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Synthesis and Toxicity Tests of *N*-Carbothioamide-3-(2,4- Dichlorophenyl)-5-(4-Hydroxy-3-Methoxyphenyl)Pyrazoline

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Abstract

Pyrazoline is an alkaloid compound which has various biological activities such as antibacterial, antifungal, antitumor and anticancer. The compound *N*-carbotioamide-3-(2,4-dichlorophenyl)-5-(4-hydroxy-3-methoxyphenyl)pyrazoline was successfully synthesized from the basic ingredients 2',4'-dichloro-4-hydroxy-3-methoxychalcone and thiosemicarbazide with sodium hydroxide catalyst at 80°C for 7 hours. The synthesized compound was characterized using ^{1}H-NMR, ^{13}C-NMR, and mass spectroscopy and had a yield of 28.03%. Based on the results of toxicity tests using the Brine Shrimp Lethality Test (BSLT) method, this compound has an LC_{50} value of 44.6 ppm and has the potential to be an antimicrobial compound.

Keywords: synthesis, pyrazoline, *N*-carbothioamide, toxicity

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1 Introduction

Pyrazoline is a natural compound in the azole group and has various biological activities, especially antibacterial, antitumor, and anticancer [1], [2], [3]. Pyrazoline belongs to a class of alkaloid compounds that are rarely found in nature [4]. The basic structure of pyrazoline is a five-ring with two nitrogen atoms adjacent to each other [5].

Pyrazoline compounds are difficult to isolate from plants and their contents are quite small [3]. Synthesis of pyrazoline derivative compounds is a way to obtain pyrazoline compounds in a shorter time and greater quantities.

Through retrosynthesis analysis, pyrazoline compounds can be synthesized using α, β-unsaturated ketones with hydrazine derivatives. The chalcone compound is an α, β-unsaturated ketone compound so it can be used as a precursor to pyrazoline compounds. The reflux synthesis method is generally used for the synthesis of pyrazoline compounds through nucleophilic addition reactions and the reaction process requires temperatures above room temperature [3].

Pyrazoline derivative compounds have anticancer activity [3], [6]. Pyrazoline compounds which have sulfur atoms in their structure will be related to their toxicity and have the potential to be anticancer candidates [6]. Synthesis of pyrazoline compounds from chalcone which has a methoxy group on the aromatic ring and thiosemicarbazide pencyclic which has a sulfur group [7]. The results obtained indicate that the pyrazoline compound which has a methoxy group and the cyclic thiosemicarbazide have anticancer potential.

Pyrazoline compounds can be synthesized with Cl, OCH_3 and OH groups on the aromatic ring [8] and carbamide groups on the pyrazoline ring [9]. These pyrazoline compounds have a toxicity LC_{50} 81.3 ppm [8] and 96.96 ppm [9]. Therefore, this research was designed with a pyrazoline analogue structure with substituted Cl, OCH_3 and OH groups on the aromatic ring and a substituted thioamide group on the pyrazoline ring.

2 Methods

2.1 Tools

The equipment used is an Erlenmeyer flask with a lid, three-neck synthetic flask, hotplate and magnetic stirrer, condenser, UV lamp (254 nm and 366 nm), vial, measuring pipette, analytical balance, NMR (JEOL Resonance 400 MHz), Mass Spectroscopy (Lockspray Waters). The ingredients used were 2,4-dichloroacetophenone (Merck), vanillin (Merck), sodium hydroxide (Merck), thiosemicarbazide (Sigma Aldrich), hydrochloric acid (Merck), n-hexane (Merck), ethyl acetate (Merck), ethanol (Merck), TLC plate GF_{254} (Merck), *Artemia salina* Leach shrimp larvae, tween 80, baker's yeast and seawater.

2.2 Synthesis of the compound 2',4'-dichloro-4-hydroxy-3- methoxychalcone [10]

Dissolve the 2,4-dichloroacetophenone compound (5 mmol) in 10 mL of ethanol into a 100 mL Erlenmeyer flask then stir. Add 5 mL of 40% sodium hydroxide. The compound 4-methoxy-3-hydroxy benzaldehyde (vanillin) (5 mmol) in 10 mL of ethanol was added to the mixture until homogeneous. This mixture is stirred for 24 hours. The TLC test was carried out after 24 hours of stirring. The reaction results were added to 10 mL of cold water and placed in a container filled with ice. Then 10% hydrochloric acid was added to neutralize the compound, the pH was measured using universal indicator paper. Next, it was washed using cold distilled water and filtered using a Buchner funnel. The resulting product is dried in a desiccator and the product mass is calculated [10].

2.3 Synthesis of N-carbotioamide-3-(2,4-dichlorophenyl)-5-(4-hydroxy-3-methoxyphenyl)pyrazoline compound

The compound 2',4'-dichloro-4-hydroxy-3-methoxychalcone (1 mmol) in 10 mL absolute ethanol was put into a three-neck synthesis flask. Add sodium hydroxide (5 mmol) to the mixture. Thiosemicarbazide compound (3 mmol) in 10 mL of absolute ethanol was added to the mixture. Then refluxed while stirring at 80°C for 7 hours. After that, it is crystallized by cooling it in a container filled with ice and storing it in the refrigerator for 24 hours. The resulting product was filtered using a Buchner funnel and stored in a glass container in a desiccator. The yield of the product was calculated and characterized using structure elucidation techniques using ^{1}H-NMR, ^{13}C-NMR and mass spectroscopy.

