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Chemistry

SYNTHESIS, IDENTIFICATION, AND EVALUATION OF ANTIBACTERIAL ACTIVITY OF SOME NEW 4,5-DIHYDRO-1*H*-PYRAZOLES, DERIVATIVES FROM SUBSTITUTED CHALCONES

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Abstract. In this work, a series of new **4**,**5**-dihydro-1*H*-pyrazole derivatives (M_{21}-M_{25}) has been synthesized successfully via the reaction of different aldehydes with ketones to produce chalcones, followed by the reaction with hydrazine and phenyl hydrazine. The prepared compounds were identified using FT-IR spectroscopy, ^{1}H NMR spectroscopy, and ^{13}C NMR spectroscopy. The biological activity of these prepared compounds was preliminarily evaluated against certain types of gram-positive (***Staphylococcus aureus)*** and gram-negative (***Escherichia coli).*** The results showed a high antibacterial effect towards both types of bacteria at high concentrations.

Key words: chalcones, hydrazine, cyclization, pyrazole, antibacterial effect.

Introduction

Pyrazole is an organic compound of the azole group with the $C_3H_3N_2H$ formula. It is a heterocycle characterized by a 5-membered ring of three carbon atoms and two adjacent nitrogen atoms, which are in ortho-substitution. Pyrazole is a weak base, with pKb 11.5 (pKa of the conjugate acid is 2.49 at 25 °C).[1] Pyrazoles are also a class of compounds that have the C_3N_2 ring with adjacent nitrogen atoms.[2] Notable drugs containing a pyrazole ring are celecoxib (Celebrex) and the anabolic steroid (Stanozolol). The term pyrazole was given to this class of compounds by German chemist Ludwig Knorr in 1883.[3] In a classical method developed by German chemist Hans von Pechmann in 1898, pyrazole was synthesized from acetylene and diazomethane.[4] Pyrazole is another example of five-membered N-heterocycles, which are useful in the medical field. Various natural products, such as pyrazofurin, contain the privileged pyrazole ring.[5]

The most recent method to synthesize the pyrazole ring is the cyclization of α-, β-unsaturated ketones known as chalcones. This cyclization reaction can be performed by the treatment of chalcones with hydrazine or phenyl hydrazine in acidic media.[6] This cyclization reaction of chalcones to pyrazole is followed by a Michael addition reaction.[7]

Pyrazole as a structural unit is of great interest in biological, medicinal, and pharmacological applications. It plays a significant role in some enzymatic and protein inhibitors,[8] as well as bioactive materials (antibacterial,[9] antioxidant,[10] and antifungal[11]). Some pyrazole compounds are candidates as active[12] and anticancer[13] drugs. It has good healing properties.[14] One of the reasons for this great attention and high attractiveness in biological and medicinal fields is related to its feature as directing and transfer groups helping to increase the reactivity due to the presence of two nitrogen atoms in the structure.[15]

This research work aims to synthesize some new pyrazolines according to the cycloaddition reaction of some chalcones and arylidenes with phenyl hydrazine and hydrazine. It also focuses on the preliminary evaluation of their antibacterial activity against *Staphylococcus aureus* and *Escherichia coli*, especially at high and low concentrations of the tested pyrazoline derivatives.

2. Experimental

2.1. Materials

The chemicals used in this work were purchased from BDH, GCC, Merck, Fluke, Alfa, and Aldrich companies.

2.2. Instruments

The uncorrected melting points were measured using an electrothermal melting point apparatus (Model 9300). To confirm and evaluate the synthesized products purity, the

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TLC techniques were employed. Activated silica gel-G fluorescent sheets with a thickness of 0.2 mm were used to track the course of reactions and determine the purity of the products. The test was carried out using several solvents, and the spots were highlighted using UV light with a wavelength of 254 nm. The FT-IR data were obtained using the FT-IR 8400s Shimadzu spectrophotometer (scale 4000-400 cm^{-1}. H^1-NMR and C^{13}-NMR spectral data were collected by a Varian instrument operating at 400 MHz, and DMSO-d^6 was used as a solvent.

2.3. Synthesis methods

2.3.1. Synthesis of Chalcones (M_1-M_6)[16]

Acetophenones with different substituted groups (0.01 mol) were mixed with different benzaldehydes or cinnamaldehydes (0.01 mol). Then, a solution of sodium hydroxide (5 g in 50 mL of distilled water) and absolute ethanol (40 mL) were added dropwise at room temperature under stirring for 30 min. The formation of the precipitate indicates the completion of the reaction. The precipitate was filtered, washed with cold distilled water (3×30 mL), dried, and recrystallized from ethanol (95 %).

2.3.2. Synthesis of dichalcones (diarylidene) derivatives (M_7-M_{12})[17]

A solution of cyclopentanone or cyclohexanone (0.005 mol) and different benzaldehydes or cinnamaldehydes (0.01 mol) was added dropwise to 30% sodium hydroxide solution under stirring at room temperature. Then, absolute ethanol (30 mL) was added, and stirring was continued for 60 min at room temperature. The obtained precipitate, filtered, washed with cold water (30×3 mL), dried, and recrystallized from absolute ethanol.

2.3.3. Synthesis of pyrazoline derivatives (M_{13}-M_{18}) and (M_{19}-M_{25})[18]

A mixture of chalcones or diarylidenes (0.004 mol), hydrazine or phenyl hydrazine (0.004 mol), 20 mL of acetic acid, and 25 mL of ethanol was dissolved and heated in reflux (78 °C) for 4 hours. The reaction mixture was poured in ice water to precipitate the product, which was then filtered and recrystallized in absolute ethanol.

2.4. Study on biological activity[19]

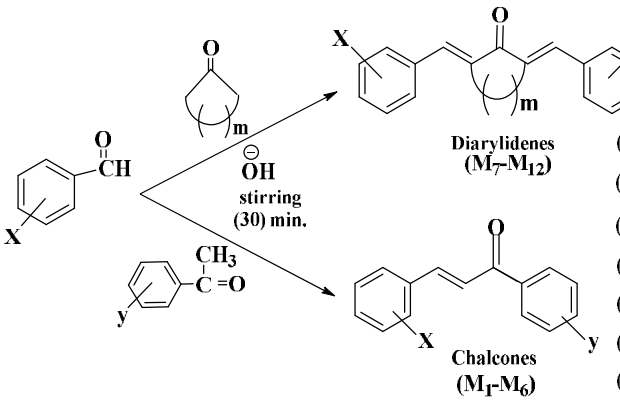
The biological activity of the synthesized compounds has been studied. The newly developed materials were used to evaluate their antibacterial effect against two types of isolated pathological bacteria: gram-positive (*Staphylococcus Aureus*) and gram-negative (*Escherichia coli*). The results were assessed according to the measured diameter of the inhibition zone. Comparisons were performed depending on amoxicillin results using a blank and control material. These isolates are pathogens of many diseases, causing mortality. *Pneumococcus* causes blood poisoning, pneumonia, bacterial meningitis, and otitis media. Meningitis is an inflammation that occurs in the three membranes that cover the brain and spinal cord. Bacterial pneumonia results in an inflammation of one or all of the lungs with bacteria in the bloodstream. The bacterial isolates were obtained in their pure form and diagnosed at the Tikrit Public Health Laboratory (Tikrit, Iraq).

