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RESEARCH ARTICLE
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Synthesis and Preliminary Pharmacological Evaluation of New Pyrazoline Derive from Different Heterocyclic Aldehydes

Shaymaa Kanaan K.[1]*, Tagreed N-A Omar[2]

[1,2]Department of Pharmaceutical Chemistry, College of Pharmacy, Baghdad University, Baghdad, Iraq

***Corresponding author:** Shaymaa Kanaan K, Department of Pharmaceutical Chemistry, College of Pharmacy, Baghdad University, Baghdad, Iraq

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ABSTRACT

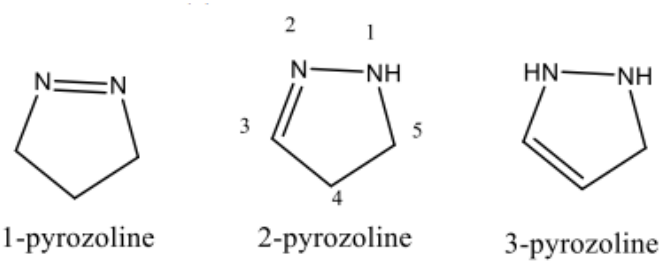
Pyrazolines, have prominent importance among various types of molecular targets attributed to their broad spectrum of pharmacological activities such as antimicrobial, anti-inflammatory, antioxidant, antiviral, anticancer activities,…. etc. in the present study a series of substituted chalcones 1(a-e) has been synthesized according to Claisen-Schmit condensation method, then by the reaction of them with hydrazine hydrate give substituted pyrazolines 2(a-e) which later react with different aromatic amines to get the final amide. All the synthesized compounds are checked by the TLC and further analyzed by the FTIR and 1HNMR and screened for their anti-inflammatory ,antibacterial and antifungal activities. All compounds showed good anti-inflammatory activity especially compounds (3a), compounds (3a) showed good antibacterial activity against gram negative bacteria, compounds (3b, 3c, 3d and 3e) showed good antibacterial activity against gram positive bacteria and compounds (3d and 3e) showed good antifungal activity.

Keywords: *chalcones, pyrazolines, nitrogen heterocyclic compounds, anti-inflammatory, antibacterial, antifungal*

INTRODUCTION

Heterocyclic compounds exhibit numerous biological activities, drawing attention to these compounds which have different heteroatoms within their structures (sulphur, oxygen, or nitrogen). Furan-2-carbaldehyde (furfural) is a heterocyclic compound whose reactivity belongs to the aldehyde functional group. Chalcone of furfural $C_5H_{12}O$ (1,3-diaryl-2-propen-1-one) has two stereochemistry forms cis and trans. Claisen-Schmidt condensation is the most popular method for the synthesis of chalcone(1).

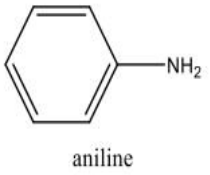
Chalcones are precursors for many biosynthesized molecules with biological importance, furthermore, chalcone's derivative Pyrazoline $C_3H_6N_2$ is a five-membered heterocyclic compound that has two adjacent nitrogen atoms with three carbon atoms in its ring with one endocyclic double bond, pyrazoline has three isomers (Figure -1) which tautomerize one into another1, 2-pyrazoline is the most stable one(2).



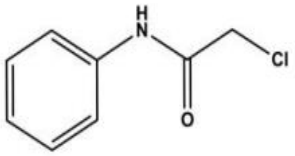
(Figure -1) of pyrazoline ring structure

Pyrazoline and its derivatives show a variety of useful biological activities such as anticonvulsant, anticancer(3) , antibacterial(4), analgesic(5), anti-inflammatory(6)'(7)'(8), antifungal(9), antidepressant(5), antimicrobial(5), antioxidant(10), radiosensitizing agents (11),antiviral(5) and monoamine oxidases (MAOs)… etc. So pyrazolines are regarding as important central core in organic synthesis, they are building blocks for molecules biologically potent.

