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SYNTHESIS, CHARACTERIZATION AND SCREENING OF PYRAZOLINE DERIVATIVES FOR ANTI-INFLAMMATORY ACTIVITY

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Keywords:

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ABSTRACT: Inflammation is a basic way in which the body reacts to infection, irritation or other injury, the key feature being redness, warmth, swelling and pain. There was a need for synthesis of anti-inflammatory agents with increased potency. Pyrazoline (dihydro pyrazole) derivatives exhibit various biological activities. These derivatives are well known for their pronounced anti-inflammatory activity. Therefore such ring systems are of great interest in medicinal chemistry. A series of novel 1, 3, 5 trisubstituted-2-pyrazoline derivatives were synthesized in a series of three steps. Firstly, acetophenone and 4-methoxybenzaldehyde were reacted in presence of sodium hydroxide with ethanol as the solvent leading to the formation of chalcones (1). These chalcones were reacted with hydrazine hydrate which formed the new cyclized product (2). Finally, the cyclized product was reacted with different substituted chlorides in presence of ethanolic solution of sodium hydroxide to give the new 1, 3, 5 trisubstituted-2-pyrazoline derivatives (3a - 3j). All the synthesized compounds were characterized by FTIR spectroscopy, UV spectroscopy and compounds of interest were characterized by (^{1}H and ^{13}C) NMR spectroscopy and LC-MS spectroscopy. These compounds were screened for *in-vivo* anti-inflammatory activity by using Carrageenan induced rat paw edema method. The compounds were tested at 50 mg/kg dose and diclofenac sodium was used as a reference standard. Compound 3d with acetyl substitution was found to be the most active compound.

INTRODUCTION: Inflammation is defined as the local response of living mammalian tissue to injury due to any agent. It is a body defense reaction in order to eliminate or limit the spread of injurious agent, followed by removal of necrosed cells and tissues.

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NSAIDs produce renal effects by at least 3 mechanisms: COX-1 dependent impairment of renal blood flow and reduction of glomerular filteration rate (g.f.r). This can worsen renal insufficiency. Juxta glomerular COX-2 (probably COX-1 also) dependent Na^{+} and water retention. Ability to cause papillary necrosis on habitual intake [1, 2].

The major mechanism of action of Non-steroidal anti-inflammatory drugs (NSAIDs) is blocking prostaglandin (PG) generation. Prostaglandins, prostacyclin (PG I_2) and thromboxane A_2 (TXA_2) are produced from arachidonic acid by the enzyme

cyclooxygenase which exists in a constitutive (COX-1) and an inducible (COX-2) isoforms; the former serves physiological 'house keeping' functions, while the latter, normally present in minute quantities, is induced by cytokines and other signal molecules at the site of inflammation. Further there is generation of PGs locally which mediate many of the inflammatory changes. However, COX-2 is constitutively present at some sites in brain and in juxtaglomerular cells: may serve physiological role at these sites. Most NSAIDs inhibit COX-1 and COX-2 non-selectively, but now some selective COX-2 inhibitors have been produced.[1]

Pyrazolines are five membered heterocyclic compounds containing two nitrogens with molecular formula $C_3H_6N_2$. The dihydropyrazoles are also called pyrazolines. 4, 5-dihydro-1*H*-pyrazole (2-pyrazoline) show a wide range of biological activity such as anti-inflammatory activity, analgesic, antipyretic, anticancer activity, CB1 receptor antagonism for obesity, antidepressant, antimicrobial, antihypertensive, antidiabetic and monoamine oxidase (MOA) inhibitory activities. Therefore such ring systems are of great interest in medicinal chemistry. Sayed MAA *et al*., reported synthesis, anti-inflammatory, analgesic, COX-1/2 inhibition activities and molecular docking study of pyrazoline derivatives [3].

MATERIALS AND METHODS: Melting points of the synthesized compounds were determined by Thiele's tube melting point apparatus. FT-IR spectra were recorded on Shimadzu IR affinity-1 spectrophotometer by using KBr pellets. The ^{1}H NMR and ^{13}C NMR were recorded on Bruker Advance II 400 NMR spectrophotometer by using $CDCl_3$ as solvent, chemical shifts are expressed as δ values (ppm). The mass spectra were recorded on Waters, Q- TOF Micromass (LC-MS). The UV spectra were recorded on Shimadzu UV-Spectrophotometer and the λ_{max} values were determined.