2.5. Preparation of test solutions for the synthesized compounds

Test solutions were prepared for the synthesized compounds using a dimethyl sulfoxide solvent (DMSO) to evaluate their biological activity against two types of bacteria. Three different concentrations (0.01, 0.001, and 0.0001 mg/mL) were prepared for each of the compounds under study. The solutions were prepared as follows: 0.1 g of the synthesized compounds was dissolved in 10 mL of DMSO solvent to obtain a concentration of 0.01 mg/mL. 1 mL of the former solution was taken and dissolved in 9 mL of DMSO to achieve the concentration of 0.001 mg/mL. To obtain the concentration of 0.0001 mg/mL, 1 mL of solution with the concentration of 0.001 mg/mL was dissolved in 9 mL of DMSO solvent.

2.6 Inhibitory activity test[20]

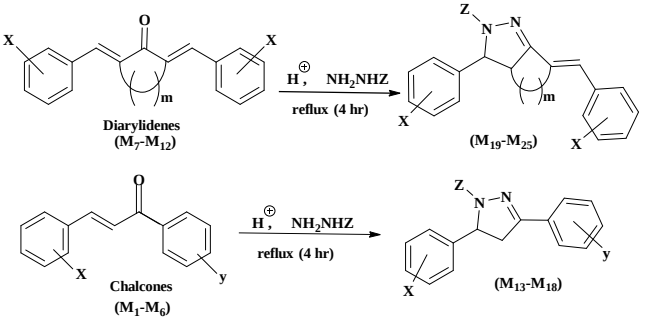
The inhibitory activity of the bacterial isolates used in this study was tested by the diffusion method in the nutrient agar medium (Muller-Hinton agar). The culture medium used for bacterial growth was prepared according to the instructions of the company, by dissolving 38 g of agar in 1 L of distilled water using a conical flask. The heating was carried out by an electric heater equipped with a magnetic stirrer, until the material was completely dissolved, reaching the boiling point, and the solution became clear with a light-yellow color. After that, the medium was sterilized using an autoclave device under pressure of 1.5 bar and a temperature of 120 °C for 20 min. Then the solution was cooled to 40 °C. The solution was poured into a Petri dish and left for 30 min to solidify at room temperature. Then the bacteria were spread in the nutrient culture medium using a sterile cotton swab (Loopful). After placing it inside the tube that contains diluted bacterial growth, the culture medium was wiped in three directions to distribute the vaccine homogeneously. After that, drilling was made in these dishes. The prepared solutions were added inside the pits using a micropipette, followed by placing the dishes in the incubator for 24 h and a temperature of 37 °C. The inhibition zone diameter of the compounds was measured using a millimeter ruler.



X = 2-OH, 4-OH, 2,4-diOH
Y = H, 4-NH_2
Z = H, Ph
m = 2, 3

(M1) (*E*)-3-(2-hydroxyphenyl)-1-phenylprop-2-en-1-one
(M2) (*E*)-1-(4-aminophenyl)-3-(2-hydroxyphenyl)prop-2-en-1-one
(M3) (*E*)-3-(4-hydroxyphenyl)-1-phenylprop-2-en-1-one
(M4) (*E*)-1-(4-aminophenyl)-3-(4-hydroxyphenyl)prop-2-en-1-one
(M5) (*E*)-3-(2,4-dihydroxyphenyl)-1-phenylprop-2-en-1-one
(M6) (*E*)-1-(4-aminophenyl)-3-(2,4-dihydroxyphenyl)prop-2-en-1-one
(M7) 2,5-bis((*E*)-2-hydroxybenzylidene)cyclopentan-1-one
(M8) 2,6-bis((*E*)-2-hydroxybenzylidene)cyclohexan-1-one
(M9) 2,5-bis((*E*)-4-hydroxybenzylidene)cyclopentan-1-one
(M10) 2,6-bis((*E*)-4-hydroxybenzylidene)cyclohexan-1-one
(M11) 2,5-bis((*E*)-2,4-dihydroxybenzylidene)cyclopentan-1-one
(M12) 2,6-bis((*E*)-2,4-dihydroxybenzylidene)cyclohexan-1-one

Scheme 1. Route of the preparing compounds M_1-M_{12}



X = 2-OH, 4-OH, 2,4-diOH Y = H, 4-NH_2 Z = H, Ph m = 2, 3

(M13) 2-(1,3-diphenyl-4,5-dihydro-1*H*-pyrazol-5-yl)phenol
(M14) 2-(3-(4-aminophenyl)-1-phenyl-4,5-dihydro-1*H*-pyrazol-5-yl)phenol
(M15) 4-(1,3-diphenyl-4,5-dihydro-1*H*-pyrazol-5-yl)phenol
(M16) 4-(3-(4-aminophenyl)-1-phenyl-4,5-dihydro-1*H*-pyrazol-5-yl)phenol
(M17) 4-(1,3-diphenyl-4,5-dihydro-1*H*-pyrazol-5-yl)benzene-1,3-diol
(M18) 4-(3-(4-aminophenyl)-1-phenyl-4,5-dihydro-1*H*-pyrazol-5-yl)benzene-1,3-diol
(M19) (*Z*)-2-(2-(3-(2-hydroxyphenyl)-2-phenyl-3,3a,4,5-tetrahydrocyclopenta [*c*]pyrazol-6(2*H*)ylidene)ethyl)phenol
(M20) (*Z*)-4-(2-(3-(4-hydroxyphenyl)-2-phenyl-3,3a,4,5-tetrahydrocyclopenta[*c*]pyrazol-6(2*H*)-ylidene)ethyl)phenol
(M21) (*Z*)-4-(2-(3-(4-hydroxyphenyl)-2-phenyl-2,3,3a,4,5,6-hexahydro-7*H*-indazol-7-ylidene)ethyl)phenol
(M22) (*Z*)-4-(2-(3-(2,4-dihydroxyphenyl)-3,3a,4,5-tetrahydrocyclopenta[*c*]pyrazol6(2*H*)-ylidene)ethyl)benzene-1,3-diol
(M23) (*Z*)-4-(2-(3-(2,4-dihydroxyphenyl)-2-phenyl3,3a,4,5-tetrahydrocyclopenta[*c*]pyrazol-6(2*H*)-ylidene)ethyl)benzene-1,3diol
(M24) (*Z*)-4-(2-(3-(2,4-dihydroxyphenyl)-2,3,3a,4,5,6-hexahydro-7*H*-indazol-7-ylidene)ethyl)benzene-1,3-diol
(M25) (*Z*)-4-(2-(3-(2,4-dihydroxyphenyl)-2-phenyl-2,3,3a,4,5,6-hexahydro-7*H*-indazol-7-ylidene)ethyl)benzene-1,3-diol

Scheme 2. Route of the preparing compounds M_{13}-M_{25}

3. Results and Discussion

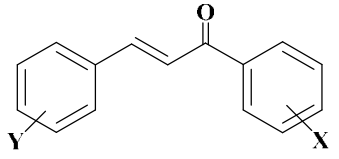
In this research, several new pyrazolines were synthesized, as shown in Schemes 1 and 2. All the newly synthesized derivatives were identified by FT-IR, and ^{1}H-NMR and ^{13}C-NMR spectra were used for the compounds applied as antibacterial materials.

