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Synthesis, Identification, and Biological Activity Investigation of New Pyrazolines Derived from Vanillin

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Abstract

This work includes the synthesis of new pyrazoline derivatives **6-20** over two steps. The first step included a condensation between vanillin and various aromatic ketones (acetophenone, *p*-nitroacetophenone, *p*-chloroacetophenone, *p*-bromoacetophenone, and 2-acetylnaphthalene) to provide the corresponding chalcone derivatives **1-5** in high yields (up to 93%). The second step involved a reaction of **1-5** with hydrazine hydrate, phenyl hydrazine, and *p*-nitrophenyl hydrazine, which gave the desired products **6-20** in yields ranging from 60 to 85%. The structures of the prepared compounds were confirmed by FT-IR and ^{1}H NMR spectroscopy. A few of the produced compounds were tested for antibacterial and antioxidant properties.

Keywords: Antibacterial, Antioxidant, Chalcone, Pyrazoline, Vanillin.

تشييد، تشخيص و فحص النشاط البيولوجي للبيرازولينات الجديدة المشتقة من الفانيلين

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الخلاصة

يتضمن هذا العمل تحضير مشتقات جديدة للبايرزول (**6–20**) على خطوتين، تضمنت الخطوة الاولى تكاثف بين الفانلين و كيتونات اروماتية مختلفة (اسيتوفينون، بارا–نايترو اسيتوفينون، بارا–كلورو اسيتوفينون، بارا–برومو اسيتوفينون و 2–استيل نفثالين) لتكوين مشتقات الجالكونات (**1–5**) بمنتوج عالي (تصل الى93%). الخطوة الثانية تضمنت تفاعل الجالكونات (**1–5**) مع هيدرات الهايدرازين، فنيل هايدرازين و بارا–نايترو فنيل هايدرازين مما اعطى المركبات المرغوبة (**6–20**) بمنتوج يتراوح من 60 الى 85%. تم تأكيد تراكيب المركبات المحضرة بواسطة مطيافية FT–IR و^{1}H NMR. تم اختبار عدد قليل من المركبات المحضرة لخصائصها المضادة للبكتيريا و مضادات الأكسدة

1. Introduction

Chalcone, a compound characterized by an α,β-unsaturated ketone moiety and two benzene rings [1], has garnered significant attention from chemists and biochemists worldwide. The unique characteristics of chalcone, influenced by the presence of the unsaturated group and specific substituents on the ring [1,2], have contributed to its broad pharmacological activity and simplicity in synthesis. This has led to extensive research on both synthetic and natural

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chalcones, exploring their potential as important synthetic intermediates for the synthesis of various heterocyclic compounds using diverse reagents. Pyrazoline is a dihydropyrazole, a five-membered heterocyclic molecule with two nitrogen atoms in close proximity and just one endocyclic double bond [3]. It has three tautomeric forms that are unsubstituted [4]. Pyrazoline serves as a crucial precursor for the synthesis of new chemical compounds with therapeutic properties [5]. Because of their role in heterocyclic synthesis and medical applications, pyrazolines are regarded as important chemicals in organic chemistry [6]. Moreover, pyrazolines possess a wide range of biological activities, such as anti-inflammatory [7,8], anti-depressant [9], antimicrobial [8,10], calcium channel blockers [11], antihypertensive [12], antitumor [13], antiviral [14], antibacterial [15], monoamine oxidase inhibitor [16], anti-HIV [17], anticancer [18], and antimalarial [19] (Figure 1). In this study, we aim to synthesize pyrazoline compounds derived from vanillin through a two-step synthetic approach. The first step involves a condensation reaction between vanillin and various aromatic ketones, resulting in the formation of corresponding chalcone derivatives. In the second step, the chalcones obtained from the first step will undergo a reaction with hydrazine and its derivatives, leading to the synthesis of the desired pyrazoline compounds. By implementing this two-step synthetic strategy, we aim to obtain diverse compounds of pyrazoline derivatives with potential applications in various fields, including medicinal chemistry and drug development.

