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Synthesis of a Five-Membered Ring Derived from Pariazoline and Evaluation of its Bactericidal Activity

Omar D.H. Al-Mouqdady [1], Athir Ibrahim Abdulkareem [2], Arwa Falih Ali [3], Mohammed Jwher Saleh [4], Jamil Nadhem Saleh [5]

[1,2] Department of Chemistry, College of Education for Pure Sciences, University of Diyala, Baqubah-32001, Iraq

[3] Bilad Al-Rafidain University-College of Pharmacy

[4,5] Ministry of Education, Salah al-Din Education Directorate, Tikrit, Iraq

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Annotation: Pyrazole is a fundamental building block containing two nearby nitrogen atoms and a five-membered heterocyclic structure. Pyrazoles are extensively employed as versatile frameworks in several chemical industry domains, such as agriculture and medicine. The significance of pyrazoles and their many biological activities—such as antituberculosis, antibacterial, antifungal, anti-inflammatory, anticancer, and antidiabetic roles—have been well discussed in earlier reviews. They have therefore garnered a great deal of interest from scholars. In this work, chalcone-based compounds were used as the nucleus in typical sublimation reactions with hydrated hydrazine to create new pyrazole compounds. Physical measurements, proton nuclear magnetic resonance spectroscopy, and infrared spectroscopy were used to verify the integrity of these molecules. Using amoxicillin as the antibiotic, their bacteriological activity against two Gram-positive and Gram-negative bacteria was also evaluated.

Keywords: Heterocyclic, Pyrazoles, biological activity.

1. Introduction

The discovery of intriguing features exhibited by several pyrazole derivatives has led to a notable surge in interest in pyrazole chemistry during the last ten years. Ludwig Knorr originally used the name "pyrazole" in 1883 [1], while Edward Buchner is credited with being the first to synthesis it in 1889 [2]. Pyrazoles are a highly valued class of compounds in chemical synthesis because they are five-membered heterocycles. They belong to one of the azole family's most studied chemical classes. Over time, a variety of synthesis methods and synthetic analogues have been documented, indicating their crucial importance in research and applications [3]. Additionally, the pyrazole fragment is an important coordination in many chemical ligands. Intriguing uses of the pyrazole structure as a guiding and transforming group in organic synthesis have been discovered recently [4,5]. Pyrazole is a fundamental component of many small molecules and has diverse applications in agriculture and medicine [6]. These compounds can act as protein glycosylating inhibitors and possess anti-inflammatory, antibacterial, antifungal, anticancer, antidiabetic, antioxidant, antidepressant, antituberculosis, and antiviral properties. Pyrazole derivatives are also used in the food industry, as cosmetic colorants, and in supramolecular and polymer chemistry [7]. These five derivatives have been used in the formulation and synthesis of pharmaceuticals approved by the US Food and Drug Administration (FDA) and have become commercially available in recent years, both patented and unpatented [8]. This pattern illustrates the widespread use of these groups in the production of novel bioactive substances.

2. Materials and Methods:

2.1. Chemicals used: All the formulation components, in addition to their solvents, were sourced from international companies such as Serdrig and BDH.

2.2. Preparation of Cyclohexenone derivatives (O6-O10).[9,10]

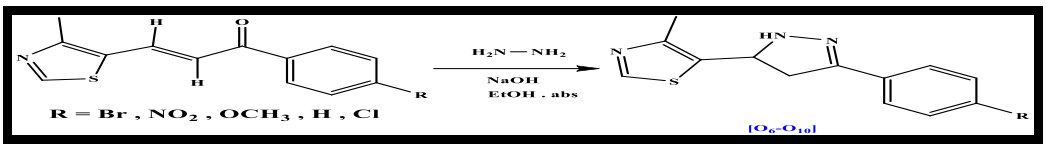
In 20 milliliters of pure ethanol, dissolve 0.001 mol of chalcone. Stir for ten minutes after adding 0.001 mol (0.29 mL) of hydrated hydrazine. As a catalyst, add 10% NaOH and let it sit for four to five hours. After adding the crushed ice, let it stand for the entire night. It neutralized the reaction media. As indicated in Table 1, track the reaction's development using a TLC plate until the mixture dried and recrystallized from the pure ethanol.