3.1. Characterization of chalcone compounds (M_1-M_6)

Chalcone compounds (M_1-M_6) were prepared via the reaction of benzaldehyde derivatives with acetophenone and its derivatives in equal molar proportions using absolute ethanol as a solvent in the presence of NaOH (40%), as shown in Scheme 1.

The IR spectra for derivatives (M_1-M_6) show the disappearance of the C-H band in the aldehyde (2750-2700 cm^{-1}) and the peak (C-H) in ketone (2900-2800 cm^{-1}). Moreover, a decrease in the frequency of the carbonyl group (C=O) in ketone appears at 1680 cm^{-1} due to the presence of the conjugated carbon-carbon double bond next to the carbonyl group in chalcone compounds.[21] Additionally, he appearance of a medium-intensity peak due to the stretching of the olefinic bond (C=C) at 1597 cm^{-1}, and (C-H) olefinic bond at 3090 cm^{-1} is observed[22]. The remaining peaks appeared at their expected locations. Table 1 shows the infrared spectra of the prepared compounds along with some physical properties.

Table 1. Some physical properties and IR spectral data of chalcones (M_1-M_6)



No.	Substituents		Physical properties				IR (KBr) cm^{-1}			
	X	Y	Yield %	Color	M. P. ^{O}C	M. Wt. g/mole	n (C-H) Arom.	n (C=O) Amide	n (C=C) Arom.	Others
M_1	2-OH	H	85	Greenish yellow	165-167	224	3150	1675	1610	O-H, N-H (3300)
M_2	2-OH	4-NH_2	78	Greenish brown	221-223	239	3100	1680	1597	O-H (3310) N-H (3373)
M_3	4-OH	H	83	Brown	175-177	224	3100	1670	1601	O-H (3169)
M_4	4-OH	4-NH_2	74	Orange	180-181	239	3110	1695	1648	O-H (3346) N-H (3455)
M_5	2,4-diOH	H	78	Dark red	152-154	240	3097	1645	1620	O-H (3298)
M_6	2,4-diOH	4-NH_2	75	Redish orange	160-161	255	3040	1643	1623	O-H (3245) N-H (3390)

3.2. Characterization of diarylidene compounds (M_7-M_{12})

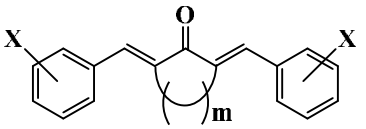
Diarylidene compounds (M_7-M_{12}) were prepared via the reaction of two moles of benzaldehyde derivatives with one mole of cyclopentanone or cyclohexanone at room temperature using absolute ethanol as a solvent in the presence of NaOH (40 %) as a catalyst, as shown in Scheme 1.

The infrared spectra of the prepared compounds revealed the absence of the (C-H) peak in aldehyde (2700-2750 cm^{-1}), and a decrease in the frequency of the stretching ketonic carbonyl group (C=O) bond at 1673 cm^{-1}, due to the presence of the conjugated carbon-carbon double bond next to the carbonyl group. We observed a medium band at a frequency (1602 cm^{-1}) corresponding to the double elastic (C=C) olefinic bonds[23] in addition to the appearance of a peak at 3100 cm^{-1} referring to the olefin (C-H) stretching bond. The other peaks are represented in Table 2 with some physical properties.

3.3. Characterization of pyrazoline compounds (M_{13}-M_{25})

Pyrazoline derivatives were prepared by condensation of chalcones (M_1-M_6) and diarylidenes (M_7-M_{12}) with hydrazine and phenylhydrazine in glacial acetic acid in equal molar ratios for chalcones and twice the molar amount for diarylidene compounds (see Scheme 1).

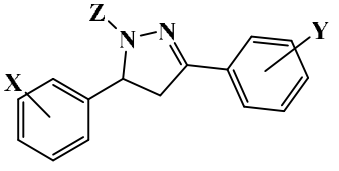
Table 2. Some physical properties and IR spectral data of chalcones (M_7-M_{12})



No.	Substituents		Physical properties				IR (KBr) cm^{-1}			
	X	m	Yield %	Color	M. P. ^{O}C	M. Wt. g/mole	n (C-H) Arom.	n (C=O)	n (C=C) Arom.	n (O-H)
M_7	2-OH	2	75	Brown	180-182	292	3093	1652	1491	3385
M_8	2-OH	3	80	Greenish yellow	300 (decomp.)	306	3100	1648	1537	3378
M_9	4-OH	2	70	Green	196-198	292	3090	1673	1602	3300
M_{10}	4-OH	3	85	Red	202-204	306	3100	1675	1658	3273
M_{11}	2,4-diOH	2	77	Blue	198-200	324	3095	1650	1645	3470
M_{12}	2,4-diOH	3	90	Violet	201-203	338	3088	1623	1600	3388

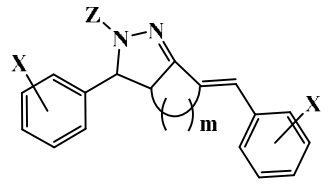
The infrared spectra of pyrazoline compounds (M_{13}-M_{25}) revealed the disappearance of the carbon bond peak with the appearance of the peak for the (C=N) bond within the pyrazoline ring at 1614 cm^{-1}. A peak at 3474 cm^{-1} referring to the stretch (N-H) within the same ring, and two peaks at 2861 cm^{-1} and 2930 cm^{-1} corresponding to the symmetrical and asymmetrical stretching of the aliphatic (C-H) bond are observed[24]. Some of the IR spectra of the pyrazoline compounds are shown in Tables 3, 4, and Figs. 1, 2.

Table 3. Physical properties and IR spectral data of pyrazolines (M_{13}-M_{18}) prepared from chalcones



No.	IR (KBr) cm^{-1}			Physical properties				Substituents			
	z	y	X	Yield %	Color	M. P. ^{O}C	M. Wt. g/mole	n (C=N)	n (C-H) aliph.	n (O-H)	n (N-H)
M_{13}	2-OH	H	Ph	68	Orange	173-175	314	1623	2929	3393	-
M_{14}	2-OH	4-NH_2	Ph	70	Redish brown	242-244	329	1607	2937	3327	3662
M_{15}	4-OH	H	Ph	75	Redish brown	220-222	314	1625	2936	3321	-
M_{16}	4-OH	4-NH_2	Ph	80	Yellow	178-180	329	1620	2935	3324	3662
M_{17}	2,4-diOH	H	Ph	85	Brown	168-170	330	1633	-	3322	-
M_{18}	2,4-diOH	4-NH_2	Ph	90	Orange	185-187	345	1640	2943	3330	3474

Table 4. Physical properties and IR spectral data of pyrazolines (M_{19}-M_{25}) prepared from diarylidene



