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RESEARCH ARTICLE

Synthesis, Characterization, and Biological Potentials of Chalcones Derivatives

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ABSTRACT

This work aims at synthesizing chalcone derivatives using Acetophenone and benzaldehyde derivatives as the primary components. The chalcone compounds [1, 2, 3, 4, 5] have been produced through Claisen-Schmidt condensation reaction, which involves combining benzaldehyde derivatives (such as 4-bromobenzaldehyde, 4-hydroxybenzaldehyde, 4-(dimethyl amino)benzaldehyde, and o-Nitro benzaldehyde) with acetophenone or its derivatives. This reaction takes place when an aqueous solution of sodium hydroxide and ethanol are combined at room temperature. The generated products were examined employing carbon-13 nuclear magnetic resonance ¹³C-NMR, proton nuclear magnetic resonance ¹H-NMR, ultraviolet (UV), and infrared (IR) spectroscopic techniques. Each compound was tested to determine how effective it was against bacteria. The agar well-diffusion approach was used to assess the antimicrobial activity. Dimethyl sulfoxide (DMSO) was used to solubilize the compounds and derivatives at a concentration of 20 mg/ml, with a concentration of 0.05 mg/ml for each derivative.

Keywords: Biological activities, Chalcones derivatives, Characterization, Claisen schmidt condensation, Synthesis

Introduction

Chalcone is the absolute name for a simple chemical scaffold that is present in many compounds of natural origin, primarily in plants. The term chalcone is derived from the Greek word *chalcos*, which means bronze color, is the source of the term chalcone. This association is due to the yellow and orange color (like bronze) of plant tissues containing these compounds. Stanislav Kostanicki and Joseph Tambor, who were the first scientists to synthesize these naturally occurring compounds with distinctive colors, first mentioned the term.¹ Besides frequently offering effortless structural adaptation, organic materials can have the capacity to possess extraordinary biological or physical characteristics.² Among such belongs the group of chalcones and their by-products.³ Chalcones are polyphenolic compounds that ascend from

plants and belong to the category of flavonoids. They contain bioactive agents, responsible for the green synthesis of metal nanoparticles. These compounds show the existence of the system of β , α -unsaturated ketone connecting two aromatic rings.⁴ Chalcones belong to benzylideneacetophenones and can be employed as important intermediates for isoflavonoid and flavonoid synthesis.⁵ The remarkable properties of chalcone derivatives are normally attributed to the existence of the moiety of β , α -unsaturated.⁶ A valuable structure-activity relationship can be made through the incorporation of diverse alternatives to the two aryl rings.⁷ For this reason, these compounds have a broad spectrum of usages in industry and pharmacology. Indeed, chalcones have been the object of several studies for different scopes, from nonlinear optics⁸ to a wide range of modulatory and cytoprotective activities. They

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exhibit biological activity that has the potency to treat various disorders such as anti-inflammatory impacts,⁹ anti-tuberculosis,¹⁰ anti-cancer,¹¹ anti-leishmanial,¹² anti-microbial,¹³ anti-malarial,¹⁴ and antifungal properties,¹⁵ as shown in Fig. 1.

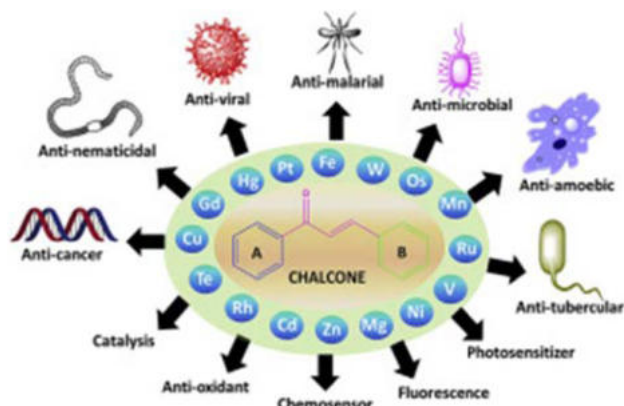


Fig. 1. Biological activity of Chalcones.

A pair of aromatic rings links an aliphatic chain of three carbon atoms, in this chemical.¹⁶ It also has a ketone group and a notably reactive ketoethylenic group (-CO-CH=CH-).¹⁷ A chalcone is a pigmented chemical with a chromophore (-CO-CH=CH-),¹⁸ as illustrated in Fig. 2.

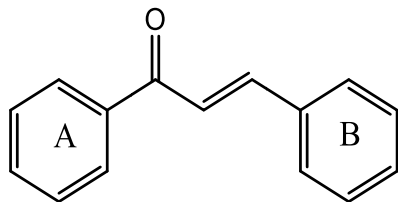


Fig. 2. Structure of chalcone.

There are plenty of methods to synthesize chalcones and their derivatives reported in literature. Among them, Claisen-Schmidt Condensation is the most frequent one.¹⁹ Chemical reactions in Heck coupling processes involve the combination of organic compounds.²⁰ Syntheses were accomplished by the Microwave Irradiation Method^{21,22} and by Solvent-Free Synthesis on Grinding.²³ Wittig reaction and Suzuki coupling are two chemical reactions that are extensively documented in the scientific literature.^{24,25} Benzaldehydes are modified by adding

substituents that underwent a chemical reaction called the Condensation of Claisen-Schmidt, which occurs between benzaldehydes and acetophenone. The process is catalyzed by sodium hydroxide, as depicted in Scheme 1.²⁶

Materials and methods

We purchased the chemicals for this process study from CDH and BDH companies without any additional purification procedures. We determined the boiling and melting points of the chemicals using a melting point apparatus. We used a UV spectrophotometer model U.V-1800 ENG240V.SOFT in conjunction with ethanol to acquire the UV-Vis spectra. We collected the infrared spectrum at the BPC-Analysis Center Laboratory in Adhamiya using a Fourier transform infrared spectroscopy system (SHIMADZU 8400). We placed a KBr disc in the apparatus to capture the spectra. This experiment made use of a Bruker USA-made ¹³C-¹H NMR nuclear magnetic resonance spectrometer operating at 400 MHz.

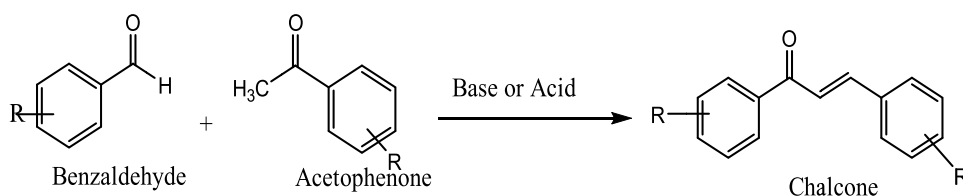
The Claisen-Schmidt synthesis method²⁷

The compounds were prepared according to the equation shown in Scheme 2, in general and according to the mechanics shown in Scheme 3.

