

Synthesis, Characterization and Preliminary Study of the Anti-Inflammatory Activity of New Pyrazoline Containing Ibuprofen Derivatives

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Abstract

New ibuprofen derivatives containing variously substituted pyrazoline have been synthesized, characterized by FT-IR and ¹H-NMR spectroscopy and evaluated preliminarily for their anti-inflammatory activity. In vivo anti-inflammatory activity of the final products (5a-f) was studied in rats using egg-white induced edema method of inflammation. All of them showed anti-inflammatory activity compared to the control group (propylene glycol) except compound 5f that showed more anti-inflammatory activity than ibuprofen.

Keywords: Ibuprofen, Pyrazoline, Anti-inflammatory

تخليق و تشخيص ودراسة اولية للفعالية المضادة للالتهاب لمشتقات جديدة للايوبروفين الحاوية على البيرازولين

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الخلاصة

تم تخليق مشتقات جديدة للايوبروفين تحتوي بيرازولين معوض بمجاميع مختلفة، و قد تم تشخيصها بمطياف الاشعة تحت الحمراء و مطياف الرنين النووي المغناطيسي للبروتون و تقييم اولي لفعاليتها المضادة للالتهاب. تم دراسة الفعالية المضادة للالتهاب للمركبات النهائية (5a-f) باستخدام طريقة الوزمة المستحدثة بزالال البيض عند الجرذان المختبرية. كل المركبات اظهرت فعالية مضادة للالتهاب مقارنة بمجموعة البروبيلين كلايكول، ماعدا المركب (5f) الذي اظهر فعالية مضادة للالتهاب اكثر من الايوبروفين. الكلمات المفتاحية: ايوبروفين، بيرازولين، مضاد التهاب.

Introduction

One of the most useful medication used in primary health care are NSAID, because of their analgesic, antipyretic and anti-inflammatory activities. Inflammation in Rheumatoid arthritis and osteoarthritis is known to be reduced by the use of NSAID, also they enhance the recovery and mobility⁽¹⁾. NSAID act by the same mechanism of action, through inhibiting cyclooxygenase enzymes COX that are responsible for the production of prostaglandins. Those enzymes are different in their regulation, distribution and biological functions⁽²⁾.

There are two isoforms of COX enzymes: COX-1 is constitutively normally expressed in most tissues, and prostaglandins controlled by COX-1 mediate cytoprotection of gastric mucosa and platelet aggregations in addition to some other physiological processes⁽³⁾. COX-2 is not detected in normal tissues and selectively induced in response to pro-inflammatory stimuli such as, cytokines and growth factors⁽⁴⁾. Classical NSAIDs, such as Ibuprofen, inhibit both isoforms of the enzymes⁽⁵⁾.

COX-3 is a splice variant/isoenzyme of COX-1 and, may have been named COX-1b. It was initially reported to be expressed in canine cerebral cortex. In humans COX-3 is found in highest concentrations in the brain and heart. The advantage of COX-3 is that it could explain the pharmacological actions of drugs such as acetaminophen and other antipyretic analgesic which are weak inhibitors of COX-1 and COX-2, but penetrate easily into the central nervous system⁽⁶⁾.

The major limitation to long term use of NSAIDs in therapy is the increased risk of gastrointestinal (GI) ulceration⁽⁷⁾. A well-accepted fact that the GI side effect of acidic NSAIDs is a result of direct irritation of gastric mucosa, because of the carboxylic group, and indirectly because of COX-1 inhibition that reduce the levels of protective prostaglandins. Inhibition of prostaglandin synthesis in the GI tract lead to increase gastric acid secretion and decrease trophic effects on epithelial mucosa⁽⁸⁾.

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In order to increase the analgesic and anti-inflammatory activity and reduce the side effects, recent researches focus on designing new compounds by derivitization of the carboxylic group of NSAID with heterocyclic systems, pyrazoline^(9,10).

