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Synthesis, Characterization, Anti-Inflammatory, and Antimicrobial Evaluation of New 2-Pyrazolines Derivatives Derived from Guaiacol

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Abstract

Pyrazolines are one of the most important nitrogen containing heterocyclic compounds that show the diversity of biological activities such as antimicrobial, anti-inflammatory, antiviral and anticancer.

The current research involves the synthesis of new 2-pyrazolines- guaiacol derivatives by using chalcones as a key intermediate. Chalcones I(a-e) was synthesized by Claisen-Schmidt Condensation method through the reaction of acetophenone with five various para substituted benzaldehyde in presence of KOH. By a two-pot reaction through the refluxing substituted chalcones I(a-e) with hydrazine hydrate in ethanol, glacial acetic acid, and guaiacol to give 2-pyrazoline-guaicol derivatives **II(a-e)**. Based on the spectral data 2-pyrazoline derivatives structures have been confirmed. The synthesized compounds were screened for their antimicrobial activity, and show moderate to high inhibition against both gram-positive and gram-negative bacteria, and a high antifungal activity for the compounds (IIb, IId, and IIe). The anti-inflammatory activity was also tested for the final compounds and it showed a good activity for both IIa and IId final compounds

Keywords: Anti-inflammatory, Antimicrobial activity, Chalcones, Guaiacol, Pyrazoline.

التوليف والتوصيف والتقييم الدوائي الأولي كمضادات للمكروبات والالتهابات لمشتقات ٢- بيرازولين- كواشيكول كمشتقات جديدة

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الخلاصة

يعتبر البيرازولين واحدة من أهم مركبات النيتروجين التي تحتوي على حلقة غير متجانسة والتي تظهر تنوعًا في الأنشطة البيولوجية مثل مضادات الميكروبات ومضادات الالتهاب والفيروسات ومضادات السرطان.

يتضمن البحث الحالي تخليق مشتقات ٢-pyrazolines الجديدة المشتقة من guaiacol باستخدام الجالكون كوسيط رئيسي. تم تصنيع Chalcones I (a-e) بواسطة طريقة Claisen-Schmidt التكثيف من خلال تفاعل الاسيتوفينون مع خمسة أنواع مختلفة من البنزيلديهايد البديل في وجود KOH. المشتقات التي تفاعلت بشكل مباشر مع الجوايكول لإعطاء مشتقات ٢-بيرازولين-جوايكول(a-e) II. بناءً على البيانات الطيفية، تم تأكيد هياكل المركبات المحضرة و وكذلك تم اختبارها ضد المكروبات (البكتريا)الموجبة والسالبة لصبغة غرام، وقد أعطت نشاطا من متوسط الى مرتفع ونشاط مضاد للفطريات الذي بين فعالية مرتفعة للمركبات(IIbو IId, IIe). - ,تم اخيرا اختبار فعالية المركبات النهائية ضد الالتهابات أيضًا وأظهرت نشاطًا جيدًا للمركبين IIa و IId.

الكلمات المفتاحية: فعالية المضادة للالتهابات، فعالية المضادة للميكروبات، الجالكونات، جوايكول، بيرازولين.

Introduction

The polyphenolic compound chalcone (1,3-diphenyl-2-propen-1-one) with the general formula (Ar-C=O-CH=CH-Ar) belongs to the flavonoids family[(1)].The general formula of chalcone $C_{15}H_{12}O$ has two stereochemistry, *cis* and *trans*, but trans (-1,3-diphenyl-2-propene-1-one) is much more reactive than *cis* isomer (1,3-diphenyl-2-propene-1-one) as shown in "Figure. 1"[(2)].

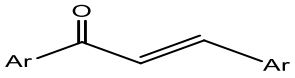

Figure 1. Chalcone structure[(3)]

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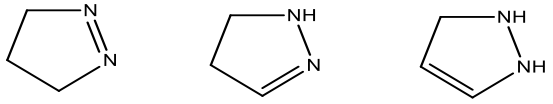
1,3-diphenyl-2-propen-1-ones are both naturally occurring and artificially prepared, and are both regarded as important intermediates in advanced chemistry in scientific research, due to the large number of replaceable hydrogens which enable a large number of derivatives to be synthesized. As a result, they are regarded as the basis for the synthesis of a wide range of compounds, involving pyrazoline, thiazine, and pyrimidine[4].

