European Journal of Chemistry 6 (4) (2015) 461-467

European Journal of Chemistry

Journal webpage: www.eurjchem.com

Synthesis, characterization and preliminary pharmacological evaluation of new non-steroidal anti-inflammatory pyrazoline derivatives

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ARTICLE INFORMATION

DOI: 10.5155/eurjchem.6.4.461-467.1321

Received: 13 September 2015
Received in revised form: 03 October 2015
Accepted: 10 October 2015
Published online: 31 December 2015
Printed: 31 December 2015

KEYWORDS

Chalcone
Synthesis
Naproxen
Pyrazoline
Anti-inflammatory
Anti-bacterial activity

ABSTRACT

A series of six pyrazoline ring derivatives as a pharmacophore were incorporated to the naproxen; to increase its size were synthesized and preliminarly evaluated as anti-inflammatory agents with expected selectivity toward COX-2 enzyme. *In vivo* acute anti-inflammatory effects of the final compounds (5a-f) were evaluated in rats using egg-white induced edema model of inflammation. The tested compounds and the reference drug (naproxen) produced significant reduction of paw edema with respect to the effect of control group (propylene glycol 50%, *v:v*). However, compound 5d and 5e show comparable effect to naproxen at all experimental time while compounds 5a, 5b and 5c produced significantly lower inhibitory effect than naproxen at time 120-240 minutes. Furthermore, compound 5f exert significantly higher paw edema reduction than naproxen at 60-240 min. Also the antibacterial activities of the final compounds were evaluated by Well Diffusion Method. All tested compounds exert significant antibacterial activity against tested Gram positive and Gram negative bacteria in comparison to dimethyl sulfoxide as control group. In comparison the antibacterial results among the tested compound 5e may regard the best one and compound 5c the lower one.

Cite this: *Eur. J. Chem.* **2015**, *6(4)*, 461-467

1. Introduction

Non-steroidal anti-inflammatory drugs (NSAIDs) are among the most widely used medications in the world, owing to their analgesic, anti-inflammatory and antipyretic properties [1,2]. Recent years, epidemiological study indicates that NSAIDs are neuroprotective [3]. Thus, prolong used reduce the risk of Alzheimer [4].

Furthermore, clinical study provides evidence that NSAIDs derivatives are promising anticancer drugs [5]. However, the use of "traditional" NSAIDs results in serious upper gastrointestinal (GI) adverse events in nearly one fourth of patients [6]. The most common side effects are the propensity of NSAIDs to induce gastric or intestinal ulceration. Thus, patients who use NSAIDs on chronic basis have about three times greater relative risk for serious adverse GI events compared to the population of non-user [7,8]. Therefore, there is a need for anti-inflammatory and analgesic drugs that will provide symptom relief without causing GI injury [9]. The NSAIDs are chemically diverse, most being organic acids, despite their structural heterogeneity, NSAIDs possess a common mode of action, which block prostaglandin synthesis largely though their inhibition of the enzyme cyclooxygenase (COX), which catalyze the transformation of arachidonic acid to prostaglandins and thromboxane [10]. Three different COX enzymes existed, known as COX-1, COX-2 and COX-3 [11].

COX-1 is a constitutive isoform found in most normal cells and tissues [12]. It is stimulated by growth factor and hormones and it has been called the housekeeping enzyme [13]. The COX-1 plays fundamental roles in the generation of PGs in homoeostasis [14] and several other physiological functions including gastric protection and control of renal blood flow [15].

COX-2 is the readily inducible form of the enzyme and is commonly associated with several pathological conditions. COX-2 is found in the heart [16], spinal cord [17], vascular endothelium, brain, kidney, bone and female reproductive system. It's also involved in certain physiological processes [18,19]. However, it is induced by inflammatory stimuli such as bacterial endotoxin and cytokines [19,20]. Increased levels of COX-2 have also been seen in diseases such as Alzheimer's disease, systemic lupus erythematous, colon, breast and pancreatic cancer, as well as diabetic neuropathy and premature labor [18,21]. Selective COX-2 inhibitors differ from traditional NSAIDs in two major ways, Coxibs are less likely to result in NSAID-induced gastropathy and they do not inhibit platelet function [22]. As a result, the major benefits of coxibs are the reduction in gastric ulcer formation and bleeding from those ulcers [23].

ISSN 2153-2249 (Print) / ISSN 2153-2257 (Online)
http://dx.doi.org/10.5155/eurjchem.6.4.461-467.1321

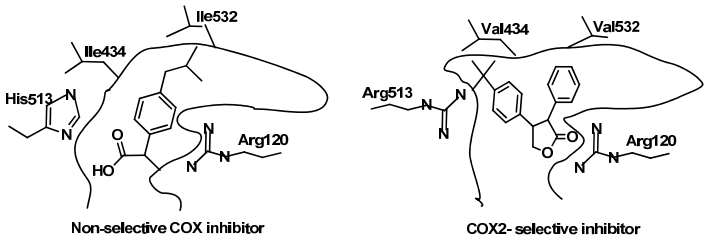


Figure 1. Difference between COX-1 and COX-2 is in size of active center [31].