2.3. Biological activity study

Following the collection of Gram-positive *Staphylococcus aureus* and Gram-negative microorganisms from Tikrit University's major labs Twenty grams of *Escherichia coli* were dissolved in half a liter of water to create the Mueller-Hunter agar culture medium. After that, the agar was heated in a sterilizer for 14 minutes at 120°C under 1.5 bar of pressure [11-15]. It was allowed to cool before being transferred onto Petri dishes and dried at 25°C [16-18]. The compounds (O6–O10) were then dissolved in DMSO at three different concentrations (0.01, 0.001, and 0.0001 mg/ml). To guarantee uniform dispersion, the dried bacteria were then swept into the plates that had been filled with the medium in three different orientations. The solutions were then poured into the three holes created by perforating the dishes with a 6 mm diameter cork hole[19-23]. The antibiotic Amoxicillin was used as a control sample, and the plates were then stored in a dedicated container at 37°C for a whole day. The findings were then measured in millimeters using a ruler [24,28].

3. Results and discussions

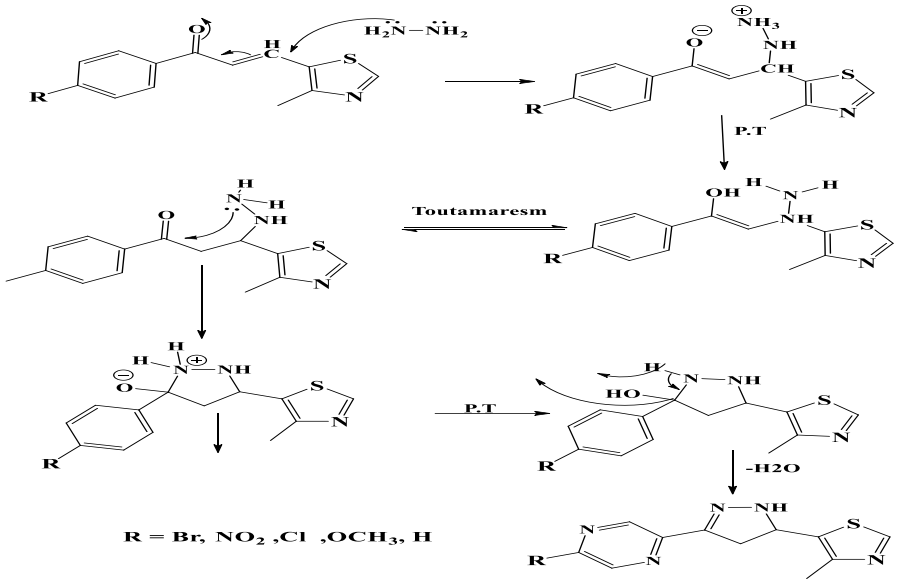
The Scheme shows the series of prepared compounds.



Scheme (1): Path of the Ready Compounds (O6-O10)

3.1. Characterization of Pyrazoline derivatives (O6-O10)

The reaction between hydrated hydrazine and chalcone substitutes in the presence of sodium hydroxide as a catalyst is depicted in the accompanying graphic [29].



Scheme (1): Mechanism of preparation of Pyrazoline derivatives [O6-O10]

Two absorption bands in the ranges of 2910–2940 cm^{-1} and 2846–2872 cm^{-1} were discovered when analyzing the Fourier transform infrared (FT-IR) spectra. The stretching of the Ar-CH bond is also responsible for the absorption band observed in the region of 3010–3072 cm^{-1}, whereas the creation of the azomethinian bond was observed in the range of 1606–1616 cm^{-1}. Additionally, two absorption bands were detected in the ranges of 1517–1581 cm^{-1} and 1457–1492 cm^{-1}, as indicated in Table 2. These bands are linked to the stretching of the aromatic (C=C) bond [30-32], as shown in Figure (1).