Chalcones also have a wide variety of pharmacological activities as anti-microbial[4], anti-inflammatory[5] There are several methods for chalcones synthesis such as: Claisen-Schmidt[6], Suzuki[7], Wittig, Friedel-Craft acylation[8] and microwave-assisted[9].

Claisen Schmidt condensation is the famous one also known as an Aldol condensation, it is a commonly employed, easy method and greater yield (60-90) %[7]

Chalcones as an α - β-unsaturated carbonyl system possess two electrophilic reactive centers, due to delocalization of electron density in the "C=C-C=O" system, allowing them to participate in addition reactions via attack to the carbonyl group (1,2-addition) or involving the -carbon (1,4-conjugate addition), leading to the synthesis of promising bioactive heterocyclic compounds such as the manufacture of 2-pyrazoline by cyclization reaction of different chalcones with hydrazine[10].

Pyrazoline and its derivatives of exhibited significant biological activities, consider as neutrophil compound[11,12] therefore it has important role in various pharmacological activities such as anti-inflammatory[13], anti-microbial[14].It is also known as dihydro pyrazole is a five-membered heterocyclic, their stability is determined by the two nitrogen atoms that are next to each other and the endo-cyclic double bond, which has a different position in each of the three isomers of pyrazoline (1-pyrazoline, 2-pyrazoline, and 3-pyrazoline), the more stable one is 2-pyrazoline "Figure. 2" [10] .



1-pyrazoline 2-pyrazoline 3-pyrazoline

Figure 2.Pyrazoline isomers[10]

The great interest in modern heterocyclic chemistry is to development of an efficient and green synthetic procedure. A classical method for the synthesis of pyrazoline was involved a two-pot technique. The technique consisted of two steps synthesis which are the preparation of chalcones followed by cyclization reaction with hydrazine to form pyrazoline or pyrazoline derivative depending upon the additive compounds[15,16]

Guaiacol will prove helpful in the design and preparation of new medications, because of its ability to inhibit the production of superoxide anion, break down the generation of hydroxyl radicals, and act as excellent free radical scavengers[17], also with antibacterial effect[18], guaiacol will be useful in the design and preparation of new medications.

The goal of this study was to synthesize 2-pyrazoline derivatives conjugated with an antioxidant (guaiacol) with the objective to improve the desired antibacterial and anti-inflammatory effects.

Materials and Methods

Chemicals and instrumentation

All chemicals have been supplied from Fluka, Sigma-aldrich, Hyperchem, BDH, Alpha chemika, and GCC. The reactions' progress was monitored by thin-layer chromatography (TLC), as a mobile phase, two solvent systems were used: n-hexane: ethyl acetate (5:2) and n-hexane: ethyl acetate: methanol (8:4:2). Electronic melting point apparatus (Stuart SMP30) was used for melting points determination. FTIR spectrophotometer (Schimadzu, Japan).^{1}HNMR spectra were obtained on BRUKER model Ultra shield 500 MHz spectrometer.

1-Chemical synthesis

The pathways involved for the synthesis of chalcones I(a-e) and targets compound II(a-e) were shown in "Scheme. 1".