Synthesis of 3-(4-methoxyphenyl)-1-phenylprop-2-en-1-one (1): A mixture of acetophenone (3.61 g, 0.03 mol) and 4-methoxybenzaldehyde (4.08 g, 0.03 mol) were dissolved in 30 ml of cold ethanol, to this solution 5 ml of 10% aqueous sodium hydroxide solution was added slowly while stirring. The reaction was stirred at room temperature for 3 h and was then cooled. The completion of the reaction was monitored by TLC using (*n*-Hexane: Ethyl acetate, 7:3) as mobile phase and visualized under UV chamber. The resulting solid was washed, dried and recrystallized from ethanol.

Yellow coloured solid, m.p. 54 °C, Yield: 92.27%, R_f value: 0.82 [*n*-hexane: Ethyl acetate; 7: 3], IR (KBr, cm^{-1}): 3014.74 (C-H aromatic), 2954.95, 2841.15 (C-H aliphatic), 1658.99 (C=O), 1598.99 (C=C alkene), 1512.19 (C=C aromatic), ^{1}H NMR ($CDCl_3$, δ ppm): 7.99-8.01 (d, 2H, Ar-H), 7.76-7.80 (d, 1H, C=C-H), 7.53-7.59 (m, 3H, Ar-H), 7.47-7.49 (d, 2H, Ar-H), 7.38-7.42 (d, 1H, C=C-H), 6.90-6.92 (d, 2H, Ar-H), 3.82 (s, 3H, O-CH_3), λ_{max}: 248.

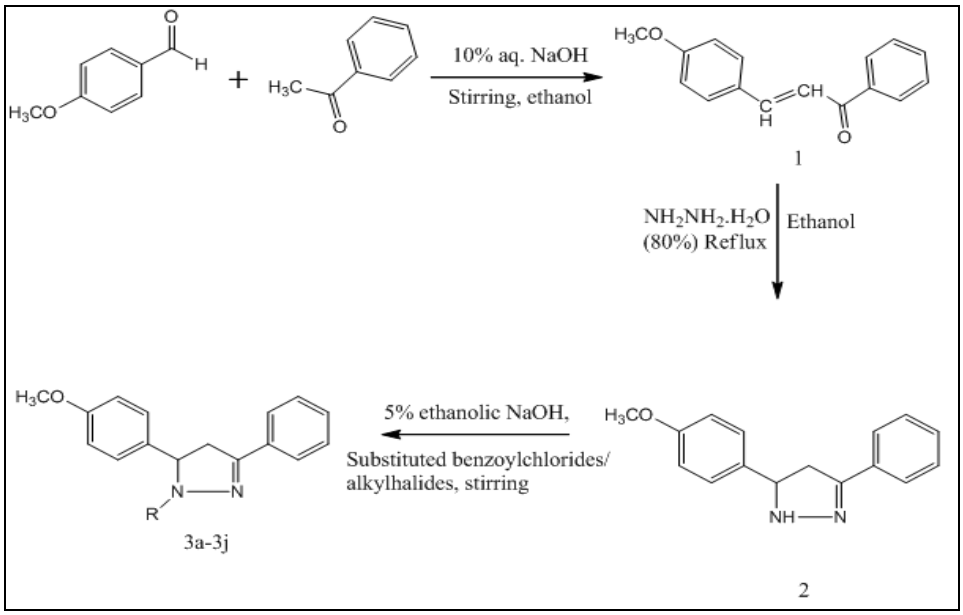
Synthesis of 5-(4-methoxyphenyl)-3-phenyl-4, 5-dihydro- 1*H*- pyrazole (2): A solution of appropriate chalcone (1, 7.14 g, 0.03 mol) and hydrazine hydrate (80%, 3.00 g, 0.06 mol) in 30 ml ethanol was refluxed for 6 h. The completion of the reaction was monitored by TLC using (*n*-Hexane: Ethyl acetate, 7:3) as mobile phase and visualized under UV chamber. The reaction mixture was cooled and kept at 0 °C overnight. The resulting solid was filtered, washed, dried and recrystallized from ethanol.

