PREPARATION AND CHARACTERIZATION OF INDOLE-3-CARBALDEHYDE-DERIVED PYRAZOLINE DERIVATIVES AND EVALUATION OF BIOLOGICAL EFFICACY

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Abstract: *The research involved the preparation of a series of chalcones [I_1-I_5] by the reaction of indole-3-carbaldehyde with acetophenone substituents in a basic medium. The prepared chalcones were then reacted with aqueous hydrazine in the presence of glacial acetic acid as a catalyst to form the pyrazoline ring [I_6-I_{10}]. The prepared compounds were formed using ethanol as the solvent. The products were validated using FT-IR, ^{1}H-NMR, and ^{13}C-NMR spectra, which revealed the formation of the studied reactions. The biological evaluation of the prepared compounds was carried out against two types of bacteria, Gram-negative Escherichia coli and Gram-positive bacteria Staphylococcus aureus, using three different concentrations. The results showed that compounds I_9 and I_{10} exhibited the highest inhibitory activity, with I_9 outperforming the standard antibiotic (ciprofloxacin) in terms of the diameter of inhibition, especially at low concentrations (0.001 mg/ml) against E. coli. Compound I_{10} had the highest activity at high concentrations (0.1 mg/ml) against the same bacteria. Compound I_9 had the highest activity against Staphylococcus aureus at high concentrations (0.1 mg/ml), where the high activity was attributed to the presence of (Br) groups in compound I_9 and (NO_2) groups in compound I_{10}. Compound I_5 showed balanced activity against both species, while the other compounds showed limited activity.*

Keywords: *heterocyclic; chalcones; pyrazoline; biological activity*
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1. Introduction

Heterocyclic compounds are currently widely used in medicinal chemistry and are essential to the development of organic synthesis. Numerous biological activities and medicinal applications depend on the ring structure that contains nitrogen as a heteroatom. Heterocyclic compounds are crucial in the development of novel structural entity classes with possible novel modes of action in medicine [1]. It is commonly recognized that these heterocyclic compounds have a variety of pharmacological characteristics, including antibacterial [2], antimalarial [3], and anticancer activities [4]. As seen in Fig. 1, pyrazoline is a five-membered, non-aromatic heterocyclic moiety that has one double bond and two nitrogen atoms at nearby positions. When both electronegative nitrogen atoms are present in the pyrazoline ring, three different isomers are created. In 1885, Knorr and Blank reported [5] that they made the pyrazoline ring by oxidizing 1,3-diphenyl-5-methylpyrazole using ethanol and salt.

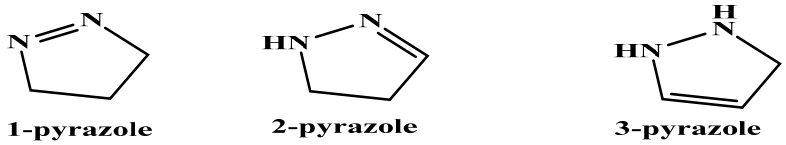


Fig. 1. Pyrazole geometries

In another publication [6], directly reacted phenylhydrazines with acrolein to create 2-pyrazoline-based derivatives. Thus far, the proposed reaction between hydrazines and α, β-

unsaturated aldehydes and ketones has been acknowledged as a simple and useful technique for creating 2-pyrazolines [7]. Auwers et al. [8] developed many substituted 2-pyrazolines and made significant contributions to the expansion of this discipline in the early decades of the 20th century. In [9], the stability of the synthesis of pyrazolines and intermediate hydrazones in the interactions of hydrazine derivatives with various aromatic and aliphatic α, β-unsaturated aldehydes and ketones was considered. Pyrazoline has a variety of bioactivities, including antimalarial [10], anticancer [11], and antibacterial [12].

This study aims to use unsaturated alpha-beta compounds as nucleophiles in the formation of new pyrazoline derivatives using an acidic medium in their preparation and to study their bactericidal activity against two types of bacteria.