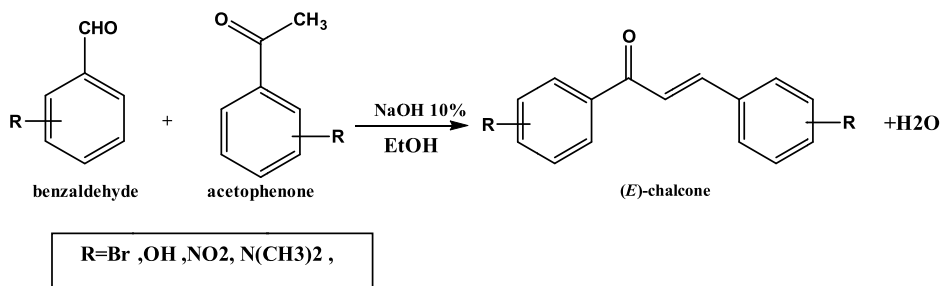
Mechanism

Creation of 3-(4-bromophenyl)-1-phenylprop-2-en-1-one (1)

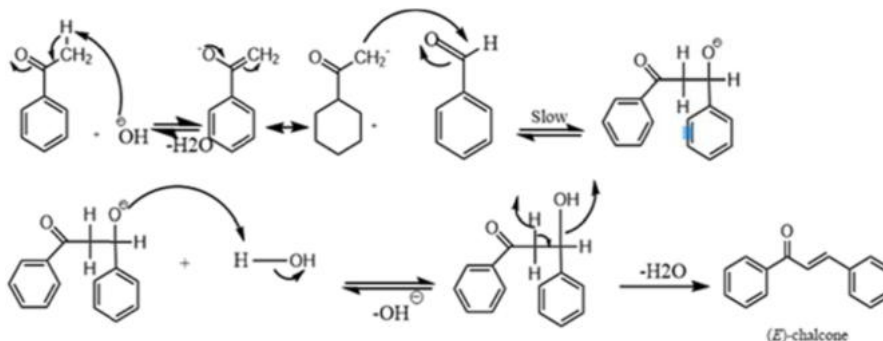
4-bromobenzaldehyde (1 gm, 0.0054 mol) was dissolved in ethanol. Acetophenone (1 mL) was also dissolved in ethanol 99% (10 mL). After a complete dissolution for both substances, sodium hydroxide (2.5 mL, 10%) was gradually added to the acetophenone. In order to stir the dissolved 4-bromobenzaldehyde, we placed the beaker on top of the magnetic stirrer. The second solution was then added dropwise for a quarter of an hour. The mixture was left undisturbed until a precipitate appeared, and then it was placed in a bath of ice. After filtering the mixture, the precipitate was bathed, allowed to



Scheme 1. The Claisen Schmidt condensation.



Scheme 2. The reaction of Chalcon synthesis.



Scheme 3. The mechanism of Chalcon synthesis.

crystallize again, and then filtered once more. Drying was used to get 3-(4-bromophenyl)-1-phenylprop-2-en-1-one. It has a point of melting of 119–124°C and a yield of 1.1 grams²⁸ as shown below in [Scheme 4](#).

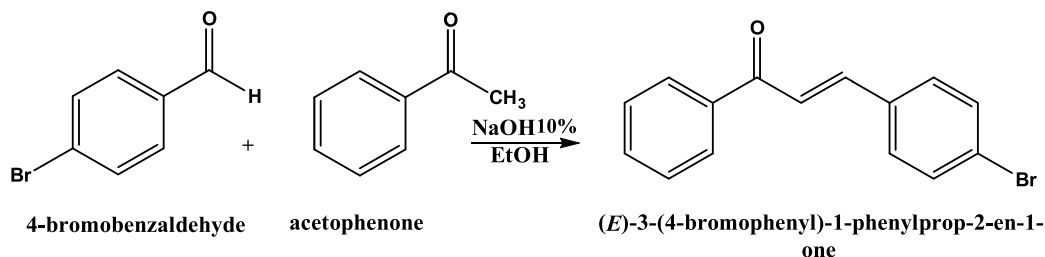
chemical 3-(4-hydroxyphenyl)-1-phenylprop-2-en-1-one was obtained by drying.²⁹ It has a melting point of 179–185°C and a mass of 2.8 grams, as illustrated in the [Scheme 5](#).

Creation of 3-(4-hydroxyphenyl)-1-phenylprop-2-en-1-one (2)

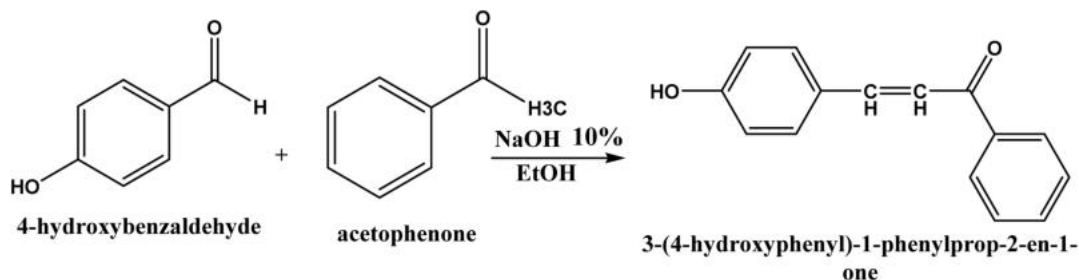
4-hydroxybenzaldehyde (2 gm, 0.0163 mol) and acetophenone (2 mL) were dissolved in ethanol. The acetophenone was progressively mixed with 5 mL of a 10% hydroxide of sodium solution once both materials had completely dissolved. A magnetic stirrer was used to stir the dissolved 4-hydroxybenzaldehyde, and the second solution was added drop by drop for 15 minutes. The mixture was then left undisturbed until a precipitate formed. After filtering and cooling in a bath of ice, the precipitate was bathed, recrystallized, and filtered again. The

Synthesis of 3-(3-hydroxyphenyl)-1-(4-nitrophenyl)prop-2-en-1-one (3)

