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Synthesis and Preliminary Anti-Inflammatory and Anti-Microbial Evaluation of New 4,5-Dihydro-1*H*-Pyrazole Derivatives[#]

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Abstract

Pyrazolines are one of five nitrogen compound classes that have been linked to a variety of pharmacological activities. The current research involves the synthesis of new pyrazoline-aniline derivatives using chalcones as a key intermediate. Chalcones were synthesized by combining Furan-2-Carbaldehyde with various acetophenones *via* Claisen-Schimidt condensation. By refluxing substituted chalcones with hydrazine hydrate in ethanol and glacial acetic acid, 2-chloro-N-phenylacetamide and 2-chloro-N-(4-nitro-phenyl)acetamide were formed, which then react with the substituted aniline to yield 4,5-dihydro-1H-pyrazole derivatives as final products. The newly synthesized compounds were characterized using FT-IR and ^{1}HNMR spectral data, and their anti-inflammatory, antibacterial, and antifungal activities were assessed. It was found that the incorporation of the pharmacophore pyrazoline into the substituted aniline improved the effectiveness of the compounds.

Keywords : Anti-inflammatory, Antimicrobial, Chalcones, Pyrazoline derivatives.

التوليف والتقييم الفعالية المضادة للالتهابات والمضادة للمكروبات لمشتقات ٤،٥-ثنائي هيدرو-١*H*بيرازول الجديدة [#]

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#المؤتمر العلمي الثاني لطلبة الدراسات العليا

[١]وزارة الصحة والبيئية ، دائرة صحة ديالى ، ديالى ، العراق

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الخلاصة

تنتمي البيرازولين إلى مركبات النيتروجين الحلقية والمعروفة بامتلاكها لمجموعة واسعة من الأنشطة الدوائية. تم تصنيع سلسلة من مشتقات البيرازولين - الأنيلين عن طريق تخليق الجالكون كوسيط رئيسي. تم تحضير الجالكون بمعاملة فيوران -٢-كاربالديهايد مع معوضات الأسيتوفينونات المختلفة بواسطة تكاثف كلايسن شميدت. تم تحضير مشتقات مختلفة من البيرازولين عن طريق إعادة االتصعيد الحراري للجالكون مع هيدرات الهيدرازين في الإيثانول و حامض الأسيتيك؛ لينتج عنه٢-كلورو-N -فينيل أسيتاميد و ٢-كلورو-N- (٤-نيتروفينيل) أسيتاميد والتي تتفاعل بعد ذلك مع معوضات الأنيلين للحصول على مشتقات ٤،٥-ثنائي هيدرو –١H- بيرازول كمركبات نهائية.تم تشخيص المركبات المحضرة حديثًا بناءً على بياناتها الطيفية للاشعة تحت الحمراء ومطياف الرنين النووي المغناطيسي للبروتون وفحصت أنشطتها المضادة للالتهابات ,وايضا كمضادات الميكروبات ؛ وقد أشار تقييم الفعالية النهائية للمركبات المحضرة, أن دمج الفارماكوفور(حامل الدواء) البيرازولين مع الأنيلين االمعوض قد عزز أنشطتها.

الكلمات المفتاحية: فعالية مضادة للالتهابات ، فعالية مضادة للميكروبات ، الجالكونات ، مشتقات البايرازولين

Introduction

Heterocyclic nitrogenous compounds and their fused analogues represent an important class of heterocyclic compounds. They exist in numerous natural products, display a wide range of biological and pharmaceutical activities**.** Pyrazolines are well known important class of five heterocyclic compounds containing two adjacent nitrogen at 1-2 positions and three carbon atoms. The three partially reduced forms of the pyrazole are 1-pyrazoline, 2-pyrazoline and 3-pyrazoline; all are having different positions of the double bonds. Among these tautomeric structures, 2-pyrazoline is the most common one [1,2] . Chalcones are important chemical building blocks in advanced chemistry, for the synthesis of a wide range of compounds with biological activity and many bioactivities, due to their highly reactive, due to the *α-β*-unsaturated keto functional group[3,4], which is responsible about many bioactivities, such as anti-inflammatory [5], anti-microbial [6], antimalarial[7] , antidiabetic[8] , anticancer [9].

Pyrazoline derivatives are electron-rich nitrogenous heterocycles, which play an important role in the diverse biological activities thus making it an important pharmacophore for carrying out further drug research (figure-1).[2].