Compound [O6]'s ^{1}H-NMR nuclear magnetic resonance spectrum revealed several signals in the range (7.90-9.15) ppm attributed to the protons of the aromatic ring, a single signal at position (7.24) ppm attributed to the (NH) group, a triple signal in the range (3.37-34074) ppm attributed to the aliphatic (CH) group, a double signals at (2.33, 2.36) ppm attributed to (CH_2) group, and a single signal in (2.55) ppm attributed to the (CH_3). as seen in Figure (2).

The carbon of the (C=N) group in the pyrazole ring appeared at (157.48) ppm, the carbon of the (C=N) in the thiazole ring appeared at (155.77) ppm, the carbons of the benzene ring appeared at (123.64-137.66) ppm, the carbon of the (CH) appeared at (56.80) ppm, the carbon of the (CH_2) appeared at (46.04) ppm), and the carbon of the (CH_3) at (29.79) ppm.

3.2. Evaluation of the Biological Activity of Prepared Compounds

The study shows that the preparation compounds exhibit a clear variation in their biological activity against the bacterial strains under investigation. As the concentration increases, the inhibition diameter increases when compared to the antibiotic used as a control sample, which demonstrated the highest activity. This indicates the compound's effectiveness as a control sample due to its ability to inhibit bacteria [33-36]. Table 1 shows that compound O7 is the most effective of the prepared compounds against gram-positive Staphylococcus aureus, with an

inhibition diameter of approximately 25 mm at high concentrations, a good percentage compared to the other compounds. At medium concentrations, the inhibition diameter was 20 mm, also the highest for gram-positive bacteria. It also showed an activity of 18 mm at low concentrations [37-41]. This may be due to the fact that the replacement group is a withdrawing group, which may increase the compound's activity. However, the inhibition percentage was low for gram-negative Escherichia coli, possibly because the cell wall of this bacterium does not allow penetration of substances. Compound O8 showed the highest activity, despite its lower activity, as mentioned earlier[42-47]. The highest inhibition was observed at 18 mM at the highest concentration, while the lowest concentration showed the highest inhibition at approximately 11 mm. At intermediate concentrations, compound O10 exhibited the highest activity, with an inhibition capacity reaching 15 mm. These results indicate that all these compounds possess good antibacterial properties, despite their variations, which are attributed to the concentration and the substituent groups on the aromatic rings [48-53]. These findings demonstrate the success of the synthesis process in producing derivatives with significant biological activity, making them a promising basis for further structural and pharmacological studies.

Table (1): A few physical characteristics of the prepared compounds (O6-O10) [54-58].

Comp. No.	R	Molecular formula	m.p. °C	Yield%	Color
O6	4-Br	$C_{13}H_{12}BrN_3O_{16}S$	194-192	67	Light Yellow
O7	4-NO_2	$C_{13}H_{12}N_3NO_2O_{16}S$	219-217	73	Brown
O8	4-OCH_3	$C_{14}H_{15}N_3O_{17}S$	210-208	69	Yellow
O9	4-H	$C_{13}H_{13}N_3O_{16}S$	199-197	71	Orange
O10	4-Cl	$C_{13}H_{12}ClN_3O_{16}S$	225-223	76	Off-Whit

Table (2): Results of FT-IR absorption for prepared compounds (O6-O10)

IR (KBr) cm^{-1}						
Comp. No.	**R**	**(C-H)ν Arom**	**ν(C-H) .Aliph.**	**ν(C=N)**	**ν(C=C) Arom.**	**Others**
O6	4-Br	3012	2931, 2846	1610	1521, 1457	ν(C-Br) 620
O7	4-NO_2	3072	2920, 2850	1616	1581, 1481	ν(N-O)1352
O8	4-CH_3	3010	2940, 2848	1606	1517, 1480	ν(C-O)1384
O9	4-H	3038	2928, 2872	1608	1529, 1492	ν(C-F) 975
O10	4-Cl	3043	2910, 2864	1614	1522 , 1462	ν(C-Cl) 786

Table (3): The biological effectiveness of the compounds generated and the control techniques (measured in mm of inhibition).

