot multicomponent reaction method. The formation of the target compound was monitored by examining the vibration of the functional group with FTIR. Figure 2 shows the FTIR spectrum of the synthesized compound which shows several vibrational peaks of the functional groups that make up the benzo[a]pyrano[2,3-c] phenazine derivative compound. The presence of a broad peak at wave number 3441 cm^{-1} indicates the stretching vibration of the O-H group, the peak at 3313 cm^{-1} and 3173 cm^{-1} is the stretching vibration of N-H_2 (primary amine), the peak at 3060 cm^{-1} is the stretching vibration of C-H, the peak at 2189 $cm^{-1}$is the C≡N vibration, the peaks at 1659 cm^{-1}, 1597 cm^{-1}, 1324 cm^{-1}, and 1230 cm^{-1} are the C=N, C=C, C-N, and C-O-C vibrations, respectively. The various vibrational bands found in the prepared product are shown in Table 1.

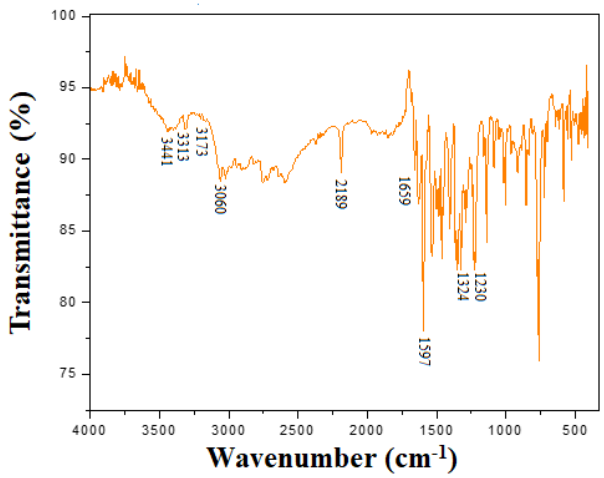

Figure 2. FTIR spectrum of the synthesized compound

Table 1. The various vibrational bands found in the prepared product

No	Functional group vibration	Wavenumber (cm^{-1})	Reference
1	O-H	3441	[26]
2	N-H_2 (primary amine)	3313 and 3173	[27]
3	C-H	3060	[27]
4	C≡N	2189	[28]
5	C=N	1659	[29]
6	C=C	1597	[28]
7	C-O-C	1230	[30]

Measurement of the maximum wavelength of the synthesized compound was carried out at a wavelength of 200-800 nm. The UV-Vis spectrum (Figure 3) showed the maximum absorption of the synthesized compound at a wavelength of 420 nm. when compared to the four bonds that make up this compound, the lawsone compound has the largest absorption wavelength at 340 nm. The maximum wavelength shift to the right is called bathochromic shift or redshift which indicates the presence of additional substituents in the synthesized compound. At the maximum absorption wavelength, a transition from n to π* occurs due to the presence of aromatic chromophore coupled with extended resonance and the addition of several other substituents.

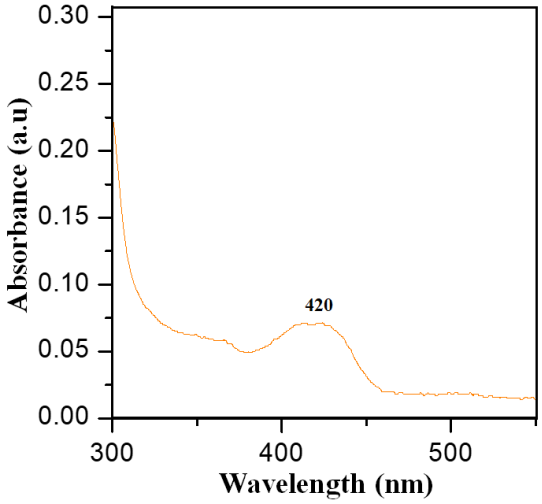

Figure 3. Maximum wavelength of the synthesized compound

Determination of the molecular weight of the synthesized compound was carried out by LCMS analysis with a column size of 2.1 µm x 150 mm, injection volume 0.5 – 1 µL, methanol solvent, with a gradient elution system. The molecular weight of the synthesized compound was analyzed using the positive ion method and the main peak was found at m/z 417 (Figure 4). This peak corresponds to the molecular weight of the target compound with the molecular formula $C_{26}H_{16}N_4O_2$ as the $[M+H]^+$ ion in the Electrospray Ionization (ESI) setting. As for the name of the synthesized compound was 3-amino-2-cyano-1-(2-hydroxyl-phenyl)-1H-benzo[a] pyrano[2,3-c] phenazine.