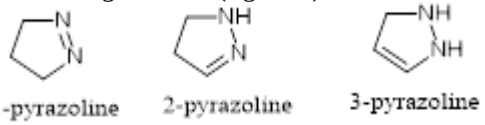

Figure 1. Isomers of pyrazoline. [2]

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Pyrazolines have been found to be an attractive pharmacophore for drug discovery in earlier studies. These heterocyclic molecules are widely distributed in nature and can be found as vitamins, alkaloids, pigments, and parts of the cells of both plants and animals[2,4]. Pyrazolines and their derivatives have been the focus of numerous activities, including : antimicrobial [10-12], anti-inflammatory [13-16], anti-cancer [17-19], antibacterial [20-22], antidiabetic [23,24], Anti-oxidants[25,26] ,Anti-malarial[27], antivirals[28-30].Based on the aforementioned biological and pharmacological profiles demonstrated by chalcone and pyrazoline, we designed our research which focuses on synthesis of different 3,5-di- phenyl-2-pyrazolines linked to aniline and substituted aniline, and evaluate their anti-inflammatory, antibacterial and antifungal activities .

Materials and Methods

Chemicals and Instrumentation

All chemicals have been supplied from Fluka ,Sigma-aldrich,Hyper chem , BDH and Riede -Dehean.The reactions' progress was monitored by thin-layer chromatography(TLC), as a mobile phase, two solvent systems were used : Ethyl acetate: n-hexane: amonia(7:2.5:0.5), ethyl acetate: n-hexane (7: 3) and ethyl acetate: n-hexane: methanol(4:6:1). Electronic melting point apparatus (Stuart SMP30) was used for melting points determination. The spectra of the samples were recorded using Attenuated total reflectance -Fourier transform infrared spectroscopy (ATR-FTIR) (Schima-dzu, Japan).^{1}HNMR spectra were obtained on BRUKER model Ultra shield 500 MHz spectro-photometer, Dimethyl sulfoxide (DMSO) used as a solvent.The above-mentioned and other devices will be used in the College of Pharmacy, University of Baghdad / according to availability Except HMR (Iran).

Chemical synthesis : *[12,15,16,20]*

General procedure for the synthesis of substituted (E)-3-(furan-2-yl)-1-phenylprop - 2-en-1-one 1(a-d)

A solution of **substituted acetophenones (0.025mol) (a-Br- 4.9g and b-NO_2- 4.1 g) were separately dissolved in ethanol (10 mL), 2- furaldehyde (0.025mol,2mL) was added to the solution, and the mixture was stirred till getting a homogeneous mixture. Then NaOH 30% (4mL) was added dropwise . The mixture was stirred in an ice bath .The resulting mass was kept in the refrigerator overnight, and on second day the crushed ice was added, neutralized by HCl, filtered and recrystallized by 70% ethanol .**

1a yield 90%, m.p. 69-70ºC, yellow crystals, $C_{13}H_9BrO_2$, **((E)-1-(4-bromophenyl)-3-(furan-3-yl)prop-2-en-1-one), M.W (277.12),** R_f (0.60), FT- **IR: ,** cm^{-1}**: 3109 aromatic (ar)** (C-H) str. vib.**, 3059** (C-H) st vib of CH, 1685,**1651(C=O) st. vib.of α,β unsaturated ketone, 1581 (C=C)st. vib. of α,β unsaturated ketone**, 1562, 1543 ar (C=C) , 1219 (C-O-C) str.vib. and **663 (C-Br) str.vib.**

1b yield 91%, m.p. 131-132ºC, dark yellow crystals, $C_{13}H_9NO_4$, ((E)-3-(furan-2-yl)-1-(4-nitrophenyl) prop -2- en-1-one)**,** M. W (243.22), R_f (0.65), FT-**IR: ,** cm^{-1}: 3143,3124 ar (C-H) str. vib.**, 3086, 3051 (C-H) st vib of CH, 1658** (C=O)st. vib**. of** α,β unsaturated ketone, 1600 (C=C)st. vib, **of α,β unsaturated ketone.**1581, 1546 ar (C=C) st. vib. , 1519 (NO_2) asymmetry, 1338 (NO_2) symmetry, 1269 (C-O-C) st. vib.and 1219 (C-N) str. vib.