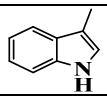
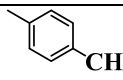
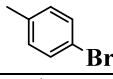
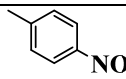
2. Experimental part

2.1. Materials. Fluka, Aldrich, and BDH supplied all of the compounds utilized in this investigation, and none of them required additional purification.

2.2. Devices used. The Electrothermal Melting Apparatus 9300 was used to measure the melting points. FT-IR 8400S Shimadzu spectrophotometer by KBr disc, 400–4000 cm^{-1} scale. Bruker equipment operating at 300 MHz produced ^{1}H-NMR and ^{13}C-NMR spectra. Using Fluka silica gel plates that were 0.2 mm thick and activated with fluorescent silica gel G, Thin Layer Chromatography (TLC) was carried out. UV light was used to see the results. The Raypa steam sterilizer (Spain) autoclave at the University of Tikrit's Advanced Microbiology Research Laboratory was utilized to sterilize the microbiological medium used in the study. In the same lab, petri dishes utilized for the microbiological investigation were incubated using a Heraeus D-63450 incubator (Germany).

2.3. Preparation of chalcones derivatives [I_1-I_5] (Scheme 1). (0.01 mol) of the ketone derivative was dissolved in a minimum amount of absolute ethanol, to which was gradually added (4 mL) of alcoholic sodium hydroxide solution (20%) with continuous stirring for 10 minutes in an ice bath. Subsequently, 0.01 mol (1.6 g) of indole-3-carbaldehyde dissolved in 20 ml of absolute ethanol was added and stirred for 3-4 hours at 20-40°C [13]. At the end of the reaction, the mixture was poured over crushed ice, where a precipitate began to form, and left in the refrigerator overnight. The reaction medium was then adjusted by adding drops of of HCl (20%), and the precipitate was filtered and recrystallized using methanol (Table 1).

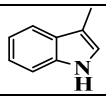
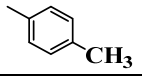
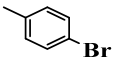
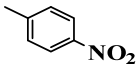
Table 1. Physical properties of chalcones derivatives [I_1-I_5]

Comp No.	Ar	Molecular Formula/ M.Wt g/mol	Color	M.P (^{0}C)	R.T hr	R_f	Yield (%)
I_1		$C_{19}H_{14}N_2O$ 286.33	yellow	160-162	3	0.72	78
I_2		$C_{17}H_{13}NO$ 247.32	white	190-192	3.5	0.63	81
I_3		$C_{18}H_{15}NO$ 261.32	yellow	177-179	3	0.68	82
I_4		$C_{17}H_{13}BrNO$ 326.22	white	183-186	3	0.70	78
I_5		$C_{17}H_{12}N_2O_3$ 292.32	Brown	167-170	4	0.65	80

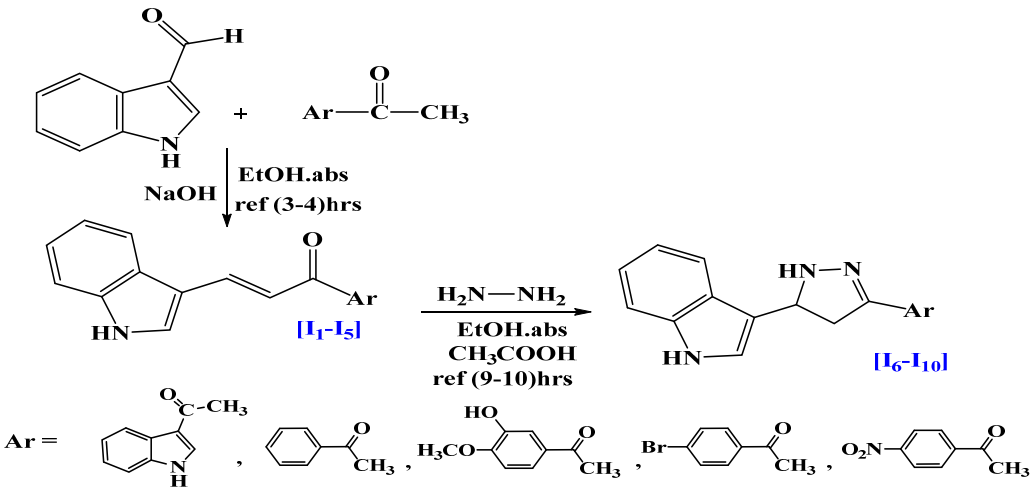
2.4. Preparation of pyrazoline derivatives [I_6-I_{10}]. Equal amounts (0.004 mol) of **[I_1-I_5]**, dissolved in a minimum amount of absolute ethanol, and were mixed with (0.004 mol, 0.5 mL) 90% aqueous hydrazine in a 100 mL round-bottom flask. Glacial acetic acid (4–5 drops) was added to the reaction mixture, and the resulting solution was stirred under heating for

