Synthesis of derivatives azomethine compounds bonded to alkoxyalted benzene and their antibacterial activity tests

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Abstract. Azomethin compound has been synthesized from addition-elimination reaction between aromatic aldehyde compounds such as benzaldehyde, verataldehyde, piperonal with primary amine compound from ethylenediamine. The presence of imine group (>C=N), and alkoxy groups such as methoxy (OCH₃) and methylenedioxy (O-CH₂-O-) affected to their activity as antibacterials. In the synthesis of methoxy substituted azomethine compound from verataldehyde with ethylenediamine to produce N,N'-Bis(3,4-dimethoxybenzylidene) ethylenediamine in the form of white powder has a melting point of 164.8–166.2°C and in about 36.98% yield. In the synthesis of methylenedioxy-substituted azomethine compound from piperonal with ethylenediamine produced N,N'-Bis(3,4-methylenedioxybenzylidene) ethylenediamine in the form of white powder has a melting point of 164.8–166.2°C and in about 82% yield. In the synthesis of non-substituted azomethine compound from benzaldehyde with ethylenediamine produced N,N'-Bis(benzylidene) ethylenediamine in the form of white powder has a melting point of 110.2–111.4°C and in about 34.82% yield. Antibacterial activity of compound N,N'-Bis(3,4-dimethoxybenzylidene) ethylenediamine, N,N'-Bis(3,4-methylenedioxybenzylidene) ethylenediamine, and N,N'-Bis(benzylidene) ethylenediamine with inhibitory zone diameter 0.25; 3.08; 0.06 mm in S.aureus bacteria (G⁺) and 3.26; 4.61; 3.48 mm in E.coli bacteria (G⁻).

1. Introduction
Azomethin compound is a compound which has an imine group (>C=N-) from the addition-elimination reaction between carbonyl compounds from aldehydes or ketones with the primary amine compound from ethylenediamine. Azomethin compound has been known to play an important role in their biological activities such as antibacterials [1]. Antibacterial activity of azomethine compound is caused by the interaction of the imine group with the bacterial cell membrane resulting in bacterial cell lysis [2]. Several studies on antibacterial activity with 1 and 2 imine groups have been reported that azomethine compound with 2 imine groups has better antibacterial activity than 1 imine group [3,4].

In this study, synthesis of azomethine derivatives bound to benzene with 2 imine group (>C=N-) and substituted by alkoxy groups including methoxy (OCH₃) and methylenedioxy (O-CH₂-O-) substituent, or without substituent. First, synthesis of methoxy-substituted azomethine compound, N,N'-Bis(3,4-dimethoxybenzylidene) ethylenediamine from verataldehyde with ethylenediamine.
second is the synthesis of methylenedioxy-substituted azomethine compound namely \(N,N'\)-Bis(3,4-methylenedioxybenzylidene) ethylenediamine from piperonal with ethylenediamine. The third is the synthesis of unsubstituted azomethine as a comparison namely \(N,N'\)-Bis(benzylidene)ethylenediamine from benzaldehyde with ethylenediamine. From the three compounds that was obtained, then it was tested as antibacterials against \(S\). \(aureus\) (G+) and \(E\). \(coli\) (G-) bacteria. By comparing the antibacterial activity of the three compounds, it can be seen the influence of the type of substituent bound to the azomethine compound. The presence of two methoxy groups in azomethine \(N,N'\)-Bis(3,4-dimethoxybenzylidene)ethylenediamine and methylenedioxy groups in azomethine \(N,N'\)-Bis(3,4-methylenedioxybenzylidene) ethylenediamine is expected to influence antibacterial activity compared to antibacterial activity. azomethine \(N,N'\)-Bis(benzylidene)ethylenediamine compounds without substituent.

