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Cyclization Reaction of 4-Nitro-3'-4'-Dimethoxychalcone and Phenylhydrazine as Antibacterial Candidate

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Abstract. Cyclization reaction of dimethoxychalcone and hydrazine was conducted by refluxing them for 6 h in glacial acetic acid. The synthesized product was characterized using ^{1}H-NMR, FT-IR and GC-Mass spectrometers. The reaction yielded red solid pyrazoline in 53.80%. The ^{1}H-NMR spectra showed proton peaks in upfield shift that identified as proton's signal of pyrazoline ring. In addition, the presence of proton peak of hydrazone showed that the reaction is predicted through 1,3-dipolar cycloaddition reaction. Pyrazoline that has been synthesized, have antibacterial activity against positive and negative bacteria. However, its activity was not good and need to be further studied.

INTRODUCTION

Infectious diseases that were caused by pathogen have become global problem health. Moreover, since it was found many cases of multi-drugs resistant bacteria. Many treatment used active compounds containing heterocycles, due to their selectivity and activity [1]. Electron rich atom, such as oxygen, sulfur, and nitrogen increase the possibility of interaction with target side. Nitrogen heterocycles have been reported for antibacterial activity[2]. The mechanism of action can be investigated from molecular docking of active compounds on target side and it was observed that their interaction with protein of bacteria were hydrogen bond, ionic and lipophilic interactions[3].

Chalcone is α,β-unsaturated ketone that have reported useful in synthesis of heterocyclic compounds [4], ie pyrazole [4, 5], pyrazoline [5, 6], oxazine [4], isoxazole [5]. Pyrazole is heterocyclic that contain two adjacent nitrogen in 6 (six) member ring which exhibited broad spectrum of biological activities such as antimicrobial [7], anti-inflammatory [8], inhibitor nNOS dan iNOS[9]. Pyrazoline is 4,5-dihydropyrazole, has been found to possess antibacterial [2], anticancer [10], anti-inflammatory [11], antifungal [2] and antimicrobial [12]. Pyrazoline can be synthesized from cyclization of chalcone and phenyl hidrazine. Synthesis of 4,5-dihydro-1H-pyrazol-5-yl -6-fluoroquinolone from α-β unsaturated ketone with hydrazine monohydrate in ethanol, yielded product in 59-76%[13]. The presence of acetic acid as catalyst can be used for increasing reactivity[14]. On the other hand, pyrazoline can be produced via Michael addition by refluxing chalcones and thiosemicarbazide under alkaline medium [15] but it was not recommended for reactants that sensitive to alkaline. However, this reaction allowed the formation of side reactions and products. This research focused on cyclization of chalcone with phenyl hydrazine to produced pyrazoline derivative as antibacterial candidate. Chalcone was synthesized from veratraldehyde and 4-nitroacetophenone via Claisen Schmidt condensation [16].

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EXPERIMENTAL

Materials

Phenylhydrazine, 3",4"-Dimethoxy-4'-nitrochalcone (1-(4'-nitrophenyl)-3-(3",4"-dimethoxyphenyl)-2-propen-1-one), glacial acetic acid, dichloromethane (DCM), dimethylsulfoxide (DMSO), n-hexane. All materials of synthesis that were used obtained with pro analysis quality (pa) from E. Merck.

Instrumentation

The ^{1}H-NMR spectrometer (JEOL, JNMECA ^{1}H 500 MHz) with TMS as internal standard, infrared spectrometer (FTIR Shimadzu Prestige 21), gas chromatography-mass spectrometers (GC-MS, Shimadzu QP-2010S), melting point was uncorrected (Electrothermal 9100).

Experimental Procedure

Dimethoxychalcone was produced by reacting 4-nitroacetophenone with veratraldehyde in ethanol with NaOH 15% (w/v) as catalyst [16]. Reaction was performed by refluxing 0.31 g (1 mmol) of chalcone, 0.32 g (3 mmol) phenylhydrazine, and 7.5 mL glacial acetic acid in 10 mL of ethanol (Fig. 1). Completion of reaction was monitored by TLC, i.e. 6 h. The solids was filtered with Buchner funnel, washed with cold aqudest and dried. The synthesis product was purified by column chr omatography, eluted with n-hexane:dichloromethane (1:1) and then characterized using FTIR, ^{1}H-NMR and GC-MS.