9–11 hours. After heating, the solution was allowed to cool and then poured over crushed ice to form a precipitate. The precipitate was separated by filtration, washed thoroughly, and recrystallized using methanol [14] (Table 2).

Table 2. Physical properties of pyrazoline derivatives [I_6-I_{10}]

Comp No.	Ar	Molecular Formula/ M.Wt g/mol	Color	M.P (^{0}C)	R.T hr	R_f	Yield (%)
I_6		$C_{19}H_{16}N_4$ 300.37	Light yellow	258-260	10	0.78	64
I_7		$C_{17}H_{15}N_3$ 261.36	Orange	243-245	11	0.71	58
I_8		$C_{18}H_{17}N_3$ 275.36	Brown	292-195	11	0.74	65
I_9		$C_{17}H_{14}BrN_3$ 340.25	Light Brown	240-242	9	0.76	60
I_{10}		$C_{17}H_{14}N_4O_2$ 306.35	yellow	227-230	10	0.72	62

2.5. Biological activity study. Two types of Gram-positive Staphylococcus bacteria and Gram-negative Escherichia coli were used from the Advanced Life Sciences Laboratory at Tikrit University. The prepared compounds were then diluted to three concentrations using dimethyl sulfoxide as a solvent (0.1, 0.01, and 0.001 mg/ml) [15, 16]. The culture medium (Mueller-Hinton agar) was then prepared by dissolving 20 g in 0.5 L of distilled water, sterilized in an autoclave at 120°C, and poured into plates after cooling. After cooling, the culture medium was wiped on the plates in three directions to distribute the bacteria in all directions. The plates were incubated at 37°C for one night, and the result was read in centimeters [17, 18].



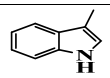
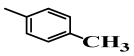
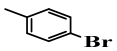
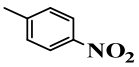
Scheme 1. Series preparation of compounds [I_1-I_{10}]

3. Results and discussion

3.1. Characterization of chalcones derivatives [I_1-I_5]. The FT-IR spectrum of compounds [I1-I5] showed a band at (1668-1662) cm^{-1} for (C=O), which alternates with (C=C), which was found at (1606-1600) cm^{-1}, making the force constant appear in a lower range; a band at (3167-3152) cm^{-1} for olefinic (=CH); a band at (3385-3348) cm^{-1} for (NH); a band at (364-3029) cm^{-1} for aromatic (Ar-CH); and a band at (3167-3152) cm^{-1} for olefinic

(=CH). It also showed a band at (3385-3348) cm^{-1} for (NH), a band at (364-3029) cm^{-1} for aromatic (Ar-CH), two bands at (1581-1508) & (1481-1460) cm^{-1} for aromatic (C=C) rings, and a band at (1264-1214) cm^{-1} for (C-N) [19]. As in Table 3 and Fig. 2.