Aniline C_6H_7N is aromatic amine has a basic behavior belong to lone pair of electrons on its nitrogen atom(12), (figure-2), a variety of chemical and pharmaceutical synthesis use aniline derivatives as useful building blocks as antitumor(13), antifungal(14), antibacterial(15) and as treatment in heart failure(16). In our work 2-chloro-N- phenylacetamide (17) is synthesized and then, it is undergo to bind with pyrazoline to get the final products the pharmacological activities are evaluated as antibacterial, antifungal and anti-inflammatory (figure-3)



(figure-2) aniline



(Figure-3) 2-chloro-N-phenylacetamide'(17)'

METHODS AND MATERIALS

Melting points were determined by Electronic melting point apparatus (Stuart SMP30). Schimadzu, Japan were used for the IR spectra of the compounds. 1H-NMR spectra were obtained on BRUKER model Ultra shield 500 MHz spectrophotometer The reactions were monitored by thinlayerchromatography (TLC), the mobile phase solvent system sused are n- hexane : ethyl acetate (2:0.5).

General synthesis of chalcones 1(a-e)

Different substituted acetophenones (0.025mole) (a- Cl- 3,3 ml, b- CH_3- 3.3 ml, c- OCH_3- 3.7gm, d- NH_2- 3.3gm, e- OH-3.4 gm) were dissolved in 10 ml ethanol, 2- furaldehyde (furfural) (0.025mole,2ml) was added to the solution, and the mixture was stirred for 15 minutes to get a homogeneous mixture. Then sodium hydroxide 30% (4ml)was added drop by drop. The mixture was stirred in an ice bath until it solidified. The resulted mass was kept in the refrigerator overnight then crushed ice was added and neutralized by 5% HCl, then filtered and recrystallized by 99% ethanol(18).

1a yelid 80%, m.p. 65-66ºC, light yellow crystals, $C_{13}H_9ClO_2$, ((E)-1-(4-chlorophenyl)-3-(furan-2-yl)prop-2-en-1-one), m.wt (232.66), Rf (0.65), FT-IR: , cm^{-1}: 3136 ar (C-H) str. vib., 3059 (C-H) st vib of CH, 1654 (C=O)st.vib. α,β unsaturated ketone,, 1589 (C=C) st.vib. of α,β

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unsaturated ketone, 1570,1546 (ar C=C) st.vib.,1222 (C-O-C)str. vib. and 736 (C-Cl) str. vib.

1b yield 70%, m.p.59-64ºC, beige crystals, $C_{14}H_{12}O_2$, ((E)-3-(furan-2-yl)-1-(p-tolyl)prop-2-en-1-one), m.wt (212.25), Rf (0.7), FT-IR: , cm^{-1}: 3151 ar (C-H) str. vib., 3051 (C-H) st vib of CH, 3035(CH) str. of CH_3, 1651 (C=O)st. vib.of α,β unsaturated ketone, 1593 (C=C) st. vib. of α,β unsaturated ketone, 1570,1550 ar (C=C) st. vib. and 1226 (C-O-C)str. vib.

1c yield 85%, m.p. 60-61ºC, beige crystals, $C_{14}H_{12}O_3$, ((E)-3-(furan-2-yl)-1-(4-methoxyphenyl)prop-2-en-1-one), m.wt. (228.25), Rf (0.69), FT-IR: , cm^{-1}:: 3132 ar (C-H) str. vib., 2970 (C-H) st vib of CH, 2920, 2843(C-H) str. of CH_3, 1654 (C=O) st. vib. of α,β unsaturated ketone , 1600, 1589 (C=C) str. vib. of α,β unsaturated ketone, 1550, 1508 ar (C=C) str. vib. and 1253, 1222 (C-O-C) st. vib.