2. Experimental

2.1. Materials and tools

The laboratory equipments are reflux tools, glass tools, funnels, magnetic stirrers (VS 130SH) and bars, chambers, analytical balance sheets (Ohaus), petri dishes, ose needles, tweezers, micropipettes 10-100 μL, spreaders, incubators (Memmert IN55), thermometer, autoclave, orbital shaker, callipers, UV 254 nm and 366 nm, Laminar Air Flow, evaporator (Buchi), melting point determinant (electrothermal melting point 1001D), UV-VIS spectrophotometer (Shimadzu UV 1280) and IR spectrophotometer (Perkin Elmer Frontier 96681).

The ingredients in this research are benzaldehyde (p.a., Merck), verataldehyde (p.a., Merck), piperonal (p.a., Merck), ethylenediamine (p.a., Merck), glacial acetic acid (p.a., Merck), methanol (p.a., Merck), ethanol (p.a., Merck), ethylacetate (p.a., Merck), n-hexane (p.a., Merck), diethyl ether (p.a., Merck), chloroform (p.a., Merck), dichloromethane (p.a., Merck), aquades, dimethyl sulfoxide (p.a., Merck), thin layer chromatography plate (Merck), whatman filter paper No. 42, yeast extract (Merck), peptone (Merck), nutrient agar (Merck), ampicillin (pharmaceutical chemistry), and 70% alcohol (Brataco).

2.2. Synthesis of \(N,N'\)-Bis (3,4-dimethoxybenzylidene) ethylenediamine from verataldehyde with ethylenediamine

A (0.67 mL, 20 mmol) ethylenediamine was dissolved in 10 mL ethanol in a three-neck round bottom flask. In other beaker glass, as much as (3,32 g, 20 mmol) verataldehyde was dissolved in 15 mL ethanol and mixed into ethylenediamine solution. Glacial acetic acid (5-10 drops) as an acid catalyst was added to the solution. Then the solution was stirred with a magnetic stirrer, stirring speed of 400 rpm and heated at 78°C for 3 hours. The reaction mixture was placed on a cold water bath for 10 minutes and filtered using Whatman filter paper and funnel. The product precipitate was then recrystallized by dissolving it in hot ethanol, filtered again, washed with cold ethanol and dried. Furthermore, the azomethine compound \(N,N'\)-Bis(3,4-dimethoxybenzylidene) ethylenediamine was weighed and analyzed using melting point test, solubility test, UV-Vis spectrophotometry and IR spectrophotometry [4].

2.3. Synthesis of \(N,N'\)-Bis (3,4-methylenedioxybenzylidene) ethylenediamine from Piperonal with Ethylenediamine

A (0.67 mL, 20 mmol) ethylenediamine was dissolved in 10 mL ethanol in a three-neck round bottom flask. In other beaker glass, as much as (3,00 g, 20 mmol) was dissolved in 15 mL ethanol and mixed into ethylenediamine solution. Glacial acetic acid (5-10 drops) as an acid catalyst was added to the solution. Then the solution was stirred with a magnetic stirrer, stirring speed of 400 rpm and heated at 78°C for 3 hours. The reaction mixture was placed on a cold water bath for 10 minutes and filtered using Whatman filter paper and funnel. The product precipitate was then recrystallized by dissolving it in hot ethanol, filtered again, washed with cold ethanol and dried. Furthermore, the azomethine
compound \(N,N'\)-Bis(3,4-methylenedioxybenzylidene) ethylenediamine was weighed and analyzed using melting point test, solubility test, UV-Vis spectrophotometry and IR spectrophotometry [4].

2.4. Synthesis of \(N,N'\)-Bis (benzylidene) ethylenediamine from benzaldehyde with ethylenediamine

A (0.67 mL, 20 mmol) ethylenediamine was dissolved in 10 mL ethanol in a three-neck round bottom flask. In other beaker glass, as much as (2.03 mL, 20 mmol) was dissolved in 15 mL ethanol and mixed into ethylenediamine solution. Then the solution was stirred with a magnetic stirrer, stirring speed of 400 rpm and heated at 78°C for 3 hours. The reaction mixture was evaporated using evaporator to remove the solvents. Then, the mixture was placed on a cold water bath for 10 minutes and filtered using Whatman filter paper and funnel. The product precipitate was then recrystallized by dissolving it in hot ethanol, filtered again, washed with cold ethanol and dried. Furthermore, the azomethine compound \(N,N'\)-Bis(benzylidene)ethylenediamine was weighed and analyzed using melting point test, solubility test, UV-Vis spectrophotometry and IR spectrophotometry [5].