Table 3. FT-IR spectrum absorption results for compounds [I_1-I_5]

Comp. No.	R	IR (KBr) cm^{-1}						
		ν(N-H) ν(C-H) Olph.	ν(C-H) Arom.	ν C=O	ν(C=C) Olph.	ν(C=C) Arom.	ν(C-N)	Others
I_1		3363 3153	3064	1664	1600	1508 1460	1253	----
I_2		3359 3159	3033	1663	1606	1581 1481	1215	---
I_3		3348 3152	3051	1668	1602	1571 1462	1244	ν(C-H) *asy*. (2935) *sym*. (2867)
I_4		3337 3161	3064	1662	1605	1562 1476	1214	ν (C-Br)632
I_5		3385 3167	3029	1664	1603	1577 1466	1264	ν(NO_2) *asy*. (1511) *sym*. (1342)

In the ^{1}H-NMR spectrum, compound [I1] showed two single signals at (8.66, 8.98) ppm attributed to (NH) in the indole rings, signals in (7.02-7.96) ppm to aromatic rings, a double signal at (6.90, 6.93) ppm to (=HC) adjacent to the indole ring, a double signal at (6.70, 6.72) ppm to (O=C-CH) adjacent to the carbonyl group, and a signal at position (2.50) ppm attributed to the protons of the solvent (DMSO-d6), as in Fig. 3.

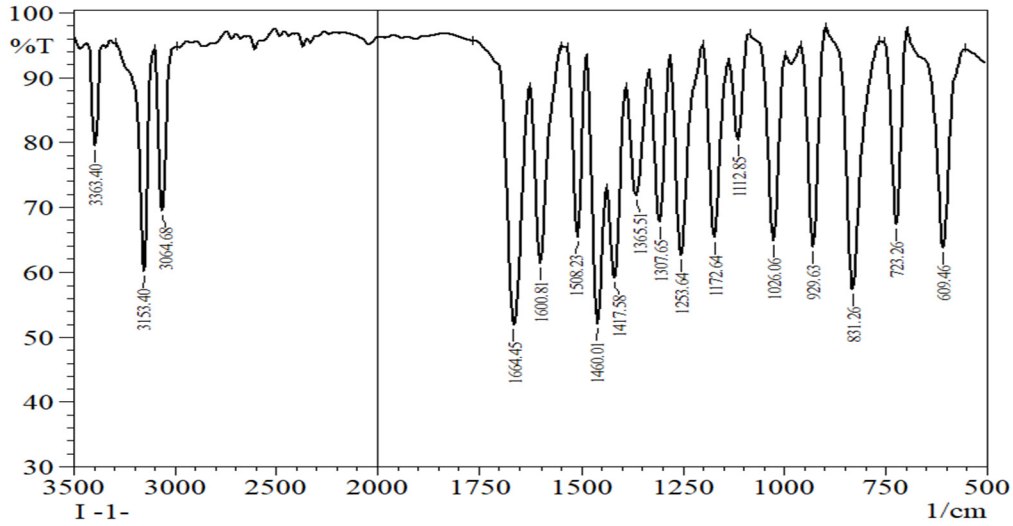


Fig. 2. FT-IR spectrum for the compound I_1

The ^{13}C-NMR spectrum of compound [I_1] showed a signal at (193.16) ppm for (C=O), a signal at (146.81) ppm for (=CH) adjacent to the aldehyde, a signal at (134.90) ppm for (O=C-CH) adjacent to the carbonyl, signals at (118.62-144.67) ppm for carbonate aromatic rings, and a DMSO-d6 solvent signal at (39.33-40.58) ppm (Fig. 4).

3.2. Characterization of pyrazoline derivatives [I_6-I_{10}].

The compounds [I_{10}-I_6] were identified using FT-IR spectroscopy. In the IR spectrum, a new band appeared due to N-H bond stretching, with lengths ranging from (3364 - 3489) cm^{-1}; while the N-H band in chalcone remained almost constant, with lengths also ranging from (3232 – 3298) cm^{-1}. The C=C and C=O bands in chalcone [I_5-I_1] disappeared, appearing in the range of 1600-1606 cm-1 and

1662-1668 cm-1, respectively.