Another benefit of the platelet sparing coxibs is their use as analgesic and anti-inflammatory agents in situations in which bleeding may limit the use of the traditional NSAIDs, such as in trauma and surgical procedures [24,25].

There are numerous biologically active hetrocycles molecules bearing nitrogen, sulphur and oxygen, always drawn the attention over the years mainly because of their biological importance [26]. Pyrazoline is five-membered heterocyclic and their derivatives have been found to have diverse pharmacological activities such as anticonvulsant, anti-inflammatory, antimicrobial, antiviral, anticancer, anti-helicobacter pylori, antitubercular, antiamoebic, antiandrogenic, hypotensive, and antihistaminic, antidiabetic, analgesic and antipyretic action [27,28].

The direction of the present work is to synthesis potential non-steroidal anti-inflammatory agents that are derivatives of naproxen by incorporating a group of pyrazoline pharmacophore in the carboxylate group of naproxen. Then evaluated them as anti-inflammatory and antibacterial agents. These newly synthesized compounds may represent potent anti-inflammatory agents and exhibit expected selectivity towards COX-2 enzyme due to their large size than its parent naproxen compound and the fact of presence the side pocket near the base of the active site of COX-2 enzyme makes its site 20% larger than that of COX-1 [29] so the active center of COX-2 can accommodate larger structures than those which are able to fit the active site of COX-1 [30] as shown in Figure 1 [31].

2. Experimental

2.1. Reagents and instrumentation

All reagents and anhydrous solvents were of analar type, were purchased from (Fluka, Switzerland, Avochem, England, Himedia, India, Sigma-Aldrich, Germany and BDH, England). Naproxen was a product of the General Company for Pharmaceutical Industries and Medical Appliances, Samarra, Iraq.

Electro thermal melting point apparatus and open capillary tubes were used to determine the melting points and are uncorrected. Thin layer chromatography was run on TLC silica gel (60) F_{254}, Merck (Germany), for checking the purity of the products as well as monitoring the progress of the reaction. Chromatograms were eluted by using two different solvent systems: A: Benzene:carbon tetrachloride:ethyl acetate (50:40:10, *v*:*v*:*v*). B: Chloroform:methanol (85:15, *v*:*v*). Compounds were revealed upon irradiation with UV light. IR spectra were recorded on a FTIR, Shimadzu 8100s spectrometer as KBr disks in College of Pharmacy, Al-Mustansiriyah University. ^{1}H NMR spectra were recorded on Bruker 500 MHz-Avance III in University of Jordan, Faculty of Science, and Department of Chemistry, Jordan. CHNS microanalysis was done using a Euro-Vector EA3000 (Italy) in College of Science, Al-Mustansiriyah University, Baghdad, Iraq. The general routes outlined in Scheme 1 were used to synthesize all compounds.

2.1.1. General procedure for synthesis of chalcone derivatives (1a-f)

Acetophenone (1.18 mL, 10 mmole) and aromatic aldehyde derivatives (**a**-**f**) (10 mmole) were added to ethanol (22 mL), then sodium hydroxide (40%, 10 mL) solution was added drop wise over 2 min. The mixture was irradiated by an ultrasonic generator in a water bath at 30-35 °C for 25 min, turbidity appeared in the mixture, which was then neutralized with 2 N HCl. The solid product formed was filtered, washed with cold water and recrystallized by ethanol [32,33], Table 1.

1,3-Diphenylpropenone (**1a**): Color: Pale yellow crystals. Yield: 94%. M.p.: 56-58 °C. R_f: A = 0.52, B = 0.39. FT-IR (KBr, ν, cm^{-1}): 1660 (C=O), 1603, 1573 (C=C aromatic). ^{1}H NMR (500 MHz, DMSO-d_6, δ, ppm): 7.45-7.96 (m, 10H, Ar-H), 8.16-8.18 (m, 2H, CH=CH). Anal. calcd. for $C_{15}H_{12}O$: C,86.51; H, 5.81. Found: C,86.70; H,5.65%.

3-(4-Chlorophenyl)-1-phenylprop-2-en-1-one (**1b**): Color: Off white crystals. Yield: 78%. M.p.: 112-114 °C. R_f: A = 0.57, B = 0.46. FT-IR (KBr, ν, cm^{-1}): 1660 (C=O), 1658, 1603 (C=C aromatic), 821 (C-Cl). Anal. calcd. for $C_{15}H_{11}ClO$: C, 74.23; H, 4.57. Found: C, 74.12; H, 4.69%.

3-(4-Nitrophenyl)-1-phenylprop-2-en-1-one (**1c**): Color: Orange powder. Yield: 70%. M.p.: 159-161 °C. R_f: A = 0.46, B = 0.36. FT-IR (KBr, ν, cm^{-1}): 1660 (C=O), 1603 (C=C aromatic), 1516 (NO_2 asymmetric), 1336 (NO_2 symmetric). Anal. calcd. for $C_{15}H_{11}NO_3$: C, 71.14; H, 4.38; N, 5.53. Found: C, 71.77; H, 4.29; N, 5.68%.