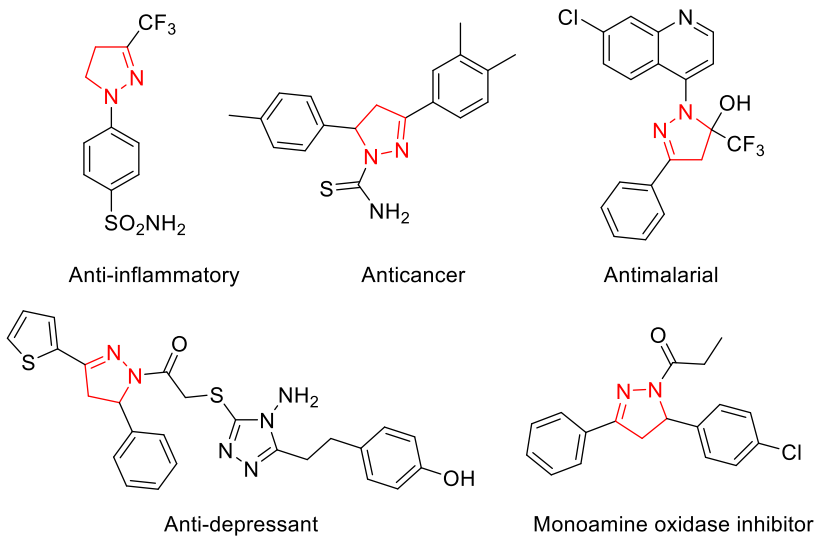


Figure 1: Examples of bioactive molecules incorporating pyrazoline rings

2. Experimental part

2.1. Materials and instrumentation

Unless otherwise specified, all chemicals were obtained from commercial sources (Merck, BDH, Sigma Aldrich, and Fluka companies) and used without further purification. A UV lamp and aqueous alkaline potassium permanganate were used to visualize TLC on Merck silica gel 60 F_{254}. The melting points were measured using Stuart Scientific SMP3 in open capillary tubes and are uncorrected. A Shimadzu 8400 FT-IR spectrometer was used to record infrared spectral data in the 500-4000 cm^{-1} region. Spectral data for ^1H NMR were captured using a Bruker AV400 spectrometer. Chemical shifts are expressed in ppm downfield from tetramethylsilane (TMS) as an internal standard or deuterated DMSO in ^1H NMR as a reference (δ_H 2.50 ppm). The antioxidant activity data were recorded using a spectrophotometer (Shimadzu UV-1800 Spectrophotometer).

2.2. Chemistry

*2.2.1. General procedure A for the synthesis of the chalcone derivatives derived from vanillin **1-5** [20]*

In a 100-mL round-bottom flask, a solution of aromatic ketone (1.19-2.14 g, 6-13 μmol, 1.0 eq.) in THF (20 mL) and sodium hydroxide solution (3 drops, 5%) were added. Vanillin (1-3 g, 6-13 μmol, 1.0 eq.) was then added to the reaction mixture before stirring at room temperature for 20-44 hours. The reaction was monitored by TLC (eluent with petroleum ether/ethyl acetate, 3:1) until no vanillin remained (*circa*. 20 hours). The organic phase was extracted with ethyl acetate (10 mL), washed with brine (10 mL), dried over anhydrous sodium sulfate, and concentrated to give title products **1**-**5**. The physical properties and FT-IR spectral data of these products are shown in Tables 1 and 2, respectively.

*2.2.2. General procedure B for the synthesis of the pyrazoline derivatives **6-20** [21]*

To a solution of compounds **1**-**5** (100-400 mg, 100-300 μmol, 1.0 eq.) in EtOH (20 mL), hydrazine hydrate, phenyl hydrazine, or *p*-nitrophenyl hydrazine (100-150 mg, 100-300 μmol, 1.0 eq.) was added dropwise. The resulting mixture was heated to reflux for 20-38 hours, as monitored by thin-layer chromatography (TLC) using a petroleum ether/ethyl acetate eluent. The mixture was then allowed to cool to room temperature before adding ice water and undergoing filtration, washing with water, and recrystallization with ethanol to provide the desired products **6**-**20**. The physical properties and FT-IR spectral data of these products are listed in Tables 1 and 2, respectively.