Pyrazolines constitute an interesting class of heterocycles due to their synthetic flexibility and effective biological activities. They have been shown to possess a broad range of physiological activities such as antimicrobial, analgesic, anti-amoebic, anticancer, antidepressant, and anti-inflammatory activities⁽¹¹⁻¹⁵⁾.

The direction of the current work is to synthesize potent non-steroidal anti-inflammatory agents that are derivatives of ibuprofen by joining a group of pyrazoline ring to the carboxylate group of ibuprofen, using spacer arm glycine ester^(16,17), and then evaluating them as anti-inflammatory agents.

Materials and Methods

Chemicals and solvents used during synthesis were of Analar type and were purchased from (Fluka, England, India and Germany). Ibuprofen (General Company for Pharmaceuticals Industries and Medical appliances, Samarra, Iraq). The progress of the reaction and checking the purity of the products were determined by thin-layer

chromatography (TLC), using silica gel G_{F254} (type 60) pre-coated aluminium sheets, Merck (Germany) exposed to UV-254nm light. Chromatograms were eluted by using three solvent systems: A /Ethyl acetate: Petroleum ether (1:1). B / Ethanol: toluene (4:6). C /Chloroform: Methanol (85:15). Melting points were measured by using Stuart SMP3 melting point apparatus in open capillary tubes, and are uncorrected. The Infrared spectra were performed using FT-IR (IRAffinity-1) spectrophotometer, Shimadzu, japan. ¹HNMR spectra were recorded on NMRReady-60 spectrometer model with tetra methylsilane as an internal standard, chemical shift were expressed as (δ = ppm) and coupling constant in (Hz).

Chemical synthesis

General method for synthesis of chalcones derivatives (1a-f)

In a 96% ethanol (22ml) acetophenone (1.18 ml, 10 mmole) and derivatives of aromatic aldehydes (a-f) (10 mmole) were dissolved (table 1). Sodium hydroxide (40%, 10 ml) solution was added gradually. The solution was stirred in an ice bath until solidified, then kept in cold condition overnight. Then the solid was diluted with cold water and acidified with 2N HCl, filtered then recrystallized by ethanol⁽¹⁸⁾.

Table (1) Aromatic aldehydes and weights used

Aldehydes' name	Product	R	Weight (gm)
benzaldehyde	I _a	H	1.06
4-chlorobenzaldehyde	I _b	Cl	1.4
4-nitrobenzaldehyde	I _c	NO ₂	1.51
4-hydroxybenzaldehyde	I _d	OH	1.22
4-methoxybenzaldehyde	I _e	OCH ₃	1.36
4-dimethylaminobenzaldehyde	I _f	N(CH ₃) ₂	1.49

1,3-Diphenylpropenone (C₁₅H₁₂O) (compound **1a**): Colour: pale yellow crystals. Yield: 90%. M.P.:56-58 °C. R_f: A=0.6. FT-IR: 3055 (CH asymmetric stretching aromatic), 3028 (CH symmetric aromatic stretching), 1662 (C=O), 1604, 1573 (C=C aromatic).

3-(4-Chlorophenyl)-1-phenylprop-2-en-1-one (C₁₅H₁₁OCl) (compound **1b**): Colour: off white powder. Yield: 80%. M.P.: 112-114 °C. R_f: A=0.5. FT-IR: 3064 (CH asymmetric aromatic stretching), 3016 (CH symmetric aromatic stretching), 1658 (C=O), 1597, 1589 (C=C aromatic), 821 (C-Cl).

3-(4-Nitrophenyl)-1-phenylprop-2-en-1-one (C₁₅H₁₁NO₃) (compound **1c**): Colour: deep yellow crystals. Yield: 75%. M.P.: 156-158 °C. R_f: A=0.54. FT-IR: 3070 (asymmetric C-H stretching aromatic), 1654 (C=O), 1589, 1577 (C=C aromatic), 1516 (NO₂ asymmetric stretching), 1334 (NO₂ symmetric stretching).

