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PREPARATION, CHARACTERIZATION, AND EVALUATION OF THE BIOLOGICAL ACTIVITY OF PYRAZOLINE DERIVATIVES PREPARED USING A SOLID BASE CATALYST

Mohammed Jwher Saleh, Jamil Nadhem Saleh
Salah al-Din Education Directorate/Iraq

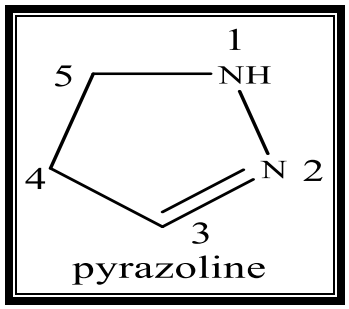
Khalid Al-Badrany
Tikrit University/College of Education for Pure Sciences/ Department of Chemistry

Absract: This study employed novel techniques for synthesizing pentacyclic rings of pyrazoline derivatives, and chalcones produced with hydrazine were reacted with the help of a solid essential catalyst. Physical techniques, including color, melting point, and spectroscopy—including infrared, proton, and carbon nuclear magnetic resonance spectroscopy and evaluation—were used to verify the validity of the structures. Some of them's biological efficacy against two kinds of bacteria—Gram-positive and Gram-negative.

Keywords: pyrazoline, chalcone, solid base catalyst. Biological activity.

1. Introduction

Pyrazoline is a five-ring heterocyclic compound consisting of two adjacent nitrogen atoms at the 2,1 position and three adjacent carbon atoms [1], as shown below:



It can be prepared by reacting chalconate with hydrazine or phenylhydrazine in an acidic medium [2] in the presence of the double bond, and the connection of the alkyl groups gives more than one geometry to the pyrazoline due to the transfer of the double bond. The solubility of the pyrazolines increases the more polar the solvent is. Studies have also shown that pyrazole derivatives have crucial biological activity for pyrazoles. Of the compounds that have important biological activities such as antibiotics [3], Antitoxic [4], Anti-inflammatory [5], Analgesic activity, antimicrobial [6-8], Antibacterial [9,10], Antioxidant [11,12], the following compound has proven effective against cancer [13]. This study aims to prepare pentacyclic rings of pyrazoline derived from chalcone using the essential catalyst as a catalyst.

2.1. Material: Without additional purification, all the compounds utilized in this investigation were acquired from BDH, Fluka, and Aldrich.

2.2. Devices used: Melting points were measured with a thermoelectric melter 9300. KBr disk at 400–4000 cm-1 scale, Shimadzu FT-IR 8400S spectrophotometer; Bruker equipment running at 400 MHz for 1H-NMR and 13C-NMR spectra. Fluka silica gel plates, with a thickness of 0.2 mm, were used in thin-layer chromatography (TLC). UV light achieved visibility after fluorescent silica gel G activated the plates.

2.3. Preparation of Pyrazoline derivatives (MH_6-MH_{10})

In a 100 ml round flask, (0.0015 mol) of chalcone was dissolved in (15 ml) of ethanol. Next, (0.00225 mol and 0.11 ml) of hydrazine were added, and it was stirred for ten minutes [14] before the catalyst was added. The mixture was prepared as (Al2O3-ONa20% by weight of chalcone) and placed in a water bath at 40°C for four to five hours. Following this, the filtrate was collected, the residue was disposed of, and the mixture was recrystallized from ethanol. Table 1 illustrates how the response was tracked using TLC plates.

Table (1): Some physical properties of Pyrazoline derivatives.