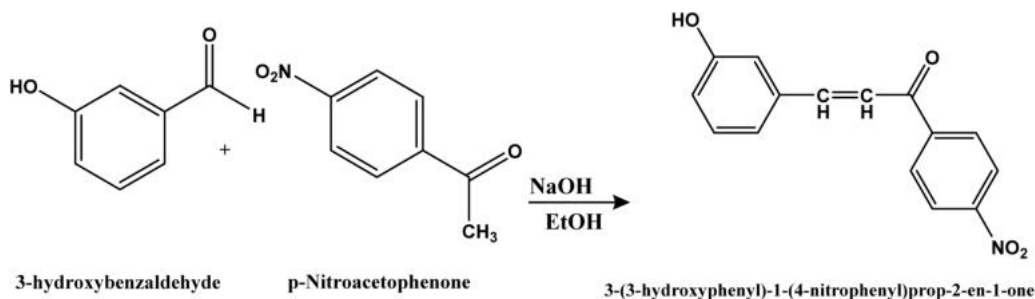
A solution was prepared via dissolving (1 gm, 0.0163 mol) of m-hydroxybenzaldehyde in 10 milliliters of ethanol, along with (1 gm, 0.006 mol) of p-Nitroacetophenone. It was also soluble in 10 mL of ethanol, a solution of 10% sodium hydroxide (NaOH) (1 mL), and 99% ethanol (5 mL) when placed on a magnetic stirrer for one hour. Afterwards, it was immersed in an ice bath until the substance underwent crystallization. The resulting solid powder was filtered and then rinsed with a mixture of ethanol and water (10 mL).³⁰ The chemical



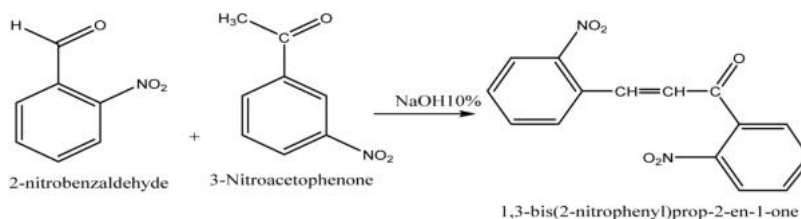
Scheme 4. The reaction for synthesis compound (1).



Scheme 5. The reaction for synthesis compound (2).



Scheme 6. The reaction for synthesis compound (3).



Scheme 7. The Reaction for Synthesis Compound (4).

3-(3-hydroxyphenyl)-1-(4-nitrophenyl)prop-2-en-1-one was obtained via recrystallization from 99% ethanol. It was then dried to give 1.1 grams of the compound with a melting point of 190–195°C, as shown in Scheme 6.

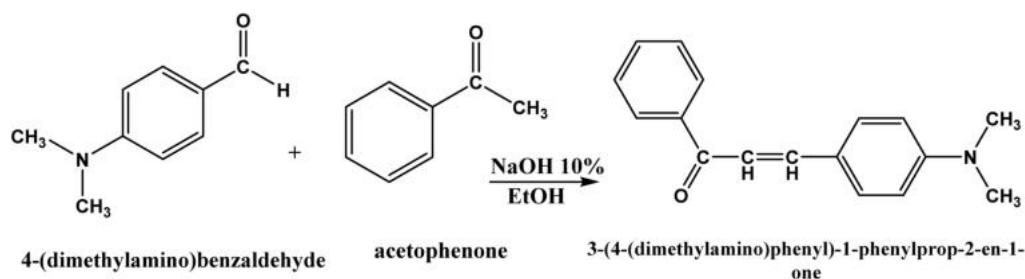
Synthesis of 1,3-bis(2-nitrophenyl)prop-2-en-1-one (4)

Initially, (1 gm, 0.0066 mol) of o-Nitrobenzaldehyde was dissolved in 10 milliliters of 99% ethanol. Then, (1 gm, 0.006 mol) of 3-Nitroacetophenone was dissolved in 10 milliliters of 99% ethanol. 2 milliliters of a 10% NaOH alkaline solution was combined with 3 units of acetophenone. Solutions 2 and 3 were combined, then gradually the mixture to be added solution 1 while stirring it on a magnetic stirrer until a precipitate was formed. Subsequently, it was immersed in a container filled with ice-cold water until the substance underwent crystallization. After filtering the resultant solid powder, it was flushed with a blend of water and ethanol (10 mL). Crystallization from 99% ethanol was yielded. The chemical 1,3-bis(2-nitrophenyl)prop-2-en-1-one was acquired

by drying and 1.4 grams of solid was yielded.³¹ The melting point of the solid was found to be between 193–197°C, as shown in Scheme 7.

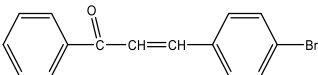
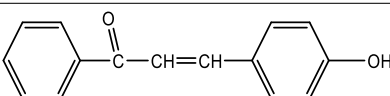
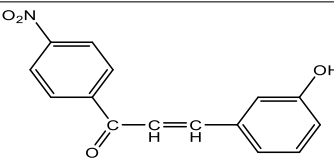
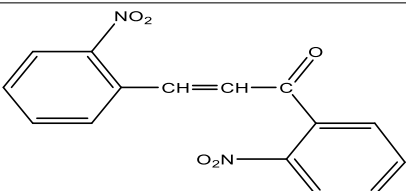
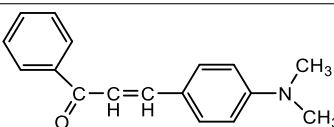
Synthesis of 3-(4-(dimethylamino)phenyl)-1-phenylprop-2-en-1-one (5)

Initially, The Author fully dissolved (1 gm, 0.0067 mol) of 4-(dimethylamino)benzaldehyde in a solution containing 10 mL of 99% ethanol. They prepared a solution by dissolving 1 mL of acetophenone in 5 mL of 99% ethanol. We injected acetophenone into a 1 mL solution of 10% sodium hydroxide. We combined the dissolved acetophenone in ethanol and base with the solution of 4-(dimethylamino)benzaldehyde. We maintained the mixture on a magnetic stirrer until it solidified. We then submerged it in ice to speed up the crystallization process. We filtered the solid precipitate and rinsed it with a mixture of 10 mL of ethanol and water. Crystallization with 99% ethanol.³² A solid with a melting point range of 301–304°C was obtained after drying 3-(4-(dimethylamino)phenyl)-1-phenylprop-2-en-1-one, as shown in Scheme 8. The chemical produced a yield of 1.7 grams.



Scheme 8. The reaction for synthesis compound (5).

Table 1. The prepared compounds' physical constants.