3-(4-Hydroxyphenyl)-1-phenylprop-2-en-1-one (C₁₅H₁₂O₂) (compound **1d**): Colour: light yellow crystals. Yield: 60%. M.P.: 182-184 °C. R_f: A=0.7. FT-IR: 3213 (OH stretching), 3070 (asymmetric CH stretching aromatic), 3032 (symmetric CH stretching aromatic), 1647 (C=O), 1597, 1554 (C=C aromatic), 1346 (OH bending), 1215 (C-O).

3-(4-methoxyphenyl)-1-phenylprop-2-en-1-one (C₁₆H₁₄O₂) (compound **1e**): Colour: yellow crystals. Yield: 80%.M.P.: 73-75°C. R_f: A=0.6. FT-IR: 3059(asymmetric CH stretching aromatic), 3017 (symmetric CH stretching aromatic), 2954(asymmetric CH stretching), 2839 (symmetric C-H stretching), 1654 (C=O), 1689, 1573(C=C aromatic), 1257(C-OCH₃), 1257 (C-O).

3-(4-(Dimethyl amino) phenyl)-1-phenylprop-2-en-1-one (C₁₇H₁₇NO) (compound **1f**): Colour: bright orange red needle like crystal. Yield: 85%. M.P.: 110-112°C. *R_f*: A=0.7. FT-IR: 2912 (asymmetric CH stretching), 2846 (symmetric CH stretching), 1647 (C=O), 1597, 1558 (C=C aromatic), 1157 (C-N).

Synthesis of ethyl amino acetate hydrochloride (C₄H₁₀NO₂Cl) (compound 2):

Glycine (2 g, 26.6 mmol) to be esterified was suspended in absolute ethanol (50 ml) and cooled to 5°C. Thionyl chloride (2.3 ml, 32 mmol) was added slowly over a period of 15 minutes, and the reaction mixture was refluxed for 3 hours. The solvent was then evaporated to dryness under reduced pressure. Finally the residue was purified by recrystallization from ethanol: diethyl ether. Colour: white crystals. Yield: 92%. M.P.: (144-145) °C, (145-146) °C Lit.. *R_f* C=0.5. FT-IR: 1743 (C=O), 1246(C-O-C), 1176 (C-N) ⁽¹⁷⁾.

Ethyl 2-(2-(4-isobutylphenyl) propanamido) acetate chemical synthesis (C₁₇H₂₅NO₃) (compound 3):

Synthesis of ibuprofen acid chloride:

Ibuprofen (1g, 4.8 mmol) was suspended in 1 ml dry chloroform and excess of thionyl chloride (1.2 ml, 16.9 mmol) was added drop wise over a period of 3-5 minutes, then refluxed for 3 hours. Evaporation under reduced pressure to remove excess gases was done. Ibuprofen acid chloride was obtained as a faint yellow oily residue⁽¹⁹⁾.

The reaction of ibuprofen acid chloride with glycine ethyl ester:

Triethylamine (TEA) (1.35 ml, 9.6mmol) was added to a suspension of glycine ethyl ester hydrochloride (0.676g, 4.8 mmol) in dry dichloromethane (DCM) (30 ml) at 25°C. The reaction mixture was stirred for 3hours. To this mixture, ibuprofen acid chloride (prepared above) dissolved in 1 ml dry chloroform was added drop wise with continuous stirring, keeping the temperature about 5°C and left stirring overnight. After evaporating the solvent under vacuum, ethyl acetate 30ml was added to the residue, filtration was done to remove the precipitate. The ethyl acetate layer was washed with 1N HCl, followed with distilled water, and then it was washed with 5% NaHCO₃ solution and distilled water. The ethyl acetate layer was dried over anhydrous magnesium sulphate, filtered, and the filtrate was evaporated under vacuum to an oily residue, that represents the compound (3). Colour: yellow oil. Yield: 80%. *R_f*: B=0.6. FT-IR: 3309 (NH amide), 1739 (C=O ester), 1654 (C=O amide) ⁽²⁰⁾.