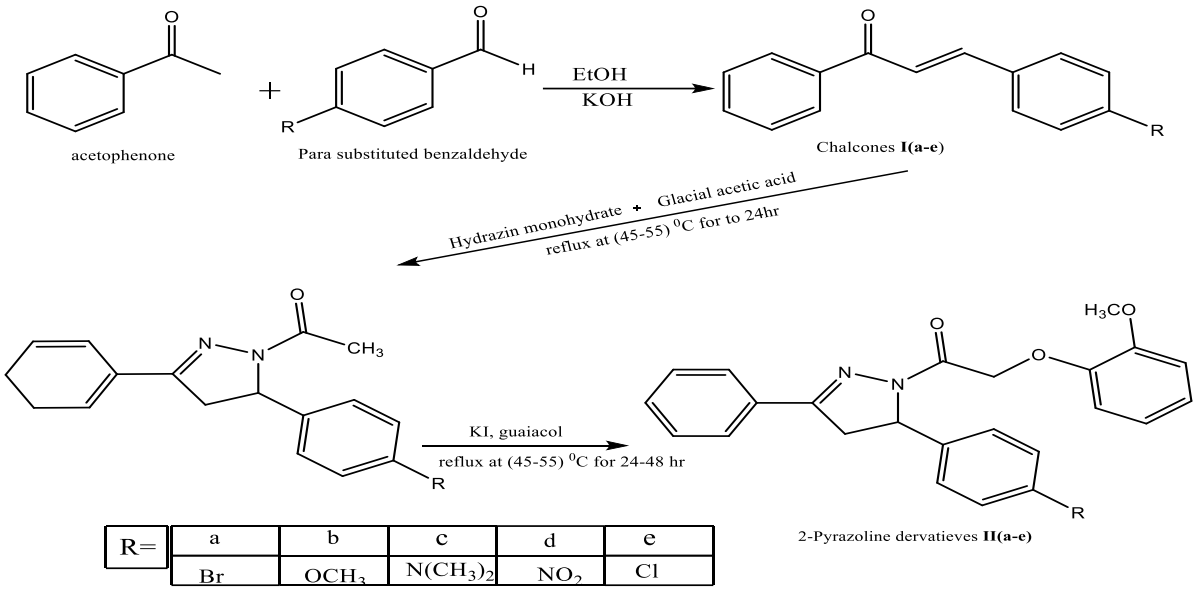
Synthesis of Chalcone Derivatives I (a-e) *[12]*

A solution of acetophenone (0.01 mol, 1.2 mL) was stirred in 99% ethanol (20 mL) for 15 min in a 250 mL round-bottom flask; then para-substituted benzaldehydes (0.01 mol) [a-Br: 2.4 g, b-OCH3: 1.2 mL, c-N(CH3)2: 1.5 g, d-NO2: 1.5 g, e-Cl: 1.4 g] was added. The reaction mixture was kept in an ice bath, after which (15 mL) 40% KOH solution was added gradually over a period of 5 minutes and the reaction mixture was allowed to stir overnight at 20-25°C.The reactions were monitored by TLC using n-hexane/ethyl acetate (5:2) as a mobile phase system. After completion of the reaction (monitored by TLC), the reaction contents were poured onto a crushed ice and neutralized with HCl (10%). The resulting product was filtered, washed with cold DW and recrystallized from a mixed solvent of n-hexane: ethanol (8:3).

Synthesis of 1,3,5-trisubstituted-2-pyrazoline II(a-e) using two – pot reaction II(a-e). *[15,*19)

Appropriate chalcones I(a-e) (0.01 mol) was dissolved in 30 mL of glacial acetic acid. To this mixture was added hydrazine monohydrate (0.02 mol, 1 mL) and the reaction mixture was refluxed for 24 hours. The reaction was monitored by TLC using n-hexane/ethyl acetate (4:1) as solvent system. Upon the completion of the reaction (monitored by TLC), the solution was then treated with KI (0.01 mol) dissolved in 10 mL (D.W) and stirred for 30 min. Guaiacol (0.01 mol, 1.12 mL) was then added, the reaction mixture was refluxed for 24-48 hours, and monitoring by TLC to ensure the completion of the reaction the mobile phase was n-hexane: ethyl

acetate: methanol (8:4:2) the reaction mixture was then poured onto crushed ice with stirring. It was left at room temperature to obtain a crystalline compound. The resulting solid was filtered and recrystallized using a two solvent system of abs. ethanol and n-hexane (3:8).



Scheme 1.S synthesis of intermediates and final compounds (IIa-e).

2-Pharmacological study

Antibacterial activity *(20–25)*

In-vitro; the antibacterial activity for all the synthesized compounds II(a-e) were done by using the agar well diffusion method. They have been screened for antibacterial activity against two-gram positive bacteria: *Staphylococcus aureus* ATCC 25923, *Streptococcus pyogenes* ATCC 12344, and two-Gram negative bacteria: *Pseudomonas aeruginosa* ATCC 27853 and *Escherichia coli* ATCC 25922 "E. coli". Candida albicans NRRL Y-12983 was used as the test organism for the antifungal activity.