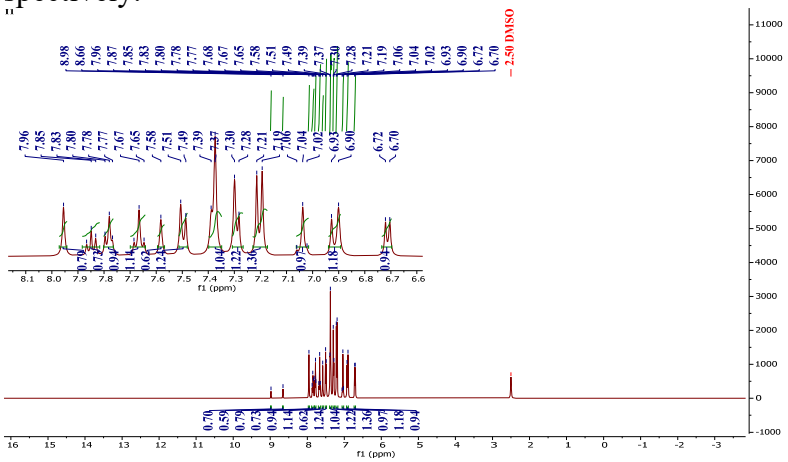


Fig. 3. ^{1}H-NMR spectrum of the compound I_1

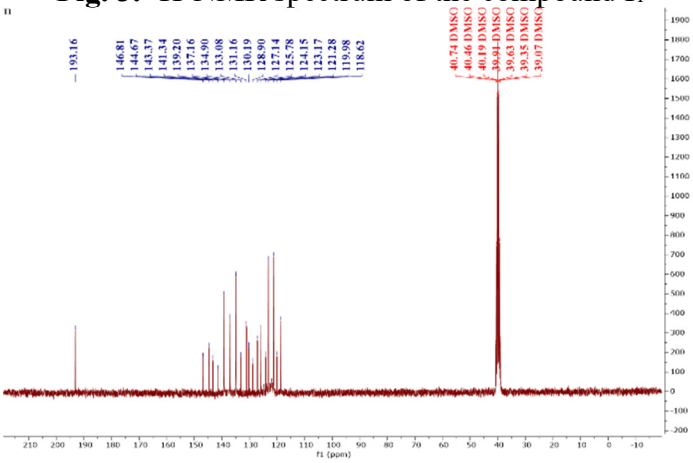
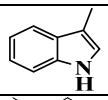
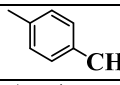
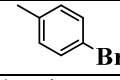
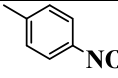


Fig. 4. ^{13}C-NMR spectrum of the compound I_1

In the range of (1620-1627) cm^{-1}, an intermediate absorption band appears due to the azomethine group (C=N) of the pyrazoline ring, and in the range of (3080-3049) cm^{-1}, an absorption band appears due to the aromatic group (CH). In addition, absorption bands appear in the range of (2966-2920) cm^{-1} and (2852-2895) cm^{-1}, which are caused by the stretching of the aliphatic C-H bond. In addition, absorption bands appear at (1561-1579) cm^{-1} and (1474-1481) cm^{-1} due to the stretching of the C=C bond of the aromatic ring, and in the range of (1232-1188) cm^{-1} they are due to the stretching of (C-N) [20]. As shown in Table 4 and Fig. 5.

Table 4. FT-IR spectrum absorption results for compounds [I_6-I_{10}]

Comp. No.	Ar	IR (KBr) cm^{-1}						
		ν(N-H)	ν(C-H) Arom.	ν(C-H) Aliph.	ν(C=N)	ν(C=C) Arom.	ν(C-N)	Others
I_6		3425 3267	3064	2945 2895	1627	1563 1481	1213	----
I_7		3392 3232	3059	2966 2891	1620	1579 1481	1232	---
I_8		3438 3298	3062	2954 2876	1625	1566 1478	1224	---
I_9		3389 3287	3049	2935 2872	1622	1561 1474	1221	ν (C-Br)612
I_{10}		3464 3234	3080	2920 2852	1624	1566 1475	1188	ν(NO_2) *asy.* (1510) *sym.* (1354)

The ^{1}H-NMR spectrum of [I7] showed a single signal at (8.78 ppm) for (NH) in the indole ring, a single signal at (8.25 ppm) for the (NH) in the pyrazoline ring, and multiple signals in (7.00-7.99 ppm) for the aromatic rings. A triple signal to (CH) at 3.65-3.69 ppm and a double signal at (3.04, 3.06 ppm) to (CH_2), in addition to the signal at (2.50) ppm to the protons of the solvent (DMSO-d^{6}) (Fig. 6).

