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SYNTHESIS AND BIOACTIVITY OF 3-(2,4-DICHLOROPHENYL)-5-(4-HYDROXY-3-METHOXYPHENYL) PYRAZOLINE

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ABSTRACT

Pyrazoline is a five-ring heterocyclic compound having 3 carbon atoms and 2 nitrogen atoms known to have some biological activities. This study aims to synthesize 3-(2,4-dichlorophenyl)-5-(4-hydroxy-3-methoxyphenyl) pyrazoline using 2',4'-dichloro-4-hydroxy-3-methoxy chalcone and hydrazine. Synthesis was performed by reflux method at 80^{o}C for 7 hours. The synthesis compounds were characterized by structural elucidation techniques. The yield of synthesised compound of 3-(2,4-dichlorophenyl)-5-(4-hydroxy-3-methoxyphenyl) Pyrazoline is pale yellow powder with 84% of purity. The result of antioxidant activity test by 2,2-diphenyl-1-picrylhydrazyl (DPPH) shown that the compound has very strong antioxidant activity, Antibacterial test was done by diffusion agar method using paper disk that shown that the compound has antibacterial activity against Gram positive bacteria (S.aureus) and Gram negative bacteria (E.coli) and toxicity test by Brine Shrimp Lethality Test (BSLT) shown that the compound has antibiotic and cytotoxic potential activity.

Keywords: synthesis, pyrazoline, antibacterial, antioxidant, toxicity

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INTRODUCTION

Pyrazoline is a five-ring heterocyclic compound having three carbon atoms and two nitrogen atoms [1]. Previous studies have suggested that pyrazolines are known to have various biological activities such as antimicrobial agents, anticancer, anti-inflammatory, antioxidant agents, analgesics, and antitumors [2].

Pyrazolines belong to the alkaloid family, but these compounds are rarely found in nature and the isolation from nature produces little yield and less varied structure compounds. Therefore, to obtain pyrazoline with a high yield is performed by synthesis. One of the methods for the shyntesis of such compound is by cyclization between α, β-unsatured ketones with hydrazine or their derivatives [3].

Heterocyclic ring with two nitrogenous bases likely to be responsible for its biological activity. 2 adjacent nitrogen atoms in the pyrazoline are known to be responsible for the antibacterial agents [4]. In addition, the antibacterial agents of pyrazoline also depend on the type of subtituents attached to their aromatic rings such as halogen groups, OH, OCH_3, NO_2 etc [5]. Substituents in aromatic rings of pyrazoline compounds such as OH group is also known to play a role in their activity as antioxidant agent. This study attempted to synthesize the desired pyrazoline which substituted two chloro groups, one methoxy group and one hydroxyl group on the aromatic ring.

MATERIALS AND METHODS

Tool

The tools used in this study are reflux apparatus, hot plate (*stirrer*), TLC plate GF_{254}, 254 nm and 366 nm UV light, Shimadu IR ShimadzunPrestige-21 , JMM JEM JNMECA 500 MHz, and MS QP2010S, UV-VIS spectrophotometer Dynamic Halo DB-20S, Laminar Air Flow, petri dish ,tweezers and Erlenmeyer.

Material

The materials used in this study are 2,4-dichloroacetophenone (Sigma Aldrich), vanillin (Merck), sodium hydroxide (Merck), hydrochloric acid (Merck), hydrazine (Sigma Aldrich), aquadest, universal indicator, absolute ethanol, methanol, 2,2-diphenyl-1-picrylhydrazyl (DPPH), NA medium, paper disk, sterile aquades and cotton. Microorganisms used in this study are gram-negative bacteria (*Echericia coli)* and gram-positive bacteria (*Staphylococcus aureus).*

Shyntesis of Chalcone

Chalcone was prepared by 5 mmol 2,4-dichloroacetophenone and 5 mmol vanillin dissolved in absolute ethanol (10 mL) with 5 mL NaOH 40% as catalyst, mixed and stirring for 24 hours at room temperature.The reaction was monitored by TLC. The reaction mixture was washed with cold water and neutralized with HCl 10%. The solid mass was filtered and dried.

Shyntesis of Pyrazoline

Pyrazoline was prepared by 5 mmol 2',4'-dichloro-4-hydroxy-3-methoxy chalcone and 5 mmol hydrazine dissolved in absolute ethanol (10 mL), mixed and stirring under reflux condition for 7 hours at 80^{o}C temperature.The reaction was monitored by TLC. The reaction mixture was washed with cold water and left overnight in Refrigerator. The solid mass was filtered and dried.The compounds were characterized using FT-IR, C-NMR, H-NMR and MS Spectroscopy.