N-(2-hydrazinyl-2-oxoethyl)-2-(4-isobutylphenyl) propanamide chemical synthesis (C₁₅H₂₃N₃O₂) (compound 4):

Compound (3) (0.8 g, 3 mmol) was dissolved in 10 ml methanol, then hydrazine hydrate (1.5 ml, 30 mmol) was added, the reaction mixture

was refluxed for 9 hours, then left over night with continuous stirring, then the solvent was evaporated under reduced pressure. White precipitate appeared upon the addition of iced water, filtered washed with cold water, dried and washed with ether for further purification. Colour: white powder. Yield: 80%. M.P.: 100-112°C. *R_f*: B=0.5. FT-IR: 3305 (NH amide), 3228 and 3190 (NHNH₂), 1678 (C=O^{2°} amide) ⁽²¹⁾.

N-(2-(3,5-diphenyl-4,5-dihydro-1H-pyrazol-1-yl)-2-oxoethyl)-2-(4-isobutylphenyl) propanamide (compounds 5 (a-f)) chemical synthesis:

Compound (4) (0.4gm, 1.44 mmol) was dissolved in absolute ethanol, together with chalcones (1a-f) of (1.44 mmol), then few drops of glacial acetic acid (GAA) was added. The reaction mixture was refluxed for 24 hours and followed by using TLC, till single spot is obtained. The solvent was evaporated to dryness under reduced pressure; the residue was triturated with petroleum ether. Then ether was added, white precipitate appeared, filtered and collect the filtrate, evaporate to dryness⁽²²⁾.

N-(2-(3,5-diphenyl-4,5-dihydro-1H-pyrazol-1-yl)-2-oxoethyl)-2-(4-isobutylphenyl) propanamide (C₃₀H₃₃N₃O₂) compound **5a**: Colour: off white powder. Yield: 60%. M.P.: 66-88°C. *R_f* A= 0.62. FT-IR: 3278 (NH amide), 1647 (C=O), 1593 (C=N). ¹HNMR (60 MHz, DMSO-*d*₆, ppm): 0.75-0.85 (6H, d, two CH₃ ibuprofen), 1.22-1.33 (3H, d, CH₃ ibuprofen), 1.76-1.86 (m, CH ibuprofen), 2.3 (2H, d, CH₂ ibuprofen), 3.63 (3H, m, CH ibuprofen and CH₂ pyrazoline), 4.21 (3H, m, CH₂ glycine and CH of pyrazoline ring), 6.94-7.44 (14H, m, Ar-H), 8.10 (1H, br. s, NH amide).

N-(2-(5-(4-chlorophenyl)-3-phenyl-4,5-dihydro-1H-pyrazol-1-yl)-2-oxoethyl)-2-(4-isobutylphenyl)propanamide (C₃₀H₃₂ClN₃O₂) compound **5b**: Colour: off white powder. Yield: 62%. M.P.: 62-65°C. *R_f* A=0.65. FT-IR: 3294 (NH amide), 1647 (C=O), 1593 (C=N), 813 (C-Cl). ¹HNMR (60 MHz, DMSO-*d*₆, ppm): 0.75-0.85 (6H, d, two CH₃ ibuprofen), 1.21-1.33 (3H, d, CH₃ ibuprofen), 1.76-1.86 (1H, m, CH ibuprofen), 2.3 (2H, d, CH₂ ibuprofen), 3.63 (3H, CH ibuprofen and CH₂ pyrazoline), 4.25 (3H, m, CH₂ glycine and CH of pyrazoline ring), 6.94-7.15 (13H, m, Ar-H), 8.12 (1H, br. s, NH amide).