No.	Substituents			Physical properties				IR (KBr) cm^{-1}			
	X	z	m	Yield %	Color	M. P. ^{O}C	M. Wt. g/mole	n (C=N)	n (C-H) aliph.	n (O-H)	n (N-H)
M_{19}	2-OH	Ph	2	58	Brown	213-215	382	1608	2861	3325	-
M_{20}	4-OH	Ph	2	76	Greenish yellow	225-227	382	1615	2889	3324	-
M_{21}	4-OH	Ph	3	73	Dark green	248-250	396	1602	2951	3303	-
M_{22}	2,4-diOH	H	2	80	Yellow	220-222	338	1633	2988	3250	3393
M_{23}	2,4-diOH	Ph	2	76	Green	221-223	414	1608	2861	3326	-
M_{24}	2,4-diOH	H	3	88	Yellow	233-235	352	1614	2927	3345	-
M_{25}	2,4-diOH	Ph	3	85	Gray	226-228	428	1614	2927	3474	-

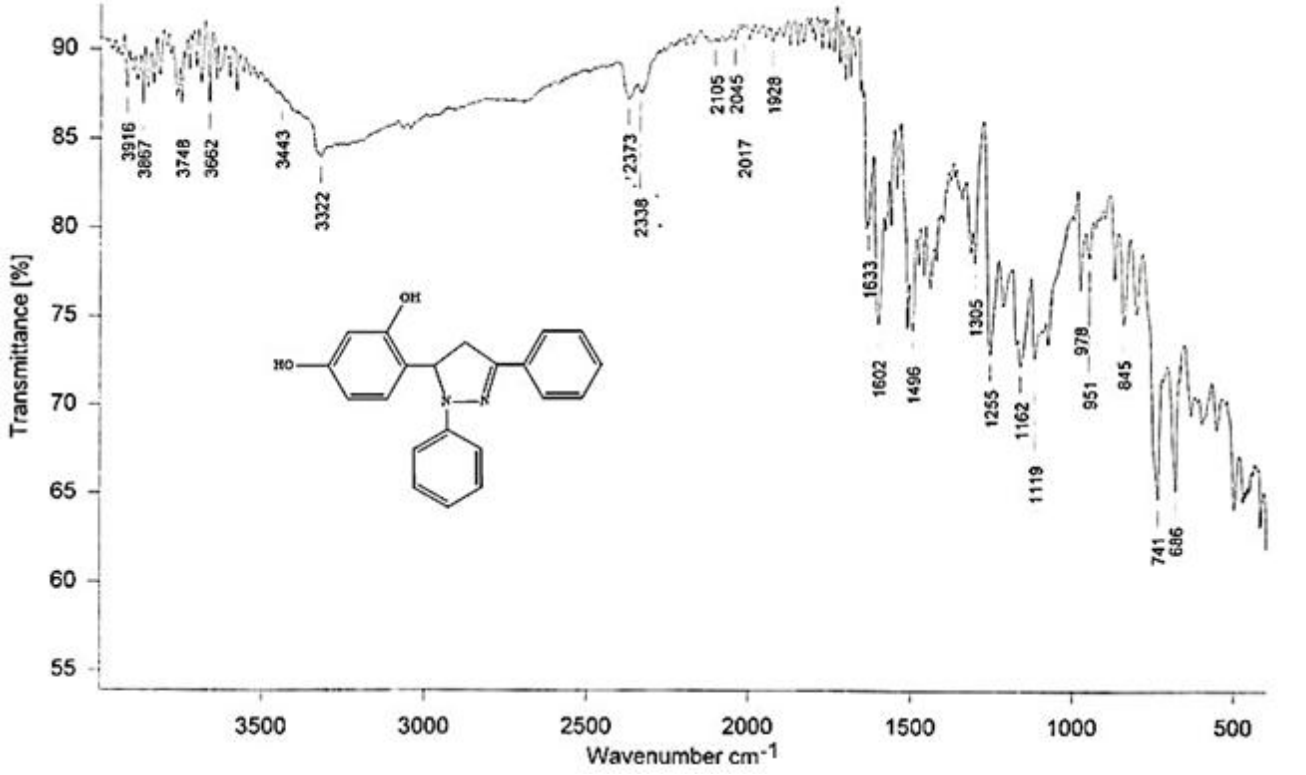


Fig. 1. FT-IR spectrum for 4-(1,3-diphenyl-4,5-dihydro-1*H*-pyrazol-5-yl) benzene-1,3-diol (M_{17})

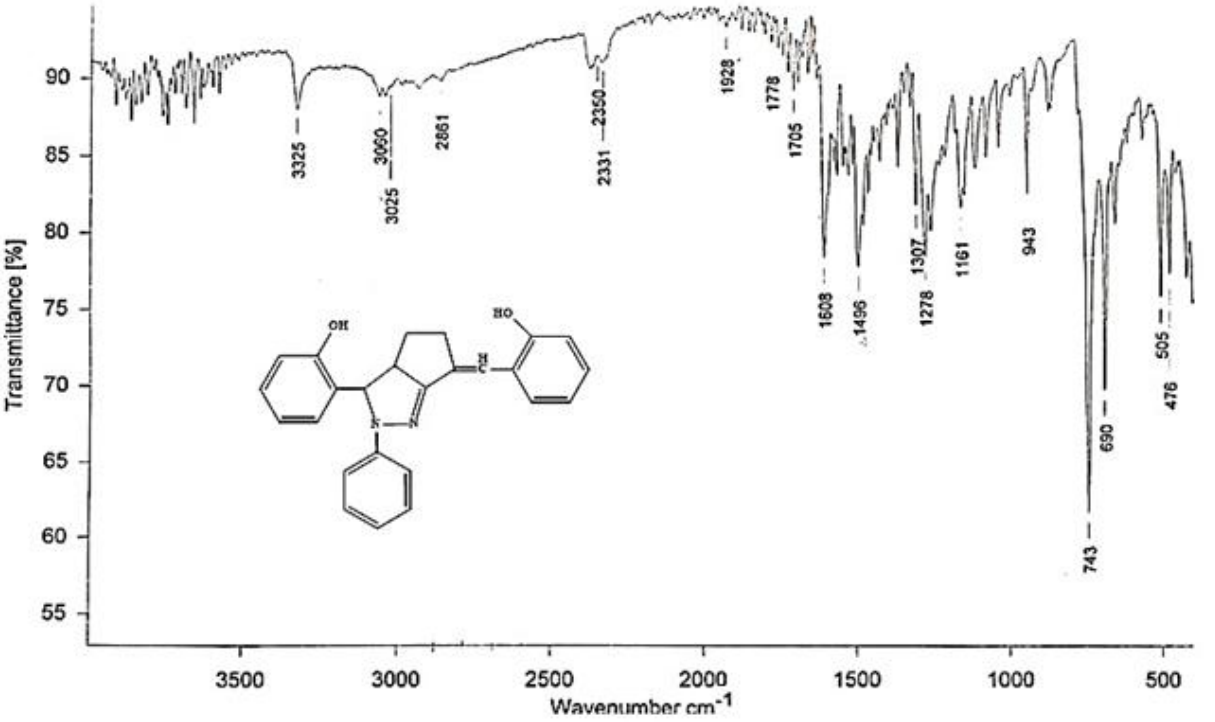


Fig. 2. FT-IR spectrum for 2-(6-(2-hydroxybenzylidene)-2-phenyl-2,3,3a,4,5,6-hexahydrocyclopenta [c] pyrazol-3-yl) phenol (M_{19})