Mueller Hinton Agar No.2 was used as the nutritional medium to sustain the bacterial and fungal cultures of the test organisms. by adding a loop's worth of the test strain to 25 ml of N-broth and incubating the mixture for 24 hours at 37°C, the bacterial strains were activated. Next, a 100 mm diameter Petri plate was filled with 28–30 mL of molten Mueller Hinton Agar No.2 and 0.2 mL of inoculum. Well diffusion method (agar well diffusion in which hole or well is created on the medium and then the extract solution is added to the well) that done by inhibited the growth of microorganisms, decreasing their shelf life and prevent from the formation of microbial colonies. The activity measurement is depended on the size of growth inhibition zone around the sample. To get started with this evaluation, the antimicrobial activity of (ciprofloxacin and amoxicillin) was chosen, while DMSO was chosen as both a negative control and a solvent, and the concentration of all compounds was 1000 µg/mL

Antifungal Activity *(17,26–28)*

The final synthesized compounds **II (a-e)** were tested for antifungal activity using also the well diffusion method such as antibacterial evaluation, with an antifungal standard agent (Fluconazole), and DMSO serving as a control and solvent.

Evaluation of the Anti-Inflammatory Activity *(29–31)*

Acute inflammation was induced by injecting undiluted egg white subcutaneously into the intra-planter side of the rat's left hind paw in order to investigate the anti-inflammatory properties of the final compounds **II(a-e).** When egg white is subcutaneously injected into a rat paw, inflammation occurs, as a result of increased tissue water and plasma protein discharge, neutrophil extravasations, and plasma extravasations, all of which are triggered by arachidonic acid metabolism. This method outperforms others by virtue of its rapid assessment by detecting inflammation early and over a short period of time, high paw sensitivity to inflammation, lack of anesthetic, cost-effectiveness, technique more comparable to human nature, and other factors.

The doses of the final synthesized compounds were determined using the general formula below:

$$\frac{\textit{Dose of reference compound}}{\textit{Molecular weight reference compound}} = \frac{\textit{Dose of tested compound\_}}{\textit{Molecular weight reference compound}}$$

Molecular weight reference compound Molecular weight reference compound 50% Propylene glycol was used as control and solvent, the dose of the final synthesized compounds with

diclofenac sodium serving as the standard drug are illustrated in "Table 1".

Table 1. Groups and dose calculation

Compounds	M.wt	Dose mg/kg
Diclofenac sodium	318	3
IIa	465.32	4.71
IIb	416.48	4.21
IIc	429.52	4.35
IId	431.45	4.37
IIe	420.89	4.26

The anti-inflammatory effect of the compounds tested was examined using an egg-white induced edema model. The paw thickness was evaluated with a vernier calliper a total of seven times, following drug administration (0, 30, 60, 120, 180, 240, and 300 minutes). Subcutaneous injection of (0.05 mL) undiluted egg-white into the plantar side of the animals' left hind paw; (30 min.) following intraperitoneal administration of the drugs or their vehicle produced significant inflammation.

Result and Discussion

1-Chemical synthesis

The physical properties, R_f and FT-IR spectral data of chalcones and final targets compound, while, ^{1}HNMR spectra data of final targets compounds are illustrated below:

I- chalcone derivatives I (a-e):

3-(4- (bromophenyl) phenyl)-1-phenylprop-2-en-1-one ($C_{15}H_{11}BrO$) (Ia): Description: Bright yellow crystal. Yield: 80%. M.P.: 100-1-04 °C. R_f =0.65. FT-IR: 3059, 3035 (str of CH Ar), 2908 (Asymmetric CH str aliphatic), 2870 (symmetric CH str aliphatic), 1654 (C=O) str, 1604 (C=C str aliphatic), 1581, 1562 (C=C str of Ar), 686 (C-Br) str.

3-(4-methoxyphenyl)-1-phenylprop-2-en-1-one ($C_{16}H_{14}O_2$) (Ib): Description: light yellow crystals. Yield: 85%. M.P.: 74-75 °C. R_f =0.55. FT-IR: 3016 (str of CH Ar), 2954, 2900 (asymmetric CH str of CH_3), 2843 (symmetric CH str. of CH_3), 1654 (C=O str), 1593(C=C str aliphatic), 1573, 1508 (C=C Ar str), 1261 (C-O-C str).

3-(4- (Dimethyl amino) phenyl)-1-phenylprop-2-en- 1-one ($C_{17}H_{17}NO$) (Ic): Description: Bright orange crystal. Yield: 90%. M.P.: 110-111°C. R_f =0.7. FT-IR: 3151 - 3028 (str. vib. of CH Ar), 2904 (Asymmetric CH str. of CH_3), 2858 (symmetric CH str. of CH_3), 1647 (C=O str), 1597 (C=C str aliphatic),1577, 1558 (C=C Ar str), 1157 (C-N str).