1d yield 80%, m.p. 97-100ºC, golden yellow greenish crystals, $C_{13}H_{11}NO_2$, ((E)-1-(4-aminophenyl)-3-(furan-2-yl)prop-2-en-1-one), m.wt. (213.22), Rf (0.70), FT-IR: , cm^{-1}: 3417, 3348 (N-H)asymmetry st. vib., 3224 (N-H) symmetry str. vib., 3136 ar (C-H) str. vib., 3035 (C-H) st vib of CH,1631 (C=O)st. vib. of α,β unsaturated ketone, 1600 (C=C) st. vib, of α,β unsaturated ketone, 1577, 1539 ar (C=C) str. vib., 1280, 1234 (C-O-C) str. vib., and 1176 (C-N)st. vib.

1e yield 75%, m.p. 128-130ºC, golden crystals, $C_{13}H_{10}O_3$, ((E)-3-(furan-2-yl)-1-(4-hydroxyphenyl)prop-2-en-1-one), m.wt. (214.22), Rf (0.64), FT-IR: , cm^{-1}: 3209 phenolic OH stretching , 3128 ar(C-H) st. vib, 3082 (C-H) st. vib. of CH, 1643 (C=O) st. vib. of α,β unsaturated ketone, 1597 (C=C) st. vib. of α,β unsaturated ketone, 1570, 1550, 1512 (ar C=C) st. vib., 1384 (OH) bending and 1276, 1226 (C-O-C)st. vib.

General synthesis of Pyrazolines 2(a-e)

Chalcone (0.01mol)(a- Cl- 2.3gm, b- CH_3- 2.1gm, c- OCH_3- 2.2gm, d- NH_2- 2.1gm, e- OH- 2.2gm), hydrazine hydrate (0.01mol,0.5ml) was mixed in 20 ml ethanol 99%, 5-8 drops of glacial acetic acid were added, then the mixture was refluxed for 8 hrs at 50 ºC, cooled, and poured over ice water. The solid separated was filtered, washed with distilled water (DW), dried, and recrystallized from ethanol, Yield:80.50%. The reaction monitored by TLC, using n-hexane: ethyl acetate (2:0.5)as a solvent system (19)'(20).

2a yield 64%, m.p. 142-149ºC, beige crystals, $C_{13}H_{11}ClN_2O$, (5-(4-chlorophenyl)-3-(furan-2-yl)-4,5-dihydro-1H-pyrazole), m.wt. (246.69), Rf (0.65), FT-IR: , cm^{-1}: 3352 (N-H)str.vib., 3255, 3205 ar(C-H) str. vib., 2972 (C-H) str, vib. of CH_2, 1585 (C=N) str of imine group overlapping with ar (C=C)str. vib., 1554, 1508 ar (C=C)str. vib., 1249 (C-O-C) str, 1149 (C-N) str. vib. and 736 (C-Cl) str. vib.

2b yield 50%, m.p. 141-142ºC, beige crystals, $C_{14}H_{14}N_2O$, (3-(furan-2-yl)-5-(p-tolyl)-4,5-dihydro-1H-pyrazole), m.wt. (226.28), Rf (0.65), FT-IR: , cm^{-1}: 3348 (N-H)str. vib., 3105 ar (C-H) str. vib., 3032,2962,2846 (C-H)str of CH_2 and CH_3 str. vib., 1612 (C=N) str. vib.of imine group, 1589 (C=N) str of imine group overlapping with ar (C=C)str. vib., 1558, 1516 ar (C=C)str.vib., 1226 (C-O-C) str. vib. and 1149 (C-N) str. vib.

2c yield 70%, m.p. 124-125ºC, beige crystals, $C_{14}H_{14}N_2O_2$, (3-(furan-2-yl)-5-(4-methoxyphenyl)-4,5-dihydro-1H-pyrazole), m.wt. (242.28), Rf (0.7), FT-IR: , cm^{-1}: 3329 (N-H)str.vib., 3132, 3113 (ar C-H) str.vib., 3001, 2962,2839 (C-H) str of CH_2 and CH_3, 1604, (C=N)str.vib.of imine group overlapping with ar (C=C)str. vib., 1562, 1516 ar(C=C) str.vib., 1346 (C-H) of CH_3 str.bending, 1257 (C-O-C) str.vib. and 1172 (C-N)str.vib.