2-(4-isobutylphenyl)-N-(2-(5-(4-nitrophenyl)-3-phenyl-4,5-dihydro-1H-pyrazol-1-yl)-2-oxoethyl) propanamide (C₃₀H₃₂N₄O₄) compound **5c**: Colour: yellow powder. Yield: 70%. M.P.: 76-78°C. *R_f* A=0.55. FT-IR: 3305 (NH amide), 1651 (C=O), 1620 (C=N), 1514 (NO₂ asymmetric stretching), 1334 (NO₂ symmetric stretching). ¹HNMR (60 MHz, DMSO-*d*₆, ppm): 0.74-0.84 (6H, d, two CH₃ ibuprofen), 1.19-1.32 (3H, d, CH₃ ibuprofen), 1.75-1.85 (1H, m, CH ibuprofen), 2.29 (2H, d, CH₂ ibuprofen),

3.63 (3H, m, CH ibuprofen and CH₂ pyrazoline), 4.36 (3H, m, CH₂ glycine and CH of pyrazoline ring), 6.93-7.82 (13H, m, Ar-H), 8.18 (1H, br. s, NH amide).

N-(2-(5-(4-hydroxyphenyl)-3-phenyl-4,5 -dihydro-1H-pyrazol-1-yl)-2-oxoethyl)-2-(4-isobutylphenyl) propanamide (C₃₀H₃₃N₃O₃) compound **5d**: Colour: light brown powder. Yield: 70%. M.P.: 82-84°C. *R_f*A=0.7. FT-IR: 3232 (NH amide), 3217 (phenolic OH), 1647 (C=O), 1604 (C=N). ¹HNMR (60 MHz, DMSO-d₆, ppm): 0.75-0.85 (6H, d, two CH₃ ibuprofen), 1.22-1.32 (3H, d, CH₃ ibuprofen), 1.8 (1H, m, CH ibuprofen), 2.31 (2H, d, CH₂ ibuprofen), 3.66 (3H, CH ibuprofen and CH₂ pyrazoline ring), 4.10 (3H, CH₂ glycine and CH pyrazoline ring), 6.81-7.43 (13H, m, Ar-H), 8.03 (1H, br. s, NH amide), 9.82 (1H, br. s, OH).

2-(4-isobutylphenyl)-N-(2-(5-(4-methoxyphenyl)-3-phenyl-4,5 -dihydro-1H-pyrazol-1-yl)-2-oxoethyl) propanamide (C₃₁H₃₅N₃O₃) compound **5e**: Colour: off white powder. Yield: 60%. M.P.: 60%. *R_f*A=0.6. FT-IR: 3294 (NH amide), 1647 (C=O), 1604 (C=N). ¹HNMR (60 MHz, DMSO-d₆, ppm): 0.75-0.85 (6H, d, two CH₃ ibuprofen), 1.22-1.33 (3H, d, CH₃ ibuprofen), 1.78-1.86 (1H, m, CH ibuprofen), 2.3-2.4 (2H, d, CH₂ ibuprofen), 3.73 (6H, m, CH ibuprofen and -OCH₃ overlapped with CH₂ pyrazoline), 4.21 (3H, CH₂ glycine and H of pyrazoline ring), 6.94-7.44 (13H, m, Ar-H), 8.05 (1H, br. s, NH amide).

N-(2-(5-(4-(dimethyl amino) phenyl)-3-phenyl-4,5 -dihydro-1H-pyrazol-1-yl)-2-oxoethyl)-2-(4-isobutylphenyl) propanamide (C₃₂H₃₈N₄O₂) compound **5f**: Colour: red powder. Yield: 65%. M.P.: 60-62°C. *R_f*A=0.55. FT-IR: 3298 (NH amide), 1647 (C=O), 1600 (C=N), 1168 (N-CH₃). ¹HNMR (60 MHz, DMSO-d₆, ppm): 0.74-0.85 (6H, d, two CH₃ ibuprofen), 1.2-1.3 (3H, d, CH₃ ibuprofen), 1.78-1.86 (1H, m, CH ibuprofen), 2.4 (2H, d, CH₂ ibuprofen), 2.9 (6H, s, N(CH₃)₂), 3.56 (3H, CH ibuprofen and CH₂ pyrazoline), 4.2 (3H, CH₂ glycine and CH pyrazoline), 6.73-7.43 (m, 13H Ar-H), 8.11 (1H, br. s, NH amide).