Comp. No.	E. Coil Conc. mg/ml			Staph. Aureus Conc. mg/ml		
	0.01	0.001	0.0001	0.01	0.001	0.0001
O6	15	10	5	19	15	10
O7	16	12	11	25	20	18
O8	18	13	10	21	16	11
O9	17	12	10	22	15	5
O10	15	15	10	20	15	8
Amoxicillin	28	23	18	35	30	20

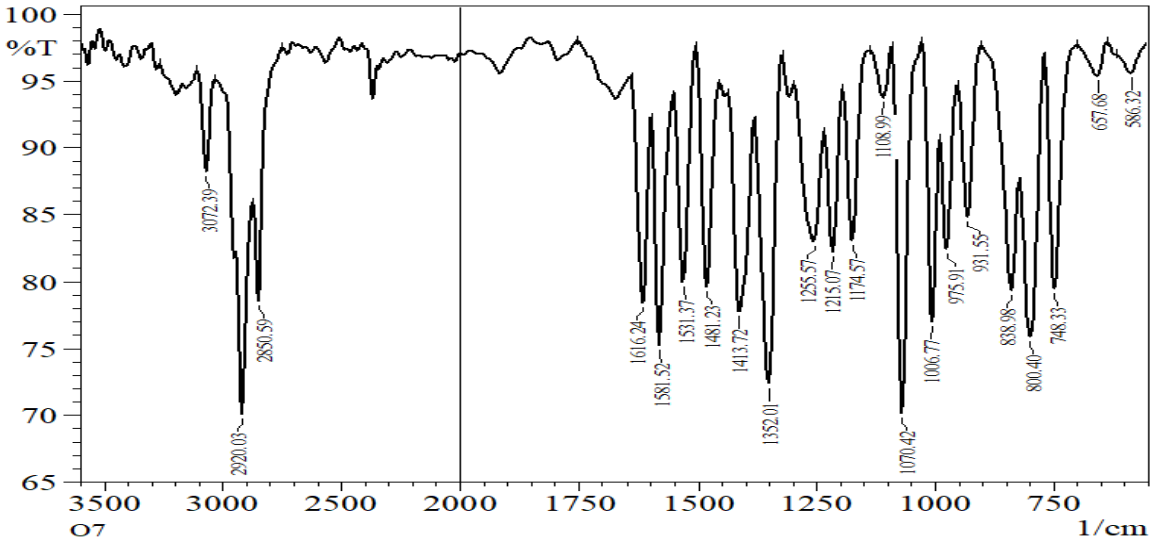


Figure (1): FT-IR of (O7).