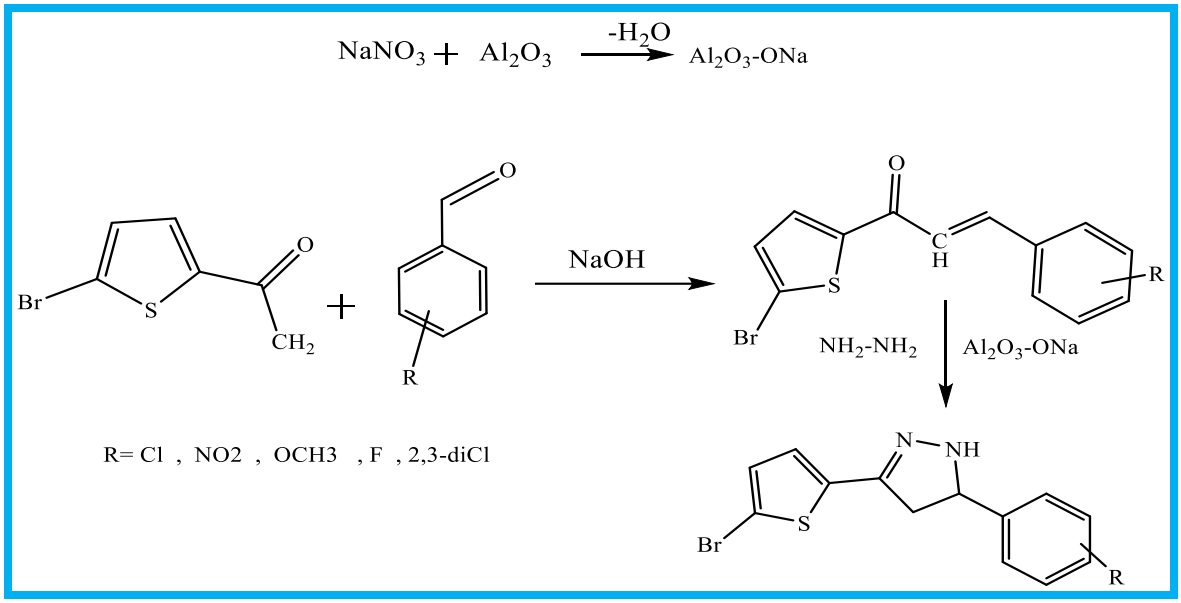
Comp. No.	R	Molecular formula	m.p. °C	Yield%	Color
MH_6	4-Cl	$C_{16}H_{12}BrClN_2$	150-152	47	Orange
MH_7	4-NO_2	$C_{16}H_{12}BrClN_3O_2$	158-160	45	Black
MH_8	4-OCH_3	$C_{17}H_{15}BrClN_2O$	187-189	51	Red
MH_9	4-F	$C_{16}H_{12}BrClN_2F$	165-168	53	Yellow
MH_{10}	2,3-dial	$C_{16}H_{11}BrCl_2N_2$	218-220	44	Brown

2.4. Biological activity study [15, 16]

This study used two pathogenic bacteria: the Gram-negative Escherichia coli and the Gram-positive Staphylococcus aureus. Multer Hinton Agar was employed as the growth medium for bacteria from the Department of Life Sciences and the College of Education for Pure Sciences. Chemical solutions of MH6, MH8, and MH10 at concentrations of (0.01, 0.001, and 0.0001) mg/ml were prepared using dimethyl sulfoxide (DMSO). The process determines and monitors the minimal inhibitory concentration (MIC). Mueller-Hinton agar was employed as the nutritional medium, and the diffusion method was performed to assess the sensitivity of the bacterial isolates used in the investigation. After being ready, the medium was autoclaved, divided across plates, and given time to solidify. Subsequently, four tiny holes were punched into each plate. It was then incubated at 37 0C for the whole day. Of the derivatives that are used. To illustrate how sensitive the used derivatives are. As the diameter grows, these derivatives depend on the inhibition diameter seen in the dishes around the holes employed. When a produced chemical exhibits inhibition, its biological activity increases; this may be compared to the antibiotics' diameter of inhibition. [17, 18].

3. Results and discussion

As seen in Scheme 1, one mole of chalcone derivatives and one and a half moles of hydrazine were reacted in this study, with the primary catalyst functioning as a catalyst and ethanol serving as a solvent to create pentacyclic rings of pyrazoline derivatives.



Scheme (1): Path of the Ready Compounds (MH_6-MH_{10})

3.1. Characterization of pyrazoline derivatives (MH_6-MH_{10})

Compounds [MH6-MH10] were diagnosed using spectroscopic methods, including the infrared (IR) spectrum because it revealed bands that appeared and absorption bands in the region of (1587-1610) cm-1, which corresponded to the (C=N) bond In the area of (3171-3221) cm-1, absorption, synergistic stretching (N-H) is responsible. Furthermore, it was observed that two absorption bands appeared due to extensive interactions (C=C) in the regions of (1517-1553) cm-1 and (1456-187) cm-1 The absorption bands at (3036-3081) cm -1 of the aromatic (ArC-H) is proportional to the width of the bands, as well as the absorption bands at (1054-1091) cm-1, which form the (N-N) bond in aromatics. (2927-2969) absorption bands due to stepwise (C-H) bond propagation in the cm-1 region, and these data are consistent with previous studies [19 as shown in Table (2) and Figures (1, 2)

Table (2): FT-IR absorption results for Pyrazoline derivatives (MH_6-MH_{10}**)**