2.5. Antibacterial activity test

Antibacterial activity test of azomethine compound was carried out by using diffusion disc method based on measuring of minimum inhibitory concentration. The compounds tested in this research were \(N,N'\)-Bis(3,4-dimethoxybenzylidene)ethylenediamine, \(N,N'\)-Bis (3,4-methylenedioxybenzylidene) ethylenediamine, and \(N,N'\)-Bis (benzyliden) ethylenediamine compound. The test solution was prepared by dissolving each azomethine compound into a dimethyl sulfoxide (DMSO) solvent with a concentration of 10 mg/ml. Dimethyl sulfoxide solution was used as a negative control, while positive control used 0.5 mg/ml ampicillin solution dissolved in a dimethyl sulfoxide solvent. The antibacterial activity tests were conducted against gram-positive bacteria \(Escherichia coli\) and gram-negative bacteria \(Staphylococcus aureus\).

3. Results and discussion

3.1. Synthesis of (3,4-dimethoxybenzylidene)ethylenediamine from veratraldehyde and ethylenediamine

Synthesis of \(N,N'\)-Bis(3,4-dimethoxybenzylidene)ethylenediamine was carried out by reacting an aromatic aldehyde from veratraldehyde (3,4-dimethoxybenzaldehyde) with primary amine from ethylenediamine. The mixture of 10 mmol ethylenediamine with 20 mmol 3,4-dimethoxybenzaldehyde was reacted by reflux method for 3 hours and glacial acetic acid as catalyst. The reaction of formation \(N,N'\)-Bis(3,4-dimethoxybenzylidene)ethylenediamine compound is presented in Figure 1.

![Figure 1. The reaction of formation \(N,N'\)-Bis(3,4-dimethoxybenzylidene) ethylenediamine from veratraldehyde with ethylenediamine](image)

The results of product \(N,N'\)-Bis (3,4-dimethoxybenzylidene)ethylenediamine in about 36.08% yield with the physical properties data presented in Table 1.
Table 1. Physical data properties of N,N'-Bis(3,4-dimethoxybenziliden)etilendiamin compound

| Characteristic       | N,N'-Bis(3,4-dimethoxybenziliden)etilendiamine |
|----------------------|--------------------------------------------------|
| Colour and odour     | White powder, it smells like verataldehyde       |
| Melting point        | 164.8 – 166.2 °C                                 |
| Solubility           | It dissolves in DMSO, ethylacetate, chloroform, dichloromethane |
|                      | It dissolves in ethanol dan methanol (on heating) |
|                      | It doesn’t dissolve in ether, n-hexane, and aquadest |

The UV-Vis analysis spectra of N,N'-Bis(3,4-dimethoxybenziliden) ethylenediamine are found at wavelengths 268.4 nm (band I) and 305.6 nm (band II). The result of UV-Vis spectra is presented in Figure 2.

![UV-Vis Spectra](image)

**Figure 2.** UV-Vis Spectra of N,N'-Bis(3,4-dimethoxybenziliden) ethylenediamine; (a) Band I = 268.4 nm; Abs= 0.615 (b) Band II = 305.6 nm; Abs= 0.295

Furthermore, the N,N'-Bis(3,4-dimethoxybenziliden)ethylenediamine compound was analyzed using IR spectrophotometry. There is an imine group (>C=N-) in the wavenumber 1638,12 cm⁻¹. The presence of imine group vibration is supported by the loss of the carbonyl (C=O) vibration in the verataldehyde compound at wavenumbers around 1681 cm⁻¹ (Rastuti et al., 2009). The IR spectra of N,N'-Bis(3,4-dimethoxybenziliden)ethylenediamine in Figure 3.