2.3. Antibacterial activity test

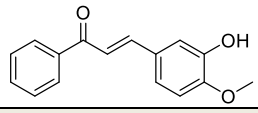
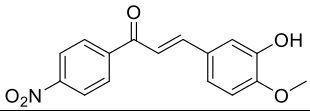
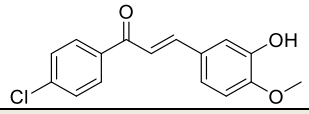
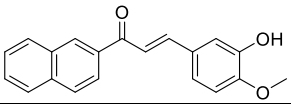
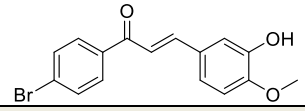
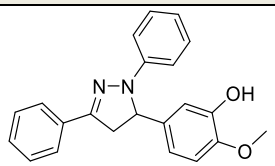
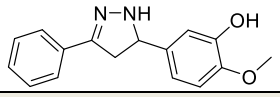
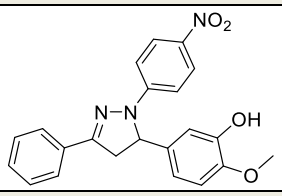
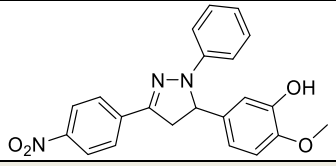
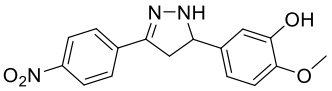
Escherichia coli and *staphylococcus aureus* were the two strains of bacteria used in the *in vitro* antibacterial activity assay of various samples [22]. The antibiotic Amikacin served as the reference compound. Both test samples and standard references were prepared by dissolving Amikacin in dimethyl sulfoxide (DMSO) to obtain a concentration of 1 mg/mL. Sterilized agar was liquefied and injected with a microbe suspension (1 mL/100 mL of media), and the mixture was poured into Petri plates to a depth of approximately 3 mm. Wells were created in the solidified agar, and the test samples and references were placed in the wells. The plates were then chilled at 5 °C for one hour before being incubated at 37 °C for 18 hours. The physical properties and FT-IR spectral data of these products are shown in Tables 1 and 2, respectively.

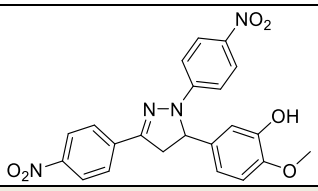
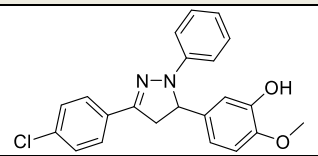
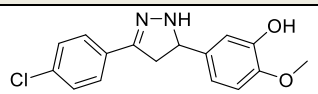
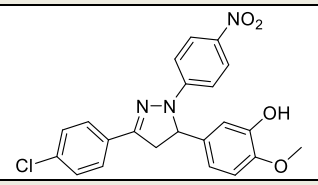
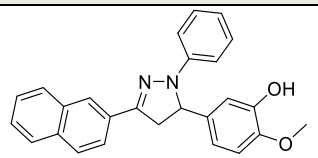
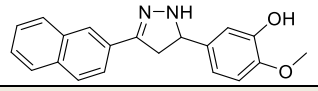
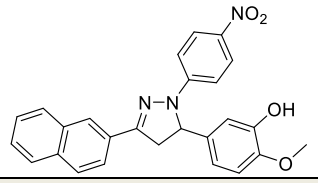
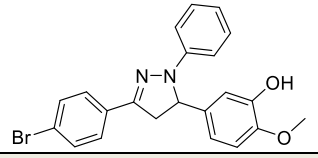
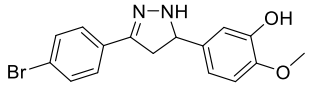
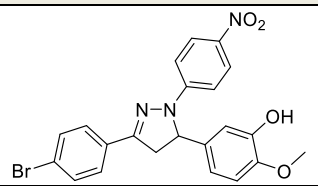
2.4. Antioxidant activity test

The scavenging activity of the produced compounds against free radicals was evaluated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) test [23]. Briefly, three concentrations of the compound (25, 50, and 100 ppm) were produced, and one milliliter of each concentration was combined with one milliliter of DPPH solution (400 mg in 100 mL). The combination was then allowed to sit at room temperature in the dark for 30 minutes. Finally, a spectrophotometer was used to test each sample's absorbance at 517 nm. The following equation was used to determine the ability to scavenge DPPH, with ascorbic acid serving as a reference.

$$I\% = (\text{Abs blank} - \text{Abs sample}) / \text{Abs blank} \times 100 \text{ [24]}$$