Comp. No.	R	ν(C-H) Aliph.	ν(C-H) Arom.	ν(N-H)	ν(C=N)	ν(N-N)	ν(C=C) Arom.	Others
MH_6	4-Cl	2935	3055	3184	1587	1091	1519,1460	ν (C-Cl) 678
MH_7	4-NO_2	2927	3081	3189	1596	1083	1541,1456	ν (N-O) 1373
MH_8	4-OCH_3	2932	3036	3221	1598	1076	1523,1471	ν (C-O)1279
MH_9	4-F	2943	3047	3199	1610	1054	1517,1465	ν (C-F) 891
MH_{10}	2,3-Cl	2969	3061	3171	1603	1055	1553,1487	ν (C-Cl)684

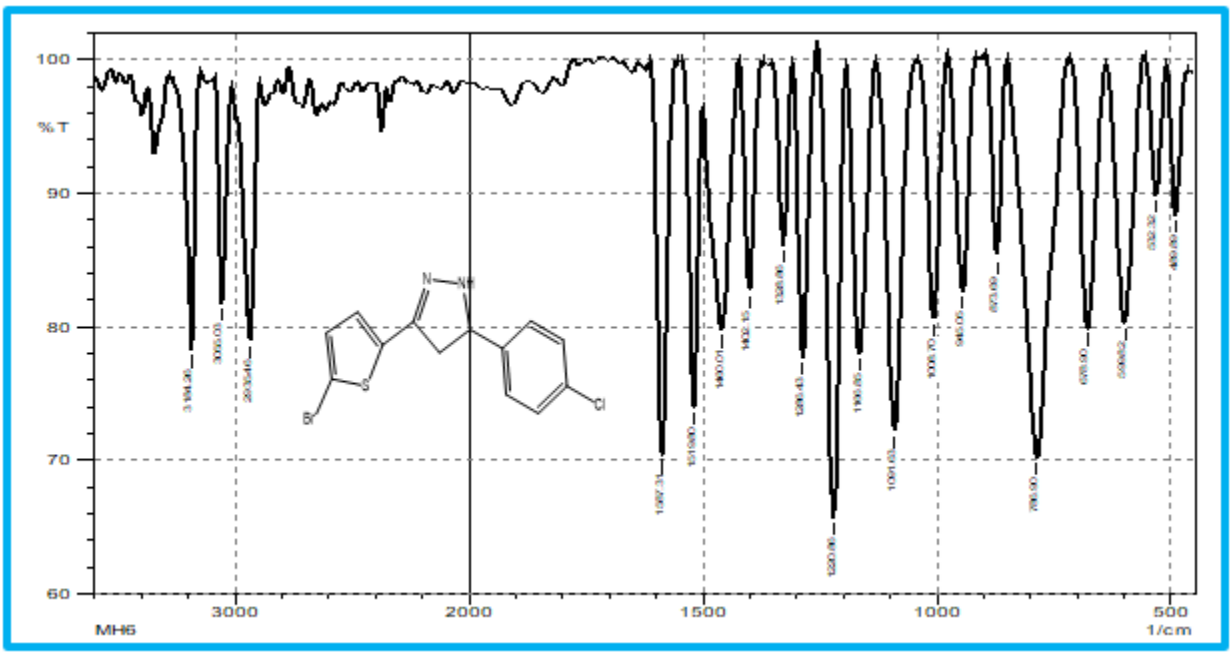


Figure (1): The compound's FT-IR spectra (MH6).

