Synthesis and antibacterial activity of 2-((1,4-dioxo-1,4-dihydronaphthalen-2-yl)amino)ethyl acetate

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Abstract. Derivatization of lawson compound (1) was done by reacting it with ethanolamine to give 2-(((2-hydroxyethyl)amino)-1,4-naphthoquinone (2), and then treating with acetic anhydride to produce the desired compound 2-((1,4-dioxo-1,4-dihydronaphthalen-2-yl)amino)ethyl acetate (3). The target compound possesses the antibacterial activity against E. coli and S. aureus.

1. Introduction
Antibacterial activity is one of the natural product properties for killing or inhibiting the growth of bacteria [1]. The necessity of antibacterial in Indonesia was high since 2008 until 2010 [2]. Moreover, there are many cases of drugs resitancy caused by bacterial mutation [3]. Nowadays, researchers have been searching for new natural products or their derivatives with potent antibacterial activities.

2-hydroxy-1,4-naphthoquinone (lawsone) is one of natural products isolated from henna leaf (Lawsonia inermis) [4]. It is usually utilized as hair dye for woman. Lawson compound has been known to show many bioactivities, such as antifungal, anti-inflammatory, antioxidant, and antibacterial [5]. The antibacterial activity is affected by the presence of two carbonyl groups at C-1 and C-4 position of naphthoquinone ring. Meanwhile, functional group at C-2 affect hydrophilicity of naphthoquinone [6]. Herein, we report two-step synthesis of 2-((1,4-dioxo-1,4-dihydronaphthalen-2-yl)amino)ethyl acetate (3) from lawson (figure 1). Then, antibacterial activity will be tested using disc diffusion method [7] against the gram-positive bacteria of S. aureus and gram-negative bacteria of E. coli.

2. Materials and methods

2.1. Materials
All chemicals were analytical grade and purchased from some chemical companies. Staphylococcus aureus and Escherichia coli were obtained from Biochemistry Laboratory, Department of Chemistry, Universitas Indonesia. The growth of bacterial was supported by nutrient broth (NB) and nutrient agar (NA) from Merck, Germany. Dimethyl Sulfoxide (DMSO) was used as solvent in antibacterial test and also as negative control, whereas chloramphenicol was used as positive control. Fourier Transform-Infrared (FT-IR) spectra were recorded on Shimadzu Prestige 21 spectrophotometer. Mass spectra were obtained from Shimadzu QP Mass Spectrometer (MS) 2010A with 70 eV electron ionization potential.

2.2. Methods

2.2.1. Synthesis of 2-((2-hydroxyethyl)amino)-1,4-naphthoquinone (compound 2). Synthesis of 2-((2-hydroxyethyl)amino)-1,4-naphthoquinone (2) was initiated by mixing of lawson (1), ethanol, and
ethanolamine as reagent. Lawsone (1 mmol) was dissolved in 20 mL ethanol in 50 mL round-bottom flask. Ethanolamine (1 mmol) was inserted into the flask. Reaction was performed at room temperature for 2 h, and product formed was monitored by Thin-Layer Chromatography (TLC). Solvent was volatilized, and then crude product was purified by column chromatography to obtain compound 2.

2.2.2. Synthesis of 2-((1,4-dioxo-1,4-dihydronaphthalene-2-yl)amino)ethyl acetate (compound 3).
Compound 2 (0.25 mmol) and acetic anhydride (5 mL) was reacted under reflux system at 115 °C for 4 h. After completion, solvent was volatilized with vacuum and crude product was refined by column chromatography to get compound 3 as red solid.

2.2.3. Antibacterial activity assay. In antibacterial activity assay, samples were dissolved in DMSO (2000 µg/mL). Chloramphenicol and DMSO were used as positive and negative control, respectively. Nutrient agar (20 mL) was mixed with nutrient broth (NB) bacterial suspension (200 µL) in which absorbance was measured within range 0.08 to 0.10 [8]. The mixture was put in the petri dish. A 6 mm disc paper was immersed in one antibacterial sample for 30 seconds and put on the hard agar. Petri dish was put inside an incubator for 48 h. Diameter of growth inhibition zone was measured to determine antibacterial activity.

3. Results and discussion

3.1. Synthesis process
Compound 2 was synthesized by utilizing the resonance properties of naphthoquinone ring. Resonance in naphthoquinone ring affected the stabilization of structure. Amine group which is good nucleophilic attacked and bonded with carbon atom at C-2 [9]. Hydroxyl group of lawsone bonded with a proton and naphthoquinone ring and released hydroxyl group as water molecule, while electron stabilized the structure immediately. Reaction yielded at 56% of 2 as red solid. We synthesized compound 2 again with water as solvent with the isolated yield of 46%.