Table 1: Some physical properties of the prepared compounds **1-20**

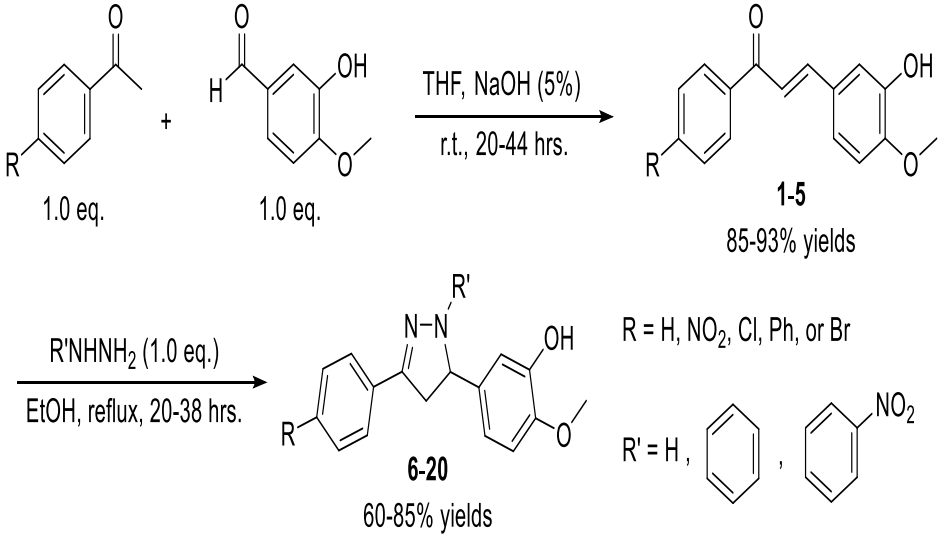
No.	Structure	Compound formula	m.p. (°C)	M.wt. (g.mol^{-1})	Color	Time (hours)	Yield (%)
1		$C_{16}H_{14}O_3$	76-78	254.28	Dark-brown	20	90
3-(3-Hydroxy-4-methoxyphenyl)-1-phenylprop-2-en-1-one							
2		$C_{16}H_{13}NO_5$	54-56	299.28	Green	38	93
3-(3-Hydroxy-4-methoxyphenyl)-1-(4-nitrophenyl)prop-2-en-1-one							
3		$C_{16}H_{13}ClO_3$	78-80	288.73	Dark-brown	38	90
1-(4-Chlorophenyl)-3-(3-hydroxy-4-methoxyphenyl)prop-2-en-1-one							
4		$C_{20}H_{16}O_3$	>350	304.35	Yellow	40	88
3-(3-Hydroxy-4-methoxyphenyl)-1-(naphthalen-2-yl)prop-2-en-1-one							
5		$C_{16}H_{13}BrO_3$	>350	333.18	Brown	44	85
1-(4-Bromophenyl)-3-(3-hydroxy-4-methoxyphenyl)prop-2-en-1-one							
6		$C_{22}H_{20}N_2O_2$	218-220	344.41	Red	38	70
5-(1,3-Diphenyl-4,5-dihydro-1H-pyrazol-5-yl)-2-methoxyphenol							
7		$C_{16}H_{16}N_2O_2$	146-148	268.32	Dark-brown	24	82
2-Methoxy-5-(3-phenyl-4,5-dihydro-1H-pyrazol-5-yl)phenol							
8		$C_{22}H_{19}N_3O_4$	148-150	389.41	Brown	24	78
2-Methoxy-5-(1-(4-nitrophenyl)-3-phenyl-4,5-dihydro-1H-pyrazol-5-yl)phenol							
9		$C_{22}H_{19}N_3O_4$	72-74	389.41	Dark-brown	20	85
2-Methoxy-5-(3-(4-nitrophenyl)-1-phenyl-4,5-dihydro-1H-pyrazol-5-yl)phenol							
10		$C_{16}H_{15}N_3O_4$	120-122	313.31	Green	20	79
2-Methoxy-5-(3-(4-nitrophenyl)-4,5-dihydro-1H-pyrazol-5-yl)phenol							