Comp no.	Name compound	M.p. ⁰ C	Color	λ max (nm)	Recryst, solvent
1	 3-(4-bromophenyl)-1-phenylprop-2-en-1-one	109–124	Faint yellow	265	EtOH
2	 3-(4-hydroxyphenyl)-1-phenylprop-2-en-1-one	179–185	Faint brown	380	EtOH
3	 3-(3-hydroxyphenyl)-1-(4-nitrophenyl)prop-2-en-1-one	190–195	Yellobrown	270	EtOH
4	 1,3-bis(2-nitrophenyl)prop-2-en-1-one	193–197	Dark Brown	265	EtOH
5	 3-(4-(dimethylamino)phenyl)-1-phenylprop-2-en-1-one	301–304	Orange	450	EtOH

Antibacterial activity

The biological effectiveness was measured by the Agar- well diffusion method as follows:^{33,34}

1. Dissolving the compounds with DMSO solution.
2. Preparing bacterial concentration 1.5×10^8 bacteria cells per ml.
3. Spreading the bacterial inoculum over the entire culture medium.
4. Making holes in the center of the agar using a sterilized drill with a diameter of 6 mm.
5. Evaluating the efficacy of various solutions by loading them onto a plate containing a single bacterial model, with a volume of 100 microliters each pit. This process is repeated for

all tested solutions, noting their respective concentrations, and studying each bacterial model employed.

6. The specimens were placed in a laboratory incubator and maintained at a temperature range of 35–37°C for 24 hours. Following the incubation time, the efficacy of the solutions was assessed by measuring the width of the zones of inhibition around the drills that were treated with the solutions. The diameter was determined using a millimeter unit with the assistance of a clear acrylic ruler. Solutions that did not exhibit any inhibition zones were deemed ineffective against the specific bacteria under investigation and were designated as “n.a.” to denote their lack of efficacy.

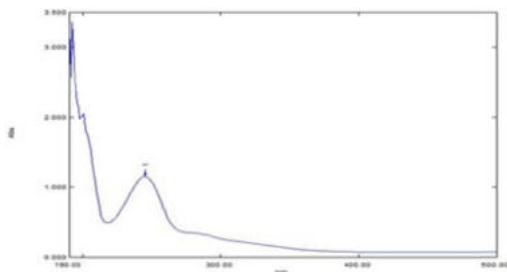


Fig. 3. UV-vis spectrum of compound 1.

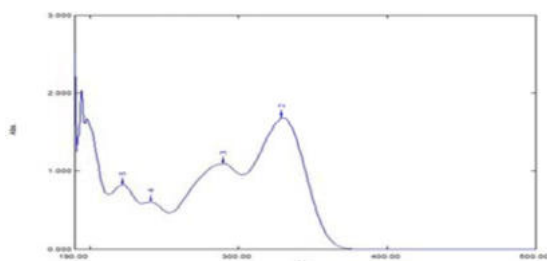


Fig. 4. UV-vis spectrum of compound 2.

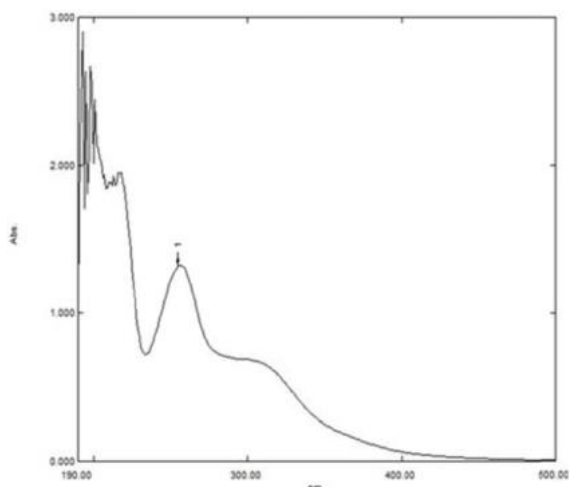


Fig. 5. UV-vis spectrum of compound 3.

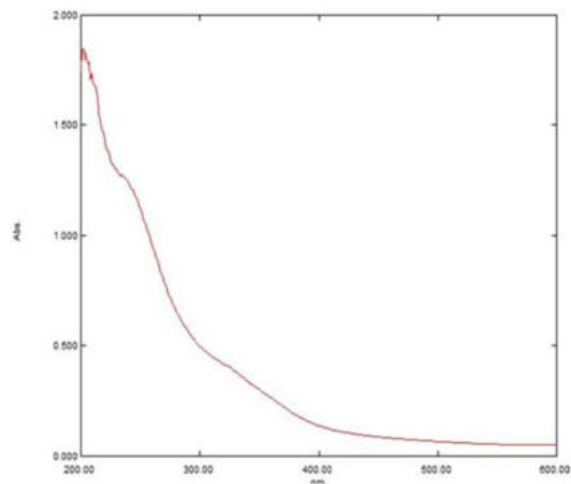


Fig. 6. UV-vis spectrum of compound 4.

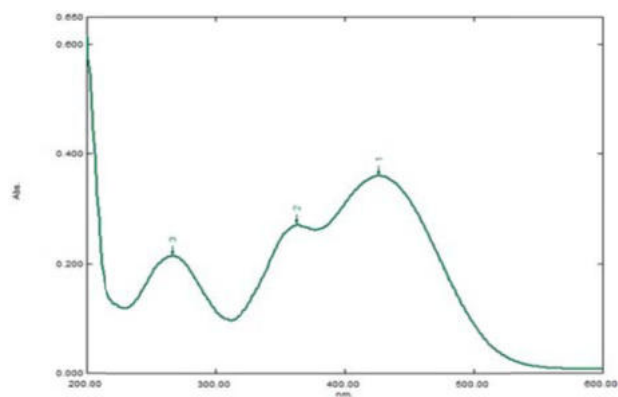


Fig. 7. UV-vis spectra of compound 5.

maxima.³⁵ As for compound 3) the absorption value at Peak is 255 nm as shown in Fig. 5. The UV-vis spectrum of compound (4) shows absorption at 300 nm as shown in Fig. 6. Compound 5 showed two absorptions 1, Peak 426 nm. 2, Peak 267 nm as shown in Fig. 7.

Results and discussion

Table 1 presents the physical and analytical information for five chemical compounds, including name of compound, melting point, color, and λ max (nm).