Pharmacology

Anti-inflammatory assessment study

In vivo, anti-inflammatory effects of the chemically synthesized products (5a-f) were assessed using egg-white induced paw oedema. Measuring the decrease of paw thickness is the basis for the evaluation of the anti-inflammatory activity of the desired compounds⁽²³⁾.

Albino rats of either sex weighing (170±10 g) were supplied by Iraqi centre for cancer and Medical genetic research and were housed in University of Technology under standardized conditions for 10 days for acclimatization. Animals

were fed commercial chow and had free access to water. Animals were taken to the laboratory, one hour before the experiment, and were separated into eight groups (each group consist of 6 rats) as follows⁽²⁴⁾:

Group A: Six rats considered as control and injected with the vehicle intra peritoneally (i.p.) (propylene glycol 50% v/v).

Group B: Six rats treated with ibuprofen as reference substance in a dose of 50mg/kg suspended in propylene glycol.

Group C-H: Six rats for each group injected with the tested compounds (5a-f), also suspended in propylene glycol⁽²⁵⁾, in doses that are illustrated in table 2.

The paw width was measured by vernier calliper at seven time periods (0, 30, 60, 120, 180, 240, and 300 min.) after drug delivery. Potent inflammation can be created by injecting of 0.05ml of undiluted egg-white subcutaneously into the planter side of the hind paw of the rats 1/2 hr. after i.p. delivery of the target compounds including standard and control.

The data was stated as the mean ± standard error of mean (SEM) and results were investigated for statistical significance using student t-test (Two sample assuming equal variances) for comparison between mean values. However, comparisons between different groups were made using ANOVA: Two factors without replication. Probability (*P*) value of less than 0.05 was considered significant.

Ibuprofen which is given in a dose of 50mg/kg, so; the doses of synthesized compounds are calculated as in table 2: M. Wt. of Ibuprofen = 206.29 (50mg / kg) / M. Wt. of reference drug = Dose / M. Wt. of the tested compound⁽²⁵⁾.

Table (2) Compounds with their molecular weight and dose

Compound	Molecular Wt.	Dose (mg/ kg)
Ibuprofen	206.29	50.00
5a	467.60	113.335
5b	502.05	121.685
5c	512.60	124.242
5d	483.60	117.21
5e	497.60	120.61
5f	510.67	123.77