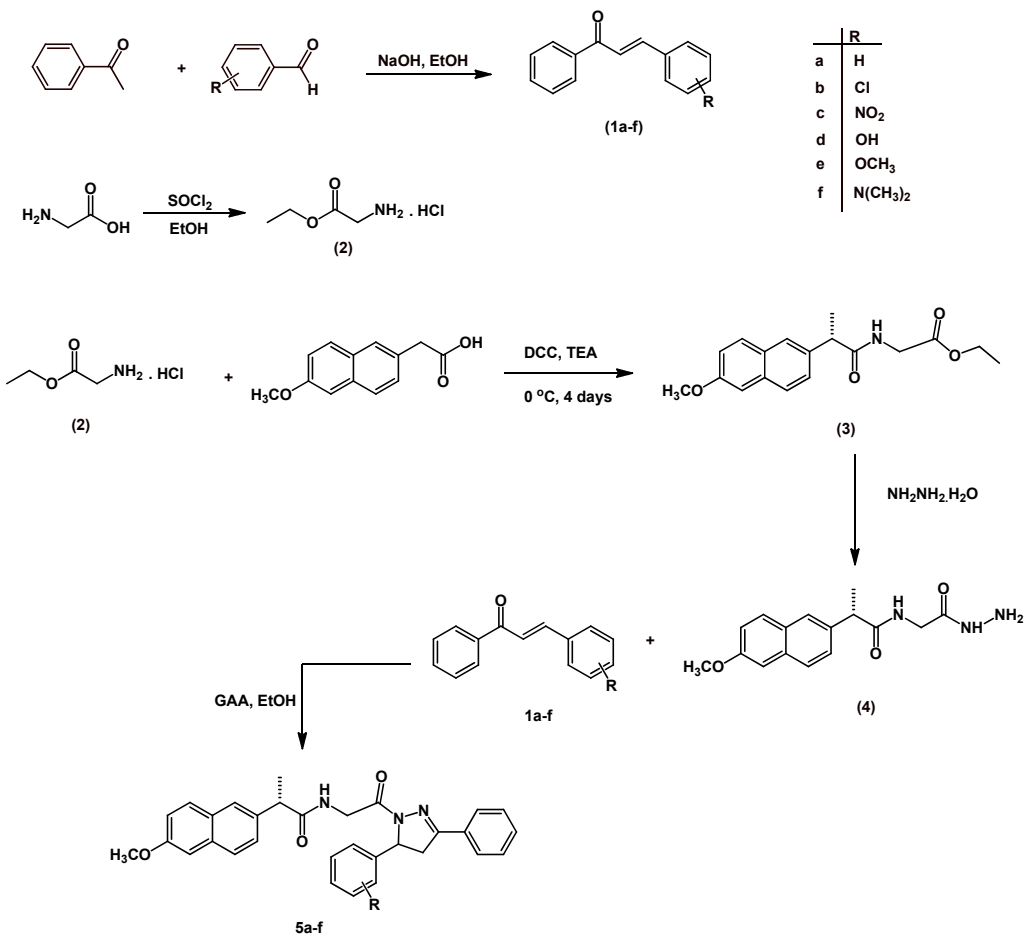
3-(4-Hydroxyphenyl)-1-phenylprop-2-en-1-one (**1d**): Color: Green-yellowish powder. Yield: 67%. M.p.: 182-184 °C. R_f: A = 0.81, B = 0.65. FT-IR (KBr, ν, cm^{-1}): 1649 (C=O), 1599, 1558 (C=C aromatic), 1348 (O-H), 1215 (C-OH). ^{1}H NMR (500 MHz, DMSO-d_6, δ, ppm): 6.86-7.75 (m, 9H, Ar-H), 8.12-8.13 (d, 2H, CH=CH). Anal. calcd. for $C_{15}H_{12}O_2$: C, 80.34; H, 5.39. Found: C, 81.22; H, 5.28%.

3-(4-Methoxyphenyl)-1-phenylprop-2-en-1-one (**1e**): Color: Pale yellow crystals. Yield: 75%. M.p.: 73-75 °C. R_f: A = 0.54, B = 0.44. FT-IR (KBr, ν, cm^{-1}): 1657 (C=O), 1601 and 1574 (C=C aromatic), 1263 (C-OCH_3). ^{1}H NMR (500 MHz, DMSO-d_6, δ, ppm): 3.82 (s, 3H, -OCH_3), 7.45-7.96 (m, 9H, Ar-H), 8.16-8.18 (d, 2H, CH=CH). Anal. calcd. for $C_{16}H_{14}O_2$: C, 80.65; H, 5.92. Found: C, 79.21; H, 5.96%.