General procedure for the synthesis of substituted 3-(furan-2-yl)-5-phenyl-4,5-dihydro-1H-pyrazole 2(a-b)

In a round bottom flask (RB) containing a mixture of different chalcones (0.01mol) (a-Br-2.7g, b-NO_2-2.4g), with hydrazine hydrate (0.01mol,0.5mL) in 20 mL abs. ethanol, and 5 drops of glacial acetic acid (GAA) were refluxed for 8 h The product was filtered and recrystallized with ethanol.**2a** yield 79%, m.p. 100-101ºC, off white crystals, $C_{13}H_{11}BrN_2O$, (5-(4-bromophenyl)-3-(furan-2-yl)-4,5-dihydro-1H-pyrazole), M.W(291.15), R_f (0.7), FT-IR: , cm^{-1}:: 3352 (N-H), 3197, 3124 ar (C-H), 2924, 2843 (C-H) str, vib. of CH_2,1608 (C=N) str. vib.1581 (C=N) str. vib. overlapping with ar (C=C), 1550, 1508 ar (C=C)str vib., 1246 (C-O-C) str, 1145 (C-N) str vib. and 671 (C-Br)str. vib.**2b** yield 81%, m.p. 134-135ºC, beige crystals, $C_{13}H_{11}N_3O_3$**,** (3-(furan-2-yl)-5-(4-nitrophenyl)-4,5-dihydro-1H-pyrazole), M.W (257.25), R_f (0.66), FT-IR: , cm^{-1}: 3332(N-H)str. vib., 3116,3062 ar (C-H)str. vib., 2908, 2885 (C-H) str. vib. of CH_2, 1593(C=N) str. vib.of imine group overlapping with ar (C=C)str. vib., 1554 ar (C=C)str. vib., 1504 (-NO_2)asymmetry, 1338 (-NO_2) symmetry, 1226(C-O-C)str.vib. and 1145 (C-N) str.vib.

Synthesis of 2-chloro-N-substituted-phenyl-acetamide I and II

Substituted anilines (0.05mol)(a-$C_6H_5NH_2$:4.5mL) and (b-4-$NO_2C_6H_4NH_2$:6.9g) were added to a mixture of glacial acetic acid (25 mL) and saturated sodium acetate solution (25 mL), then by drop wise addition of (0.06 mol,4,8mL) chloroacetyl chloride to the mixture. Following stirring the mixture in an ice bath, until the reaction was complete (TLC), the product was filtered and recrystallized with abs ethanol.

I yeild 85%, C_8H_8ClNO, 2-chloro-N-phenylacetamide**,** m.p. 88-90ºC, pretty white crystals, M.W (169.61), R_f (0.65), FT-IR: , cm^{-1}: 3267 (N-H) str. vib., 3143, 3097 ar (C-H) str. vib., 2947 (C-H) str. vib. of CH_2, 1670 amide (C=O) str.vib., 1600, 1554 ar (C=C)str. vib., 1192, 1172 (C-N) str. vib. and 748 (C-Cl)str. vib.

II yeild 90%, $C_8H_7ClN_2O_3$, 2-chloro-N-(4-nitrophenyl)acetamide , m.p 135-137ºC, yellow crystals, M.W (214.61), R_f (0.64), FT-IR: , cm^{-1}: 3275 (N-H) str. vib.. 3105 (ar C-H) str. vib., 2947 (C-H) str. vib. of CH_2, 1681 amide (C=O) str. vib., 1597, 1562 ar (C=C) str. vib., 1511(-NO_2) asym str. vib., 1334(-NO_2) sym str. vib. 1172 (C-N) str. vib. and 748 (C-Cl) str. vib.

Synthesis of N-(substituted phenyl)-2-(3, 5-diphenyl-4,5-dihydro1H-pyrazol-1-yl)acetamide 3(a-d)

Compounds 2(a-d) (0.05mol) (a-Br-14.5g and b-NO_2-12.8g and 2-chloro-N-substitut-ed-phenyl acetamide (0.05mol) (I:H- 8.4g and II: NO_2-11.4g) were refluxed for 4-6 h in the presence of triethylamine (TEA) (0.001ml), and 1,4- dioxane (15 ml), as a solvent. The mixture was then poured into crushed ice. The product was washed with 1% K_2CO_3 and cold water, before being recrystallized with abs.ethanol.