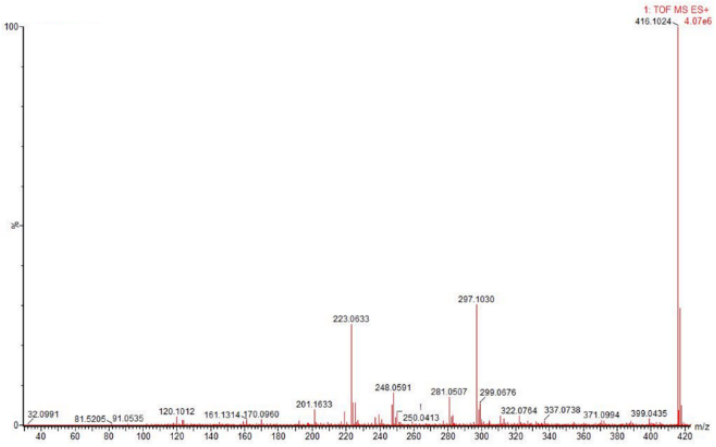

Figure 4. Mass spectrum of the synthesized compound

Based on mass spectrum analysis, several fragments of the compound $C_{26}H_{16}N_4O_2$ were found. The fragments are located at m/z 399, 322, 297, and 233. The proposed structure of the fragments is shown in Figure 5.

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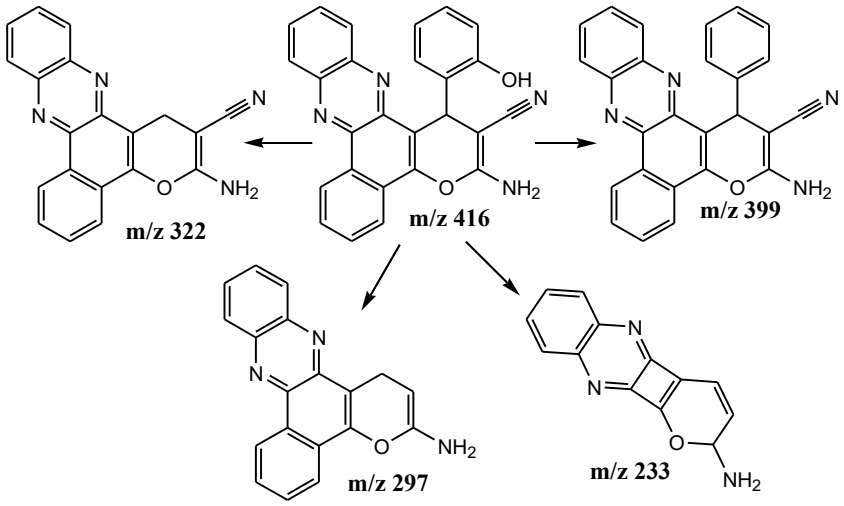

Figure 5. Several fragments of synthesized compound from mass spectrum analysis

The proposed reaction mechanism in the synthesis of 3-amino-2-cyano-1-(2-hydroxyl-phenyl)-1H-benzo[a]pyrano[2,3-c] phenazine in Figure 6 begins with a condensation reaction between lawsone (1) and o-phenylenediamine (2) compounds. The reaction of these two substrates forms a phenazine ring (3) where the carbon on the carbonyl group is attacked by a nucleophile (o-phenylenediamine) and releases two H_2O molecules. Furthermore, 2-hydroxybenzaldehyde (4) and malononitrile (5) form olefins (6) with the help of caffeine as a catalyst. In the last step, the phenazine ring (3) reacts by addition with the olefin (6) to form an intermediate (7) which is cyclized to form the target compound (8).

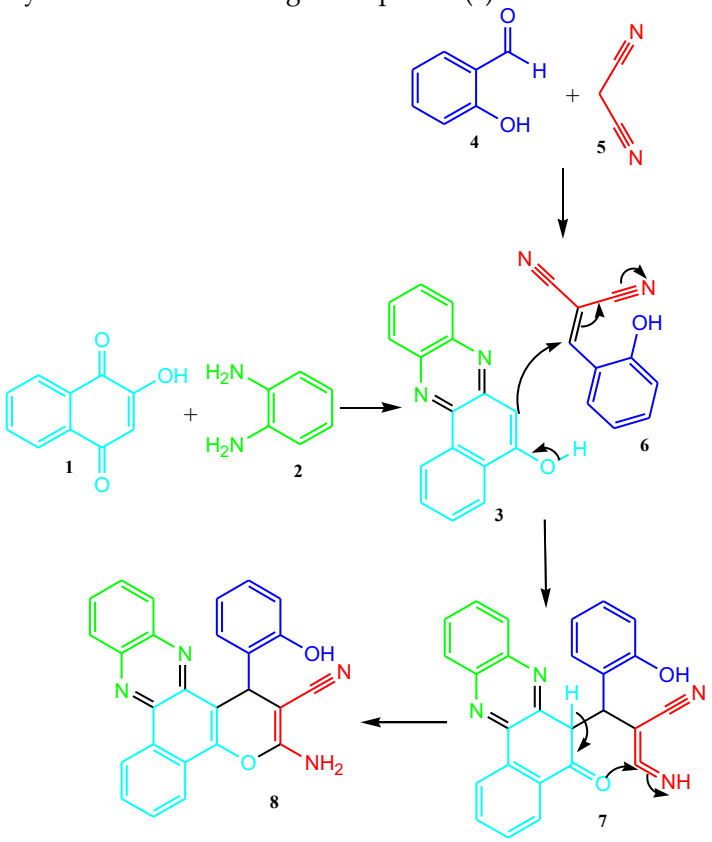

Figure 6. The proposed reaction mechanism of the synthesized compound

The optimization of reaction conditions to get the best yield is done by varying the concentration of catalyst (5, 10, 15, 20% w/w), reaction temperature (room temperature, 50°C, boiling point of solvent), and reaction time (15, 30, 45, 60 minutes). The optimum conditions were obtained by using a 15% w/w caffeine