White coloured solid, m.p. 96 °C, Yield: 93.52%, $R_f$value: 0.72 [*n*-hexane: Ethyl acetate; 7:3], IR (KBr, cm^{-1}): 3340.71 (NH), 3034.03 (C-H aromatic), 2939.52, 2839.22 (C-H aliphatic), 1606.70 (C=N), 1510.26 (C=C), ^{1}H NMR ($CDCl_3$, δ ppm): 7.64-7.67 (d, 2H, Ar-H), 7.31-7.38 (m, 3H, Ar-H), 7.24-7.27 (d, 2H, Ar-H), 6.83-6.86 (d, 2H, Ar-H), 4.82-4.87 (dd, 1H, pyrazole C_5-H), 3.76 (s, 3H, O-CH_3), 3.37-3.44 (dd, 1H, pyrazole C_4-H), 2.97-3.03 (dd, 1H, pyrazole C_4-H), λ_{max}:227.5.

Synthesis of 1, 3, 5-trisubstituted-2-pyrazoline derivatives (3a- 3j): Pyrazoline (2, 1.26 g, 0.005 mol) was addedin 20 ml 5% ethanolic sodium hydroxide solution in a small conical flask. To the above solution substituted benzoyl chloride/ alkyl halide (0.02 mol) was added with constant shaking and cooling (if necessary). The reaction mixture was stirred vigorously for 5 - 10 mins until the odour of substituted benzoyl chloride / alkyl halide

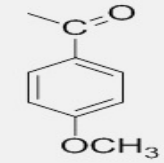
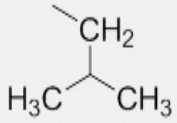
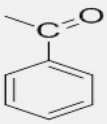
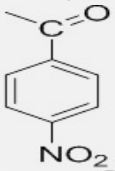
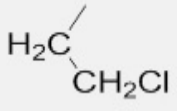
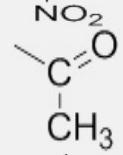
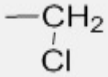
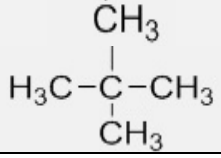
disappears. The solid thus formed was filtered off, washed with cold water and recrystallized from ethanol.

[5-(4-methoxyphenyl)-3-phenyl-4, 5-dihydro-1*H*-pyrazol-1-yl](4-methoxyphenyl) methanone (3a): Cream coloured solid, m.p. < 300 °C, Yield: 94.56, IR (KBr, cm^{-1}): 3055.24 (C-H aromatic), 2924.09 (C-H aliphatic), 1625.99 (C=O), 1604.77 (C=N), 1514.12 (C=C), ^{1}H NMR ($CDCl_3$, δ ppm): 6.83-8.11 (m, 13H, Ar-H); 5.7-5.79 (dd, 1H, pyrazole C_5-H); 3.83 (s, 1H, O-CH_3); 3.73 (s, 1H, O-CH_3); 3.66-3.71 (dd, 1H, pyrazole C_4-H); 3.13-3.17 (dd, 1H, pyrazole C_4-H); ^{13}C NMR ($CDCl_3$, δppm): 165.65 (1C,C=O); 159.03 (1C,C=N); 142.25, 134.35, 132.41, 131.59, 130.30, 128.93, 128.77, 128.37, 127.60, 127.10, 126.75, 126.66, 126.59, 125.75, 124.15, 114.30, 114.17, 112.94 (18C, Ar-C); 60.93 (1C, CH-N); 55.43, 55.38 (2C, C-O); 41.52 (1C, pyrazole CH_2), MS: m/z = 387.2 (M^+), λ_{max}: 309.5.