Antibacterial Test

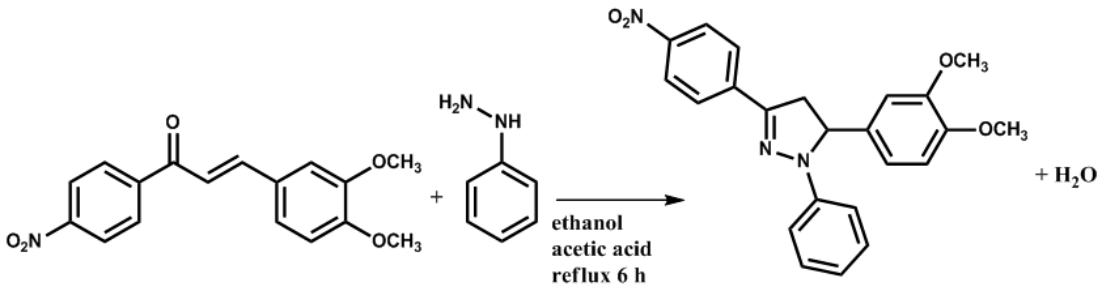


FIGURE 1. Reaction of cyclization chalcone

The culture medium used nutrient agar. It sterilized by autoclaving at 15 psi and 121 °C for 15 minutes. Well-diffusion was used for antibacterial test against Gram positive and negative bacteria, i.e. *Staphylococcus aureus*, *Bacillus cereus*, *Bacillus subtilis*, *Escherichia coli* and *Shigella flexneri*. Positive control was tetracycline (100 ppm) and negative control was dimethyl sulfoxide (DMSO 99.9%). The sample was tested at 100, 300, 500 and 1000 ppm in DMSO.

RESULTS AND DISCUSSION

Characterization of Synthetic Product

1-phenyl-3-(4'-nitrophenyl)-5-(3',4'-dimethoxyphenyl)-2-pyrazoline. Red solid. Yield 53.80%. M.p. : 166-167 °C. ^{1}H-NMR (500 MHz, $CDCl_3$) δ (ppm) : 3.16 (dd, 1H, J = 7.75, 17.20 Hz, H_a pyrazoline ring), 3.82 (s, 3H, OCH_3), 3.86 (s, 3H, OCH_3), 3.86 (dd, 1H, J = 4.55, 12.30 Hz, H_b pyrazoline ring), 5.35 (dd, 1H, J = 7.80, 12.60 Hz, H_x pyrazoline ring), 6.78 (d, 1H, J = 1.95 Hz, Ar-H), 6.83 (d, 1H, J = 8.45 Hz, Ar-H), 6.86 (dd, 1H, J = 1.95, 7.80 Hz, Ar-H), 6.87 (d, 1H, Ar-H), 7.12 (d, 2H, J = 7.75 Hz, Ar-H), 7.21 (t, 2H, J = 7.80 Hz, Ar-H), 7.82 (d, 2H, J =

8.45 Hz, Ar-H), 8.24 (d, 2H, J = 9.10 Hz, Ar-H). FTIR (cm^{-1}, KBr) : 2924, 2854 (C-H pyrazoline ring), 1597 (C=N pyrazoline ring), 1558 (C=C Ar), 1512, 1342 (-NO_2), 1257, 1072 (C-N pyrazoline ring), 1026 (C-O ether). GC-MS (purity 77.11%). MS relative intensity (m/z): 403 (M^+, base peak), 373, 266, 164, 91.

Cyclization reaction of dimethoxychalcone and phenyl hidrazine produced red solid product. Based on characterization of synthesized product, it can be predicted that product was a pyrazoline compound. The geminal (H_a and H_b) and vicinal (H_x) protons of pyrazoline ring closure can be identified from ^{1}H-NMR spectra as doublet of doublet peaks in upfield shift. The upfield doublet of doublet signals at 3.23-3.58 ppm (H_a) and 3.80-3.96 ppm (H_b) were identified as characteristic of pyrazoline's geminal proton(H_b). In addition, the vicinal proton (H_x) appeared at 5.57-5.74 ppm [17]. The ^{1}H-NMR spectra of product (shown in Fig. 2) presented geminal protons at signals 1 and 4. The vicinal proton of CH (H_x) that coupled by geminal protons attached to C_4 of pyrazoline ring predicted as signal 5. The protons of aromatic ring that have 2H integration appeared as 12 and 13, respectively, while the protons of aromatic ring 2 appeared as 6, 7 and 8 signals. Signal 8 matched to proton at C_6 aromatic ring 2, coupled by two protons that were not equivalent. Signal 9 that overlapped with 8 corresponding to proton of aromatic ring 3, while signal 10 and 11 predicted as protons of aromatic ring 3.