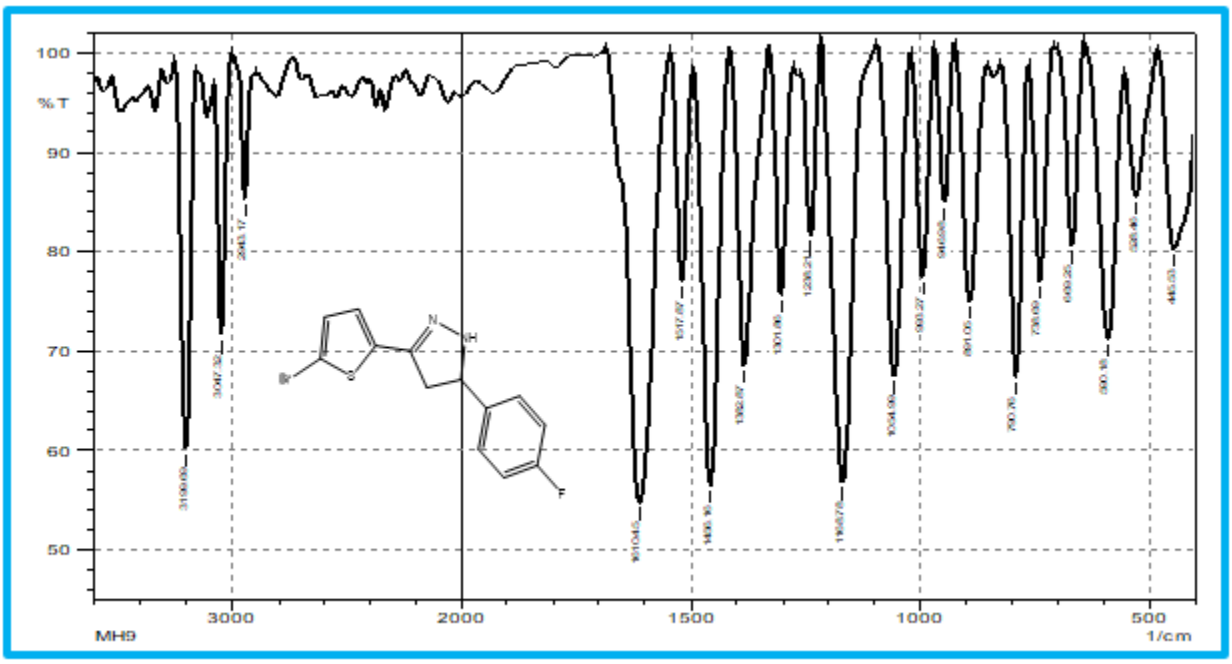


Figure (2): The compound's FT-IR spectra (MH9).

The nuclear magnetic resonance (1H-NMR) spectrum of compound (MH9) revealed the appearance of a triplet signal in the range (396-3.98) belonging to the proton of the (CH) group attached to the (NH) group in the pyrazoline and a doublet signal in the range (3.43-3.45) ppm. It belongs to the group proton (CH2) in the pyrazoline ring, and in addition, a multiple signal and a signal belonging to the group proton (NH) were detected at (8.96) ppm. A Numerous signal is attributed to the protons of the aromatic ring in the range of (6.56-7.84) ppm [20]. The protons in the solvent (DMSO-d6) cause a signal to appear at ppm (2.51), as in Figure (3).

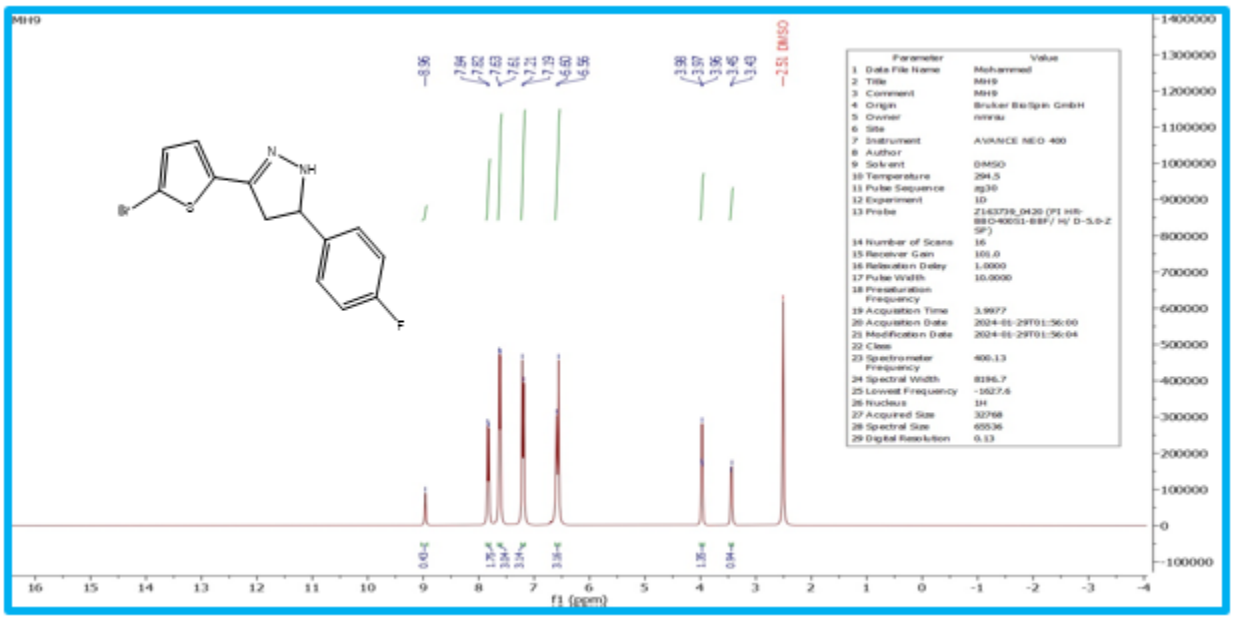


Figure (3): 1-H NMR spectra of the substance (MH6).