2d yield 60%, m.p. 122-124ºC, dark yellow crystals, $C_{13}H_{13}N_3O$, (4-(3-(furan-2-yl)-4,5-dihydro-1H-pyrazol-5-yl)aniline, m.wt. (227.27), Rf (0.6), FT-IR: , cm^{-1}: 3456, 3317(N-H)str. vib., 3209, 3093 ar (C-H), 2962 (C-H) str. vib. of CH_2, 1639, 1604 (C=N) of imine group overlapping with ar (C=C)str. vib., 1585, 1519 ar (C=C), 1284 (C-O-C)str.vib.and 1130 (C-N)str. vib.

2e yield 40%, m.p. 138-140ºC, beige crysrals, $C_{13}H_{14}N_2O_2$, (4-(3-(furan-2-yl)-4,5-dihydro-1H-pyrazol-5-yl)phenol), m.wt. (228.25), Rf (0.6), FT-IR: , cm^{-1}: 3321 (N-H) overlapping with (O-H) str.vib., 3174, 3120 ar (C-H)st. vib., 2943, 2889 (C-H) str. vib. of CH_2, 1600(C=N) str. vib.of imine group overlapping with ar (C=C)str. vib., 1581, 1512 ar (C=C) str. vib., 1230 (C-O-C) str. vib. and 1145 (C-N)str. vib.

Synthesis of 2-chloro-N-substituted-phenyl-acetamide(21)

A mixture of glacial acetic acid (25 ml) and (25 ml) of a saturated solution of sodium acetate, aniline ($C_6H_5NH_2$0.05mol, 4.5ml) were added. (0.06 mol,4,8ml) chloro- acetyl chloride was added drop by drop to the mixture which then stirred in an ice bath, until the constitution of a white mass product, the product was filtered and washed with 50% aqueous acetic acid, then it was washed with DW.

2-chloro-N-phenylacetamide, C_8H_8ClNO, yeild 85%, m.p. 88-90ºC, pretty white crystals, m.wt. (169.61), Rf (0.65), FT-IR: , cm^{-1}: 3205 (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.

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

3,5-diphenyl-4, 5-dihydro-1H-pyrazole (a- Cl-12.3gm, b- CH_3- 11.3gm, c- OCH_3- 12.1gm, d-NH_2-11.3 gm, e- OH- 11.4gm) (0.05mol), 2-chloro-N-substituted-phenyl acetamide (H-8.4gm) (0.05mol) and triethylamine(TEA) (0.005 ml) in 1,4- dioxane (15 ml) were refluxed for 4-6 hours in 50-60 ºC. Then the mixture was poured into crushed ice. The product was washed with 10% K_2CO_3 and then it was washed with cold water(21).

For final synthesis compounds 3(a-e) percent yield, physical data and FT-IR characteristic absorption bands, 1 H NMR are given below.

3a yeild 70%, m.p 99-100 ºC, light yellow crystals, (2-(5-(4-chlorophenyl)-3-(furan-2-yl)-4,5-dihydro-1H-pyrazol-1-yl)-N-phenylacetamide), m.wt. (379.84), Rf (0.55), FT-IR: , cm^{-1}: 3267 (N-H) str. vib. of 2º amide, 3143, 3097 ar (C-H)str. vib., 2947 (C-H) str. vib. of CH_2, 1670 (C=O)str. vib. of amide, 1600 ar(C=N)str. vib., 1558, 1500 ar(C=C)str. vib., 1292, 1249 (C-O-C) str. vib., 1192 , 1176 (C-N) str. vib., and 748 (C-Cl)str. vib.1 H NMR (δ ppm) 3.01-3.10(1H,dd, CH_2 of pyrazoline ring), 3.42-3.52 (1H, dd, CH_2 of pyrazoline ring), 3.77 (2H, s, methylene group α to C=O of amide group), 3.97-4.04(1H, dd, CH of methine group of pyrazoline), 6.41-6.51(1H, m,proton of furan ring), 6.66(1H, d, proton of furan ring), 7.02-7.14(3H, m, protons of ring A), 7.34(2H, d, protons of ring B), 7.45 (2H, d, protons of ring B) 7.67(2H, d, protons of ring A)7.97(1H, d, proton of furan ring), 10.36(1H, s, proton of amide).