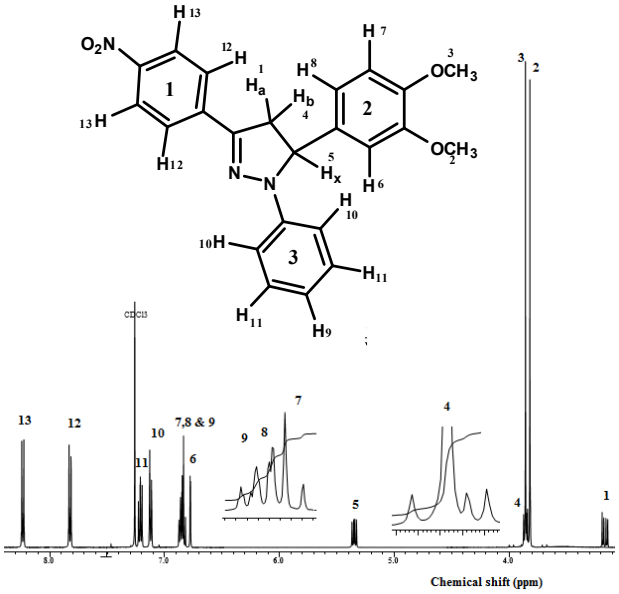


FIGURE 2. ^{1}H-NMR spectra of synthetic product

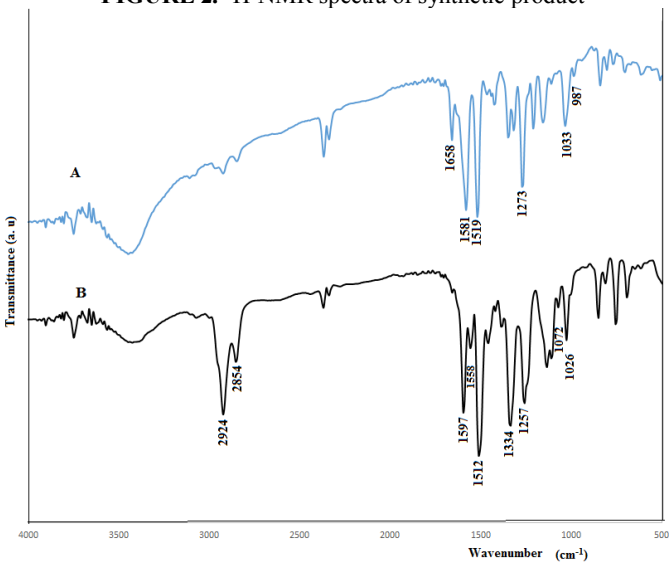
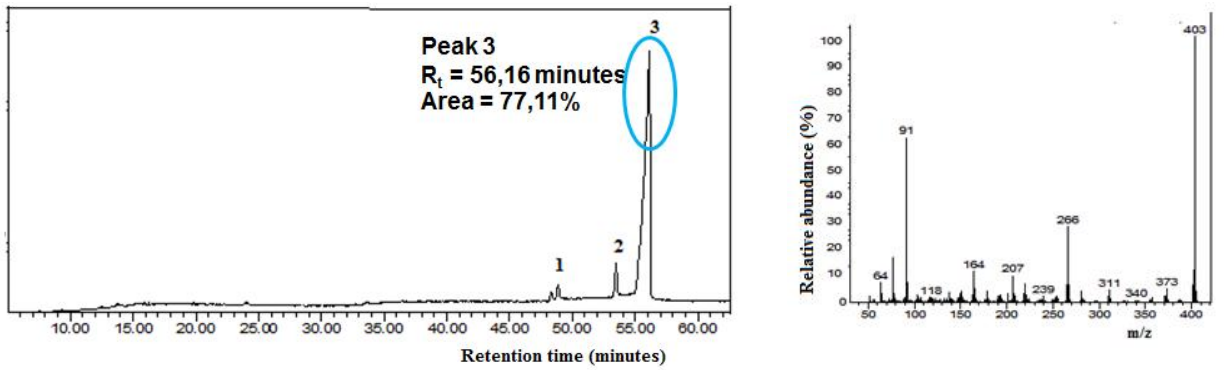


FIGURE 3. IR spectra of chalcone (A) and product of cyclization (B)

Further analysis by FT-IR spectra showed adsorption bands of C=N and C-N groups from pyrazoline ring. It presented as strong band of ν(C=N) at 1597 cm^{-1} and C-N stretching vibration of C-N appeared weak adsorption at 1072 cm^{-1}. The loss of absorption band ν(C = C) of enone group form dimethoxychalcone as reactant reinforced the formation of pyrazoline ring [18]. The FT-IR spectra of reactant and product shown in Fig. 3.