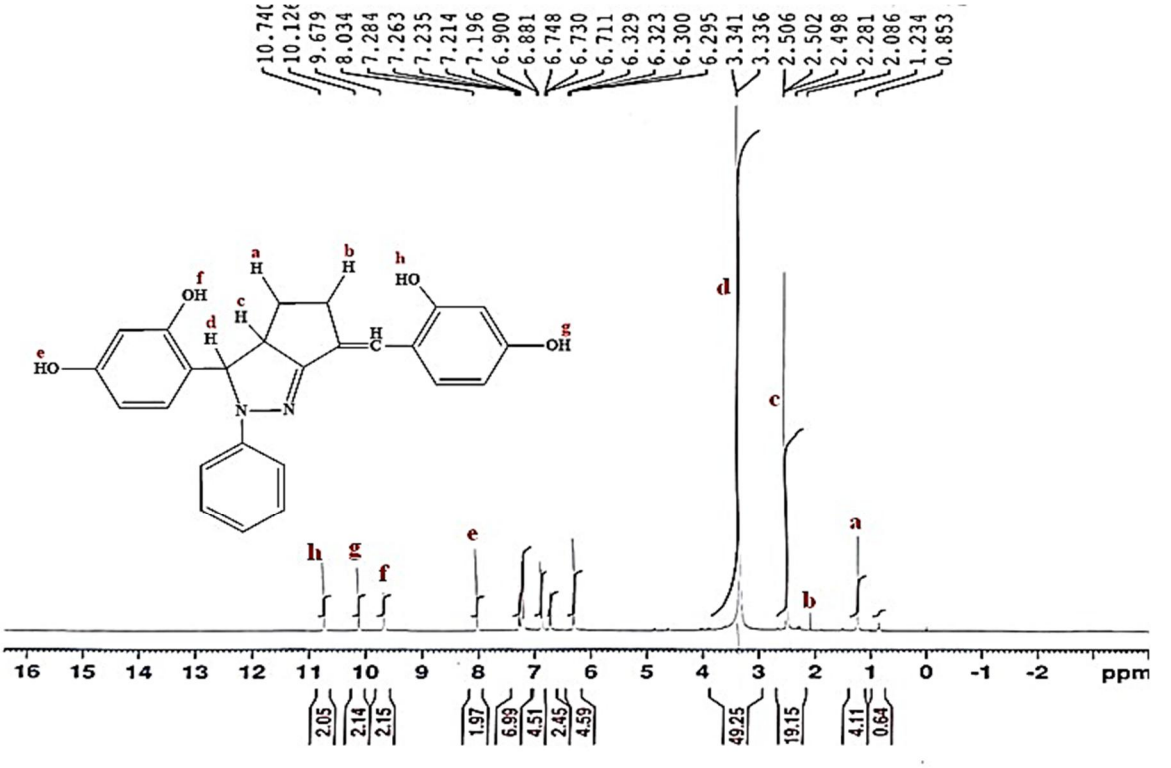


Fig. 3. [1]H-NMR spectrum for 4-(6-(2,4-dihydroxybenzylidene)-2-phenyl-2,3,3a,4,5,6-hexahydrocyclopenta[c] pyrazol-3-yl) benzene-1,3-diol (M_{23})

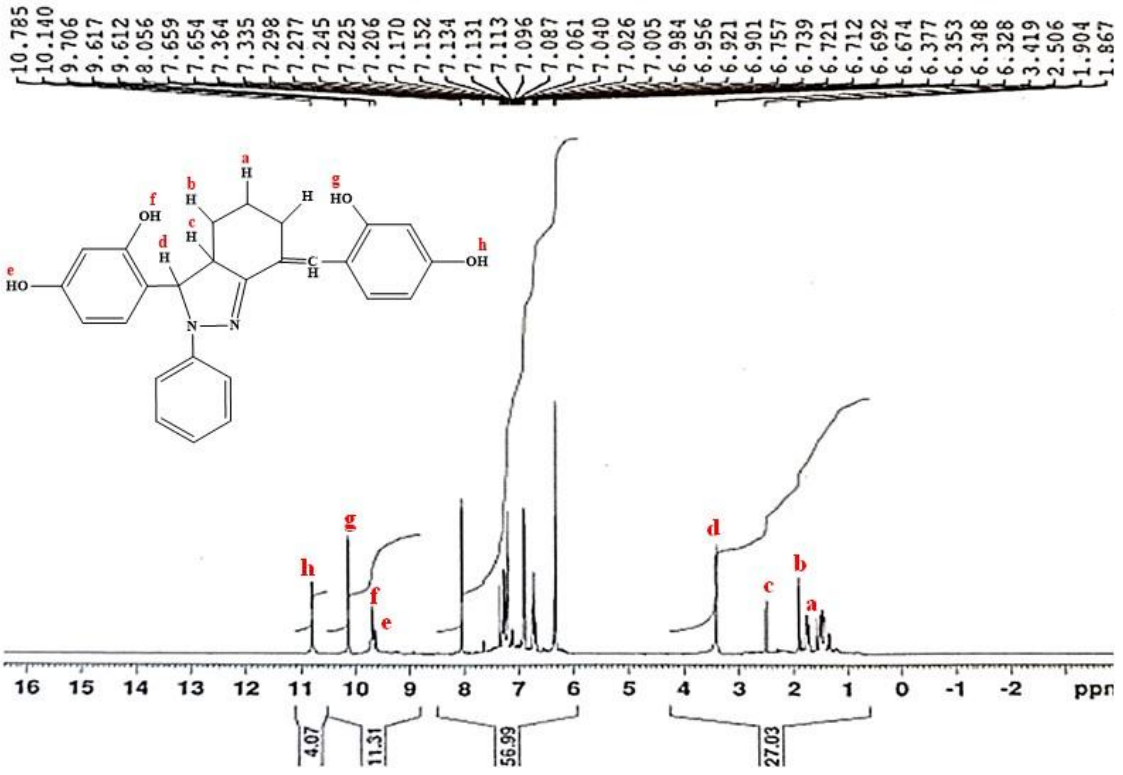


Fig. 4. ^{1}H-NMR spectrum for 4-(7-(2,4-dihydroxybenzylidene)-2-phenyl-3,3a,4,5,6,7-hexahydro-2H-indazol-3-yl) benzene-1,3-diol (M_{25})

Some pyrazoline compounds were characterized using the ^{1}H-NMR and ^{13}C-NMR. The ^{1}H-NMR spectra for compounds (M_{17}, M_{19}, M_{23} and M_{25}) show the important protons of aliphatic, phenolic and aromatic benzene ring protons (Figs. 3, 4, and Table 5. The ^{13}C-NMR spectra for some pyrazoline compounds (M_{17}, M_{19}, M_{23} and M_{25}) show important signals (Figs. 6, 7, and Table 6), which confirm their structures[25].