3-(4-Nitrophenyl)-1-phenylprop-2-en-1-one ($C_{15}H_{11}NO_3$) (Id): Description: Yellow to brown powder. Yield: 90%. M.P.: 155-156 ^{o}C, R_f =0.59, FT-IR: 3132, 3070 (str. vib. of CH Ar), 2900, 2870 (asymmetric and symmetric str of CH aliphatic),1658 (C=O str), 1608 (C=C str of aliphatic), 1573,1527 (C=C Ar str), 1510, 1350 (NO2 asymmetric and symmetric str. respectively)

(E)-3-(4-chlorophenyl)-1-phenylprop-2-en-1-one ($C_{15}H_{11}ClO$) (Ie): Description: Off-wight to yellow crystals. Yield: 95%. M.P:110- 112 ^{o}C, R_f =0.45. FT-IR: 3059 (Str of CH Ar), 3028,2889 (asymmetric and symmetric str of CH aliphatic), 1658 (C=O str), 1600 (C=C str aliphatic), 1573,1527 (C=C Ar str), 740 (C-Cl str)

II-Final targeted compounds:

1-(5-(4-bromophenyl)-3-phenyl-4,5-dihydro-1H-pyrazol-1-yl)-2-(2-methoxyphenoxy) ethan-1-one ($C_{24}H_{21}BrN_2O_3$) (IIa): Description: Off-white crystal. Yield: 70%. M.P.: 150-152°C. R_f =0.5. FT-IR: 3143, 3062 (str of CH aromatic), 2927 (Asymmetric CH str. of CH_3), 2839 (symmetric CH str. of CH_3), 1666 (C=O str), 1593 (C=N str overlap with C=C aromatic) 1566, 1542 (C=C Ar str), 1303 (C-O-C str), 690 (C-Br str).

^{1}HNMR (δ ppm) 3.12 (1H, dd, CH of CH_2 of pyrazoline ring), 3.72 (1H, dd, CH of CH_2 of pyrazoline ring), 3.88(3H, s, OCH3 group), 4.84 (2H, s, CH_2 group), 5.54 (1H, dd, CH of pyrazoline ring), 6.88 (2H, d, Ar protons ring c), 7.12 (2H, d, aromatic protons ring C), 7.42 (2H, d, meta to Br ring B), 7.50-7.64 (5H, m, aromatic protons ring A), 7.72 (2H, d, aromatic protons ortho to Br ring B).

2-(2-methoxyphenoxy)-1-(5-(4-methoxyphenyl)-3-phenyl-4,5-dihydro-1H-pyrazol-1-yl) ethan-1-one ($C_{25}H_{24}N_2O_4$) (IIb): Description: Pale brown crystal. Yield: 78%. M.P.: 168-170°C. R_f =0.7. FT-IR: 3059, 3035 (str. of CH Ar), 2947 (Asymmetric CH str. of CH_3), 2831 (symmetric CH str. of CH_3), 1647 (C=O str),1597 (C=N str) ,1570, 1543, 1523 (C=C Ar str), 1246 (C-O-C str), 1157 (C-N str).

^{1}HNMR (δ ppm) 3.14(1H, dd, CH of CH_2 of pyrazoline ring), 3.72(1H, dd, CH of CH_2 of pyrazoline ring), 3.84 (3H, s, OCH_3 group ring C),3.90 (3H, s, OCH_3 group ring B), 4.38 (2H, s, CH_2 group), 5.58 (1H, dd, CH of pyrazoline ring), 6.81 (2H, d, aromatic protons ring C), 6.94 (2H, d, aromatic protons ring C), 7.22 (2H, d, aromatic protons meta to OCH_3 ring B),7.42 (2H, d, aromatic protons ortho to OCH_3 ring B) 7.71-7.75 (5H, m, aromatic protons ring A)

1-(5-(4-(dimethyl amino) phenyl)-3-phenyl-4,5-dihydro-1H-pyrazol-1-yl)-2-(2-methoxy-phenoxy) ethan-1-one ($C_{26}H_{27}N_3O_3$) (IIc): Description: Beige crystal. Yield: 80%. M.P.: 185-187°C. R_f =0.55. FT-IR: 3170, 3074 (str of CH Ar), 3024, 2900 (Asymmetric CH str. of CH_3), 2816 (symmetric CH str. of CH_3), 1662 (C=O str.), 1620 (C=N str), 1589, 1566, 1527 (C=C Ar str), 1234 (C-O-C str), 1157 (C-N str).