3a yeild 80%, m.p 122-123 ºC, yellow crystal, $C_{21}H_{18}BrN_3O_2$**,** (2-(5-(4-bromophenyl)-3-(furan-2-yl)-4,5-dihydro-1H-pyrazol-1-yl)-N-phenylacetamide), M.W (424.30), R_f (0.65), FT-IR: , cm^{-1}: 3267 (N-H) str. vib. of 2º amide, 3143 ar (C-H)str. vib., 3097 (C-H) str. vib. of CH_2, 1670 (C=O)str. vib. of amide, 1600 ar(C=N)str. vib., 1558, 1504 (C=C)str. vib., 1292, 1249 (C-O-C) str. vib., 1192, 1976 (C-N) str. vib., and 690(C-Br)str. vib

^{1}H NMR (δ ppm) 3.08 (1H,dd, CH_2 of pyrazoline ring), 3.26 (1H, dd, CH_2 of pyrazoline ring), 3.46 (2H, s, methylene group α to C=O of amide group), 4.00(1H, dd, CH of methine group of pyrazoline), 6.21-6.34(1H, m,proton of furan ring), 6.45(1H, d, proton of furan ring), 7.07-7.17 (3H, m, protons of ring A), 7.34(2H, d, protons of ring B), 7.63 (2H, d, protons of ring B) 7.76(2H, d, protons of ring A)7.91(1H, d, proton of furan ring), 10.37(1H, s, proton of amide).

3b yeild 87%, m.p 156-158ºC, dark yellow, $C_{21}H_{18}N_4O_4$, (2-(3-(furan-2-yl)-5-(4-nitrophenyl)-4,5-dihydro-1H-pyrazol-1-yl)-N-phenylacetamide), M.W (390.40), R_f (0.63), FT-IR: , cm^{-1}: 3336 (N-H)str. vib. 2º amide, 3147, 3132 ar(C-H)str. vib., 1670 (C=O)str. vib. of amide, 1600 ar(C=N)str. vib. overlapping with (C=C) str. vib., 1558 ar (C=C)str. vib., 1516 (-NO_2)asym str. vib., 1342 (-NO_2)sym str. vib., 1249, 1192 (C-O-C) str. vib. and 1176, 1107 (C-N) str. vib.

^{1}H NMR (δ ppm) 3.25(1H, dd, CH_2 of pyrazoline ring), 3.63 (1H, dd, CH_2 of pyrazoline ring), 4.35 (2H, s, methylene group α to C=O of amide group), 5.13 (1H, dd, CH of methane group of pyrazoline group), 6.41-6.48 (1H, m,proton of furan ring), 6.59(1H, d, proton of furan ring), 7.04-7.15(3H, m, protons of ring A), 7.34(2H, d, protons of ring B), 7.64 (2H, d, protons of ring A), 7.85(1H, d, protons of furan ring), 8.02(2H, d, protons of ring B), 10.10(1H, s, proton of amide).

3 c yeild 83%, m.p 165-167ºC, dark brown crystals, $C_{21}H_{17}BrN_4O_4$, 2-(5-(4-bromophenyl)-3-(furan-2-yl)-4,5-dihydro-1H-pyrazol-1-yl)-N-(4-nitrophenyl)acetamide, M.W (469.30), R_f (0.61), FT-IR: , cm^{-1}: 3282 (N-H) str.vib.,of 2ºamide, 3163-3105 ar (C-H)str. vib., 2943 (C-H) str. vib. of CH_2, 1678 amide (C=O)str. vib., 1620 ar(C=N)str. vib., 1585-1566 ar(C=C)str. vib., 1504(-NO_2) asym str., 1330(-NO_2) sym str., 1296, 1253 (C-O-C) str. vib., 1172(C-N) str.vib. and 668(C-Br)str. vib.

^{1}H NMR (δ ppm) 3.18 (1H,dd, CH_2 of pyrazoline ring), 3.72 (1H, dd, CH_2 of pyrazoline ring), 3.99(2H, s, methylene group α to C=O of amide group), 4.20 (1H, dd, CH of methane group of pyrazoline in methylene group), 6.22-6.37 (1H, m,proton of furan ring), 6.46(1H, d, proton of furan ring), 7.04(2H, d, protons of ring B),7.51(2H, d, protons of ring B),7.69 (2H, d, protons of ring A), 7.90 (1H, d,proton of furan ring), 8.21 (2H, d, protons of ring A), 10.58 (1H, s, proton of amide).

3d yeild 85%, m.p 160-161ºC, brown crystals, $C_{21}H_{17}N_5O_6$, 2-(3-(furan-2-yl)-5-(4-nitrophenyl)-4,5-dihydro-1H-pyrazol-1-yl)-N-(4-nitrophenyl)acetamide, M.W (435.40), R_f (0.58), FT-IR: , cm^{-1}: 3336 (N-H)str. vib. of 2ºamide, 3147, 3097ar(C-H)str. vib., 2904, 2885 (C-H) str. vib of CH_2, 1670 (C=O)str. vib. of amide, 1597 ar(C=N)str. vib. overlapping with (C=C) str. vib., 1558 ar-(C=C)str. vib., 1504 (-NO_2)asym st, 1338 (-NO_2)sym st, 1288, 1249 (C-O-C) str. vib. and 1188, 1145 (C-N) str. vib.