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catalyst at the boiling point of the solvent for 45 minutes time reaction. The yield of the product obtained at the optimum condition was 67.2%.

The antioxidant activity of benzo [a]pyrano[2,3-c] phenazine derivative was carried out by DPPH assay which was measured by UV-Vis spectrophotometer at a wavelength of 517 nm. Based on the calculation of the IC_{50} value using a linear regression relationship between the logarithm of concentration and %inhibition, it was found that the IC_{50} value of the synthesized compound was 14.26 ppm (strong antioxidant ≤50 ppm). At this concentration, the synthesized compound was able to reduce 50% of DPPH free radicals. This number is much smaller than the IC_{50} value of the lawsone compound as the precursor used, which was 241.71 ppm (moderate antioxidant 101-250 ppm). Thus, the synthesized compound had better antioxidant capacity than lawsone compound. The comparison of antioxidant activity of the lawsone and the synthesized compound is shown in Table 2.

Table 2. The comparison of antioxidant activity of lawsone and synthesized compound

Concentration	%Inhibition	
	Lawsone	Synthesized compound
5 ppm	26.33	40.82
10 ppm	30.47	50.59
50 ppm	32.54	58.28
100 ppm	42.60	63.01
300 ppm	56.21	65.38
IC_{50} (ppm)	241.71	14.26

3. CONCLUSION

The benzo[a]pyrano[2,3-c] phenazine derivative compound has been successfully synthesized using lawsone, o-phenylenediamine, malononitrile, and 2-hydroxybenzaldehyde as the precursor through the one-pot multicomponent reaction with caffeine catalyst. The structural elucidation of the synthesized compounds was carried out by FTIR, Uv-Vis spectrophotometer, and LCMS. The experimental data showed that the synthesized compound was 3-amino-2-cyano-1-(2-hydroxyl-phenyl)-1H-benzo[a]pyrano[2,3-c] phenazine with the molecular formula $C_{26}H_{16}N_4O_2$. The optimum conditions for the reaction were obtained at 15% w/w caffeine catalyst at the boiling point of solvent for 45 minutes time reaction. The ability of the synthesized compound as an antioxidant was classified as good with an IC_{50} value of 14.26 ppm, compared to lawsone compound with an IC_{50} of 241.71 ppm.

4. MATERIALS AND METHODS

4.1. Materials and instruments

All chemicals and reagents in this study were used for analytical grade without any further purification. These include lawsone (2-hydroxy-1,4-naphthoquinone), o-phenylenediamine, malononitrile, 2-hydroxybenzaldehyde, caffeine (1,3,7-trimethylpurine-2,6-dione), DPPH (2,2-diphenyl-1-picrylhydrazyl), ethanol, and methanol. Structure elucidation of the product was analyzed for its functional group vibration, maximum wavelength, and molecular weight by Fourier Transform InfraRed (FTIR; Shimadzu Prestige 21), Ultraviolet-Visible Spectrophotometer (UV-Vis; Shimadzu 2600), and Liquid Chromatography Mass Spectrometer (LCMS; Agilent Technologies 7890A-5975C), respectively.

4.2. Synthesis of benzo[a]pyrano[2,3-c] phenazine derivative compound

Synthesis of the benzo[a]pyrano[2,3-c] phenazine derivative was carried out by mixing 1 mol of lawsone, 1 mol of o-phenylenediamine, 1 mol of malononitrile, and 1 mol of 2-hydroxybenzaldehyde in a reflux flask with ethanol as a solvent. The general procedure of benzo[a]pyrano[2,3-c] phenazine derivative synthesis is shown in Figure 1. The reaction conditions were varied with the catalyst concentration, reaction temperature, and reaction time. After completion, the synthesized product was purified by recrystallization with ethanol.

4.3. Antioxidant activity test (DPPH assay)

Determination of antioxidant activity was carried out using the dpph assay method [31,32]. Concentration series of sample solution (5; 10; 50; 100; 300 ppm) in methanol solvent and DPPH solution with

a concentration of 100 µM. 2 mL of each sample solution was reacted with 1 mL of DPPH and incubated for 30 minutes at room temperature in dark conditions. The absorbance of the solution was measured by a UV-Vis spectrophotometer at a wavelength of 517 nm. The IC_{50} value was calculated by linear regression relationship between the logarithm of concentration and %inhibition with the formula:

$$\%\text{inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \text{ x } 100\%$$

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