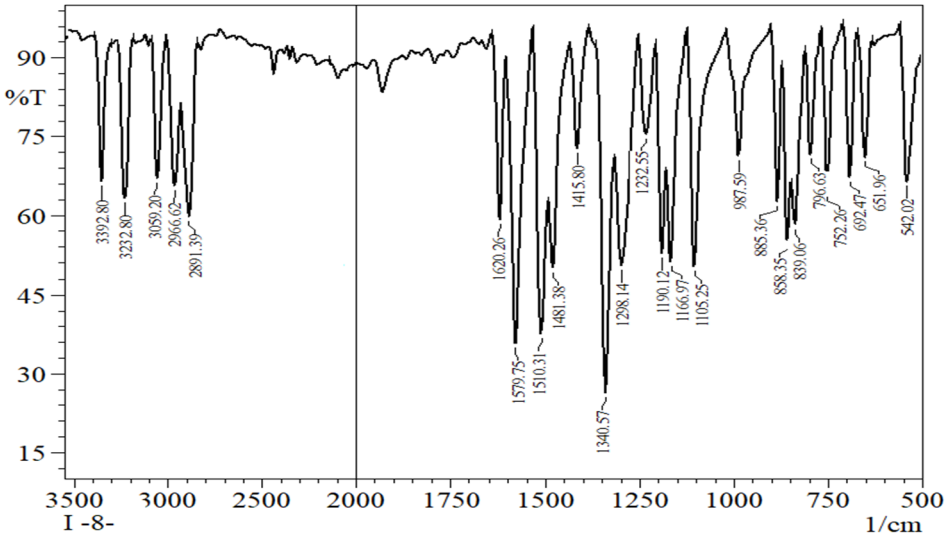


Fig. 5. FT-IR spectrum for the compound I5

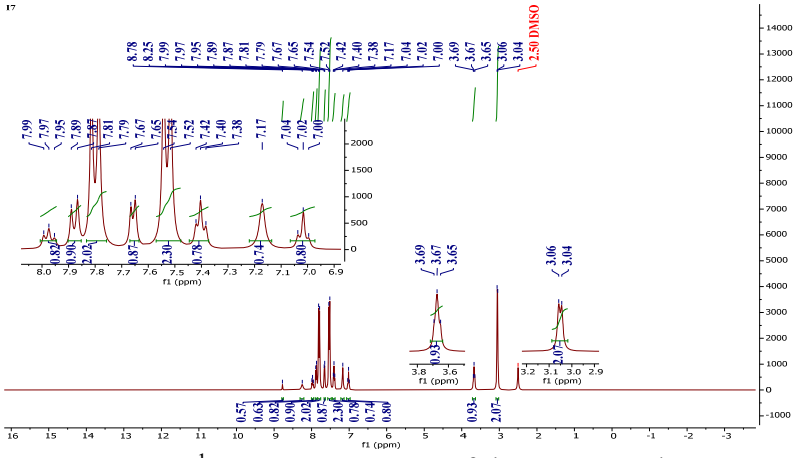


Fig. 6. ^{1}H-NMR spectrum of the compound I7

When studying the ^{13}C-NMR spectrum of the compound [I7], carbon of the (C=N) in the pyrazoline ring was found to have a signal at (158.39) ppm. Additionally, signals from the aromatic rings' carbons were found to have an appearance in the range of 121.11-141.04 ppm, the carbon of the (CH) group at 49.40 ppm, the carbon of the (CH_2) group at 42.30 ppm, and the carbon of the solvent (DMSO-d6) showed up in the range of 39.35-40.61 ppm. As shown in Fig. 7.