Table 5. ^{1}H-NMR data of some pyrazolines (M_{17}, M_{19}, M_{23} and M_{25})

Comp. No.	(^{1}H-NMR) δ = ppm
M_{17}	For (H_a) singlet at δ = 2.51 ppm (pyrazoline ring), (H_b) singlet at δ = 3.41ppm (pyrazoline ring), (H_c) singlet at δ = 10.14 ppm (phenolic protons), (H_d) singlet at δ = 10.78 ppm (phenolic protons), multiplet for aromatic ring protons at δ = (6.32-8.05) ppm.
M_{19}	For (H_a) triplet at δ = 1.89 ppm (cyclopentane ring), (H_b) doublet at δ = 2.50 ppm (cyclopentane ring), (H_c) doublet at δ = 3.36 ppm (pyrazoline protons), (H_d) singlet at δ = 10.41 ppm (phenolic protons), (H_e) singlet at δ = 10.53 ppm (phenolic protons), multiplet for olefinic and aromatic ring protons at δ = (6.75-8.15) ppm.
M_{23}	For (H_a) singlet at δ = 1.23, 2.08 ppm (cyclopentane ring), (H_b) singlet at δ = 2.28, 2.49 ppm (cyclopentane ring), (H_c) tetralet at δ = 2.50 ppm (pyrazoline protons), (H_d) doublet at δ = 3.34 ppm (phenolic protons), (H_e) singlet at δ = 8.03 ppm (phenolic protons), (H_f) singlet at δ = 9.67 ppm (phenolic protons), (H_g) singlet at δ = 10.12 ppm (phenolic protons), (H_h) singlet at δ = 10.74 ppm (phenolic protons), multiplet for olefinic and aromatic ring protons at δ = (6.29-7.28) ppm.
M_{25}	For (H_a) pentalet at δ = 1.86 ppm (cyclohexane ring), (H_b) tetralet at δ = 1.90 ppm (cyclohexane ring), (H_c) tetralet at δ = 2.50 ppm (pyrazoline protons), (H_d) doublet at δ = 3.40 ppm (pyrazoline protons), (H_e) singlet at δ = 9.61 ppm (phenolic protons), (H_f) singlet at δ = 9.70 ppm (phenolic protons), (H_g) singlet at δ = 10.14 ppm (phenolic protons), (H_h) singlet at δ = 10.78 ppm (phenolic protons), multiplet for olefinic and aromatic ring protons at δ = (6.32-8.05) ppm.

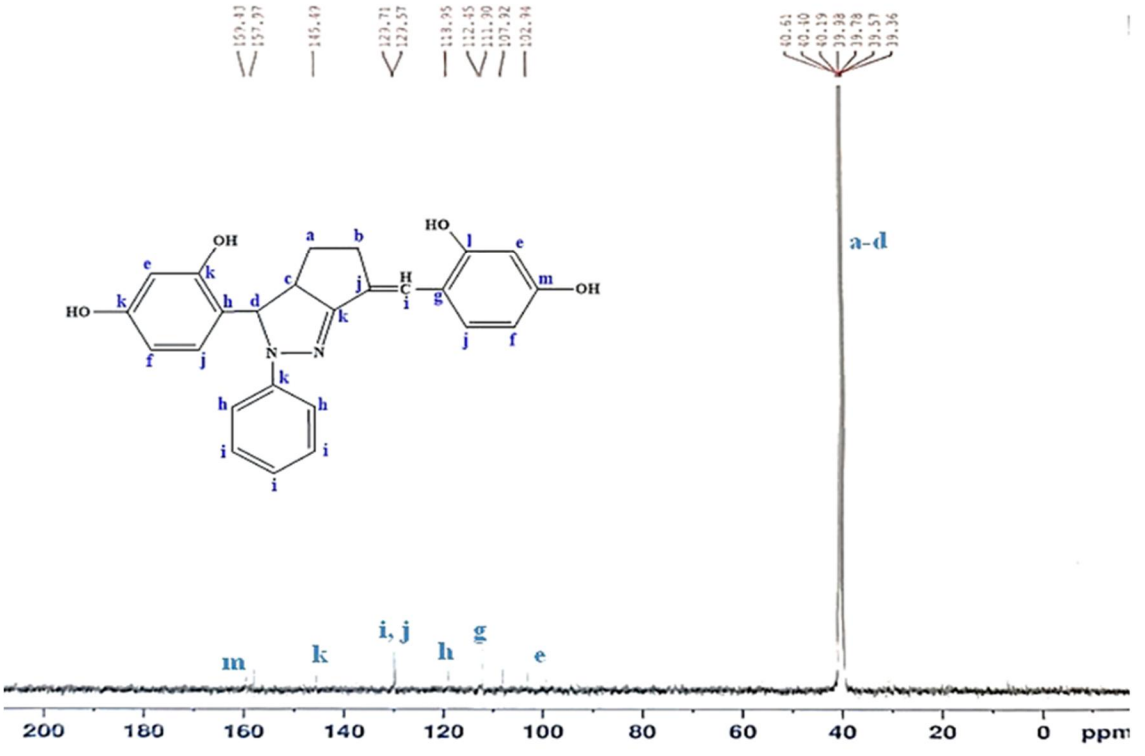


Fig. 6. ^{13}C-NMR spectrum for 4-(6-(2,4-dihydroxybenzylidene)-2-phenyl-2,3,3a,4,5,6-hexahydrocyclopenta[c] pyrazol-3-yl) benzene-1,3-diol (M_{23})

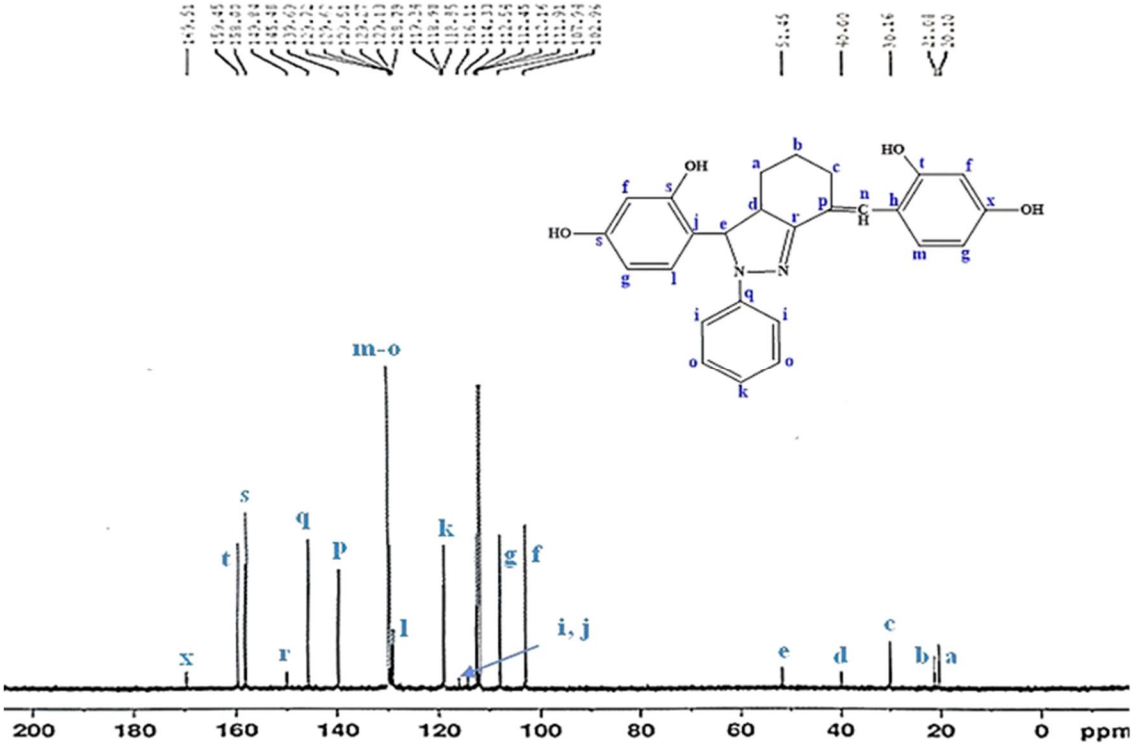


Fig. 7. ^{13}C-NMR spectrum for 4-(7-(2,4-dihydroxybenzylidene)-2-phenyl-3,3a,4,5,6,7-hexahydro-2H-indazol-3-yl) benzene-1,3-diol (M_{25})