![IR Spectra](image)

**Figure 3.** IR Spectra of N,N'-Bis(3,4-dimethoxybenziliden)ethylenediamine

3.2. Synthesis of N,N'-Bis(3,4-methylenedioxybenziliden)ethylenediamine from piperonal and ethylenediamine

Synthesis of N,N'-Bis(3,4-methylenedioxybenziliden)ethylenediamine was carried out by reacting an aromatic aldehyde from piperonal (3,4-methylenedioxybenzaldehyde) with primary amine from ethylenediamine. The mixture of 10 mmol ethylenediamine with 20 mmol 3,4-methylenedioxybenzaldehyde was reacted by reflux method for 3 hours and glacial acetic acid as catalyst. The reaction of formation N,N'-Bis(3,4-methylenedioxybenziliden)ethylenediamine compound is presented in Figure 4.
Figure 4. The reaction of formation \( \text{N,N'}-\text{Bis(3,4-methylenedioxy benzylidene) ethylenediamine} \) from veratraldehyde with ethylenediamine.

The results of product \( \text{N,N'}-\text{Bis(3,4-methylenedioxy benzylidene) ethylenediamine} \) in about 67.88% yield with the physical properties data presented in Table 2.

**Table 2. Physical data properties of \( \text{N,N'}-\text{Bis(3,4-methylenedioxy benzylidene) ethylenediamine} \)**

| Characteristic         | \( \text{N,N'}-\text{Bis(3,4-methylenedioxy benzylidene) ethylenediamine} \) |
|------------------------|-----------------------------------------------------------------------------|
| Colour and odour       | Greenish-white powder, it smells like piperonal                              |
| Melting point          | 176.3 – 177.4 °C                                                            |
| Solubility             | It dissolves in DMSO, ethylacetate, chloroform, dichloromethane              |
|                        | It dissolves in ethanol dan methanol (on heating)                            |
|                        | It doesn’t dissolve in ether, n-hexane, and aquadest                        |

The UV-Vis analysis spectra of \( \text{N,N'}-\text{Bis(3,4-methylenedioxy benzylidene) ethylenediamine} \) is found at wavelengths 268.4 nm (band I) and 305.6 nm (band II). The result of UV-Vis spectra is presented in Figure 5.

Figure 5. UV-Vis spectra \( \text{N,N'}-\text{Bis(3,4-methylenedioxy benzylidene) ethylenediamine} \) (a) Band I = 267.8 nm ; Abs= 0.551 (b) Band II = 313.8 nm ; Abs= 0.258

Furthermore, the \( \text{N,N'}-\text{Bis(3,4-methylenedioxy benzylidene) ethylenediamine} \) compound was analyzed using IR spectrophotometry. There is an imine group (\( >\text{C}=\text{N}^- \)) in the wavenumber 1642.00 cm\(^{-1}\). The presence of imine group vibration is supported by the loss of the carbonyl (\( \text{C}=\text{O} \)) vibration in the piperonal compound at wavenumbers around 1674 cm\(^{-1}\) (Syamsudin, 2018). The IR spectra of \( \text{N,N'}-\text{Bis(3,4-methylenedioxy benzylidene) ethylenediamine} \) in Figure 6.
3.3. Synthesis of N,N’-Bis(benzylidene)ethylenediamine from Benzaldehyde and Ethylenediamine

Synthesis of N,N’-Bis(benzylidene)ethylenediamine was carried out by reacting an aromatic aldehyde from benzaldehyde with primary amine from ethylenediamine. The mixture of 10 mmol ethylenediamine with 20 mmol 3,4-methylenedioxybenzaldehyde was reacted by reflux method for 3 hours. The reaction of formation N,N’-Bis(benzylidene)ethylenediamine compound is presented in Figure 7.