11		$C_{22}H_{18}N_4O_6$	190-192	434.41	Red	25	75
5-(1,3-Bis(4-nitrophenyl)-4,5-dihydro-1H-pyrazol-5-yl)-2-methoxyphenol							
12		$C_{22}H_{19}ClN_2O_2$	78-80	378.86	Brown	28	70
5-(3-(4-Chlorophenyl)-1-phenyl-4,5-dihydro-1H-pyrazol-5-yl)-2-methoxyphenol							
13		$C_{16}H_{13}O_2ClN_2$	164-168	300.74	Dark-brown	30	65
5-(3-(4-Chlorophenyl)-4,5-dihydro-1H-pyrazol-5-yl)-2-methoxyphenol							
14		$C_{22}H_{16}O_4ClN_3$	218-220	421.84	Red	30	67
5-(3-(4-Chlorophenyl)-1-(4-nitrophenyl)-4,5-dihydro-1H-pyrazol-5-yl)-2-methoxyphenol							
15		$C_{26}H_{22}N_2O_2$	124-126	394.47	Brown	35	60
2-Methoxy-5-(3-(naphthalen-2-yl)-1-phenyl-4,5-dihydro-1H-pyrazol-5-yl)phenol							
16		$C_{20}H_{18}N_2O_2$	110-112	318.38	Light-brown	35	62
2-Methoxy-5-(3-(naphthalen-2-yl)-4,5-dihydro-1H-pyrazol-5-yl)phenol							
17		$C_{26}H_{21}N_3O_4$	178-180	439.47	Red	35	60
2-Methoxy-5-(3-(naphthalen-2-yl)-1-(4-nitrophenyl)-4,5-dihydro-1H-pyrazol-5-yl)phenol							
18		$C_{22}H_{19}BrN_2O_2$	216-218	423.31	Brown	30	63
5-(3-(4-Bromophenyl)-1-phenyl-4,5-dihydro-1H-pyrazol-5-yl)-2-methoxyphenol							
19		$C_{16}H_{15}BrN_2O_2$	222-224	347.21	Dark-brown	28	70
5-(3-(4-Bromophenyl)-4,5-dihydro-1H-pyrazol-5-yl)-2-methoxyphenol							
20		$C_{22}H_{18}BrN_3O_4$	Dec. 220	468.31	Brown	30	62
5-(3-(4-Bromophenyl)-1-(4-nitrophenyl)-4,5-dihydro-1H-pyrazol-5-yl)-2-methoxyphenol							