^{1}HNMR (δ ppm) 3.00 (6H, s, 2xCH_3), 3.10 (1H, dd, CH of CH_2 of pyrazoline ring), 3.78 (1H, dd, CH of CH_2 of pyrazoline), 3.98 (3H, s, OCH_3), 4.46 (2H, s, CH_2), 5.42 (1H, dd, CH of pyrazoline ring), 6.65 (2H, d, ortho to $N(CH_3)_2$ group), 6.98 (2H, d, aromatic protons ring C),7.46 (2H, d, aromatic protons ring C) 7.65(2H, d, meta to $N(CH_3)_2$ group), 7.77-7.81 (5H, m, aromatic protons ring A).

2-(2-methoxyphenoxy)-1-(5-(4-nitrophenyl)-3-phenyl-4,5-dihydro-1H-pyrazol-1-yl) ethan-1-one ($C_{24}H_{21}N_3O_5$) (IId): Description: Yellow to brown crystal. Yield: 75%. M.P.: 175-177°C. R_f =0.65. FT-IR: 3190, 3032 (str of CH Ar), 2889 (Asymmetric CH str. of CH_3), 2835 (symmetric CH str. of CH_3), 1643 (C=O str), 1597 (C=N str overlap with C=C Ar) 1568, 1527(C=C Ar str), 1500, 1346(asymmetric and symmetric str respectively of NO_2), 1226 (C-O-C str), 1157 (C-N str.).

^{1}HNMR (δ ppm) 3.16 (1H, dd, CH of CH_2 of pyrazoline), 3.85 (1H, dd, CH of CH_2 of pyrazoline), 3.95 (3H,s ,OCH_3 group), 4.45 (2H,s , CH_2 group), 5.68 (1H,dd, CH of pyrazoline ring), 6.86 (2H, d, aromatic protons ring C), 7.03 (2H, d, CH_2 aromatic protons ring C), 7.26-7.33 (3H, m, aromatic protons ring A), 7.48 (2H, d, aromatic protons meta to NO_2 ring B), 7,75 (2H, d, aromatic protons ring A), 8.09 (2H, d, ortho to NO_2, ring B)

1-(5-(4-chlorophenyl)-3-phenyl-4,5-dihydro-1H-pyrazol-1-yl)-2-(2-methoxyphenoxy) ethan-1-one($C_{24}H_{21}ClN_2O_3$) (IIe): Description: Beige crystals. Yield: 65%, M.P:146-148, R_f = 0.6, FT-IR: 3059, 3032 (str of CH Ar), 3005 (Asymmetric CH str of CH_3), 2927 (symmetric CH str of CH_3), 1647 (C=O str), 1597 (C=N str) 1570, 1543, 1518 (C=C Ar str), 1246, (C-O-C str) 752 (C-Cl str).

^{1}HNMR (δ ppm) 3.12 (1H, dd, CH of CH_2 of pyrazoline), 3.74 (1H, dd, CH of CH_2 of pyrazoline), 3.92 (3H,s ,OCH_3 group), 4.44 (2H,s , CH_2 group), 5.55 (1H,dd, CH of pyrazoline ring), 6.89 (2H, d, aromatic protons ring C), 7.00 (2H, d, aromatic protons ring C), 7.36 (2H, d, aromatic protons ortho to Cl -ring B), 7.44 (2H, d, aromatic protons meta to Cl -ring B), 7,69 (2H, d, aromatic protons ortho to pyrazoline ring A), 7.72-7.76 (3H, m, aromatic protons, ring A)

Antibacterial activity

Antibacterial activity was evaluated for the final synthesized compounds **II(a-e**) against gram-negative and gram-positive bacteria. As shown in "Table 2" all of the synthesized products have a moderate to high inhibitory impact against Gram-negative and Gram-positive bacteria, compounds **IIa, IId** and **IIe** which substituted by (Br, NO_2, Cl) respectively, have the most potent antibacterial activity against Gram -ve bacteria while compound **IIb, IIc** which substituted by (OCH_3, $N(CH_3)_2$) respectively show a high antibacterial activity against Gram +ve bacteria ,the antibacterial activity of the final synthesized compounds is due to the incorporation of guaiacol and different functional groups attached to aromatic ring-B

Table2. Antibacterial activity of final compounds (IIa-e).