![Figure 7](image)

**Figure 7.** The reaction of formation N,N’-Bis(benzylidene)ethylenediamine from benzaldehyde and ethylenediamine

The results of product N,N’-Bis(benzylidene) ethylenediamine in about 34.82% yield with the physical properties data presented in Table 3.

| Characteristic     | N,N’-Bis(benzylidene)ethylenediamine |
|--------------------|-------------------------------------|
| Colour and odour   | Orange solid, it smells like benzaldehyde |
| Melting point      | 110.2 – 111.4°C                      |
| Solubility         | It dissolves in DMSO, ethylacetate, chloroform, dichloromethane |
|                    | It dissolves in etanol dan metanol (on heating) |
|                    | It doesn’t dissolve in ether, n-hexane, and aquadest |

The UV-Vis analysis spectra of N,N’-Bis(3,4-benzylidene)ethylenediamine is found at wavelengths 243.4 nm (band I). The result of UV-Vis spectra is presented in Figure 8.

![Figure 6](image)

**Figure 6.** IR Spectra of N,N’-Bis(3,4-methylenedioxybenzylidene)ethylenediamine
Figure 8. UV-Vis Spectra of N,N’-Bis(benzylidene)ethylenediamine (a) Band I = 243.4 nm; Abs = 0.551

Furthermore, the N,N’-Bis(benzylidene)ethylenediamine compound was analyzed using IR spectrophotometry. There is an imine group (>C=N-) in the wave number 1643.84 cm\(^{-1}\). The presence of imine group vibration is supported by the loss of the carbonyl (C=O) vibration in the benzaldehyde compound at wavenumbers around 1703 cm\(^{-1}\) (Tolstorozhev et al., 2012). The IR spectra of N,N’-Bis(3,4-methylenedioxybenzylidene)ethylenediamine in Figure 9.

Figure 9. IR Spectra of N,N’-Bis(benzylidene)ethylenediamine

3.4. Antibacterial activity test

Antibacterial activity of azomethine compound was tested against *Escherichia coli* (gram-negative bacteria) and *Staphylococcus aureus* (gram-positive bacteria). This antibacterial testing was done by disc diffusion method. It was done by a filter paper containing a number of active compounds placed on the surface of the solid medium which had previously been inoculated by the bacteria on the surface of the medium.

The compound tested in this research are N,N’-Bis(3,4-dimethoxybenzylidene)ethylenediamine, N,N’-Bis(3,4-methylenedioxybenzylidene) ethylenediamine, and N,N’-Bis(benzylidene)ethylenediamine and each compound was tested at concentration 10 mg/ml. While positive control was ampicillin at concentration 5 mg/ml and negative control was dimethyl sulfoxide (DMSO).

Antibacterial activity of azomethine compound is caused by the presence of imine groups (>C=N-). The presence of imine groups will interfere with the normal process of bacterial cells by forming hydrogen bonds between the active centre on the bacterial cell membrane with nitrogen at the imine group of the azomethine compound. The hydrogen bond will affect the permeability of the cell wall and cytoplasmic membrane. The disturbance of permeability cell walls and cytoplasmic membranes can cause an imbalance of macromolecules and ions in cells so that the cell becomes lysis [2]. The antibacterial activity test results of azomethine compound were indicated by the measurement of compound inhibition zone diameter (mm) which is presented in table 4.
Table 4. Inhibitory zone diameter of antibacterial activity

| No | Compounds                | Concentration (mg/ml) | Inhibitory zone diameter (mm) |
|----|--------------------------|-----------------------|-------------------------------|
|    |                          |                       | S.aureus (G⁺) | E.coli (G⁻) |
|    |                          |                       | 12 hours | 24 hours | 12 hours | 24 hours |
| 1  | (+)                      | 5                     | 4,05     | 3,14     | 20,05    | 24,25    |
| 2  | (-)                      | 10                    | 0        | 0        | 0        | 0        |
| 3  | P1                       | 10                    | 0,61     | 0,06     | 3,44     | 3,48     |
| 4  | P2                       | 10                    | 0,39     | 0,25     | 2,02     | 3,26     |
| 5  | P3                       | 10                    | 3,38     | 3,08     | 3,72     | 4,61     |