^{1}H NMR (δ ppm) 3.18 (1H,dd, CH_2 of pyrazoline ring), 3.62(1H, dd, CH_2 of pyrazoline ring), 4.28 (2H, s, methylene group α to C=O of amide group), 5.01(1H, dd, CH of methine group of pyrazoline in methylene group), 6.40-6.47 (1H, m,proton of furan ring), 6.86(1H, d, proton of furan ring), 7.84(1H, d,proton of furan ring), 8.01-8.12(4H, m, protons of ring A and B), 8.29-8.45 (4H, m, protons of ring A and B) and 9.97 (1H, s, proton of amide).

Anti-inflammatory activity (31-34)

The anti-inflammatory activity was evaluated by paw-edema method with egg albumin-induced the effectiveness of synthetic chemicals in reducing rat paw edema in comparison to conventional compounds is the basis for this study.

Method

Six groups of albino rats weighing (160±10 g) were housed in the same location and under standardized conditions. Each group was consisting of 6 rats.

Group A: six animals were injected 3mg/ kg of diclofenac sodium [31,34] intraperitoneally (i.p).

Group B: six animals were injected propylene glycol (50% v/v) as a negative control (i.p).

Groups (C, D, E and F) six animals were administered injection intraperitoneal propylene Glycol suspension of prepared compounds in the doses mentioned in table (1).
The doses of final synthesized compounds were calculated by using the general formula below depending on diclofenac sodium as standard drug.

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

Table 1.Groups and dose calculation

Compound	Mwt	Dose mg/ kg
Control	-	-
Diclofenac	318	3
4a	424.30	4
4b	390.40	3.6
4c	469.30	4.4
4d	435.40	4

All rats receiving subcutaneous injection of (0.05 ml) of undiluted egg-white intra-per-itoneally into their left hind paws to induce inflammation. Paw thickness was measured using a Venier calliper at intervals of 0,30, 60, 120,180, 240, and 300 minutes.To compare mean values and test for statistical significance, the student T test (two samples, assuming equal variances) was used. Standard deviation (SD) of all data collected for this experiment was reported. The ANOVA: two components without replication was used for comparing data from the various groups; a significant result is defined, as a p-value (probability) of less than 0.05.

Antimicrobial & antifungal activity [12,20,35]

The synthetic compounds' antibacterial activity was investigated at the University of Baghdadis/ Girls College of Science, Department of Biology, and Bacterial Preparation Lab. The Well Diffusion Method has been used to perform a preliminary antimicrobal activity.The antmicrobial activity of the synthesized compounds has been investigated in vitro against (*Candida albicans*) fungi and (*Staphylococcus aureus, Streptococcus pyogenes*) as gram positive bacteria and(*Pseudomonas aeruginosa, Escherichia coli*), as gram negative bacteria. Amoxicillin and Ciprofloxacin were chosen as references for antibacterial activity.A reference for the anti-fungal action was fluconazole.The synthesized compounds were studied using (1000 µg/mL) concentration dissolved in dimethyl sulfoxide (DMSO). The zone of inhibitions were measured after 24 h incubation at 37°C.