3-(4-(Dimethyl amino) phenyl)-1-phenylprop-2-en-1-one (**1f**): Color: Orange crystals. Yield: 80%. M.p.: 111-113 °C. R_f: A = 0.59, B = 0.49. FT-IR (KBr, ν, cm^{-1}): 1649 (C=O), 1599, 1562 (C=C aromatic), 1172 (N-CH_3). ^{1}H NMR (500 MHz, DMSO-d_6, δ, ppm): 3.02 (s, 6H, N(CH_3)$_2$), 6.75-7.72 (m, 9H, Ar-H), 8.10-8.11 (m, 2H, CH=CH). Anal. calcd. for $C_{17}H_{17}NO$: C, 81.24; H, 6.82; N, 5.57. Found: C, 83.13; H, 7.03; N, 5.85%.

2.1.2. Synthesis of ethyl amino acetate hydrochloride (2)

Thionyl chloride (0.8 mL, 11 mmole) was added gradually to absolute ethanol (10 mL) cooled to (0 °C). 2-Aminoacetic acid (0.75 g, 10 mmol) was suspended in the reaction mixture and subjected to ultra-sonication at room temperature for 45 min.



Scheme 1

On completion of the reaction, the solvent was removed under reduced pressure and the residue was purified by recrystallization from methanol: diethyl ether [34]. Color: White crystals. Yield: 90%. M.p.: 145-147 °C. R_f: A = 0.55, B = 0.48. FT-IR (KBr, ν, cm^{-1}): 1748 (C=O ester), 1250 (C-O-C ester), 1172 (N-CH_3). Anal. calcd. for $C_4H_{10}ClNO_2$: C, 34.42; H, 7. 22; N, 10.03. Found: C, 33.65; H, 7.16; N, 10.32%.

2.1.3. Synthesis of (2S)-ethyl-2-[2-(6-methoxynaphthalen-2-yl)-propanamido] acetate (3)

Compound **2** (2.8 g, 20 mmole), triethylamine (3 mL, 21 mmole) and naproxen (4.6 g, 20 mmole) were dissolved in dry dichloromethane (DCM) (40 mL). The reaction mixture was stirred at 0 °C for 30 min.To this solutiondicyclohexyl carbodiimide (DCC) (4.13 g, 20 mmole) in dry DCM (10 mL) was added slowly in a drop wise manner. Reaction mixture was stirred for 3 days at 0 °C. Precipitated dicyclohexylurea was filtered off and the solvent was distilled off under reduced pressure. The product obtained was dissolved in ethyl acetate (30 mL) and filtered. Ethyl acetate layer was washed with 10% aqueous solution of sodium bicarbonate (3×30 mL) and distilled water (3×30 mL). Ethyl acetate layer was dried with anhydrous magnesium sulphate and filtered to get a clear solution of product in ethyl acetate. Solvent was evaporated under reduced pressure and the crude product was recrystallized by using hexane: ethyl acetate [35]. Color: Off White powder. Yield: 65%. M.p.: 84-86 °C. R_f: A = 0.66, B = 0.55. FT-IR (KBr, ν, cm^{-1}): 3293 (NH amid), 1740 (C=O ester), 1649 (C=O amide). ^{1}H NMR (500 MHz, DMSO-d_6, δ, ppm): 1.14 (t, 3H, OCH_3 ester), 1.24-1.26 (d, 3H, CH_3 naproxen), 3.80-3.84 (m, 3H, CH naproxen and CH_2 glycine), 3.87 (s, 3H, -OCH_3 naproxen), 4.04-4.08 (q, 2H, CH_2 ester), 7.14-7.79 (m, 6H, Ar-H), 8.40 (br. s, 1H, NH amide). Anal. calcd. for $C_{18}H_{21}NO_4$: C, 68.55; H, 6. 71; N, 4.44. Found: C, 69.61; H, 6.79; N, 4.59%.

2.1.4. Synthesis of (2S)-N-(2-hydrazinyl-2-oxoethyl)-2-(6-methoxy-naphthalen-2-yl) propanamide (4)

Compound **3** (1.00 g, 3 mmole) was dissolved in 15 mL methanol and hydrazine hydrate (90%) (0.7 mL, 14 mmole) was added. The reaction mixture was stirred at room temperature overnight. On the next day, the solvent has been removed under reduced pressure and the crude product was washed with ether under stirring to afford the product in pure state [35]. Color: Yellow powder. Yield: 78%. M.p.: 158-160 °C. R_f: A = 0.75, B = 0.50. FT-IR (KBr, ν, cm^{-1}): 3339 and 3277 ($NHNH_2$), 1678(C=Oamidic), 1645(C=Oamide). ^{1}H NMR (500 MHz, DMSO-d_6, δ, ppm): 1.41-1.42 (d, 3H, CH_3 naproxe), 3.55-3.73 (d, 2H, CH_2 glycine), 3.81-3.87 (q, 1H, CH naproxen), 3.90 (s, 3H, -OCH_3 naproxen), 4.20 (br. s, 2H, NH_2 hydrazide), 7.13-7.83 (d, 6H, Ar-H), 8.20 (br. s, 1H, NH amide), 9.01(s, 1H, NH hydrazide). Anal. calcd. for $C_{16}H_{19}N_3O_3$: C, 63.77; H, 6. 36; N, 13.94. Found: C, 62.89; H, 6.25; N, 14.04%.