UV-Vis spectra for compounds

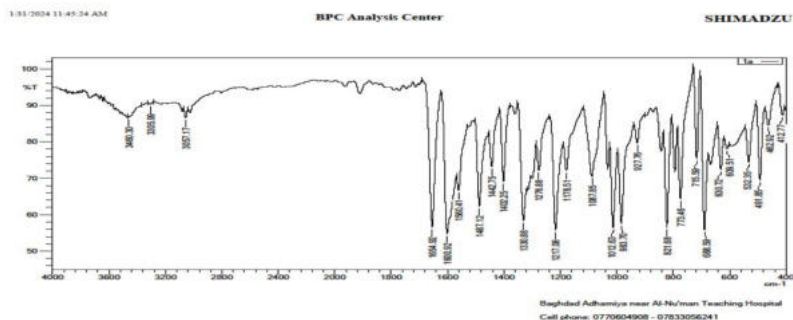
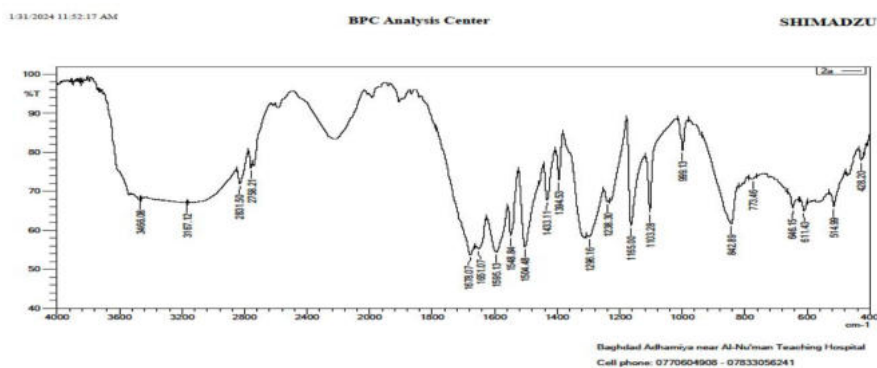
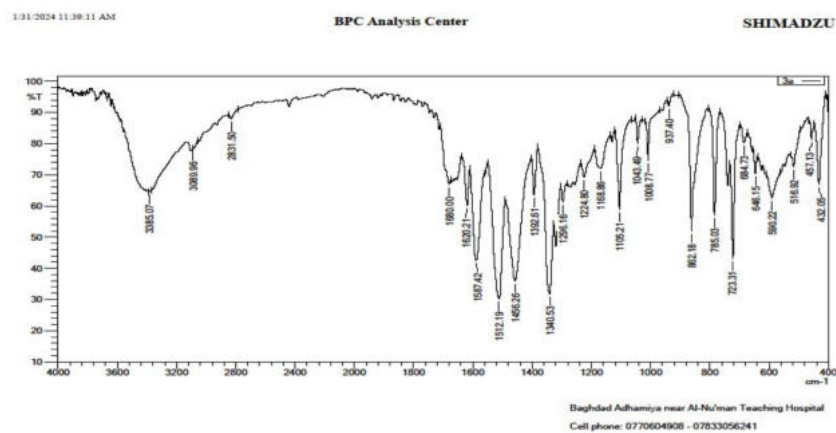
The UV-Visible spectra of (1) in Fig. 3 exhibits strong absorption at (265 nm) attributed to the $\pi \rightarrow \pi^*$ transition and (2) in Fig. 4. Absorption at (380 nm) is due to the $n \rightarrow \pi^*$ transition a peak with a high intensity band formed with absorption

FT-IR spectra for new compounds

The synthesized compounds were analyzed utilizing FTIR technology, the findings are shown in Table 2. The spectra of the compounds revealed a band at $(1654) \text{ cm}^{-1}$, which coincides with the carbonyl group's (C=O) stretching vibration. The peaks seen at $(3074) \text{ cm}^{-1}$, $(3057) \text{ cm}^{-1}$, $(2089) \text{ cm}^{-1}$, correspond to the vibrations caused by stretching of (C-H) bonds in aliphatic, alkene, and aromatic compounds, respectively. At $1665\text{--}1660 \text{ cm}^{-1}$, the bands correspond to stretching vibrations in the C=C bond^{36,37} as displayed in Figs. 8 to 12.

Table 2. Exhibits the compound's Fourier Transform Infrared (FT-IR) spectrum.

Comp. No.	C-H aromatic	C=C aromatic	C=C olefinic	C=O	Others
1	3057 cm^{-1}	1560 cm^{-1}	1600 cm^{-1}	1654 cm^{-1}	C-Br 688 cm^{-1}
2	3167 cm^{-1}	1548 cm^{-1}	1665 cm^{-1}	1678 cm^{-1}	OH 3466 cm^{-1}
3	3089 cm^{-1}	1587 cm^{-1}	1620 cm^{-1}	1680 cm^{-1}	OH 3385 cm^{-1}
4	3074 cm^{-1}	1527 cm^{-1}	1560 cm^{-1}	1618 cm^{-1}	NO ₂ 1512 cm^{-1}
5	2900 cm^{-1}	1597 cm^{-1}	1647 cm^{-1}	1680 cm^{-1}	NO ₂ Asymmetric 1450 cm^{-1} NO ₂ Symmetric 1344 cm^{-1} C-N 1224 cm^{-1}

**Fig. 8.** FT-IR spectrum of compound (1).**Fig. 9.** FT-IR spectrum of compound (2).**Fig. 10.** FT-IR spectrum of compound (3).

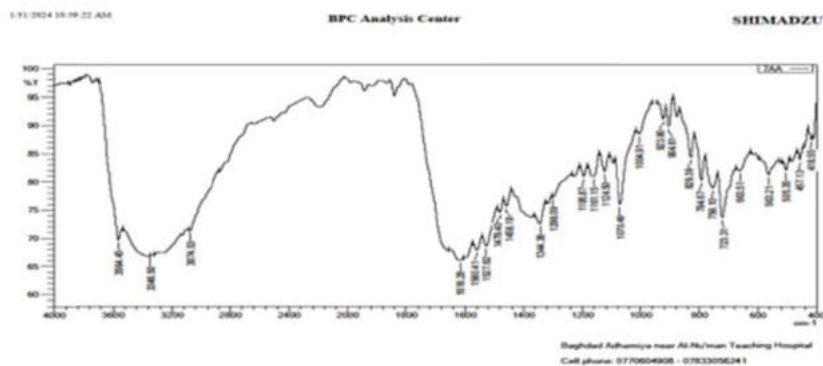


Fig. 11. FT-IR spectrum of compound (4).

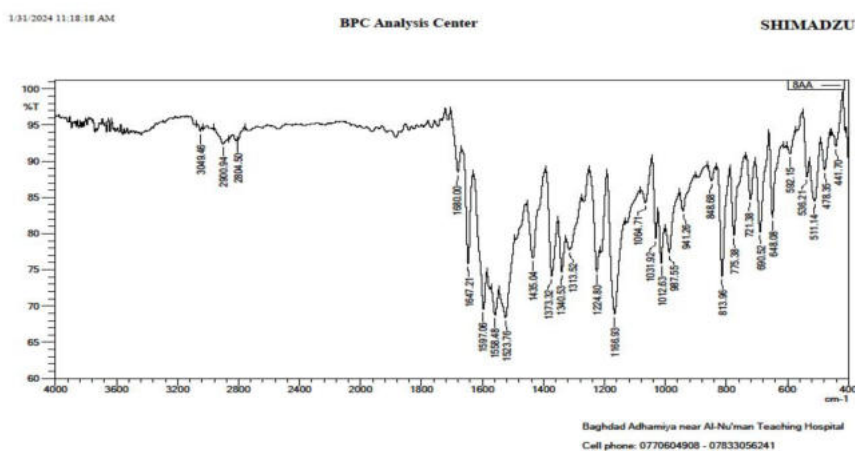


Fig. 12. FT-IR spectrum of compound (5).