Bioactivity Test

Antibacterial activity of pirazoline was examined by the disc-diffusion method using Nutrient Agar medium. Microorganisms used in this study are gram-negative bacteria (*Echericia coli)* and gram-positive bacteria (*Staphylococcus aureus).* Thebacterial (24 h) cultures from the slantswere diluted with sterile NaCl 0.9 % and mixed thoroughly to prepare a clear homogeneous suspension. The solutions of pirazoline (1; 2.5; 5; 7,5; and 10 %) were prepared in aquadest. Tween were used as a negative control. Sterile paper discs (6 mm diameter) were dripped with the 20 µL sample and were placed on the agar plates uniformly inoculated with the test microorganisms suspension. Then incubated in the incubator at 37°C. The diameter for the zones of inhibition around the paper disk was measured after incubated for 24 hours.

Antioxidant activity of pirazoline was examined by *2,2-diphenyl-1-picrylhydrazyl* (DPPH) radical method. Stock (100 ppm) solution of pirazoline have been diluted in metanol to obtain 2, 4, 6, 8 and 10 ppm concentration. 2 mL sampels and 2 mL DPPH

40 ppm were mixed and incubated for 30 minutes without exposing to light. Mixture of methanol and DPPH 40 ppm used as blank solution. The absorbance of each solution was measured with UV-Vis Spectroscopy using 514 nm wavelength. The percentage scavenging of test samples at each concentration were calculated using the following formula

$$\frac{\text{Blank absolbance} - \text{Sample absorbance}}{\text{Blank absorbance}} \times 100$$

The IC_{50} was calculated using the Microsoft excel.

Toxicity of pirazoline was examined by Brine Shrimp Lethality Test (BSLT) method. Stock (1000 ppm) solution of pirazoline have been diluted in sea water to obtain 20, 40, 60, 80 and 100 ppm concentration. Tween were used as a negative control. sampels and control solutions were placed into each calibrated vial, and 10 shrimp larvae (*Artemia salina* Leach) were added to each test and control vial. Observed mortality of shrimp larvae after 24 hours. The test result calculated the value of LC_{50} by using Reed and Muench analysis.

RESULTS AND DISCUSSION

3-(2,4-dichlorophenyl)-5-(4-hydroxy-3-methoxy phenyl) pyrazoline is synthesized by a cyclization between 2',4'-dichloro-4-hydroxy-3-methoxy chalcone and hydrazine for 7 hours under reflux conditions at 80 °C. The reaction was monitored by TLC using eluen n-hekesan : etil asetat (7:3) observed with UV light 254 nm and 366 nm showed Rf value of compound is 0.42. 3-(2,4-dichlorophenyl)-5-(4-hydroxy-3-methoxy phenyl) pyrazoline produced in pale yellow powder with 84% yield. The synthesized pyrazoline structure was characterized using FT-IR, C-NMR, H-NMR and MS spectroscopy.

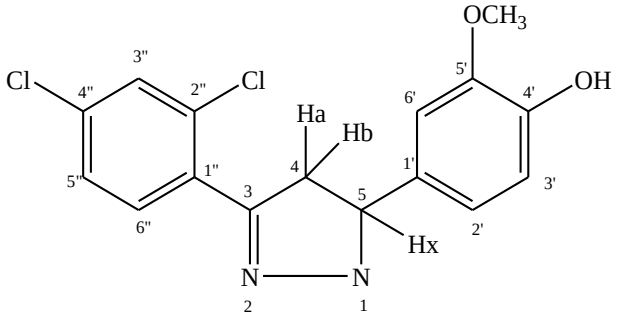

Figure 1. Structure and numbering of Prazoline

The IR band at 617 cm-1 suggesting the presence of (C-Cl) group. The IR band at 1126 cm-1 suggesting the presence of (C-O phenol)group, the band at 1165 cm-1 suggesting the presence of (O-CH3) group, the band at 1527 cm-1 suggesting the presence of (C=C aromatic) group, the band at 3255 cm-1 suggesting the presence of (C-H aromatic) group, the band at 1257 cm-1 suggesting the presence of (C-N) group, the band at 1597 cm-1 suggesting the presence of (C=N) group, the band at 3294 cm-1 suggesting the presence of (N-H) group, and the band at 3417 cm-1 suggesting the presence of (O-H) group.

Analysis using mass spectroscopy shown that the molecular weight is indicated by the mass spectrum calculated as $C_{16}H_{14}N_2O_2Cl_2$ m / z 336 according to the molecular weight of the compound.

Table 1 shows the interpretation of NMR of pyrazoline. The C NMR spectrum shows that the number of carbon atoms corresponds to the number of carbon atoms of the synthesized pyrazoline. The chemical shift at δ 150.1 ppm shows the presence of C = N, δ 44.1 ppm and 65.4 ppm showed C4 and C5 on pyrazoline and δ 56.1 ppm indicated carbon in OCH3. The overall interpretation of NMR data can be seen in table 1.