Compounds	Conc. µg/mL	*Zone of inhibition in mm*			
		Gram -ve		*Gram +ve*	
		E. coli	*Pseudomonas aeruginosa*	*Staphylococcus Aurous*	*Streptococcus pyogenes*
IIa	10^3	35 mm	28 mm	4 mm	8 mm
IIb	10^3	13 mm	4 mm	8 mm	42 mm
IIc	10^3	-----	8 mm	30 mm	38 mm
IId	10^3	38 mm	35 mm	12 mm	5 mm
IIe	10^3	15 mm	25 mm	-----	12 mm
Ciprofloxacin	10^3	28 mm	42 mm	50 mm	47 mm
Amoxicillin	10^3	20 mm	25 mm	45 mm	32 mm
DMSO	10^3	------	------	------	------

The tested compound is considered Highly active when Inhibition zone (more than15mm), moderately active when Inhibition zone in between (10-15mm), slightly active when Inhibition zone in between (5-10 mm), and inactive when inhibition zone (less than 5).[12]

Antifungal activity

Antifungal activity of the final synthesized compounds IIa (a-e) is show in "Table 3". Compounds IIb and IId exert high antifungal activity compared with standard, while compound IIe has the same standard activity, their antifungal activity is, due to incorporation of guaiacol and different functional groups attached to the aromatic ring B[26]

Table 3.Antifungal activity of final synthesized compounds II(a-e).

compounds	Conc. µg/mL	Zone of inhibition in (mm)
		Candida albicans
IIa	10^3	------
IIb	10^3	32 mm
IIc	10^3	------
IId	10^3	35 mm
IIe	10^3	20 mm
Fluconazole	10^3	20 mm
DMSO	10^3	------

Anti-inflammatory Activity[32,33]

Comparison between the effect of standard (diclofenac sodium) and control (propylene glycol)

There was no significant difference in the alleviation of paw edema between the control group and the standard group at the start or after half an hour. As shown in "Table 4", diclofenac sodium caused a significant percent reduction ($P<0.05$) in paw edema after 2, 3, 4, and 5 hours, when compared to propylene glycol[28].

Anti-inflammatory Effect of Tested Compounds:

The effect of tested compounds on egg-white induced edema as an indicator of anti-inflammatory activity is shown in "Table 4"and "Figure. 3". The intra-plantar injection of egg white into the rat hind paw triggered progressive edema, which attained a maximum (measured in millimeters) after 1 hour. The degree of anti-inflammatory effect achieved through intra-peritoneal injection of tested compounds varied in this study.

Compounds **II(a-e)** produced significant reductions in paw edema, when compared to the effect of propylene glycol 50%v/v (control group)

At (0, 0.5. and,1 hr) time there is no significant difference between the tested compounds (IIa- IId), and the standard, but the good activity and high significant reduction pow thickness comparable with standard and control was shown after 2 to 5 hrs due to their substitution with electron with-drawing groups[20,21].

While the similar effect in pow thickness reduction of standard shown by compound (IIe). Finally compounds (IIb, IIc) have a moderate effect in reduce the paw thickness reduction.

Table 4.Anti-inflammatory activity of the final compounds II(a-e)

Time (hr.)	0 hr.	0.5 hr.	1 hr.	2 hr.	3 hr.	4 hr.	5 hr.
control	4.5±0.02	4.76±0.03	5.9±0.03	7.53±0.04	7.96±0.05	6.85±0.02	6.73±0.09
standard	4.45±0.02	4.61±0.03	5.88±0.03	6.94±0.05*	6.4±0.03*	6.15±0.02*	5.68±0.01*
IIa	4.46±0.01	4.6±0.02	6.01±0.01	6.18±0.02*^{b}	5.6±0.03*^{b}	5.36±0.01*^{b}	5.01±0.02*^{b}
IIb	4.51±0.01	4.76±0.02	5.99±0.04	7.11±0.02*	6.99±0.01*	6.99±0.02*	6.62±0.03*
IIc	4.48±0.02	4.65±0.01	6.03±0.01	6.8±0.01*	6.75±0.02*	6.66±0.03*	6.53±0.01*
IId	4.43±0.01	4.68±0.01	5.95±0.04	6.65±0.03*	5.85±0.01*^{a}	5.2±0.04*^{b}	5.05±0.04*^{b}
IIe	4.53±0.01	4.66±0.01	5.85±0.04	6.49±0.01*^{a}	6.35±0.01*	5.98±0.02*	5.66±0.01*