Results and Discussion

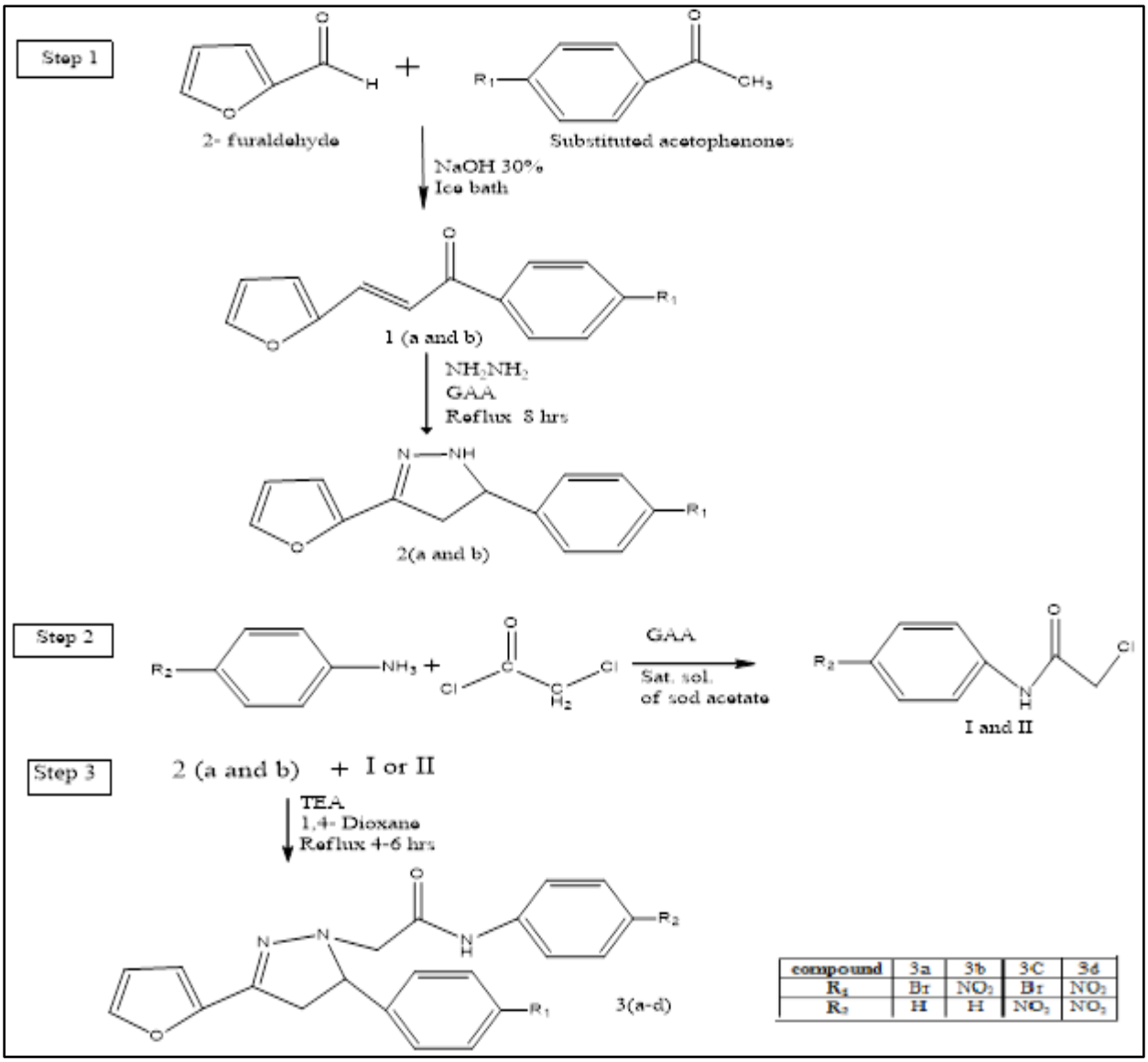
1-Chemical synthesis: [12,15,16,20]

All the steps involved in synthesis of intermediates and targets compound were shown in(Scheme 1). The physical properties for both of intermediates and targets compound and Rf are listed in Table-2. The FT-IR spectral data are given in Table 3. While, 1H-NMR spectra are listed in Table 4. All the steps involved in the synthesis of intermediates and targeted compounds were shown in (Scheme-1).

2-Anti-inflammatory activity [31-34]

In vivo, anti-inflammatory activities of the final synthesized products (3a-d) were assessed using egg-white induced paw edema. The evaluation of the anti-inflammatory activity is based on measuring the decrease of paw thickness and using diclofenac sodium as standard

Six groups of rats weighing (160±10) were used. The tested, standard, and control compounds are injected into rats, which are then subcutaneously injected with 0.05 ml undiluted egg white after 30 minutes. The paw thickness was measured at seven time intervals (0, 30, 60, 120, 180, 240, and 300 minutes). Table 4 and figure-2 show that at 120, 180, 240, and 300 minutes, the standard and tested compounds produced a significant percent reduction ($p \leq 0.05$) in paw edema compared to the control. All synthesized compounds (3a, 3b, 3c, and 3d) showed considerable decrease in paw edema compared to standard diclofenac sodium (3mg/kg) as shown in table 2.