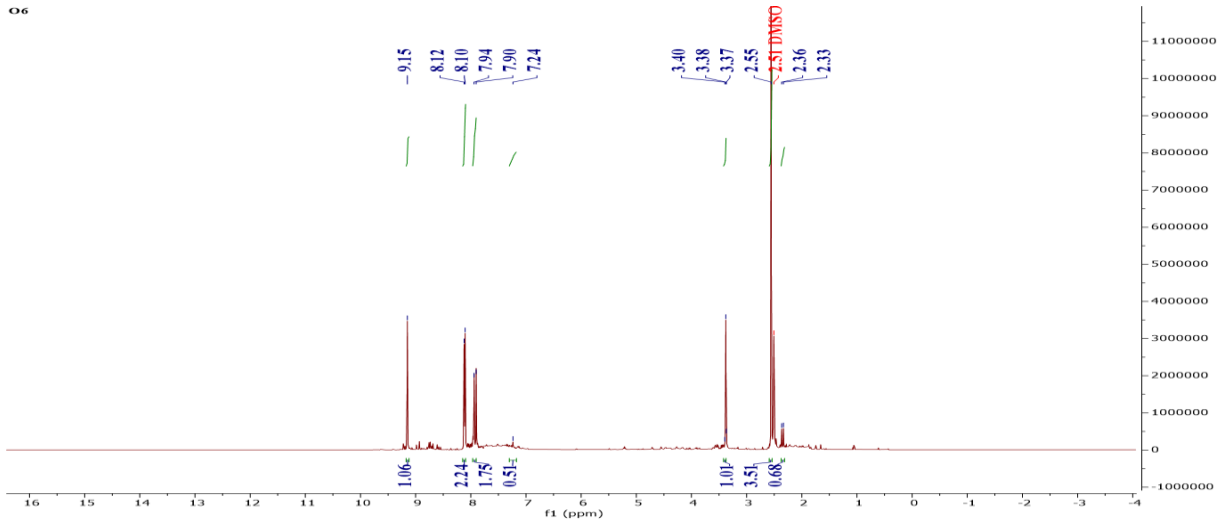


Figure (2):1H-NMR of (O6).

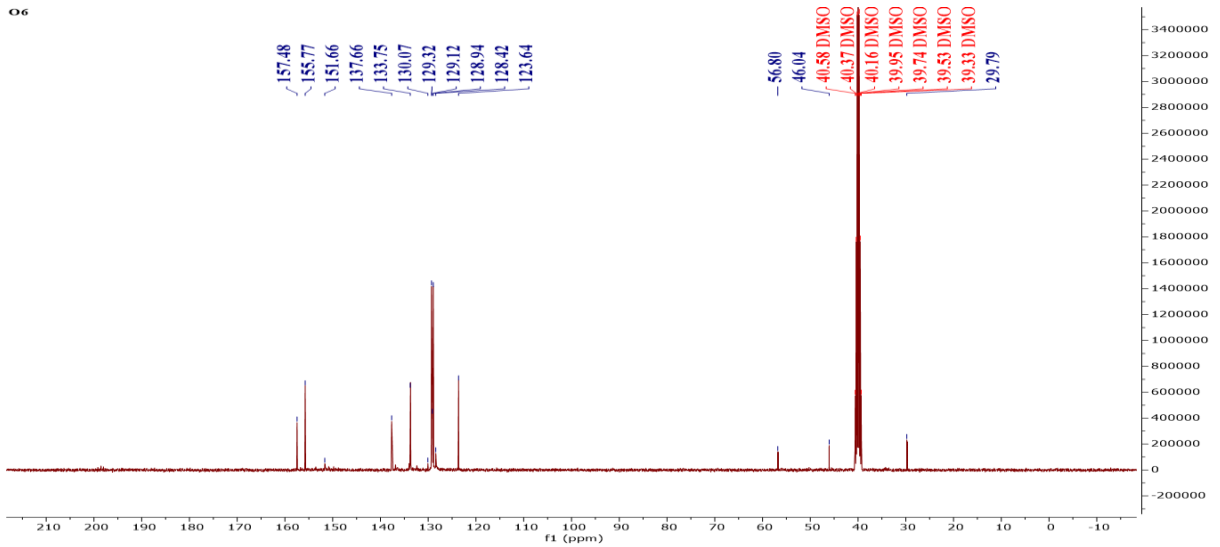


Figure (3):^{13}C- NMR of (O6).

Conclusions

A well-formed five-membered pyrazole ring is created when hydrated hydrazine reacts with alpha-beta substituents. Spectroscopic measurements verified the authenticity and precision of

the findings. When compared to the antibiotic amoxicillin, these compounds showed a high yield, superior purity, and strong potency against the bacterial species under investigation.

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