2.1.5. General procedure for synthesis of (2S)-N-(2-(3,5-diaryl-4-5-dihydro-1H-pyrazol-1-yl)-2-oxoethyl)-2-(6-methoxynaphthalen-2-yl) propanamid (5a-f)

Table 1. Aromatic aldehyde's name and products.

Compound	Aldehyde	Products No	R	Quantity (g)
a	Benzaldehyde	1a	H	1.06
b	4-Chlorobenzaldehyde	1b	Cl	1.40
c	4-Nitrobenzaldehyde	1c	NO_2	1.51
d	4-Hydroxybenzaldehyde	1d	OH	1.22
e	4-methoxybenzaldehyde	1e	OCH_3	1.36
f	4-Dimethylaminobenzaldehyde	1f	$N(CH_3)_2$	1.49

The product has been synthesized by dissolving a mixture of one chalcone derivatives (**1a-f**) (1 mmole) and compound **4** (0.30 g, 1 mmole) in 20 mL of ethanol, after 15 min, the mixture has been refluxed. A catalytic glacial acetic acid has been added and the contents allowed to be refluxed for 24 hrs. The reaction time was considered by performing TLC to obtain single spot. The reaction mixture has been cooled and transfers it in to 20 mL of cold water in order to precipitate the product. The product has been washed twice in cold water, filtered and then dried. After that, the re-crystallized process has been done from hot ethanol.

(2S)-N-(2-(3,5-Diphenyl-4,5-dihydro-1H-pyrazol-1-yl)-2-oxo ethyl)-2-(6-methoxynaphthalen-2-yl) propanamide (**5a**): Color: Off white powder. Yield: 70%. M.p.: 68-70 °C. R_f: A = 0.61, B = 0.49. FT-IR (KBr, ν, cm^{-1}): 3296 (NH amide), 1653 (C=O amide), 1604 (C=C Aromatic), 1533 (C=N amide), 1267 (Ar-O-C). ^{1}H NMR (500 MHz, DMSO-d_6, δ, ppm): 1.41-1.43 (d, 3H, CH_3 naproxe), 3.49-3.73 (m, 3H, CH_2 pyrazoline and CH of naproxen), 3.86 (s, 3H, -OCH_3 naproxen), 4.23 (br.s, 2H, CH_2 glycine), 5.28 (br. s, 1H, CH pyrazoline), 7.13-8.20 (m, 16H, Ar-H), 8.52 (br. s, 1H, NH amide). Anal. calcd. for $C_{31}H_{29}N_3O_3$: C, 75.74; H, 5.95; N, 8.55. Found: C, 76.24; H, 5.82; N, 8.74%.

(2S)-N-(2-(5-(4-Chlorophenyl)-3-phenyl-4, 5-dihydro-1H-pyrazol-1-yl)-2-oxoethyl)-2-(6-methoxynaphthalen-2-yl)propan amide (**5b**): Color: Off white powder. Yield: 63%. M.p.: 78-80 °C. R_f: A = 0.65, B = 0.54. FT-IR (KBr, ν, cm^{-1}): 3292 (NH amide), 1653 (C=O amide), 1606 (C=C Aromatic), 1541 (C=N amide), 1265 (Ar-O-C), 813 (C-Cl). ^{1}H NMR (500 MHz, DMSO-d_6, δ, ppm): 1.42-1.44 (d, 3H, CH_3 naproxe), 3.52-3.71 (m, 3H, CH_2 pyrazoline and CH of naproxen), 3.86 (s, 3H, -OCH_3 naproxen), 4.00 (br.s, 2H, CH_2 glycine), 5.38 (br. s, 1H, CH pyrazoline), 7.13-7.99 (m, 13H, Ar-H), 8.09-8.32 (m, 2H, Ar-H), 8.41 (br. s, 1H, NH amide). Anal. calcd. for $C_{31}H_{28}ClN_3O_3$: C, 70.78; H, 5.37; N, 7.99. Found: C, 71.98; H, 5.24; N, 8.19%.

(2S)-2-(6-Methoxynaphthalen-2-yl)-N-(2-(5-(4-nitrophenyl) -3-phenyl-4, 5-dihydro-1H-pyrazol-1-yl)-2-oxoethyl)propanami de (**5c**): Color: Pale orange powder. Yield: 61%. M.p.: 85-88 °C. R_f: A = 0.55, B = 0.45. FT-IR (KBr, ν, cm^{-1}): 3294 (NH amide), 1658 (C=O amide), 1604 (C=C Aromatic), 1572 (C=N amide), 1516 (NO_2 asymmetric), 1342 (NO_2 symmetric), 1265 (Ar-O-C). ^{1}H NMR (500 MHz, DMSO-d_6, δ, ppm): 1.41-1.43 (d, 3H, CH_3 naproxe), 3.48-3.71 (m, 3H, CH_2 pyrazoline and CH of naproxen), 3.86 (s, 3H, -OCH_3 naproxen), 4.00 (br.s, 2H, CH_2 glycine), 5.21 (br. s, 1H, CH pyrazoline), 7.13-8.30 (m, 15H, Ar-H), 8.41 (br. s, 1H, NH amide). Anal. calcd. for $C_{31}H_{28}N_4O_5$: C, 69.39; H, 5. 26; N, 10.44. Found: C, 68.25; H, 5.33; N, 10.56%.