3b yeild 65%,m.p 99-100ºC, beige crystal, $C_{22}H_{21}N_3O_2$, (2-(3-(furan-2-yl)-5-(p-tolyl)-4,5-dihydro-1H-pyrazol-1-yl)-N-phenylacetamide), m.wt. (359.43), Rf (0.45), FT-IR: , cm^{-1}: 3120 (N-H) str. vib. of 2º amide, 3032 ar (C-H)str. vib., 2920, 2885 (C-H)str of CH_2 and CH_3, 1678 (C=O)str. vib. of amide, 1604 ar(C=N)str. vib., 1550, 1504 ar(C=C)str. vib., 1280, 1226 (C-O-C)str.vib. and 1180, 1149 (C-N) str. vib.

1 H NMR (δ ppm) 3.01(3H, s, CH_3-Ring B), 3.12-3.22 (1H,dd, CH_2 of pyrazoline ring), 3.52-3.57 (1H, dd, CH_2 of pyrazoline ring), 3.71 (2H, s, methylene group α to C=O of amide group), 4.75-4.84(1H, dd, CH of methine group of pyrazoline), 6.27-6.34(1H, m, proton of furan ring), 6.55(1H, d, proton of furan ring), 6.80 (2H, d, protons of ring B), 7.06 (2H, d, protons of ring B), 7.21-7.42(3H, m, protons of ring A), 7.61 (2H, d, protons of ring A), 7.73(1H, d, proton of furan ring) and 10.17(1H, s, proton of amide).

3c yeild 75%,m.p 106-107ºC, beige crystals, $C_{22}H_{21}N_3O_3$, (2-(3-(furan-2-yl)-5-(4-methoxyphenyl)-4,5-dihydro-1H-pyrazol-1-yl)-N-phenylacetamide), m.wt. (375.43), Rf (0.4), FT-IR: , cm^{-1}: 3267 (N-H)str. vib. of 2º amide , 3205, 3143 ar(C-H)str. vib., 3097 (C-H) str of CH_3, 1670 (C=O)str. vib. amide, 1600 ar(C=N) str. vib., 1558, 1512, 1500 ar(C=C) str. vib., 1342 (C-H) of CH_3 str. vib., 1292, 1249 (C-O-C) str. vib.and 1172 (C-N)str. vib.

1 H NMR (δ ppm) 3.01-3.4.12(1H,dd, CH_2 of pyrazoline ring), 3.26-3.30 (1H, dd, CH_2 of pyrazoline ring), 3.50(3H, s, CH_3-Ring B), 3.67 (2H, s, methylene group α to C=O of amide group), 3.73-4.78(1H, dd, CH of methine group of pyrazoline in methylene group), 6.32-6.42(1H, m,proton of furan ring), 6.86(1H, d, proton of furan ring), 7.97 (2H, d, protons of ring B), 7.26 (2H, d, protons of ring B),7.43- 7.54(3H, m, protons of ring A), 7.75(2H, d, protons of ring A)7.95(1H, d, proton of furan ring) 10.35(1H, s, proton of amide).