Table 3. ¹H-, ¹³C-NMR data for prepared chalcone derivatives.

Compound no	Structure	¹ H NMR and ¹³ C NMR spectral data (δ, ppm)
1		¹ H NMR (400 MHz, DMSO) δ 8.23 (d, J = 6.9 Hz, 1H), 8.01 (d, J = 9.8 Hz, 1H), 7.79 – 7.70 (m, 1H), 7.68 – 7.52 (m, 2H). ¹³ C NMR (75 MHz, DMSO) δ 133.93 (d, J = 27.2 Hz), 131.10, 129.89 – 128.40 (m), 40.79, 40.51, 40.23, 39.95, 39.67, 39.40, 39.12.
2		¹ H NMR (400 MHz, DMSO) δ (ppm) 9.21 (s, 1H), 9.2 (dd, J = 8.3, 1.6 Hz, 1H), 7.30 (d, J = 16 Hz, 1H), 7.27 (m, 2H), 6.12 (d, J = 16 Hz, 1H), 6.09 (m, 3H). ¹³ C NMR (75 MHz, DMSO) δ (ppm) 186.62, 120.34, 118.78, 40.67, 40.39, 40.10, 39.83, 39.55, 39.27, 38.99.
3		¹ H NMR (400 MHz, DMSO) δ 8.37 (s, 1H), 8.23 (d, J = 8.7 Hz, 9H), 8.11 (d, J = 8.6 Hz, 10H), 8.04 (d, J = 8.9 Hz, 1H), 7.86 (d, J = 15.6 Hz, 1H), 7.75 (d, J = 12.5 Hz, 1H), 6.45 (s, 2H). ¹³ C NMR (75 MHz, DMSO) δ 128.32, 123.66, 40.76, 40.49, 40.07 (d, J = 20.9 Hz), 39.65, 39.24 (d, J = 21.1 Hz).
4		¹ H NMR (400 MHz, DMSO) δ 8.60 (s, 1H), 7.75 (d, J = 32.3 Hz, 5H), 7.49 (s, 18H), 7.31 (s, 7H), 7.15 (s, 25H), 6.99 (s, 27H), 6.50 (d, J = 63.4 Hz, 85H), 5.78 (s, 10H), 5.36 (s, 7H), 5.18 (s, 7H). ¹³ C NMR (75 MHz, DMSO) δ 147.83, 135.96, 129.35, 124.06 (d, J = 19.5 Hz), 40.65, 40.37, 40.09, 39.81, 39.53, 39.25, 38.98.
5		¹ H NMR (400 MHz, DMSO) δ 8.13 (s, 1H), 8.10 (s, 1H), 7.72 (s, 1H), 7.69 (s, 2H), 7.66 (s, 1H), 7.61 (d, J = 7.2 Hz, 1H), 7.58 – 7.49 (m, 2H), 6.73 (d, J = 9.0 Hz, 2H). ¹³ C NMR (75 MHz, DMSO) δ 189.09, 152.44, 145.70, 132.97, 130.19 (d, J = 163.0 Hz), 122.42, 116.46, 111.84 (d, J = 51.6 Hz), 40.80, 40.52, 40.24, 40.14, 39.96, 39.69, 39.41, 39.13.)

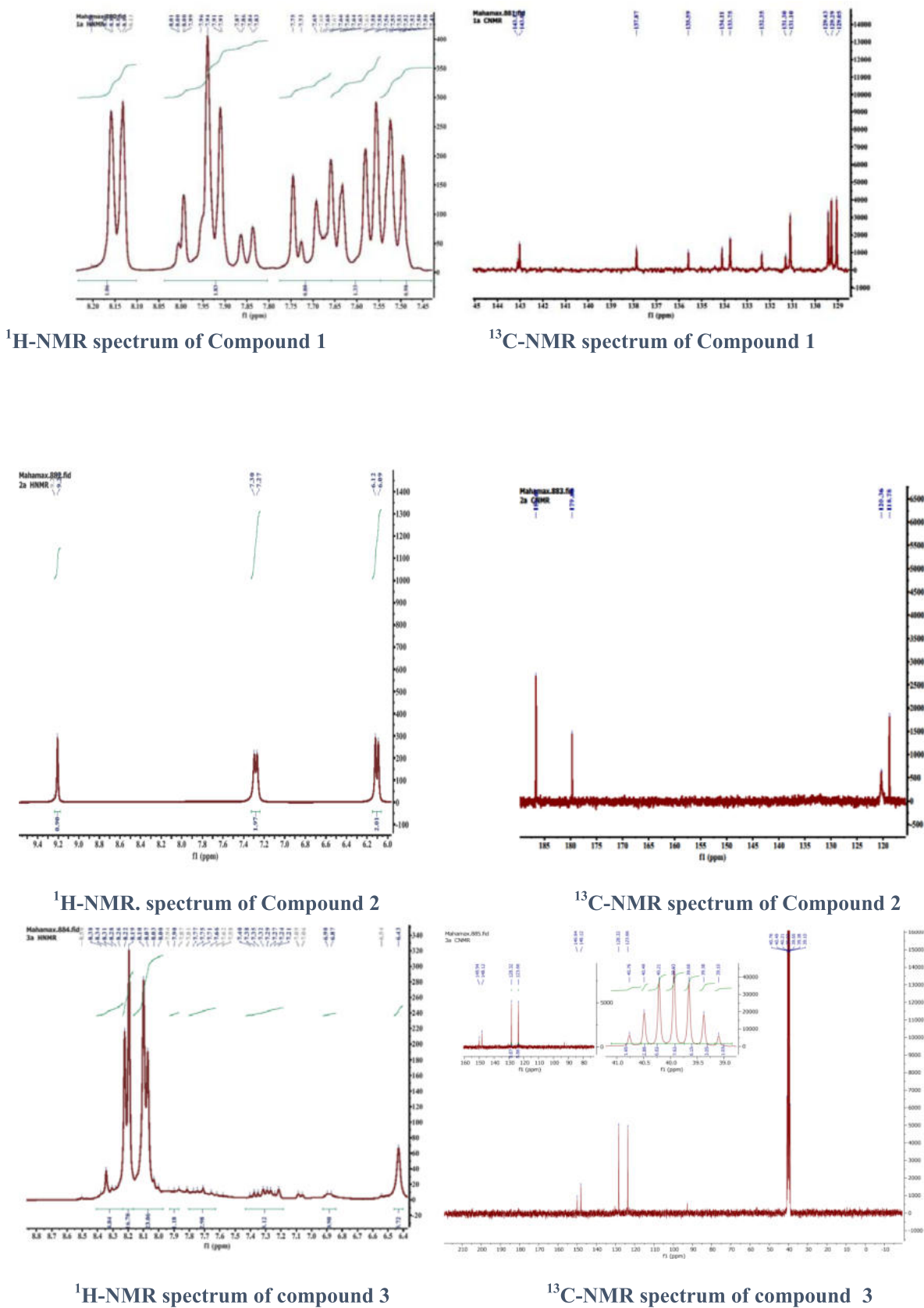


Fig. 13. ¹H-, ¹³C-NMR spectrum of compounds (1), (2) and (3).