Table 6. ^{13}C-NMR data of some pyrazolines (M_{17}, M_{19}, M_{23} and M_{25})

Com. No.	(^{13}C-NMR) δ = ppm
M_{17}	For (C_a) at δ = 39.32 ppm (pyrazoline carbon), (C_b) at δ = 50.15 ppm and (C_c) at δ = 102.55 ppm (all these for the pyrazoline carbons), (C_d) at δ = 107.93 ppm (aromatic carbon), (C_e) at δ = 119.90 ppm (aromatic carbon), (C_f) at δ = 112.44 ppm (aromatic carbon), (C_g) at δ = 119.97 ppm (aromatic carbon), (C_h) at δ = 129.01, 129.61, 129.72, 130.19 ppm (aromatic carbons), (C_i) at δ = 139.66 ppm (aromatic carbon), (C_j) at δ = 145.47 ppm (pyrazoline carbon), (C_k) at δ = 157.99 ppm (C-OH), (C_l) at δ = 159.44 ppm (C-OH).
M_{19}	For (C_a) at δ = 39.37 ppm, (C_b) at δ = 39.58 ppm and (C_c) at δ = 40.00 ppm (all these for the cyclopentane carbons), (C_d) at δ = 40.62 ppm (pyrazoline carbon), (C_e) at δ = 112.19 ppm (aromatic carbon), (C_f) at δ = 112.79 ppm (aromatic carbon), (C_g) at δ = 116.36 ppm (aromatic carbon), (C_h) at δ = 119.43 ppm (aromatic carbon), (C_i) at δ = 119.85 ppm (aromatic carbon), (C_j) at δ = 120.95 ppm (pyrazoline carbon), (C_k) at δ = 127.77 ppm (aromatic carbon), (C_l) at δ = 128.59 ppm (aromatic carbon), (C_m) at δ = 129.11 ppm (aromatic carbon), (C_n) at δ = 129.65 ppm (aromatic carbon), (C_o) at δ = 129.75 ppm (olefinic carbon attached to cyclopentane ring), (C_p) at δ = 129.95 ppm (cyclopentane carbon), (C_q) at δ = 137.74 ppm (pyrazoline carbon), (C_r) at δ = 145.22 ppm (C-OH), (C_s) at δ = 156.13 ppm (C-OH).
M_{23}	For (C_a) at δ = 39.36 ppm, (C_b) at δ = 39.57 ppm and (C_c) at δ = 40.19 ppm (all these for the cyclopentane carbons), (C_d) at δ = 40.61 ppm (pyrazoline carbon), (C_e) at δ = 102.94 ppm (aromatic carbon), (C_f) at δ = 107.92 ppm (aromatic carbon), (C_g) at δ = 111.90 ppm (aromatic carbon), (C_h) at δ = 118.95 ppm (aromatic carbon), (C_i) at δ = 129.57 ppm (olefinic and aromatic carbons), (C_j) at δ = 129.71 ppm (aromatic and cyclopentane carbons), (C_k) at δ = 145.49 ppm (pyrazoline carbon and C-OH), (C_l) at δ = 157.97 ppm (C-OH).
M_{25}	For (C_a) at δ = 21.08 ppm, (C_b) at δ = 20.10 ppm and (C_c) at δ = 30.16 ppm, , (C_d) at δ = 40.00 ppm (all these for the cyclohexane carbons), (C_e) at δ = 51.45 ppm (pyrazoline carbon), (C_f) at δ = 102.96 ppm (aromatic carbon), (C_g) at δ = 107.94 ppm (aromatic carbon), (C_h) at δ = 111.91 ppm (aromatic carbon), (C_i) at δ = 116.11 ppm (aromatic carbon), (C_j) at δ = 119.34 ppm (aromatic carbon), (C_k) at δ = 120.79 ppm (aromatic carbon), (C_l) at δ = 128.79 ppm (aromatic carbon), (C_m) at δ = 129.13 ppm (aromatic carbon), (C_n) at δ = 129.27 ppm (olefinic carbon), (C_o) at δ = 129.51 ppm (aromatic carbon), (C_p) at δ = 139.69 ppm (cyclohexane carbon), (C_q) at δ = 145.40 ppm (aromatic carbon), (C_r) at δ = 149.89 ppm (pyrazoline carbon), (C_s) at δ = 158.00 ppm (C-OH), (C_t) at δ = 159.45 ppm (C-OH), (C_x) at δ = 169.51 ppm (C-OH).

3.4. Evaluation of biological activity for pyrazolines (M_{13}-M_{25})

The antibacterial activity for pyrazolines (M_{13}-M_{25}) was studied using two types of bacteria, gram-positive bacteria (*Staphylococcus Aureus)* and gram-negative bacteria (*Escherichia coli)*. The results indicate that the most of the pyrazoline compounds show a good antibacterial activity toward the used bacteria at (0.01 mg/mL) compared to (0.001 and 0.0001) mg/mL due to the high concentration effect. The inhibition growth area of the tested compounds revealed higher effectiveness than blank and control materials (amoxicillin). The concentration of the pyrazoline compounds does not affect the diameter of the inhibition zones, but rather the magnitude of the effect, as shown in the photos of the dishes, where the color of the inhibition zones darkens with increasing concentration. The results of studying antibacterial activity are shown in Table 7 and Figs. 8,9,10.

Table 7. Diameter of the inhibition growth area (mm) of pyrazoline compounds (M_{13}-M_{25})

Comp. No.	The inhibition zone growth diameter (mm)		
	Conc. mg/mL	*Staphylococcus Aureus*	*Escherichia coli*
1	2	3	4
M_{13}	0.01	16	19
	0.001	13	15
	0.0001	11	13
M_{14}	0.01	24	22
	0.001	22	19
	0.0001	18	17
M_{15}	0.01	19	26
	0.001	17	22
	0.0001	12	13
M_{16}	0.01	19	23
	0.001	13	20
	0.0001	11	18
M_{17}	0.01	0.01	18
	0.001	0.001	16
	0.0001	0.0001	13

Table 7. (Continuation).