3d yeild 75%, m.p 112-114ºC, yellow greenish crystals, $C_{21}H_{20}N_4O_2$, (2-(5-(4-aminophenyl)-3-(furan-2-yl)-4,5-dihydro-1H-pyrazol-1-yl)-N-

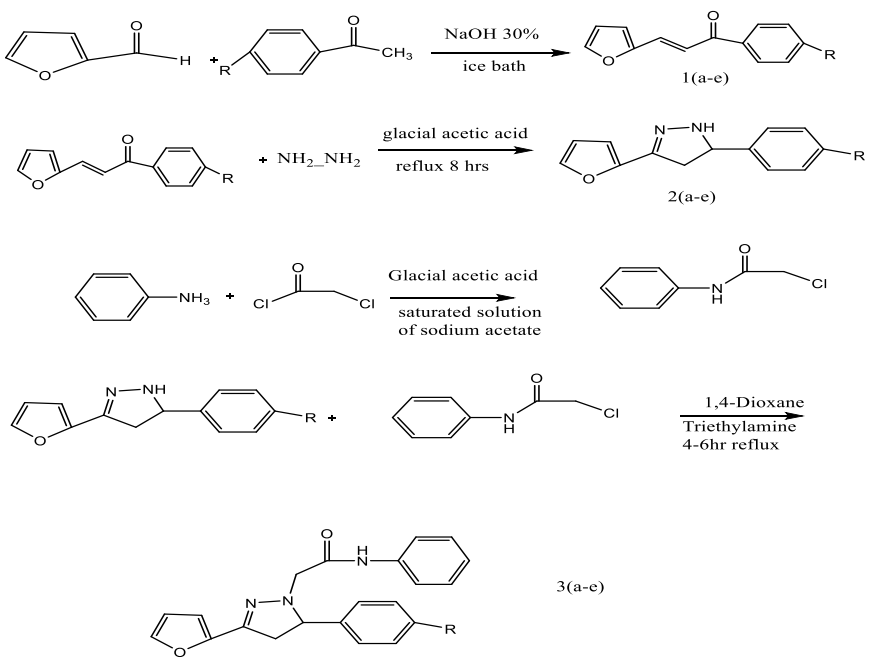
phenylacetamide), m.wt. (360.42), Rf (0.55), FT-IR: , cm^{-1}: 3456, 3317 (N-H) str. vib., 3093 ar(C-H) str. vib., 2947, 2885 (C-H) str. vib. of CH_2, 1639 (C=O)str. vib.of amide, 1604 ar (C=N)str. vib., 1585, 1519 ar (C=C) str. vib. and 1284, 1249 (C-O-C) str. vib. and 1184, 1149 (C-H) str. vib.

1 H NMR (δ ppm) 2.94-3.00(1H,dd, CH_2 of pyrazoline ring), 3.14-3.17 (1H, dd, CH_2 of pyrazoline ring), 3.30(2H, s, methylene group α to C=O of amide group), 3.50-3.53(1H, dd, CH of methane group of pyrazoline group), 4.50(2H, d, 2protons of amine), 6.06(2H, d, protons of ring B), 6.35-6.38 (1H, m,proton of furan ring), 6.66(1H, d, proton of furan ring), 7.05(2H, d, protons of ring B), 7.20-7.29(3H, m, protons of ring A),7.53(2H,d protons of ring B), 7.73(1H, d, proton of furan ring), 10.15(1H, s, proton of amide).

3e yeild 70%, m.p 95-97ºC, golden crystals, $C_{21}H_{19}N_3O_3$, (2-(3-(furan-2-yl)-5-(4-hydroxyphenyl)-4,5-dihydro-1H-pyrazol-1-yl)-N-phenylacetamide), m.wt. (361.40), Rf (0.53), FT-IR: , cm^{-1}: 3267, 3205 (N-H) str. vib.of 2º amide, 3143,3101 ar (C-H)st. overlapping with (O-H), 2993, 2951 (C-H) str of CH_2, 1670 (C=O)str. vib. of amide, 1600 ar (C=N) str. vib., 1554, 1512 ar (C=C)str. vib., 1273, 1249 (C-O-C) str. vib., and 1168 (C-N) str. vib.