(2S)-N-(2-(5-(4-Hydroxyphenyl)-3-phenyl-4, 5-dihydro-1H-pyrazol-1-yl)-2-oxoethyl)-2-(6-methoxynaphthalen-2-yl)propan amide (**5d**): Color: Light brown powder. Yield: 58%. M.p.: 87-89 °C. R_f: A = 0.70, B = 0.58. FT-IR (KBr, ν, cm^{-1}): 3298 (NH amide), 1649 (C=O amide), 1604 (C=C Aromatic), 1545 (C=N amide), 1346 (O-H), 1267 (Ar-O-C). ^{1}H NMR (500 MHz, DMSO-d_6, δ, ppm): 1.41-1.43 (d, 3H, CH_3 naproxe), 3.57-3.74 (m, 3H, CH_2 pyrazoline and CH of naproxen), 3.86 (s, 3H, -OCH_3 naproxen), 4.27 (br.s, 2H, CH_2 glycine), 5.20 (br. s, 1H, CH pyrazoline), 6.66-8.28 (m, 15H, Ar-H), 8.44 (br. s, 1H, NH amide), 10.22 (br. s, 1H, OH). Anal. calcd. for $C_{31}H_{29}N_3O_4$: C, 73.35; H, 5.76; N, 8.28. Found: C, 74.09; H, 5.67; N, 8.41%.

(2S)-2-(6-Methoxynaphthalen-2-yl)-N-(2-(5-(4-methoxy phenyl)-3-phenyl-4, 5-dihydro-1H-pyrazol-1-yl)-2-oxoethyl) propanamide (**5e**): Color: Off white powder. Yield: 68%. M.p.: 100-102 °C. R_f: A = 0.62, B = 0.51. FT-IR (KBr, ν, cm^{-1}): 3298 (NH amide), 1655 (C=O amide), 1604 (C=C Aromatic), 1573 (C=N amide), 1261 (Ar-O-C). ^{1}H NMR (500 MHz, DMSO-d_6, δ, ppm): 1.42-1.44 (d, 3H, CH_3 naproxe), 3.58-3.83 (m, 3H, CH_2 pyrazoline and CH of naproxen), 3.86 (s, 6H, -OCH_3 naproxen, OCH_3), 4.00 (br.s, 2H, CH_2 glycine), 5.25 (br. s, 1H, CH pyrazoline), 7.14-8.29 (m, 15H, Ar-H), 8.41 (br. s, 1H, NH amide). Anal. calcd. for $C_{32}H_{31}N_3O_4$: C, 73.68; H, 5.99; N, 8.06. Found: C, 74.79; H, 6.13; N, 8.25%.

(2S)-N-(2-(5-(4-(Dimethylamino)phenyl)-3-phenyl-4, 5-di hydro-1H-pyrazol-1-yl)-2-oxoethyl)- 2-(6-methoxynaphthalen-2-yl) propanamide (**5f**): Color: Orange powder. Yield: 54%. M.p.: 115-117 °C. R_f: A = 0.59, B = 0.45. FT-IR (KBr, ν, cm^{-1}): 3294 (NH amide), 1653 (C=O amide), 1603 (C=C Aromatic), 1523 (C=N amide), 1267 (Ar-O-C). ^{1}H NMR (500 MHz, DMSO-d_6, δ, ppm): 1.43-1.44 (d, 3H, CH_3 naproxe), 2.99 (s, 6H, $N(CH_3)_2$), 3.60-3.76 (m, 3H, CH_2 pyrazoline and CH of naproxen), 3.86 (s, 3H, -OCH_3 naproxen), 4.29 (br.s, 2H, CH_2 glycine), 5.27 (br. s, 1H, CH pyrazoline), 7.14-8.30 (m, 15H, Ar-H), 8.47 (br. s, 1H, NH amide). Anal. calcd. for $C_{33}H_{34}N_4O_3$: C, 74.13; H, 6.41; N, 10.48. Found: C, 73.69; H, 6.50; N, 10.67%.

2.2. Pharmacology

2.2.1. Anti-inflammatory evaluation study

In vivo, acute anti-inflammatory effects of the chemically synthesized compounds **5a-f** were evaluated in egg-white induced paw edema [36]. Their evaluation for ant-inflammatory activity based on measuring the decreases of paw thickness.

2.2.1.1. Methods

2.2.1.1.1. Animals

Albino rats of either sex weighing (170±10 g) were supplied by Iraqi Center for Cancer and Medical Genetic Research and were housed in College of Pharmacy, University of Al-Mustansiriyah under standardized conditions for 10 days for acclimatization. Animals were fed commercial chaw and had free access to water. Animals were brought to the laboratory, one hour before the experiment, and were divided into eight groups (each group consist of 6 rats) as follows:

Group A: Six rats served as control and treated with the vehicle (propylene glycol 50%, *v*:*v*).