3.5. Evaluation of the Biological Activity of Prepared Compounds. Table 5 showed that the compounds had good activity against *Staphylococcus aureus* and *Escherichia coil* strains at dilute concentrations (0.1, 0.01, 0.001) mg/ml, as the diversity of activity in the compounds was related to the type of substituent groups (R) associated with the structural structure of the compounds. Compound I10 recorded the highest activity against the negative bacteria *Escherichia coil* with an inhibition diameter reaching 4.3 cm at the high concentration of 0.1 mg/ml, followed by compound I9 with high activity at the low concentration of 0.001 cm, as the inhibition diameter reached 4.2 cm, outperforming these compounds over *Ciprofloxacin* at the mentioned concentration [21-26]. Against gram-positive bacteria *Staphylococcus aureus*, these two compounds also had the highest activity compared to the rest of the compounds.

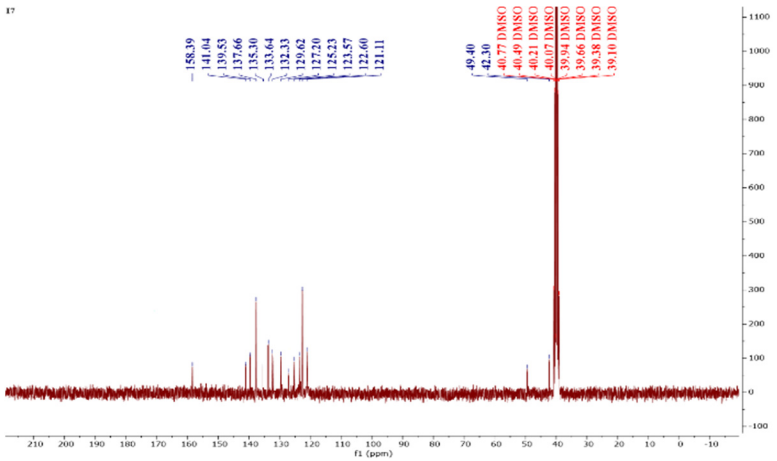


Fig. 7. ^{13}C-NMR spectrum of the compound I_7

Table 5. Biological effectiveness of prepared compounds and control treatments (inhibition in *cm*)

Comp. No.	*Escherichia coil*			*Staphylococcus aureus*		
Conc. mg/ml	0.1	0.01	0.001	0.1	0.01	0.001
I_1	1.3	1.0	0.7	1.9	1.5	0.8
I_2	1.2	1.3	1.8	1.0	0.5	0.5
I_3	1.6	1.2	0.7	1.7	1.0	1.0
I_4	2.0	1.7	1.3	1.9	1.7	1.3
I_5	2.1	2.1	1.5	2.4	2.0	1.6
I_6	1.9	1.6	1.0	1.8	1.0	0.5
I_7	1.8	1.3	1.0	2.1	1.5	1.5
I_8	1.3	1.0	0.5	1.6	1.2	0.8
I_9	1.5	1.5	4.2	4.8	3.1	1.5
I_{10}	4.3	1.8	2.1	2.6	1.0	0.9
Ciprofloxacin	3.5	3	2.1	3.8	3.2	2.7

Compound I_9 showed the highest activity against wave bacteria at the high concentration of 0.1 mg/ml, as the inhibition diameter reached 4.8 cm, surpassing the antibiotic, which inhibited it at the same concentration of 3.8 cm. This was followed by compound I_{10}, with an inhibition diameter of 2.6 cm. As shown in Fig.s 8 and 9. This high activity is attributed to the nature of the organic groups in these two compounds.