1 H NMR (δ ppm) 3.14-3.21(1H,dd, CH_2 of pyrazoline ring), 3.46-3.53 (1H, dd, CH_2 of pyrazoline ring), 3.86 (2H, s, methylene group α to C=O of amide group), 5.32-5.40(1H, dd, CH of methane group of pyrazoline group),6.43-6.50(1H, m, proton of furan ring), 6.81 (2H, d, aromatic proton of ring B) 6.93(1H, d, proton of furan ring), 7.08 (2H, d, protons of ring B), 7.30(2H, d, protons of ring A), 7.49 (3H, d, protons of ring A), 7.67 (1H, d,proton of furan ring), 9.75(1H, s, proton of hydroxide), 10.12(1H, s, proton of amide).

Scheme



R	a- Cl	b- CH_3	c- OCH_3	d- NH_2	e- OH

RESULTS AND DISCUSSION

Chemistry

Chalcones 1(a-e) were synthesized by Claisen-Schmitt condensation through reacting furan with substituted acetophenones in ethanol with NaOH 30% solution. Chalcones were characterized FTIR that shows appearance of C=O stretching at (1658- 1647). Pyrazolines 2(a-e) were synthesized by refluxing of each chalcone with hydrazine hydrate in ethanol with drops of galacial acetic acid characterized by FTIR by appearance of all bands of C=N stretching at(

1585-1612), the two another intermediates (2-chloro-N-substituted-phenyl-acetamide)were synthesized by reaction of aniline with chloro acetyl chloride characterized by FTIR that shows appearance of C=O stretching at (1670-1681), The final compounds 3(a-e) were synthesized by refluxing the pyrazolines with 2-chloro-N-substituted-phenyl-acetamide in 1,4-dioxan and using of TEA, amide formation were characterized by FTIR that shows appearance of C=O stretching at (1639-1678) and ^{1}HNMR by appearance of 10.37 (1H , s, NH Proton of amide group). The appearance of the pair of doublet of doublet of methylene Protons of pyrazoline and the methine appeared as a doublet of doublet due to vicinal coupling with two magmatically nonequivalent protons of pyrazoline's methane.

Anti-inflammatory activity

A lot of synthesized Pyrazoline derivatives were proved that they have anti-inflammatory effect(22), the final synthesized compounds 3(a-e) were tested to evaluate their anti-inflammatory activity with diclofenac sodium as standard , This done by paw - edema method (egg-albumine induced acute odema), using eleven groups of rats weighing (170±10 g). They injected the tested, standard, and control compounds, then they injected with 0.05 ml undiluted egg white intraperitoneally by subcutaneous injection after 30 minutes. Determing the paw thickness decreasing was at seven time intervals (0, 30, 60, 120, 180, 240 and 300 min) . At time 120, 180, 240 and 300 min the standard and the tested compounds produced significant percent reduction ($p \leq 0.0001$) in paw edema compared to control, as in table 1.

In this research, the injection of tested compounds resulted in varying degrees of reducing inflammation. All synthesized compounds showed reduction in paw thickncess compared with 50% v/v propylene glycol (control group). The compounds (3b, 3c 3d and 3e) showed reduction of the paw edema closed to the diclofenac sodium (standard)(3mg/kg)., while the compounds (3a) produced a considerable decrease in paw edema in relation to diclofenac sodium (standard).

All data gathered for this work were expressed as the mean SD (standard deviation), the findings were examined for statistical significance by using the student T test (two sample assuming equal variances) in order to compare between mean values. ANOVA: Two factors without replication was used to compare data from the different groups, a significant value was defined as a P value (probability) of less than 0.05.

TABLE 1: Anti-inflammatory activity of final synthesized compounds on egg-albumin induced paw edema in rat.