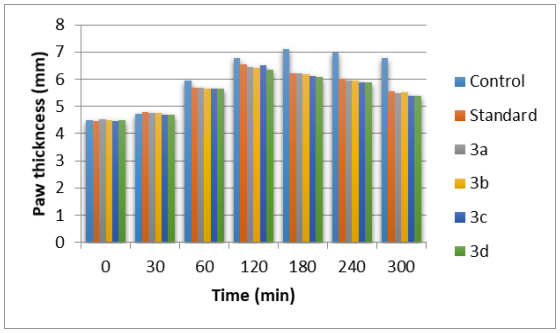


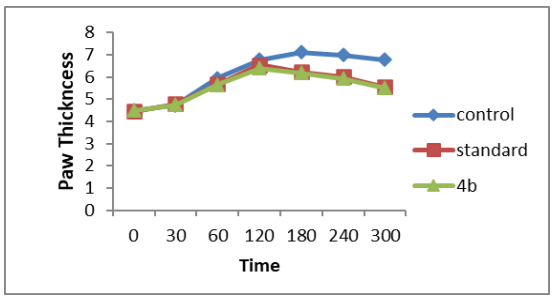
Scheme-1. Synthesis of intermediates and targeted compounds 3(a-d).

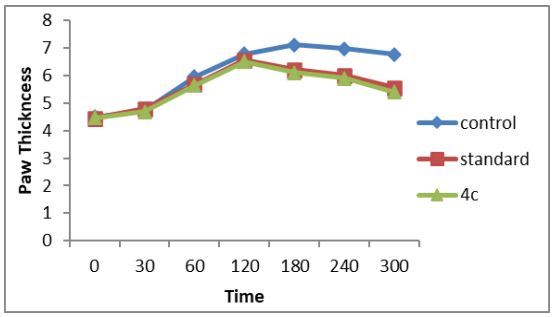
Table 2.Anti-inflammatory activity of the final synthesized compounds (3a-3d) on egg-albumin induced paw edema in rat.

Time (min)	Paw Thickness (mm)					
	Control	Standard	3a	3b	3c	3d
0	4.49±0.06	4.45±0.02	4.51±0.02	4.49±0.01	4.47±0.02	4.49±0.03
30	4.72±0.02	4.79±0.12	4.76±0.06	4.75±0.05	4.68±0.06	4.7±0.04
60	5.95±0.03	5.68±0.04	5.69±0.07	5.66±0.05	5.64±0.02	5.66±0.07
120	6.78±0.05	6.55±0.02*	6.44±0.04*	6.40±0.04*	6.50±0.01*	6.34±0.02*
180	7.11±0.03	6.22±0.01*	6.20±0.02*	6.19±0.02*	6.10±0.05*[a]	6.12±0.02*[a]
240	6.98±0.02	6.01±0.01*	5.95±0.02*	5.94±0.01*	5.90±0.03*[b]	5.80±0.01*[b]
300	6.77±0.11	5.55±0.02*	5.55±0.01*	5.51±0.03*	5.39±0.07*[b]	5.38±0.03*[b]

Different testing groups' non-identical superscripts (a, b) are evaluated as significantly different ($p \leq 0.05$).Data are expressed as mean ± SEM of mm paw thickness , n= number of animal ,time (0) is time of injection of tested compounds time (30) nim is time of injection of egg-white (induced of paw edema) , *significantly different with control ($p \leq 0.05$)







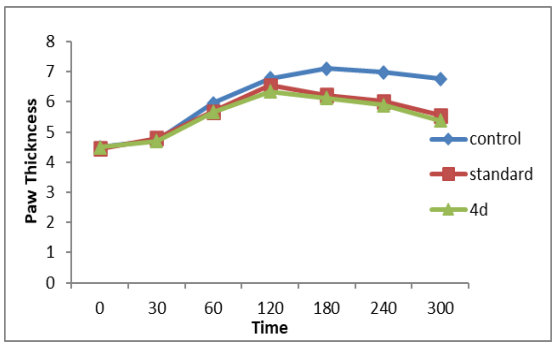


Figure2. Curves illustrates anti-inflammatory activities of final compounds in comparison with control and standard

3-Antimicrobial & antifungal activities. *(12,20,35)*

The final synthesized compounds were tested against Gram-negative bacteria of Escherichia coli and Pseudomonas aeruginosa, and Gram-positive bacteria of Staphylococcus aureus and Streptococcus pyogenes, using Ciprofloxacin and Amoxicillin, as references for antibacterial activity and dimethyl sulfoxide (DMSO) as a solvent. Table 6 depicts the millimeter-measured zone of inhibition. As shown in table-5 and figure-3, the final synthesized compounds **3a-3d** displayed superior antibacterial activity against Gram -ve bacteria over Gram +ve bacteria. Compound **3c** was the most potent against Gram -ve bacteria.