A signal was observed at the ppm site (37.92) corresponding to the carbon of the pyrazoline group (CH2), a signal at ppm (56.52) corresponding to the (CH) carbon, a signal at ppm (160.98) corresponding to the azomethine group (C=N)[21], and a multiple signals at ppm (121.81-163.64) corresponding to the carbonate of the aromatic ring when examining the 13C-NMR of the compound [MH9].

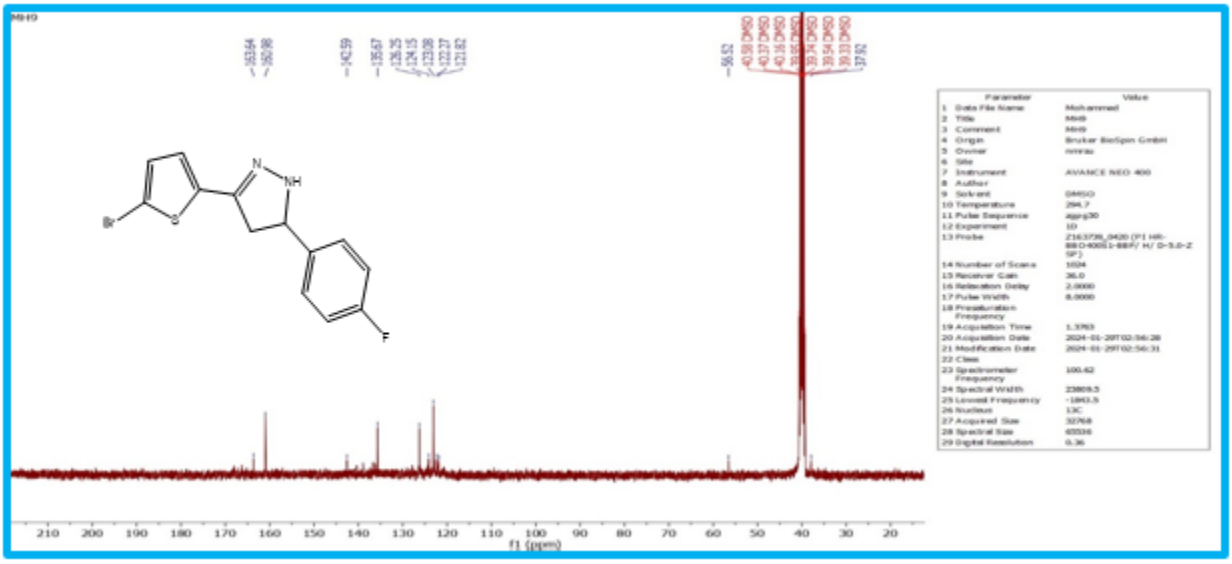


Figure (4): 13C-NMR spectrum of the compound (MH6).

3.2. Evaluation of the Biological Activity of Prepared Compounds

Escherichia coli and Staphylococcus aureus were the two types of bacteria used in this dissertation to investigate the biological activity of the compounds created. Compounds with non-homogeneous rings show different biological activity against Gram-positive and Gram-negative bacteria. These bacteria were chosen since they are known to cause various disorders. Furthermore, these microorganisms' antibiotic resistance patterns vary [22]. The biological activity of the compounds generated was assessed by measuring the inhibition zone width and utilizing the agar well diffusion method [23]. The results indicate that the synthesized compounds had varying degrees of ability to hinder the growth of Gram-positive and Gram-

negative bacteria. The substances showed significant inhibition activity against Escherichia coli and extraordinary inhibitory effect against Staphylococcus aureus[24,25]. A concentration of 0.01 milligrams per milliliter increased inhibition percentages in a dose-dependent connection between concentration and inhibition. as presented in Table (3).

Table (3): Biological efficacy of produced substances and control methods (measured in millimeters 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
MH6	22	10	--	15	10	10
MH8	18	15	13	30	20	15
MH10	20	15	10	15	10	--
Amoxicillin	25	16	14	25	21	18

4. Conclusions

When chalconate derivatives react with substances that have the right functional groups, heterocyclic pentacyclic rings are frequently produced. The majority of the produced compounds exhibited antibacterial activity and could limit bacterial growth, according to the bioanalysis results. They demonstrated higher biological activity when comparing several of these compounds to the antibiotics used as control samples. Measurements conducted using spectroscopy and physical methods showed how accurately the synthesized chemicals were composed.

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