Time (min)	paw thickness (mm)						
	Control	Standard	3a	3b	3c	3d	3e
0	4.49±0.06	4.45±0.02	4.48±0.02	4.49±0.01	4.46±0.02	4.47±0.03	4.46±0.01
30	4.72±0.02	4.79±0.12	4.72±0.05	4.75±0.05	4.8±0.03	4.77±0.03	4.8±0.05
60	5.95±0.03	5.68±0.04	5.63±0.09	5.68±0.02	5.72±0.01	5.7±0.07	5.65±0.03
120	6.78±0.05	6.55±0.02*	6.34±0.10*	6.58±0.01*	6.2±0.02*	6.71±0.03*	6.56±0.05*
180	7.11±0.03	6.22±0.01*	6.12±0.03*	6.2±0.04*	6.42±0.05*	6.31±0.01*	6.22±0.04*
240	6.98±0.02	6.01±0.01*	5.95±0.04**	6.03±0.01*	6.22±0.04*	6.12±0.05*	6.02±0.01*
300	6.77±0.11	5.55±0.02*	5.41±0.07**	5.54±0.03*	5.58±0.04*	5.6±0.05*	5.51±0.01*

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 ($p \leq 0.05$)
**significantly different with diclofenac sodium ($p \leq 0.05$)

Microbiology

The newly synthesized compounds 3(a-e) were tested for their antifungal activity against Candida albicans and antibacterial activity by

well diffusion assy, against gram negative bacteria of Escherichia coli and Pseudomonas aeruginosa; Staphylococcus aureus and Streptococcus pyogenes, as a gram positive bacteria and, Ciprofloxacin and Amoxicillin, was used as references for antibacterial activity, dimethyl sulfoxide (DMSO) was used as solvent, The zone of inhibition illustrated in table 2 was measured by millimeter.

Antibacterial activity

The results shows that the anti-microbial assessment of the final synthesized compounds with the incorporation of electron-donating groups NH_2 and OH display more activity to gram (-ve) bacteria while the compounds incorporate than the electron-withdrawing groups Cl , NO2 display more activity against gram (+ve) bacteria. When Inhibition zone (more than15mm), the synthesized compound is considered highly active, when Inhibition zone in between (10-15 mm) the compound considers moderately active, while when Inhibition zone in between (5-10 mm) the compound considers slightly active, and inactive when inhibition zone (less than 5)(23).

Antifungal activity

By using well diffusion method, the anti-fungal activity evaluation of the final synthesized compounds was done against candida albicans and Fluconazole was used as reference and the DMSO was used asa solvent and control. The table-2 illustrates the zone of inhibition. The compounds 3a,3e, 3g and 3i show a good anti-fungal activity in dose 1000 µg/Ml.

TABLE 2: Inhibition zone of final compounds

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	32mm	27mm	25mm	12mm
3b	10^3	15	7mm	10mm	15mm	13mm
3c	10^3	25mm	12mm	25mm	22mm	10mm
3d	10^3	20	21mm	30	29mm	15mm
3e	10^3	26mm	25mm	33mm	30mm	17mm
Ciprofloxacin	10^3	-	42mm	50mm	47mm	-
Amoxicillin	10^3	-	25mm	45mm	32mm	-
Fluconazole	10^3	-	-	-	-	20mm
DMSO	Control & solvent	0	0	0	0	

CONCLUSION

1-The chemical synthesis of a new pyrazoline linked to2-chloro-N-phenylacetamide compounds has been achieved successfully. 2-Physical properties (melting point and description), FT-IR, 1H-NMR spectra have been checked for the identification and characterization of the synthesized compounds and the results confirm their chemical structure. 3- While compounds 3b, 3c, 3d and 3e showed anti-inflammatory activity similar to diclofenac sodium (3mg/kg), compounds 3a showed significant anti-inflammatory efficacy when compared to diclofenac sodium, in vivo anti-inflammatory evaluation of all investigated compounds and the reference medication diclofenac sodium generated a considerable reduction of paw thickness in comparison with the that of propylene glycol 50%v/v (control group). 4- The anti-microbial investigation of the final synthesized compounds with the incorporation of electron-donating groups NH_2 and OH shown more inhibition activity to gram (+ve) bacteria while the compound incorporate the electron-withdrawing groups Cl display more inhibition activity against gram (-ve) bacteria. 5-The antifungal activity assessment of the final synthesized compounds with the incorporation of electron-donating groups NH_2 and OH display little more inhibitory effect against candida albicans in compared with electron-withdrawing group Cl.

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