2.4 Toxicity test

The synthesized compounds were made into concentration series, namely 20, 40, 60 and 80 ppm. Prepare a calibrated 10 mL vial. Then to each vial, varying concentrations of synthetic compounds and 2 mL of seawater were added and shaken until dissolved. A total of 10 *Artemia salina* Leach shrimp larvae and a drop of yeast solution were put into a vial, and then seawater was added to the calibration limit mark of 10 mL. The toxicity level was determined by counting the number of shrimp larvae that were still alive and dead after 24 hours after being placed under an incandescent lamp. The test was replicated 3 times with the same treatment for each concentration. Data were analyzed using the Reeds and Muench analysis method to obtain LC_{50} values.

3 Results and Discussion

Pyrazoline derivative compounds are obtained by reacting the 2',4'-dichloro-4-hydroxy-3-methoxychalcone compound and the thiosemicarbazide compound with a sodium hydroxide catalyst. The cyclic formation reaction of the chalcone compound into pyrazoline by thiosemicarbazide begins with the nitrogen atom in the NH_2 of the thiosemicarbazide compound which is more nucleophilic than sulfur attacking the carbon atom in the carbonyl group of the chalcone compound. Next, the NH atom next to NH_2 attacks Cβ which is electropositive, forming 2 C-N bonds and forming cyclic ring 5. The final stage of the reaction is the dehydration stage releasing H_2O to form a C=N bond. The reaction scheme can be seen in Figure 1.

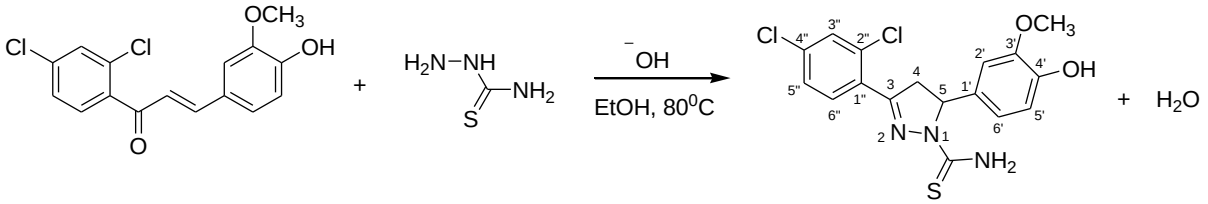

Figure 1. Reaction of the compound *N*-carbotioamide-3-(2,4-dichlorophenyl)-5-(4-hydroxy-3-methoxyphenyl)pyrazoline.

The compound obtained from the synthesis of pyrazoline derivatives was *N*-carbotioamide-3-(2,4-dichlorophenyl)-5-(4-hydroxy-3-methoxyphenyl)pyrazoline in the form of a brownish-yellow powder with a yield of 28.03%.

Compound of *N*-carbotioamide-3-(2,4-dichlorophenyl)-5-(4-hydroxy-3-methoxyphenyl) pyrazoline**, ^{1}H-NMR ($CDCl_3$, ppm, 400 MHz);** δ_H 3.33 (1H, *dd*, *J* = 3.4 ; 17.6 Hz, H4*a*); 3.86 (3H, *s*, OCH_3); 3.97 (1H, *dd*, *J* = 11.2 ; 18 Hz, H4*b*); 5.94 (1H, *dd*, *J* = 3.2; 11.6 Hz, H5); 6.69 (1H, *dd*, *J* = 2 ; 8 Hz, H6'); 6.76 (1H, *d*, *J* = 1.6 Hz, H2'); 6.85 (1H, *d*, *J* = 8.8 Hz, H5'); 7.30 (1H, *dd*, *J* = 2 ; 8.4 Hz, H5"); 7.46 (1H, *d*, *J* = 2.4 Hz, H3"); 7.64 (1H, *d*, *J* = 8.4 Hz, H6"). **^{13}C-NMR ($CDCl_3$, ppm, 100 MHz)**; δ_C 45.9 (C-4); 56.0 (OCH_3); 63.6 (C-5); 108.6 (C-2'); 114.8 (C-5'); 118.1 (C-6'); 127.6 (C-5"); 128.5 (C-3"); 131.1 (C-1"); 131.2 (C-1'); 133.4 (C-6"); 133.9 (C-2"); 136.9 (C-4"); 145.2 (C-4'); 146.7 (C-3'); 154.5 (C-

3); 177.2 (C=S). **ESI-MS (m/z)**: 396.0358 ($M+H^+$).