Results and Discussion

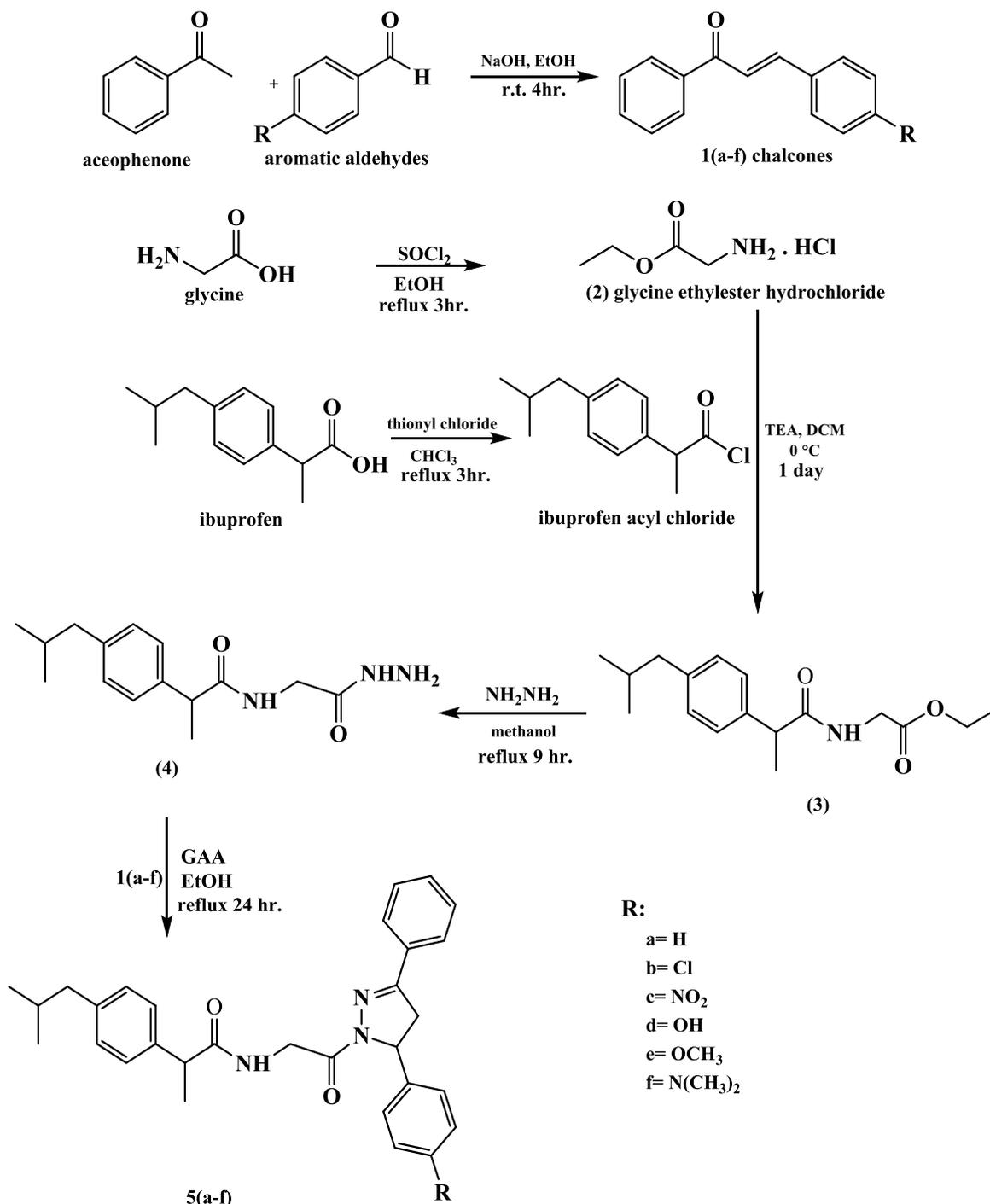
Chemistry

Chalcones (**1a-f**) were synthesized based on aldol condensation by reacting aromatic aldehydes with acetophenone in ethanol with NaOH 40% solution. Chalcones were characterized by FTIR that shows appearance of C=O stretching at (1662-1647). Glycine ethyl ester HCl (**2**) was prepared by refluxing glycine with thionyl chloride in absolute

ethanol. FTIR shows characteristic band of ester C=O stretching at (1743). Compound (3) was synthesized from reacting ibuprofen acyl chloride with compound (2), and was characterized by FTIR to show NH stretching at (3305), C=O stretching of ester at (1739) and C=O stretching of amide at (1654).

Compound (4) was prepared by refluxing compound (3) with hydrazine hydrate in methanol.

FTIR shows characteristic band of hydrazide of NHNH_2 stretching at 3224 and 3190. The final product (5) was synthesized by refluxing chalcones with compound (4) in ethanol using glacial acetic acid as a catalyst. The appearance of bands C=N stretching at (1620-1593), were characteristic of pyrazoline derivatives. All the steps involved in synthesis of targets compound were shown in (Scheme 1).



Scheme (1) synthesis of intermediates and targets compounds

The anti-inflammatory activity evaluation of the tested compounds

The anti-inflammatory activity of the tested compounds has been evaluated in comparison with their vehicle (control group) and ibuprofen. Table 3 explains the effect of tested compounds 5a-f in comparison to control and ibuprofen.