The H-NMR spectrum shows the typical proton of the pyrazoline. The chemical shift of δ 3.18 ppm (Ha) appears in the doublet of doublet peak with the

coupling constant (Jax = 9 Hz and Jab = 16.5 Hz), δ 3.55 ppm (Hb) appears in peak doublet of doublet with coupling constant (Jbx = 10.5 Hz and Jba = 16.5 Hz), and 4.88 ppm (Hx) appear in triplet peak, showing protons on C4 and C5. Chemical shifts δ 6.95 ppm (d, 1H), δ 6.86 ppm (d, 1H) and δ 6.82 ppm (dd, 1H) showed protons on C-2 ', C-5' and C-6 '. The chemical shift δ 7.39 ppm (d, 1H), δ 7.24 ppm (dd, 1H) and δ 7.61 ppm (d, 1H) showed protons on C-3 ", C-5" and C-6 ". 3.89 ppm chemical shift with singlet peak indicates 3 protons at OCH_3.

Table 1. Interpretation of C-NMR and H-NMR Spectra of Pyrazoline

Atom Number		δH (ppm)	Integration and multiplicity type	δC (ppm)
NH		-	-	-
2		-	-	-
3		-	-	150,1
4	Ha	3,18 (J_{ax}=9 Hz and J_{ab}=16,5 Hz)	1H, *dd*	44,1
	Hb	3,55 (, J_{bx}=10 Hz and J_{ba}=16,5 Hz)	1H, *dd*	
5	Hx	4,88	1H, *t*	65,4
1’		-	-	131,0
2’		6,95 (, J=1,5 Hz)	1H, *d*	108,6
3’		-	-	147,4
4’		-	-	147,0
5’		6,86 (J=8,0 Hz)	1H, *d*	114,5
6’		6,82 (J= 8,25 ; 1,75 Hz)	1H, *dd*	119,6
1”		-	-	130,9
2”		-	-	135,0
3”		7,39 (, J= 2 Hz)	1H, *d*	130,4
4”		-	-	145,5
5”		7,24 (J= 8,75;1,75 Hz)	1H, *dd*	127,4
6”		7,61 (J=8,5 Hz)	1H, *d*	134,0
OCH_3		3,89	3H, s	56,1
OH		-	-	-

Table 2. Results of Measurement of Inhibition Zones by the Agar Diffusion Method

Concentration (%)	Zone of Inhibition	
	S.Aureus (mm)	*E.coli* (mm)
1	7.13	6.38
2,5	7.59	8.65
5	8.15	10.26
7.5	8.60	11.03
10	8.18	11.01

The pirazoline were screened againt *S.aureus* (Gram positive) and *E.coli* (Gram negative) organisme using agar difution method. Pirazoline have shown antibacterial activity with zone of inhibition between 7-11 mm.

Heterocyclic ring with two nitrogenous bases likely to be responsible for

its biological activity. Heterocyclic compounds with nitrogen rings such as pyrazolines are able to inhibit bacterial growth. Quartener ammonium compounds are known to damage cell membranes by lowering surface tension, inactivating enzymes and denaturing cell proteins so that pyrazoline compounds have the ability to inhibit the growth of all bacteria used in antibacterial activity tests [2].

The results of antioxidant activity of pirazoline has been known that the IC_{50} value is 6.38 ppm so it can be concluded that the antioxidant activity of the compound of is very strong. The presence of substituted hydroxy groups in aromatic rings and NH group on pyrazolines leads to antioxidant activity. The presence of an OH group attached to an aromatic ring has the ability to absorb free radicals by donating its hydrogen atom and forming a phenoxyl radical that can stabilize itself through the resonance process.

The results of toxicity test of pyrazoline determined by Reed and Muench analysis method showed potential toxicity with LC_{50} value is 81,3 ppm. This value indicates that at that concentration the compound is able to kill shrimp larvae up to 50% of the population. Based on studies conducted by Meyer, chemical compounds are said to be potentially active when having LC_{50} values less than 1000 ppm. According to [6] a compound have the potential as antitumor or anticancer if it has $LC_{50} \leq 30$ ppm. The synthesized pyrazoline derived compounds have an LC_{50} value of 81.3 ppm (<1000 ppm) indicating that the compound has potential as antibacterial.

CONCLUSION

Based on the results of the research, it can be concluded that the compound 3-(2,4-dichlorophenyl) -5- (4-hydroxy-3-methoxyphenyl) pyrazoline has been successfully synthesized and has antibacterial activity, antioxidant activity and toxicity.

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