(+)=ampisilin; (-)=DMSO
P1 = N,N'-Bis(benzylidene)ethylenediamine
P2 = N,N'-Bis(3,4-dimethoxybenzylidene)ethylenediamine
P3 = N,N'-Bis(3,4-methylenedioxybenzylidene)ethylenediamine

Based on table III.4 that the antibacterial activity of compound N,N'-Bis(3,4-dimethoxybenzylidene)ethylenediamine looks the smallest compared to N,N'-Bis(benzylidene)ethylenediamine. In the structure of compound N,N'-Bis(3,4-dimethoxybenzylidene)ethylenediamine has 2 methoxy groups (-OCH₃) which are substituted in the meta and para positions. The substituent gives steric effect to the reactivity of synthesis. The steric effect of both methoxy groups in azomethine N,N'-Bis(3,4-dimethoxybenzylidene)ethylenediamine which is difficult to penetrate the bacterial cell membrane so that the inhibitory process against bacteria becomes smaller. This is supported by research conducted by Alcaraz et al. (2000) reported that the addition of methoxy groups could reduce the antibacterial activity of azomethine compounds. From this explanation, it can be concluded that the presence of methoxy groups can reduce the antibacterial activity of azomethine compound. In compound N,N'-Bis(benzylidene)ethylenediamine doesn’t have a substituent in the structure of the azomethine compound so that the antibacterial activity is only affected by the imine group (>C=N-). In azomethine N,N'-Bis(3,4-methylenedioxybenzylidene)ethylenediamine has the highest antibacterial activity compared to N,N'-Bis(benzylidene)ethylenediamine and N,N'-Bis(3,4-dimethoxybenzylidene)ethylenediamine. This is because the compound N,N'-Bis(3,4-methylenedioxybenzylidene)ethylenediamine has a cyclic methylenedioxy group (-O-CH₂-O-) which is an electron booster. This impulse of electrons from the methylenedioxy group gives a positive mesomeric effect on the aromatic ring so that the aromatic ring is more electronegative. As a result, the N atoms in the imine group (>C=N-) are more electronegative. The free electrons of the N atom of the imine group can bond hydrogen more strongly with the active center of the bacterial cell membrane so that the inhibition of the bacteria becomes stronger.

4. Conclusion
From the results of this research, it can be concluded that synthesis of alkoxy-substituted azomethine compounds were carried successfully. Synthesis of methoxy-substituted azomethine compound from veratraldehyde with ethylenediamine produce N,N'-Bis(3,4-dimethoxybenzylidene) ethylenediamine in the form of white powder with a melting point 164,8–166,2°C and in about 36,08% yield. Synthesis of methylenedioxy-substituted azomethine compound from piperonal with ethylenediamine produce N,N'-Bis(3,4-methylenedioxybenzylidene) ethylenediamine in the form of greenish-white powder with a melting point 176,3–177,4°C and in about 67,88% yield. Synthesis of unsubstituted azomethine compound from benzaldehyde with ethylenediamine produce N,N'-Bis(benzylidene)ethylenediamine in the form of orange solid with a melting point 110,2–111,4°C and in about 34,82% yield. Azometin compound showed antibacterial activity against gram-negative bacteria Escherichia coli and gram-positive bacteria Staphylococcus aureus. The largest antibacterial activity is N,N'-Bis(3,4-methylenedioxybenzylidene)ethylenediamine compound and the smallest antibacterial activity is N,N'-Bis(3,4-dimethoxybenzylidene)ethylenediamine. Antibacterial activity of compound N,N'-Bis(3,4-
dimethoxybenzylidene)ethylenediamine, \( N,N'\)-Bis(3,4-methylenedioxy benzylidene)ethylenediamine, and \( N,N'\)-Bis(benzylidene)ethylenediamine with inhibitory zone diameter 0.25; 3.08; 0.06 mm in \textit{S.aureus} bacteria (G\( ^+ \)) and 3.26; 4.61; 3.48 mm in \textit{E.coli} bacteria (G\( ^- \)).

**Acknowledgement**

We are grateful to financial support from “RPP”- Grant, Diponegoro University No. 474-30/UN7.P4.3/PP/2019

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