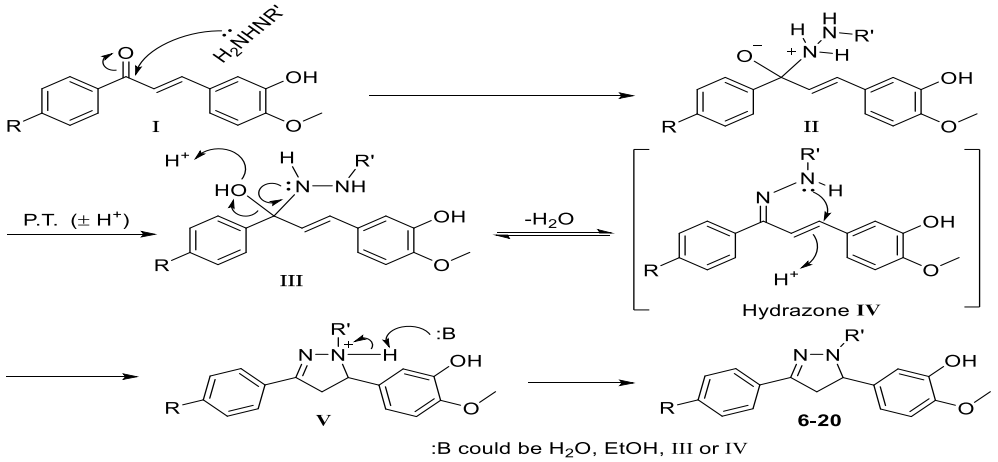
3. Results and discussion

3.1. Chemistry

Chalcone derivatives **1-5** were synthesized in the first part of our study through the aldol condensation reaction between aromatic ketones and vanillin (Scheme 1). The FT-IR spectral analysis of compounds **1-5** revealed the absence of the aldehydic hydrogen signal of vanillin at 2750-2850 cm^{-1}, indicating its successful conversion. Additionally, new absorption bands in the range of 1668-1730 cm^{-1} were observed, corresponding to the stretching vibrations of the carbonyl groups (C=O) in the chalcone structure. Furthermore, the appearance of new absorptions in the range of 1591-1684 cm^{-1} indicated the presence of C=C double bonds in the alkene groups of the synthesized compounds [25]. Two absorption bands at 1593 and 1344 cm^{-1} appeared at compound **2** due to the asymmetric and symmetric stretching absorptions of the NO_2 group, respectively [26]. Table 2 displays all FT-IR spectral data for compounds **1-5**. The second part is the cyclization of chalcone derivatives **1-5** with hydrazine and its derivatives to give pyrazoline derivatives **6-20** (Scheme 1). Each product of **6-20** is likely to be a mixture of two enantiomers. The mechanism of pyrazoline derivatives **6-20** formation *via* cycloaddition reaction is suggested in Scheme 2. The FT-IR spectra of the compounds **6-20** revealed the disappearance of the absorption bands of the C=O and C=C groups and the appearance of new absorption bands at 1622-1688 cm^{-1} for the C=N group of the pyrazoline ring [27]. All FT-IR spectral data for compounds **6-20** are shown in Table 2. The ^{1}H NMR spectral data of compounds **2** and **3** showed singlet signals at 9.57 and 9.73 ppm due to the O-H proton of the aromatic ring. Multiple signals at 8.16-7.16 ppm and 7.40-6.93 ppm are for the aromatic protons [25]. The two protons of the alkene bond appeared at 6.81-6.78 ppm in compound **2** and at 6.80-6.67 ppm in compound **3** [28]. The protons of the methoxy group appeared at 3.88 and 3.74 ppm in compounds **2** and **3**, respectively. The ^{1}H NMR spectral data of compounds **10** and **13** confirmed the formation of the pyrazoline ring *via* the disappearance of the alkene protons and the appearance of new bands at 3.58-2.30 ppm and 3.69-2.77 ppm, which belong to the C-H and CH_2 protons for the pyrazoline ring. All the ^{1}H NMR spectral data are shown in Table 3.



Scheme 1: Synthesis of pyrazoline derivatives using vanillin

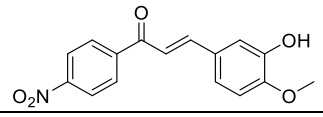
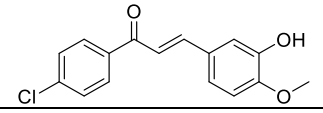
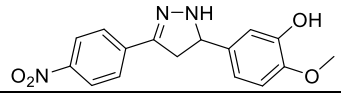
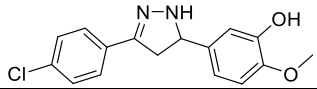


Scheme 2: Suggested mechanism for the generation of pyrazolines **6-20** *via* cycloaddition reaction

Table 2: FT-IR spectral data (υ, cm^{-1}) of compounds **1-20**

No.	C=O Ketone	C=C Aliphatic	C=C Aromatic	C=N Imine	C-H Aromatic	C-H Aliphatic	O-H Vanillin	Other bands
1	1720	1684	1578	-	3003	2932	3441	-
2	1693	1607	1526	-	3049 3007	2922	3431	1593 (NO_2 asym.) 1344 (NO_2 sym.)
3	1668	1591	1512	-	3024	2978	3182	1028 (C-Cl)
4	1724	1657	1582	-	3005	2970	3450	-
5	1730	1668	1591	-	3069 3022	2945	3180	1028 (C-Br)
6	-	-	1599	1676	3061	2922	3423	-
7	-	-	1599	1624	3076 3005	2938	3396	3431 (NH)
8	-	-	1593	1672	3070 3007	2937	3364	1547 (NO_2 asym.) 1308 (NO_2 sym.)
9	-	-	1601 1556	1682	3086	2937	3342	1506 (NO_2 asym.) 1335 (NO_2 sym.)
10	-	-	1593	1688	3076	2930	3478	1518 (NO_2 asym.) 1344 (NO_2 sym.)
11	-	-	1593	1686	3074 3005	2922	3364	1514 (NO_2 asym.) 1307 (NO_2 sym.)
12	-	-	1599 1580	1622	3005	2937	3481	1032 (C-Cl)
13	-	-	1601 1583	1624	3084 3003	2937	3435	3481 (NH) 1032 (C-Cl)
14	-	-	1593	1678	3074 3005	2935	3362	1514 (NO_2 asym.) 1308 (NO_2 sym.) 1036 (C-Cl)
15	-	-	1557	1639	3034	2964	3433	-
16	-	-	1576	1668	3061	2972	3418	-
17	-	-	1576	1672	3005	2966	3410	1514 (NO_2 asym.) 1308 (NO_2 sym.)
18	-	-	1601 1556	1639	3001	2974	3439	1022 (C-Br)
19	-	-	1558	1641	3026	2970	3437	3456 (N-H) 1022 (C-Br)
20	-	-	1601	1639	3025	2982	3433	1556 (NO_2 asym.) 1340 (NO_2 sym.) 1022 (C-Br)