Purity of product analyzed by GC-MS that shown in Fig.4. Mass spectra showed peak 3 was pyrazoline where molecular ions (M^+) appeared as base peaks at m/z 403. Peak 3 have relative abundance of 77.11% with retention time at 56.16 minutes. From the characterization of product, it can be concluded that the product of cyclization chalcone and phenyl hidrazine was pyrazoline. Due to the characterization of product, it can be predicted that reaction through the 1,3-dipolar cycloaddition mechanism. The mechanism reaction started with nucleophilic attack from nitrogen atom of hydrazine to carbonyl group of chalcone. The acid catalyst had role to the next step, The next step was dehidration or the loss of water molecule to form intermediate (hydrazone) with which can be more faster in the presence of an acid catalyst. The formation of hydrazone was indicated by the proton signal at 1.25 ppm at ^{1}H-NMR spectra. Furthermore, hydrazone undergo cyclization by nitrogen atom attack on C_β of chalcone. This step was known as rate determining step[19], so can be said that electronegativity of C_β from enone group as reactant affected the rate of the cyclization reaction.



(a) (b)

FIGURE 4. Analysis product with GC-MS, Chromatogram (a), Mass spectra (b)

Antibacterial Test

The in-vitro antibacterial test of the compound synthesized was shown in Fig. 5 and Table 1. Pyrazoline has two methoxy groups attached to the aromatic ring 2 at meta-para positions and a nitro group from aromatic ring 1. The inhibition zone of pyrazoline showed that the compound had antibacterial activity against all bacteria. Pyrazoline showed the lowest activity against *E. Coli*. If there was comparison of activity with the positive control, i.e. tetracycline, the pyrazoline had pottentially against *S. flexneri*. This phenomenon indicated that the pyrazoline had a polarity corresponding to the five strains of bacteria tested, that could diffuse to inhibit the growth of bacterial cells. However, its activity was not good and need to be further studied. Mechanism of the antibacterial action against *S.aureus* and *S. Flexneri* that exhibited in this studies need to be clarified with further research.

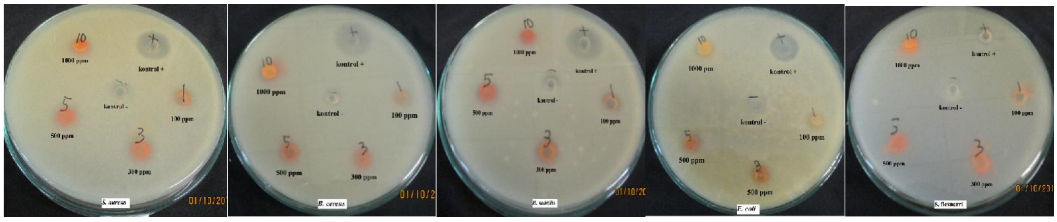


FIGURE 5. Antibacterial test of pyrazoline

TABLE 1. Zone Inhibition of Pyrazoline

Concentration (ppm)	Zone of Inhibition (mm)				
	Staphylococcus aureus	***Bacillus cereus***	***Bacillus subtilis***	***Escherichia coli***	***Shigella flexneri***
100	5.25	4.25	4.75	3.25	5.50
300	8.25	5.50	5.50	3.75	4.75
500	7.75	6.50	5.75	4.75	5.75
1000	6.25	6.50	4.75	3.50	5.25
Control (-)	-	-	-	-	-
Control (+)	18.75	20.75	20.25	13.75	7.75

Control (+): tetracycline 100 ppm, control (-): DMSO, (+): -): did not show zone inhibition

CONCLUSION

The cyclization reaction of chalcone and phenyl hidrazine produced heterocyclic compound, namely pyrazoline. It has been successfully synthesized in 53.80% yield, via 1,3-dipolarcycloaddition reaction based on the presence of hydrazone proton signal in ^{1}H-NMR spectra. Pyrazoline showed antibacterial activity againts tested bacteria. It had the highest zone inhibition of 8.25 mm (at 300 ppm) against *S. aureu*s and has pottentially as antibacterial agent against *S. flexnerri*. However, the activity was still low when compared to positive controls and it was need to be further studied.

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