Non-identical superscripts (a, b) among different tested group are regarded significantly different ($p < 0.05$). (*) significantly different compared to control ($p < 0.05$). Data are expressed in mm paw thickness as mean ± SD. Time (0) is the time of i.p. injection of diclofenac sodium, tested compounds and propylene glycol. Time (30) is the time of injection of egg white to induce edema.

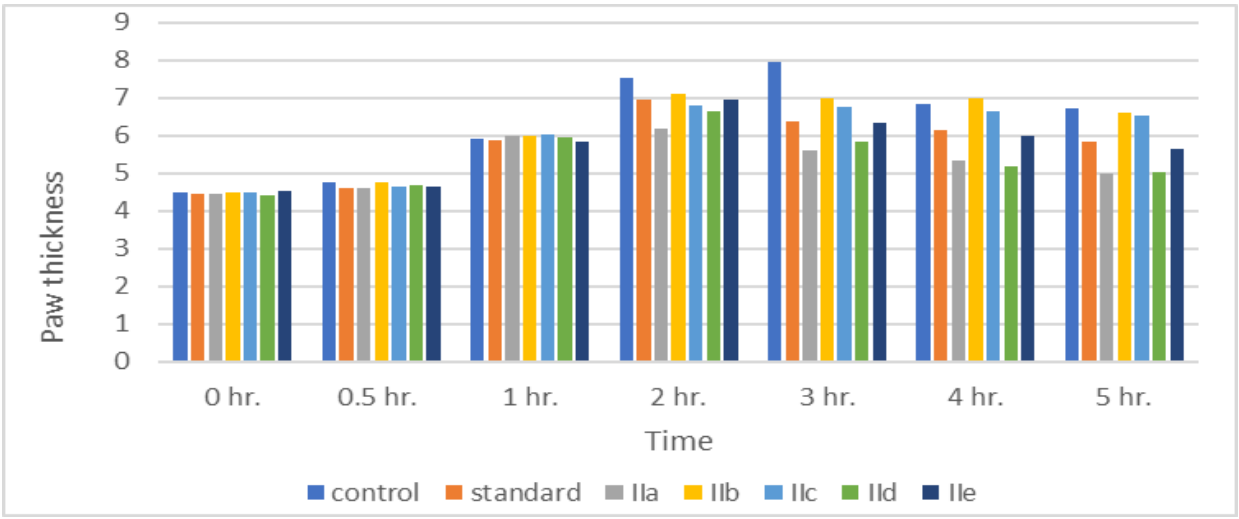


Figure 3. Anti-inflammatory activity of the final synthesized compounds

Conclusion

New pyrazoline derivatives were successfully synthesized, and their chemical structures are being validated using FTIR and ^{1}HNMR spectroscopy.

Potent antibacterial activity against Gram + ve bacteria was showed when the compound substituted with electron donating group (OCH_3, $N(CH_3)_2$), while compounds revealed a significant antibacterial activity against Gram -ve bacteria when substituted with electron withdrawal group (Br, NO_2, Cl). In addition, **IIb** and **IId** which substituted by (OCH_3, NO_2) respectively proved potent antifungal activity against *Candida albicans*. In terms of anti-inflammatory properties, as a result of the *in vivo* anti-inflammatory evaluation, the thickness of the paw edema was significantly reduced. The anti-inflammatory effects of the compounds **II**a and **IId** substituted by **(**Br and NO_2) respectively seemed significant.

Competing of Interest

The authors declare that they have not known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper

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Ethics Statements

The authors declare that their study does not need ethical approval from an ethics committee

Author Contribution

Both authors contributed to: the research study design and practical application of the research strategy for the preparation of target compounds for which FTIR and ^{1}HNMR tests were conducted on, and interpretation of their results. As well as conducting antimicrobial and anti-inflammatory tests and discussing their results; Also, both authors reviewed the complete research writing in terms of scientific and linguistic formulation.

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