Group B: Six rats treated with (s)-naproxen as reference substance in a dose of 50mg/kg suspended in propylene glycol [37,38].

Group C-H: Six rats/group treated with the tested compounds **5a-f**, respectively in doses that determined below, also suspended in propylene glycol.

2.2.1.1.2. Calculations for dose determination

50 mg / kg / 230 = Dose / M.Wt. of the tested compound ((s)-Naproxen = 230.26 g/mol), Table 2.

2.2.1.1.3. Experimental design

The anti-inflammatory activity of the tested compounds was studied using the egg-white induced edema model.

Table 2. Compounds with their molecular weight and dose.

Compounds	Molecular weight, g	Dose mg/kg
(S)-Naproxen	230.26	50.00
5a	491.59	106.74
5b	526.03	114.22
5c	536.58	116.51
5d	507.58	110.21
5e	521.60	113.26
5f	534.65	116.09

Table 3. The anti-inflammatory effect of control, naproxen and compounds **5a-f** on egg-white induced paw edema in rat [#].

	Compounds	Time (min)						
		0	30	60	120	180	240	300
Paw Thickness (mm) / n=6	Control	4.85±0.05	5.83±0.06	6.58±0.06	6.96±0.03	6.81±0.06	6.71±0.02	5.39±0.01
	Naproxen	4.82±0.04	5.72±0.05	6.51±0.05	5.81±0.05 *a	5.44±0.06 *a	5.14±0.06 *a	4.93±0.02 *
	5a	4.87±0.06	5.74±0.02	6.56±0.06	6.31±0.01 *b	5.72±0.03 *b	5.43±0.02 *b	5.06±0.04 *
	5b	4.81±0.03	5.75±0.06	6.51±0.01	6.21±0.04 *b	5.76±0.06 *b	5.51±0.02 *b	4.99±0.05 *
	5c	4.81±0.02	5.75±0.01	6.50±0.03	6.17±0.06 *b	5.73±0.05 *b	5.47±0.06 *b	5.03±0.06 *
	5d	4.78±0.01	5.79±0.06	6.49±0.04	5.84±0.03 *a	5.46±0.02 *a	5.16±0.05 *a	5.06±0.06 *
	5e	4.82±0.06	5.74±0.02	6.49±0.03	5.91±0.02 *a	5.41±0.04 *a	5.15±0.01 *a	4.99±0.06 *
	5f	4.84±0.01	5.82±0.02	5.77±0.04 *	5.43±0.05 *c	5.08±0.06 *c	4.86±0.03 *c	4.94±0.08 *

[#] Non-identical superscripts (a, b and c) among different tested compounds are considered significantly different ($p < 0.05$).
* Significantly different compared to control ($p < 0.05$). Data are expressed in mm paw thickness as mean ± SEM. n= number of animals. Time (0) is the time of i.p. injection of naproxen, tested compounds and propylene glycol. Time (30) is the time of injection of egg white (induction of paw edema).

The paw thickness was measured by vernea at seven time intervals (0, 30, 60, 120, 180, 240, and 300 min) after drug administration. Acute inflammation was produced by a sub-cutaneous injection of 0.05 mL of undiluted egg-white into the plantar side of the left hind paw of the rats; 30 min after intra-peritoneal administration of the drugs or their vehicle.

2.2.1.2. Statistical analysis

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

2.2.2. Antimicrobial activity

A preliminary antibacterial activity has been carried out according to Well Diffusion Method: The prepared compounds have been studied for their antimicrobial activity *in vitro* against four tested bacteria (*Staphylococcus aureus*, *Bacillus*, as Gram positive bacteria and *Pseudomonas aeruginosa*, *Escherichia coli*, as Gram negative bacteria) were clinically activated and maintained on nutrient agar medium for testing the antibacterial activity. Naproxen was used as a reference drug for antibacterial activity.

2.2.2.1. Sensitivity assay

Well diffusion assay was carried out by using bacterial suspension of about 1.5×10^6 CFU/mL with turbidity standard (number 0.5). This was used to inoculate by swabbing the surface of Mueller Hinton agar (MHA) plates. Excess liquid was air-dried under a sterile hood. In each agar plate of tested bacteria four wells were made and 100 µL of each concentration was added in it. The plates were incubated at 37 °C for 24 hrs. The assessment of antibacterial activity were based on measurement of the diameter of inhibition zone formed around the well [39] thus, showed that the zone of inhibition mostly increased with the increasing of concentration of the tested compounds.

3. Result and discussions

3.1. Anti-inflammatory evaluation study

3.1.1. 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 naproxen. Table 3 explains the effect of tested compounds **5a-f** in comparison to control and naproxen.