The ^{1}H-NMR (400 MHz, $CDCl_3$**)** spectrum of *N*-carbotioamide-3-(2,4-dichlorophenyl)-5-(4-hydroxy-3-methoxyphenyl)pyrazoline showed the presence of 12 proton signals. At δ_H 3.33 ppm and δ_H 3.97 ppm there are 2 different protons. H4a proton (3.33; dd; J = 3.4; 17.6 Hz; 1H) and H4b proton (3.97; dd; J = 11.2; 18 Hz; 1H). The H4a proton shows mutual coupling with the H4b proton with coupling constants of 17.6 and 18 Hz. This coupling constant shows that the H4a and H4b protons are geminal. H4a and H4b protons also experience coupling with H5 protons (5.94; dd; J = 3.2; 11.6 Hz; 1H) where H4a and H4b protons are visually bound to H5 with coupling values of 3.4 and 11.2 Hz respectively. Proton H5 shows a doublet of doublet spectrum, this shows that H5 also couples H4a and H4b with coupling constants of 3.2 and 11.6 Hz. The H5 proton is the Hx proton on the pyrazoline ring. These protons form the ABX system which shows the characteristic features of the pyrazoline ring. Therefore, this proves that a pyrazoline ring has been formed.

The ^{1}H-NMR (400 MHz, $CDCl_3$) at δ 3.86 ppm (3H, s) shows a singlet peak which is the 3 protons of the OCH_3 group. At δ_H (6.69; dd; J = 2; 8 Hz; 1H) shows the aromatic proton H6' which is located in an ortho position to the proton in H5' (6.85; d; J = 8.8 Hz; 1H) and meta position towards the proton in H2' (6.76; d; J = 1.6 Hz; 1H). Meanwhile, δ_H (7.30; dd; J = 2; 8.4 Hz; 1H) shows the aromatic proton H5" which is located in an ortho position to the proton H6" (7.64; d; J = 8.4 Hz; 1H) and the meta position towards the H3” proton (7.46; d; J = 2.4 Hz; 1H). NH_2 and OH protons do not appear in the ^{1}H-NMR spectrum which can be caused by using $CDCl_3$ solvent in the ^{1}H-NMR analysis. Chemical shift and spectrum of NH_2 and OH protons in ^{1}H-NMR can depend on the type of solvent. However, NH_2 and OH protons can be proven from the molecular weight in the mass spectrum which is by the molecular weight of the compound being synthesized.

The ^{13}C-NMR (100 MHz, $CDCl_3$**)** of *N*-carbotioamide-3-(2,4-dichlorophenyl)-5-(4-hydroxy-3-methoxyphenyl)pyrazoline spectrum shows the presence of 17 chemically inequivalent carbon atom signals. There are 12 C=C sp^2 signals at the δ_C between 108-133 ppm which is typical of the sp^2 carbon atom signals from 2 benzene rings. The signal at δ_C 154.5 ppm (C-3) shows the sp^2 carbon imine (C=N) and at δ 177.2 ppm shows the thioamide carbon (H_2N-C=S) on the pyrazoline ring residue. There are 3 C-C sp^3 signals at δ_C 45.9; 63.6 and 56.0 ppm. Chemical shift of 56.0 ppm represents the carbon of OCH_3 groups. Chemical shifts of 45.9 ppm (C-4) and 63.6 ppm (C-5) show a typical C-C sp^3 signal from the pyrazoline ring.

The mass spectrum of the pyrazoline compound shows that the molecular weight m/z obtained is 396.0358 g/mol and is by the molecular weight of this compound. Based on the results of ^{1}H-NMR, ^{13}C-NMR and mass spectroscopy data analysis, it can be confirmed that the compound *N*-carbotioamide-3-(2,4-dichlorophenyl)-5-(4-hydroxy-3-methoxyphenyl)pyrazoline with the chemical formula $C_{17}H_{15}Cl_2N_3O_2S$ has successful. synthesized.

Toxicity tests are carried out to determine the toxicity of synthetic compounds and obtain LC_{50} values. The method used is the Brine Shrimp Lethality Test (BSLT) method for *Artemia salina* Leach larvae. The results of the toxicity test analyzed using the Reeds and Muench method showed potential toxicity with an LC_{50} value is 44.60 ppm. A chemical compound can have antimicrobial activity if it has an LC_{50} is 30-200 ppm [11] and anticancer if it has an LC_{50} value is <30 ppm [11], [12]. Therefore, the compound *N*-carbotioamide-3-(2,4-dichlorophenyl)-5-(4-hydroxy-3-methoxyphenyl)pyrazoline has the potential as an antimicrobial [11].

4 Conclusions

The compound of *N*-carbotioamide-3-(2,4-dichlorophenyl)-5-(4-hydroxy-3-methoxyphenyl)pyrazoline has been successfully synthesized from the basic ingredients 2',4'-dichloro-4-hydroxy-3-methoxychalcone and thiosemicarbazide using a sodium hydroxide catalyst with a yield of 28.03%. This compound has an LC_{50} value is 44.60 ppm and has potential as an antimicrobial.

5 Declarations

5.1 Acknowledgments

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5.2 Author Contributions

The names of the authors listed in this journal contributed to this research.

5.3 Conflicts of Interest

The authors declare no conflict of interest.

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