Table 3: ^{1}H NMR spectral data (δ, ppm) of compounds **2**, **3**, **10** and **13**

No.	Compound structure	^{1}H NMR spectral data (δ, ppm)
2		9.57 (s, 1H, OH), 8.16-7.16 (m, 7H, Ar-H), 6.81-6.78 (m, 2H, CH=CH), 3.88 (s, 3H, CH_3)
3		9.73 (s, 1H, OH), 7.40-6.93 (m, 7H, Ar-H), 6.80-6.67 (m, 2H, CH=CH), 3.74 (s, 3H, CH_3)
10		9.72 (s, 1H, OH), 8.54-6.54 (m, 8H, NH + Ar-H), 3.81 (s, 3H, CH_3), 3.58-3.05 (m, 1H, CH), 2.58-2.30 (m, 2H, CH_2)
13		9.75 (br. s, 1H, OH), 8.57 (s, 1H, NH), 7.45-6.71 (m, 7H, Ar-H), 3.83 (s, 3H, CH_3), 3.69-2.77 (m, 3H, CH + CH_2)

3.2. Antibacterial activity

The antibiotic Amikacin was used as a control in this test, and DMSO was used as a solvent. Some of the tested compounds showed activity against both gram-positive and gram-negative bacteria. The results showed that compound **14** was the best inhibitor of positive bacteria, and this compound was less effective against negative bacteria. Compounds **6** and **11** were equally effective against both types of bacteria. Compound **13** had a moderate impact on both types of bacteria, as shown in Table 4.

Table 4: Antibacterial activity of selected tested compounds (**6, 11**, **13**, and **14**) measured by inhibition zone diameter (mm)

Compound number	Gram-negative bacteria (*Escherichia coli*)	Gram-positive bacteria (*Staphylococcus aura*)
6	14	8
11	14	8
13	12	10
14	8	24
DMSO	8	8
Amikacin	34	40

3.3. Antioxidant activity

The antioxidant activity of compounds **6**, **11**, **13**, and **14** was evaluated using the spectrophotometric DPPH assay at three different concentrations (25, 50, and 100 mg/mL). The scavenging activity against DPPH radicals was measured, and the results were compared to ascorbic acid as a reference. Compound **13** exhibited superior antioxidant activity at a 50% concentration, surpassing the performance of ascorbic acid. Similarly, compounds **6**, **11**, and **14** demonstrated higher antioxidant activity than ascorbic acid at a 25% concentration. All data are listed in Table 5.

Table 5: Free radical-scavenging activity (%) for some of the prepared compounds **6**, **11**, **13**, and **14**

Compound number	Inhibition (%)for the concentrations (mg/mL)		
	100%	50%	25%
6	83.93	88.58	86.95
11	83.63	88.13	90.09
13	89.78	91.74	70.27
14	77.32	75.37	83.63
Ascorbic acid	93.54	89.25	80.95

4. Conclusion

In conclusion, the successful synthesis of new pyrazolines derived from vanillin in two steps has been accomplished. The utilization of aldol condensation and cyclization reactions resulted in the formation of the desired pyrazoline compounds. Several of the synthesized compounds exhibited promising antioxidant and antibacterial properties. Among the tested compounds, **14** demonstrated significant inhibition against gram-positive bacteria (*Staphylococcus aureus*). Compounds **6**, **11**, and **13** also displayed moderate inhibitory effects on gram-negative bacteria (*Escherichia coli*). Furthermore, the compounds were assessed for their antioxidant activity and showed remarkable results, surpassing the performance of ascorbic acid. Compound **13** exhibited potent antioxidant activity at a concentration of 50%, while compound **11** displayed high antioxidant activity at a concentration of 25%. Additionally, compound **13** demonstrated strong scavenging effects against the tested free radicals at a concentration of 100%. These findings highlight the potential of the synthesized pyrazoline compounds as valuable candidates for further exploration in the fields of antibacterial and antioxidant research.

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