3.1.2. Comparative study

All tested compounds exerted significant reduction of paw edema in comparison to the effect of propylene glycol 50%v/v (control group). The effect of naproxen and all tested compounds started at time 120 min except **5f** which has been started at 60 min. thus, indicate fast onset of action. The effect of tested compounds and naproxen continued until the end of experiment. Compound **5d** and **5e** showed comparable effect to naproxen at all experimental time while compounds **5a**, **5b** and **5c** produced significantly lower inhibitory effect than naproxen at time 120-240 min. Surprisingly, compound **5f** exert significantly higher paw edema reduction than naproxen at 60-240 min. All tested compounds have been showed comparable effect to that of naproxen at time 300 min.

3.1.3. Percent of inhibition in paw edema thickness

The percent of inhibition of paw edema thickness at each time interval was calculated from the mean effect in control and treated animals according to the equation [40]:

$$\%\text{Inhibition} = [Vc - Vt / Vc] \times 100 \quad (1)$$

Where Vc and Vt are the mean paw thickness of the control group and tested group (at t-time zero) respectively [40,41]. The comparison among the naproxen, compounds **5a-f** was shown in Table 4.

3.2. Antibacterial activity

Naproxen and ciprofloxacin as reference, DMSO as control and the synthesized compounds **5a-f** were screened for their antibacterial activity studies against *Pseudomonas aeruginosa*, *Escherichia coli*, *Bacillus* and *Staphylococcus aureus* at concentrations of 62.5, 125, 250 and 500 µg/mL except the control which used pure. Table 5 illustrates the inhibition zone in millimeter for each concentration of all tested compounds.

Table 4. Percent inhibition of naproxen, compounds **5a-f** on egg-white induced paw edema in rats.

Time (min)	Percent of inhibition, %						
	Naproxen	5a	5b	5c	5d	5e	5f
60	2.3	2.3	1.7	3.2	1.1	3.4	46.2
120	53.0	31.7	33.6	35.5	49.7	48.3	72.0
180	68.3	56.6	51.5	53.0	65.3	69.8	87.7
240	82.7	69.8	62.3	64.5	79.5	82.2	98.9
300	79.6	64.8	66.6	59.2	48.1	68.5	81.4

Table 5. Antibacterial activity of synthesized compounds **5a-f**.

Compounds and standard	Concentration (µg/mL)	Zone of inhibition (mm)			
		Gram negative		Gram positive	
		Escherichia coli	*Pseudomonas aeruginosa*	*Bacillus*	*Staphylococcus aureus*
5a	500.0	20	19	29	18
	250.0	7	19	24	10
	125.0	12	14	23	7
	62.5	-	-	23	-
5b	500.0	16	20	23	21
	250.0	12	21	25	12
	125.0	7	10	19	-
	62.5	7	8	17	-
5c	500.0	13	17	21	16
	250.0	10	14	20	8
	125.0	9	13	19	8
	62.5	10	12	15	-
5d	500.0	15	19	24	15
	250.0	13	14	24	13
	125.0	11	10	26	10
	62.5	11	-	20	8
5e	500.0	23	25	32	14
	250.0	15	15	20	7
	125.0	11	11		
	62.5	11	7	16	-
5f	500.0	16	22	22	20
	250.0	15	20	20	16
	125.0	13	15	20	14
	62.5	13	11	20	-
Naproxen	500.0	-	12	-	-
	250.0	-	-	-	-
	125.0	-	-	14	-
	62.5	-	-	-	-
Ciprofloxacin	500.0	35	18	26	25
	250.0	33	23	30	25
	125.0	30	19	26	25
	62.5	28	13	26	25
DMSO	Pure	-	-	-	-

In general, all tested compounds showed an interesting activity against Gram positive and Gram negative bacteria especially *Bacillus, Staphylococcus aureus, Pseudomonas aeruginosa*and *Escherichia coli.* These tested compounds exert significant antibacterial activity in comparison to DMSO as control group. In comparison to standard compound (Ciprofloxacin), tested compound exert lower effect against *Escherichia coli* and nearly comparable effect against *Pseudomonas aeruginosa*. Also, the tested compounds showed a comparable antibacterial effect against *Bacillus* and lower activity against *Staphylococcus aureus.* In comparison the antibacterial results among the tested compounds **5e** might be considered as the most effective one and **5c** as the lower effective one which might lead to the conclusion that electron withdrawing substitutes have generally lower antibacterial effect than electron donating substitutes.

4. Conclusions

Anti-inflammatory study that has been used egg-white induced edema model of inflammation has been shown that the incorporation of pyrazoline pharmacophore into a naproxen maintained or enhanced it is anti-inflammatory activity. Furthermore the antibacterial activity has been studied by well diffusion method showed significant activity against Gram positive and Gram negative bacteria especially *Bacillus, Staphylococcus aureus, Pseudomonas aeruginosa*and *Escherichia coli.*

Acknowledgements

The authors are thankful to the laboratory for pharmaceutical Chemistry, Al-mustansiriyah University, Baghdad, Iraq, for providing the necessary facilities to conduct research work. We would also like to acknowledge the support received from assistant Prof.Dr. Ali Hussein Alwan and Prof.Dr. Tarek Aboul-Fadl while conducting the research work.

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