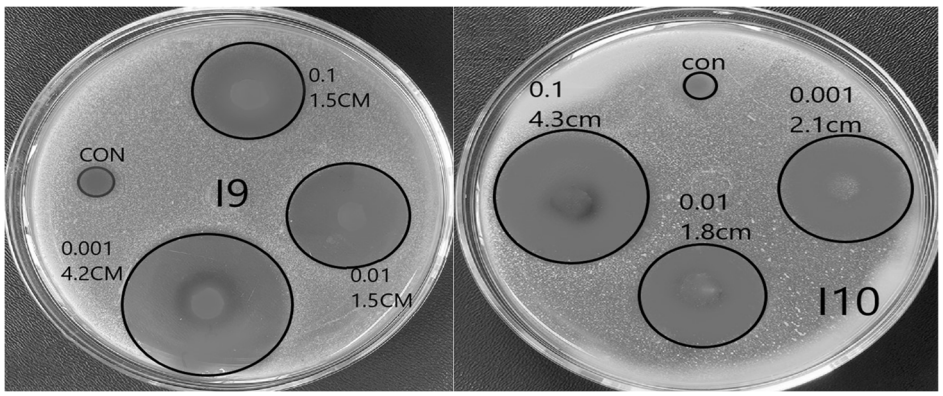


Fig. 8. Inhibitory activity of compounds (I_9, I_{10}) against *E.coli*

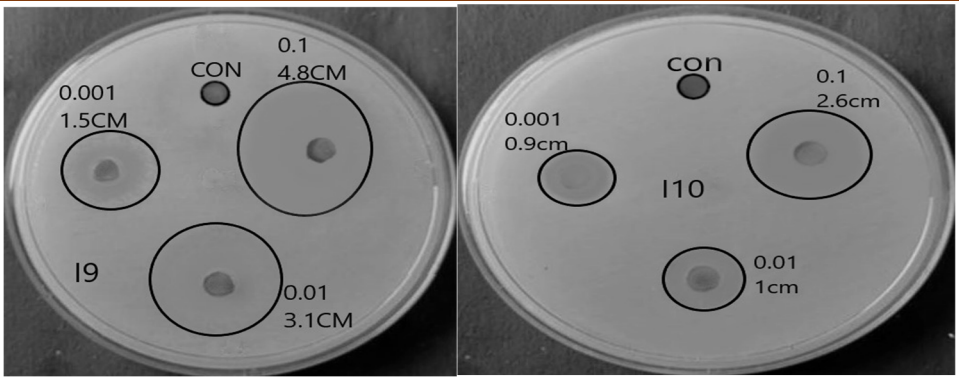


Fig. 9. Inhibitory activity of compounds (I_9, I_{10}) against *S. aureus*

Compound I_{10} contains a nitro group (NO_2), which is an electron-withdrawing group that enhances the compound's interaction with the bacterial structure, strengthening hydrogen and electrostatic bonds with active sites in proteins or the cell wall [27-30]. The structure–activity relationship (SAR) analysis revealed that the presence of electron-withdrawing and halogenated substituents had a notable impact on antibacterial activity. For instance, compound I9 contains a bromine atom (Br), a halogen group known to engage in non-covalent interactions such as halogen bonding, thereby enhancing the stability of the compound within the bacterial binding pocket. Similarly, compounds I4 and I5, which also possess functional groups capable of forming stabilizing interactions, demonstrated enhanced antibacterial activity in comparison to their unsubstituted analogues. In contrast, compounds lacking such substituents, particularly those devoid of electron-withdrawing or halogenated groups, exhibited markedly reduced antibacterial efficacy. These findings support the hypothesis that strategic incorporation of functional groups—such as nitro and bromine moieties—can modulate the molecular interactions within the biological target site, thereby enhancing antimicrobial potency [31-33].

4. Conclusions

The reaction of chalcone compounds with functional groups such as hydrazine hydrate yields pyrazoline-derived pentameric rings. The accuracy and validity of the compounds have been confirmed by spectroscopic measurements such as FT-IR and ^{1}H&^{13}C-NMR and physical parameters such as melting point and color. The prepared compounds demonstrated notable antibacterial potential. In particular, compounds I9 and I10 exhibited the highest inhibitory activity against the tested bacterial strains, in several cases surpassing the reference antibiotic ciprofloxacin at specific concentrations. The enhanced biological activity of compound I9 can be attributed to the presence of the bromo-substituent (Br), which significantly increased its effectiveness against both Gram-positive and Gram-negative bacteria. Similarly, the strong activity of compound I10 is associated with the nitro substituent (NO_2), which plays a key role in improving antibacterial performance. In contrast, the remaining compounds showed moderate to variable activity, depending on the bacterial type.

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