Discussion

All tested compounds employed imperative reduction of paw oedema in comparison to the effect of propylene glycol 50%v/v (control group). The effect of Ibuprofen and all tested compounds started

at time 120 min except 5f which has started at 60 min. indicating rapid onset of action. The effect of target compounds and Ibuprofen continued till the end of experiment. Compound 5d and 5e showed comparable effect to Ibuprofen at all experimental time while compounds 5a, 5b and 5c produced significantly lower inhibitory effect than Ibuprofen at time 120-240 minutes. Remarkably, compound 5f exerted significantly higher paw edema reduction than ibuprofen at 60-240 min. All tested compounds have showed comparable effect to that of ibuprofen at time 300 minutes.

Table (3) The anti-inflammatory effect of control, ibuprofen and compounds 5a-f on egg-white induced paw edema in rat #

Paw Thickness (mm) / n=6	Compound	Time (min)						
		0	30	60	120	180	240	300
	Control	4.80±0.05	5.79±0.06	6.52±0.06	6.90±0.03	6.78±0.06	6.65±0.02	5.33±0.01
	Ibuprofen	4.78±0.04	5.70±0.05	6.42±0.05	5.70±0.05 ^{*a}	5.39±0.06 ^{*a}	5.13±0.06 ^{*a}	4.87±0.02 ^{*a}
	5a	4.84±0.06	5.70±0.02	6.51±0.06	6.27±0.01 ^{*b}	5.69±0.03 ^{*b}	5.39±0.02 ^{*b}	5.01±0.04 ^{*a}
	5b	4.77±0.03	5.71±0.06	6.42±0.01	6.16±0.04 ^{*b}	5.70±0.06 ^{*b}	5.47±0.02 ^{*b}	4.92±0.05 ^{*a}
	5c	4.78±0.02	5.71±0.01	6.41±0.03	6.21±0.06 ^{*b}	5.71±0.05 ^{*b}	5.42±0.06 ^{*b}	4.97±0.06 ^{*a}
	5d	4.76±0.01	5.75±0.06	6.44±0.04	5.81±0.03 ^{*a}	5.43±0.02 ^{*a}	5.14±0.05 ^{*a}	5.02±0.06 ^{*a}
	5e	4.79±0.06	5.70±0.02	6.45±0.03	5.83±0.02 ^{*a}	5.38±0.04 ^{*a}	5.13±0.01 ^{*a}	4.99±0.06 ^{*a}
	5f	4.81±0.01	5.79±0.02	5.75±0.04 [*]	5.40±0.05 ^{*c}	5.05±0.06 ^{*c}	4.87±0.03 ^{*c}	4.84±0.08 ^{*a}

#Non-identical superscripts (a, b and c) among different tested compounds are regarded significantly different ($p < 0.05$); *significantly different compared to control ($p < 0.05$). Data are expressed in mm paw thickness as mean \pm SEM. n= number of rats. Time (0) is the time of i.p. injection of ibuprofen, tested compounds and propylene glycol. Time (30) is the time of injection of egg white to induce edema.

Percent of inhibition in paw oedema thickness

The percent of inhibition of paw oedema thickness at each time interval was calculated from the mean effect in control and tested animals according to the equation⁽²⁶⁾:

$$\% \text{ Inhibition} = [V_c - V_t / V_c] \times 100$$

Where V_c and V_t are the mean paw thickness of the control group and tested group (at t-time zero) respectively. The comparison among the ibuprofen, compounds 5a-f was presented in table 4.

Table (4) Percent of inhibition of inflammation for ibuprofen and compounds 5a-f on egg-white induced paw oedema in rats.

Time (min.)	Ibuprofen	5a	5b	5c	5d	5e	5f
60	4.6%	2.9%	4%	5%	2.3%	3.5%	45%
120	56%	32%	34%	32%	50%	50%	72%
180	69%	57%	53%	53%	66%	70%	88%
240	81%	70%	62%	65%	79%	81%	97%
300	83%	68%	72%	64%	51%	62%	92%

Conclusion

New pyrazoline containing ibuprofen derivatives have been synthesized and characterized successfully. All of the synthesized compounds demonstrated anti-inflammatory activity in the control group of rats. Out of them, compound 5f showed better anti-inflammatory activity than that of ibuprofen, since it has electron donating group $N(CH_3)_2$.

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