1	2	3	4
M_{18}	0.01	25	22
	0.001	20	16
	0.0001	15	13
M_{19}	0.01	19	22
	0.001	15	18
	0.0001	12	13
M_{20}	0.01	21	20
	0.001	16	18
	0.0001	13	14
M_{21}	0.01	19	19
	0.001	15	17
	0.0001	13	15
M_{22}	0.01	18	19
	0.001	14	17
	0.0001	12	14

Table 7. (Continuation).

M_{23}	0.01	18	17
	0.001	14	15
	0.0001	12	13
M_{24}	0.01	25	20
	0.001	21	18
	0.0001	18	15
M_{25}	0.01	25	22
	0.001	17	18
	0.0001	15	16
Amoxicillin	0.01	19	25
	0.001	17	20
	0.0001	25	16

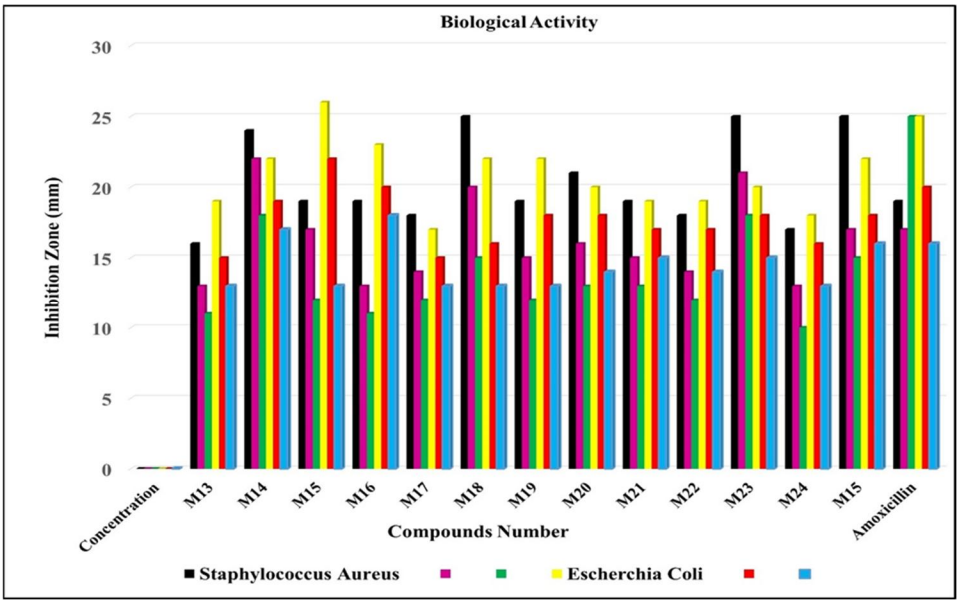
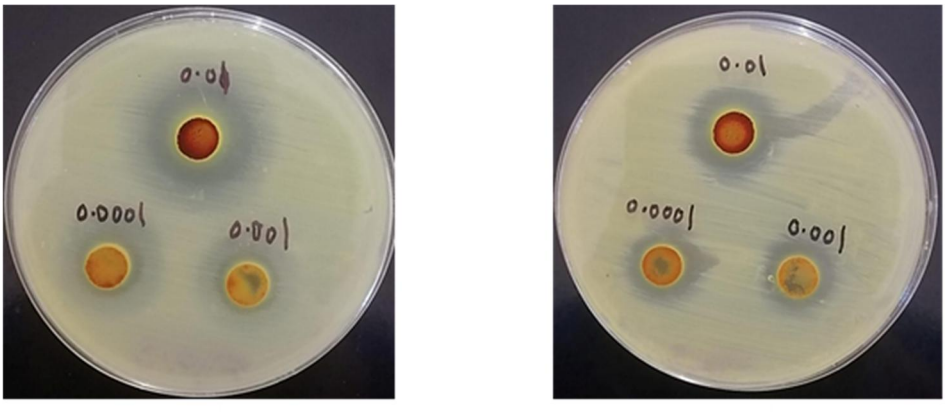
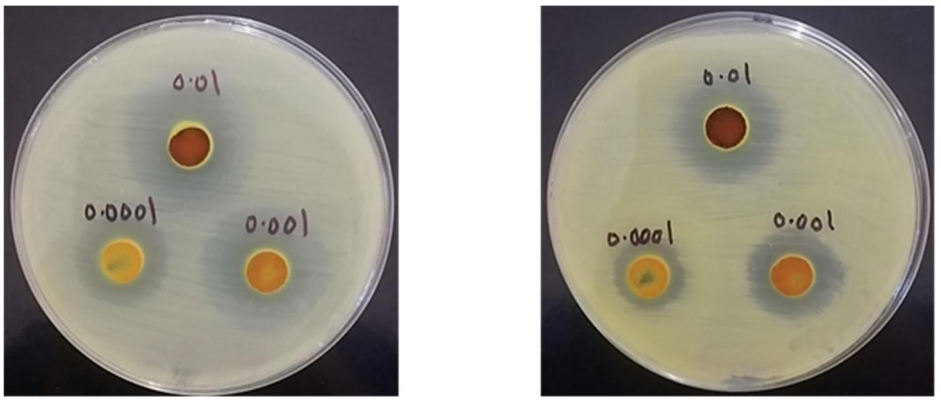


Fig. 8. Antibacterial activity against *Staphylococcus Aureus* and *Escherichia coli*



a b

Fig. 9. Images of the inhibition area for M_{23} against (a): *Staphylococcus aureus* and (b): *Escherichia Coli*



a b

Fig. 10. Images of the inhibition area for M_{25} against (a): *Staphylococcus aureus* and (b): *Escherichia Coli*

4. Conclusions

Several new pyrazoline derivatives were successfully synthesized via cycloaddition reactions between selected diarylidenes or chalcones and hydrazine or phenylhydrazine. The chemical structures of the obtained pyrazolines were confirmed by spectral analysis. Biological evaluation demonstrated that all synthesized compounds exhibited notable antibacterial activity against the tested bacterial strains at all applied concentrations (0.01, 0.001, and 0.0001 mg/mL). Notably, the compounds showed particularly strong antibacterial effects against *Staphylococcus aureus* and *Escherichia coli*, especially at the highest concentration (0.01 mg/mL).

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СИНТЕЗ, ІДЕНТИФІКАЦІЯ Й ОЦІНКА АНТИБАКТЕРІАЛЬНОЇ АКТИВНОСТІ ДЕЯКИХ НОВИХ 4,5-ДИГІДРО-1*H*-ПІРАЗОЛІВ, ПОХІДНИХ ЗАМІЩЕНИХ ХАЛКОНІВ

Анотація. *У цій роботі успішно синтезовано серію нових похідних 4,5-дигідро-1H-піразолу (M21-M25) через реакції різноманітних альдегідів з кетонами для отримання халконів з подальшою реакцією останніх з гідразином і фенілгідразином. Синтезовані сполуки були ідентифіковані за допомогою FT-IR спектроскопії, ^{1}H ЯМР і ^{13}C ЯМР спектроскопії. Біологічна активність синтезованих сполук була попередньо оцінена щодо певних типів грампозитивних (Staphylococcus aureus) і грамнегативних (Escherichia coli) бактерій. Результати показали високий антибактеріальний ефект проти обох типів бактерій за високих концентрацій.*

Ключові слова: *халкони, гідразин, циклізація, піразол, антибактеріальний ефект.*