Compound 3 was synthesized by acetylation of hydroxyl group. Resonance of anhydride acetic gave a place of carbon atom to be attacked by hydroxyl group. This affected the oxygen atom to withdraw electron and release as acetic acid immediately, while electron stabilized the structure [10]. Yield percentage of the reaction was found to be 52% as red solid.

3.2. Characterization of products
In FT-IR spectrum of 2-((2-hydroxyethyl)amino)-1,4-naphthoquinone (2), a medium broad peak appeared from 3200 cm⁻¹ to 3600 cm⁻¹ was interpreted as overlapping of secondary amine (-NH₂) and hydroxyl groups (-OH). A sharp peak appeared at 1725 cm⁻¹ and a weak peak located at 1650 cm⁻¹ were assumed as identity of carbonyl (C=O) and C=C double bond, respectively. FT-IR spectrum of 2-((1,4-dioxo-1,4-dihydronaphthalene-2-yl)amino)ethyl acetate (3) showed a different functional group than the former product. A medium sharp peak appeared at 3333 cm⁻¹ was assumed as secondary amine group (-NH) [11]. Moreover, at wavenumber of 1050 cm⁻¹, a weak sharp peak attributed to C-O-C functional group. The FT-IR spectra of compound 2 and 3 can be seen in figure 2.

In MS characterization, both products were measured to ensure the relative molecular mass corresponding to structure of compound. Figure 3a shows MS spectrum of 2-((2-hydroxyethyl)amino)-1,4-naphthoquinone (compound 2). The m/z value of compound 2 was 217. This number was matched with Mr of the desired product having molecular formula of C₁₉H₁₆NO₂. For the MS characterization of 2-((1,4-dioxo-1,4-dihydronaphthalene-2-yl)ethyl acetate (compound 3), the m/z value of this compound was found to be 259. The figure 3b shows MS spectrum of 2-((1,4-dioxo-1,4-
Table 1. Result of antibacterial activity test

| Antibacterial Sample                  | S. aureus (mm) | E. coli (mm) |
|---------------------------------------|---------------|--------------|
| Positive control (Chloroamphenicol)   | 16.1          | 12.2         |
| Negative control (DMSO)               | No activity   | No activity  |
| Compound 1                            | 11.0          | 20.0         |
| Compound 2                            | 9.8           | 17.1         |
| Compound 3                            | 10.0          | 10.8         |

Figure 2. FT-IR Spectrum of lawsone derivates: (a) 2-(2-hydroxyethyl)amino)-1,4-naphthoquinone; (b) 2-((1,4-dioxo-1,4-dihydronaphthalene-2-yl)amino)ethyl acetate

Figure 3. Mass spectra of (a) 2-((2-hydroxyethyl)amino)-1,4-naphthoquinone (compound 2) and (b) 2-((1,4-dioxo-1,4-dihydronaphthalene)amino)ethyl acetate (compound 3)

dihydronaphthalene-2-yl)amino)ethyl acetate (3). This number was matched with Mr of product having formula of C_{14}H_{13}NO_{4}. Both products have an odd m/z number which indicate the presence of nitrogen atom within the structures [13].

3.3. Antibacterial activity test

Disc diffusion method is one of the antibacterial tests where the measurement of growth inhibition zone is the indicator of antibacterial activity. Therefore, antibacterial activity is measured by the ability to inhibit or kill a bacterial which form an inhibition zone [7,8]. Classification of antibacterial
activity ability listed by diameter of growth inhibition zone. Very strong: ≥ 30 mm; strong: 21–29 mm; medium: 16–20 mm; weak: 6–15 mm; and no activity ≤ 6 mm [6].

In antibacterial test against *S. aureus*, all tested compounds showed weak activity (table 1). Compound (1) was able to form 11 mm inhibition zone, while (2) and (3) were able to form 9.8 and 10 mm, respectively. In another antibacterial test against *E. coli*, the activity of compound (1) and (2) were stronger than compound (3). Lawsone (1) was able to form 20 mm inhibition zone, while compound (2) and (3) were able to form 17.1 and 10.8 mm, respectively. These results prove that lawsone derivates have antibacterial activity against *S. aureus* and *E. coli*.

4. Conclusions
We have successfully synthesized lawsone derivatives (compound 2 and compound 3) in medium yields. These compounds showed antibacterial activity against *S. aureus* and *E. coli* at 2000 μg/mL sample concentration. However, derivative products have weaker antibacterial activity than lawsone (1). It may be due to the solubility effect of the derivatives that could limit the interaction with bacteria.

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