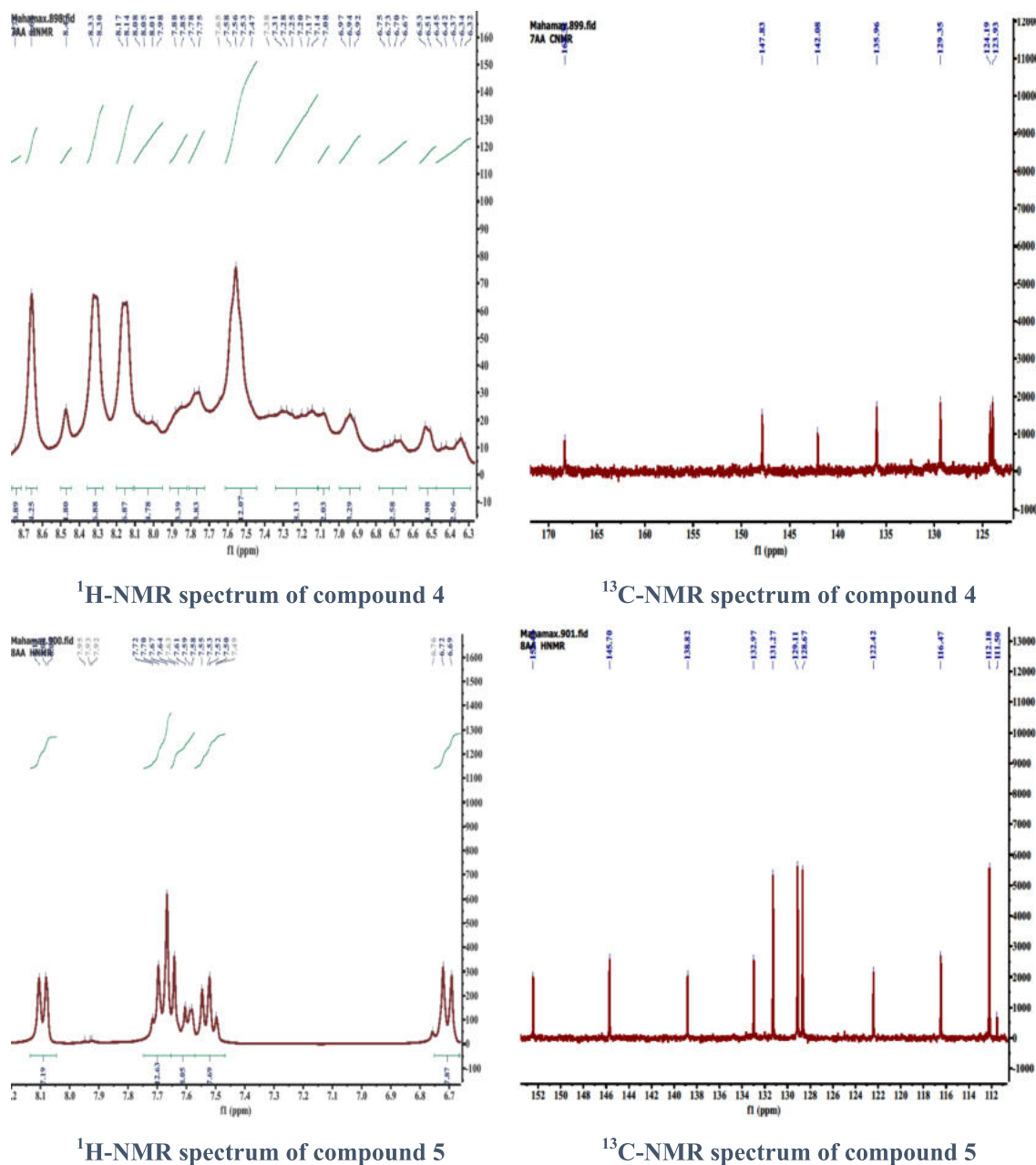


Fig. 14. ¹H-, ¹³C-NMR spectrum of compounds (4) and (5).

¹H-, ¹³C-NMR for prepared compounds

An intense singlet of chalcone's OH signals was observed in the ¹H-NMR spectra of the compounds in the range of 12.79–12.92 ppm. All compounds displayed summits in the range 6.88 to 7.94 ppm, which were attributed to phenyl protons. At 6.9–7.9 ppm, the sign is attributed to two protons of alkene became visible and consequently combined with the aromatic protonic multiplet. At 1.25 ppm, the methyl proton signal became visible as a singlet. The compounds' ¹³C-NMR spectra showed a distinct singlet at 193.65–193.74 ppm, Table 3, which is

attributed to the –C¹/₄O carbon. At 163.54–163.61 ppm, the phenolic C–OH carbon signal became visible. The aromatic carbon multiplets at 114.51–145.45 ppm were identified. At 115.45–140.58 ppm, the alkene carbons became visible and consequently combined with the aromatic carbons,^{38,39} as shown in Figs. 13 and 14.

Antimicrobial efficacy

The control used for antibacterial activity were amoxicillin and ciprofloxacin. The compounds examined had a significant inhibitory effect, compound

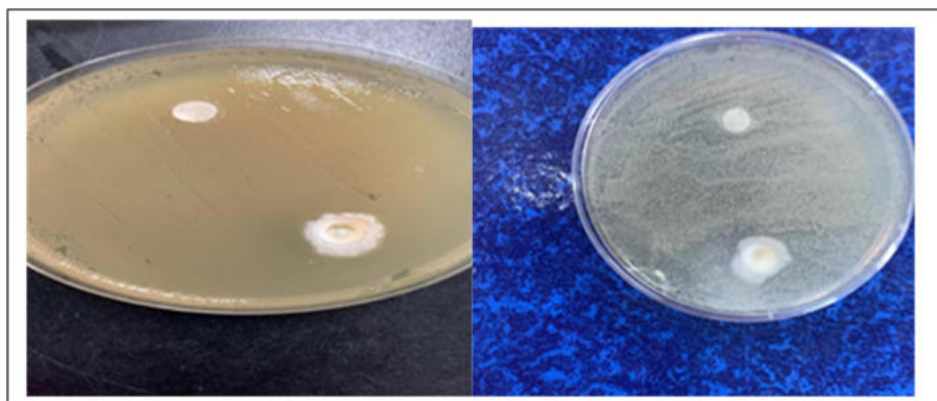


Fig. 15. Sample (1) against *E. coli* and *S. aureus*.