Antifungal activity

The anti-fungal activity of the final compounds against Candida albicans was evaluated using Fluconazole as a reference, DMSO as a solvent and control, and the well diffusion method. Table-6 depicts the zone of inhibition (mm). Figure-3 shows that the compounds **3a,3b,** and **3d** had good antifungal activity at a concentration of 1000 µg/mL.

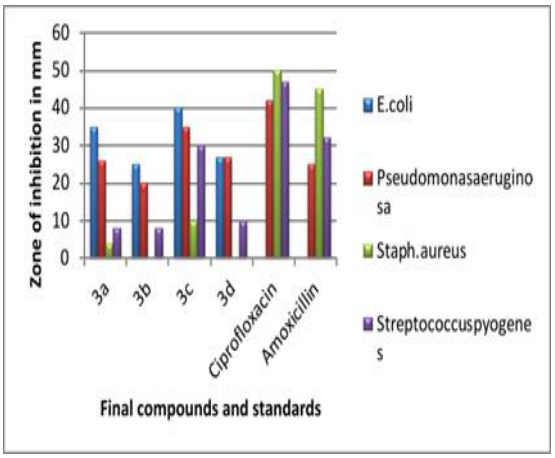


Figure 3. Antibacterial activity of final compounds and standard

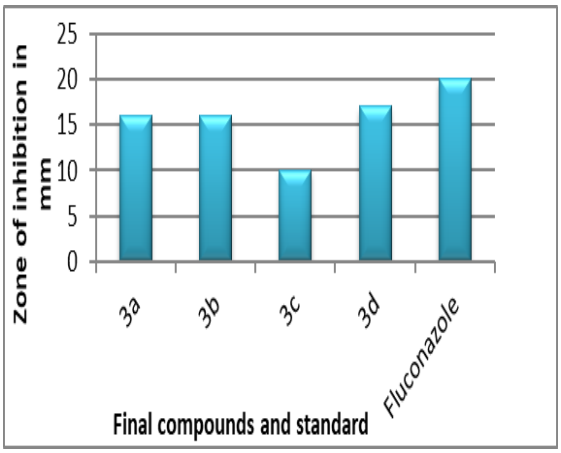


Figure 4. Antifungal activity of final compounds and standard

Table 3.Antimicrobial activity as of final compounds as Inhibition zone

Compound	Conc. µg/ml	Zone of inhibition in mm				
		Gram negative		Gram positive		
		E. coli	*Pseudomonas aeruginosa*	*Staph. aureus*	*Streptococcus pyogenes*	*Candida albicans*
3a	10^3	35mm	26mm	4mm	8mm	16mm
3b	10^3	25mm	20mm	-	8mm	16mm
3c	10^3	40mm	35mm	10mm	30mm	10mm
3d	10^3	27mm	27mm	-	10mm	17mm
Ciprofloxacin	10^3	25mm	42mm	50mm	47mm	-
Amoxicillin	10^3	20mm	25mm	45mm	32mm	-
Fluconazole	10^3	-	-	-	-	20mm
DMSO	Control & solvent	0	0	0	0	

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). [21]

Conclusion

New 4,5-dihydro-1H-pyrazole derivatives 3(a-d) were successfully synthesized and characterized by spectral data (FTIR and 1HNMR) and the structures were consistent with the data.

The anti-inflammatory activity of the target compound 3(a-d) was evaluated using egg white induced edema method. All synthesized compounds show effect comparable to the standard (Diclofenac), in reducing paw edema in rats especially 3a and 3b which substitutions with (Br and NO_2) respectively were seemed significant anti-inflammatory effect.

All the target compound have been evaluated for their antibacterial activity against four test organisms :(***Staphylo-coccus aureus, Streptococcus pyogenes***) as gram positive bacteria and(***Pseudomonas aeruginosa, Escherichia coli***) and antifungal activity against Candida albicans by Well Diffusion Method. The results of this study have been compared by taking Amoxicillin and Ciprofloxacin as references. The compounds 3(a-d) substituted with electron withdrawal groups (Br and NO_2) were showed potent antibacterial activity against Gram -ve bacteria more than towards Gram + ve bacteria.

Also the result data of antifungal activity showed that 3a, 3b and 3d compounds which substituted with (Br and NO_2) were proved potent antifungal activity against Candida albicans. The results of antifungal evaluation have been compared by using fluconazole as a reference.

Competing of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appesred 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.

Authors Contribution

Both authors contribute 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. Aa well as conducting antimicrobial and ant-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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