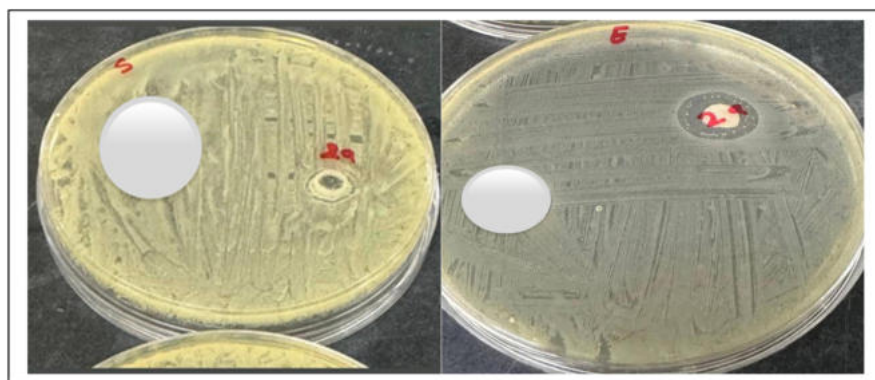


Fig. 16. Sample (2) against *E. coli* and *S. aureus*.

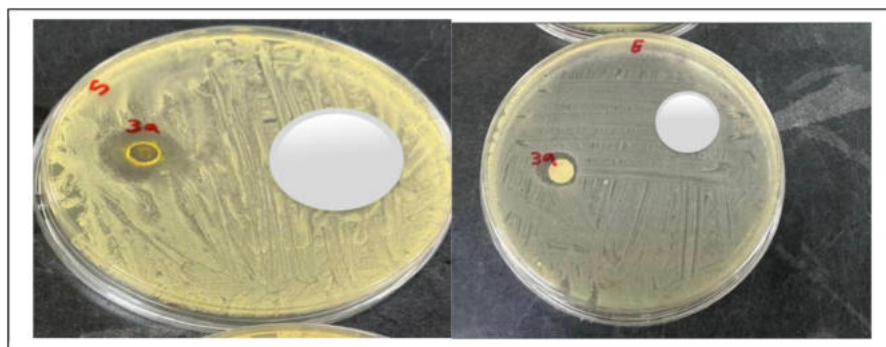


Fig. 17. Sample (3) against *E. coli* and *S. aureus*.

1 had similar effects on both Gram-positive and Gram-negative bacteria, and had no inhibitory effect on either type of bacteria as shown in Fig. 15. Furthermore, compounds 2 and 4 had a fairly strong inhibitory effect on Gram-negative bacteria only as shown in Fig. 16. Compound 3 showed the highest level of efficacy against Gram-positive and Gram-negative bacterial species as shown in Fig. 17, compound 3 was yellow color shown in Fig. 18.

Gram-positive bacteria were selectively inhibited by compound 5, and Gram-negative bacteria were inactive. Amikacin, an antibiotic, was used as a control, while DMSO was used as a solvent.⁴⁰ Table 4 shows the antibacterial activity of the tested prepared compounds. Table 4 Zone of inhibition of compounds 1, 2, 3, 4, and 5 on Gram-negative and Gram-positive bacteria.



Fig. 18. Images of some of the synthesized components.

Table 4. Antibacterial activity of the tested prepared compounds.

Compound Number	Concentrate	<i>E.coli</i>	<i>S.aureus</i>
1	0.05 ml	n.a	n.a
2	0.05 ml	15 mm	n.a
3	0.05 ml	9 mm	15 mm
4	0.05 ml	15 mm	n.a
5	0.05 ml	n.a	10 mm

Conclusion

In this research, chalcone derivatives were prepared by Claisen-Schmidt method, which is a successful method for preparing chalcones. In order to confirm the chemical structure of the prepared compounds, they were characterized by several techniques, namely UV-Vis, IR - Spectroscopy, $^1\text{H-NMR}$, $^{13}\text{C-NMR}$, and their biological activity as antibacterial compounds was studied. Some derivatives showed activity against *E. coli* and *S. aureus* bacteria, while others were ineffective.

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Author's declaration

- Conflicts of Interest: None.
- We hereby confirm that all the figures and tables in the manuscript are ours. Besides, figures and images which are not ours have been given the

permission for re-publication attached with the manuscript.

- No animal studies are present in the manuscript.
- No human studies are present in the manuscript.
- Ethical Clearance: The project was approved by the local ethical committee at University of Baghdad.

Author's contribution

A.J.H conducted the practical side of the research, analyzed the results and wrote the manuscript. H.H. J & K.A.A conceived idea, supervised the research, contributed to the analysis of the results, and revised and proofread the manuscript.

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تحضير مشتقات الجالكون وتشخيصها ودراسة فعاليتها البيولوجية

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الملخص

يهدف هذا البحث إلى تحضير مشتقات الجالكون باستخدام مشتقات الأستوفينون ومشتقات البنزالديهايد كمكونات أساسية. تم إنتاج مركبات الجالكون [1، 2، 3، 4، 5] من خلال تفاعل تكثيف كلايزن-شميت، والذي يستلزم دمج مشتقات البنزالديهايد (مثل مثل 4-بروموبنزالدهيد، 4-هيدروكسي بنزالدهيد، 4-ثنائي ميثيل أمينو) بنزالدهيد، وأونيترو بنزالدهيد) مع الأستوفينون أو مشتقاته. يحدث هذا التفاعل عندما يتم مزج محلول مائي من هيدروكسيد الصوديوم والإيثانول في درجة حرارة الغرفة. تم تشخيص المركبات الناتجة باستخدام التقنيات الطيفية للأشعة تحت الحمراء (IR)، والأشعة فوق البنفسجية (UV)، والرنين المغناطيسي النووي البروتوني (¹H-NMR)، والرنين المغناطيسي النووي للكربون-13 (¹³C-NMR). تم إخضاع كل مركب للاختبار للتأكد من فعاليته المضادة للبكتيريا. النشاط المضاد للبكتيريا باستخدام تقنية الأجار للانتشار الجيد. تم إذابة المشتقات في ثنائي ميثيل سلفوكسيد (DMSO) بتركيز محدد. 20 ملغم/مل، بتركيز كل مشتق 0.05 ملغم/مل.

الكلمات المفتاحية: الفعالية البيولوجية، مشتقات الكالكون، التوصيف، تكاثف كلايسن شميدت، التحضير.