SCHEME 1: SYNTHESIS OF 1, 3, 5-TRISUBSTITUTED-2-PYRAZOLINE DERIVATIVES (3a- 3j)

TABLE 1: STRUCTURES OF PYRAZOLINE DERIVATIVES (3a-3j)

Compound	R / Ar	Compound	R / Ar
3a		3f	
3b		3g	
3c		3h	
3d		3i	
3e		3j	

[5-(4-methoxyphenyl)-3-phenyl-4, 5-dihydro-1*H*-pyrazol-1-yl] (phenyl)methanone (3b): Yellow coloured solid, m.p. < 300 °C, Yield: 93.47%, IR (KBr, cm^{-1}): 3030.17 (C-H aromatic), 2922.16 (C-H aliphatic), 1633.71 (C=O), 1597.06 (C=N), 1552.70 (C=C), ^{1}H NMR ($CDCl_3$, δ ppm): 7.24-7.72 (m, 14H, Ar-H); 5.77-5.81 (dd, 1H, pyrazole C_5-H); 3.88 (s, 3H, O-CH_3), ^{13}C NMR ($CDCl_3$, δppm): 165.28 (1C, C=O); 160.92 (1C, C=N); 142.15, 134.42, 134.06, 130.95, 130.50, 130.07, 129.99, 129.39, 129.09, 128.50, 128.16, 127.38, 127.29, 127.09, 126.67, 126.47, 125.30, 123.36 (18C, Ar-C); 60.46 (1C, CH-N); 55.06 (1C, C-O); 40.19 (1C, pyrazole CH_2), MS: m/z = 356.1 (M^+), λ_{max}: 222.

[5-(4-methoxyphenyl)-3-phenyl-4, 5-dihydro-1*H*-pyrazol-1-yl](4-nitrophenyl) methanone (3c): Greenish yellow coloured solid, m.p. < 300 °C, Yield: 94.26%, IR (KBr, cm^{-1}): 3022.45 (C-H aromatic), 2929.87 (C-H aliphatic), 1639.49 (C=O), 1597.06 (C=N), 1519.91, 1340.53 (NO_2), λ_{max}: 272.50.

1-[5-(4-methoxyphenyl)- 3- phenyl-4, 5-dihydro-1*H*-pyrazol-1-yl]ethanone (3d): Orange coloured solid, m.p. 100 °C, Yield: 83.24%, IR (KBr, cm^{-1}): 3055.24 (C-H aromatic), 2951.09 (C-H aliphatic), 1633.71 (C=O), 1597.06 (C=N), 1550.77 (C=C), λ_{max}: 260.

1-*tert*-butyl-5-(4-methoxyphenyl)-3- phenyl- 4, 5-dihydro-1*H*-pyrazole (3e): Yellow coloured solid, m.p. 140 °C, Yield: 93%, IR (KBr, cm^{-1}): 3066.82 (C-H aromatic), 2954.95 (C-H aliphatic), 1595.13 (C=N), 1550.77 (C=C), λ_{max}: 228.

5- (4-methoxyphenyl)- 1- (2- methylpropyl)- 3-phenyl-4, 5-dihydro-1*H*-pyrazole (3f): Yellow coloured solid, m.p. 142 °C, Yield: 82.62%, IR (KBr, cm^{-1}): 3057.17 (C-H aromatic), 2908.65 (C-H aliphatic), 1595.13 (C=N), 1550.77 (C=C), λ_{max}: 246.

5- (4- methoxyphenyl)- 1- pentyl- 3-phenyl-4, 5-dihydro-1*H*-pyrazole (3g): Yellow coloured solid, m.p. 138 °C, Yield: 80.16%, 3012.81 (C-H aromatic), 2937.59 (C-H aliphatic), 1595.13 (C=N), 1550.77 (C=C), λ_{max}: 229.

1-(2-chloroethyl)-5-(4-methoxyphenyl)-3-phenyl-4, 5-dihydro-1*H*-pyrazole (3h): Yellow coloured solid, m.p. 102 °C, Yield: 81.13%, 3057.17 (C-H aromatic), 2954.95 (C-H aliphatic), 1598.99 (C=N), 1573.91 (C=C), 779.24 (C-Cl), λ_{max}: 328.5.

1-(chloromethyl)-5-(4-methoxyphenyl)-3-phenyl-4, 5-dihydro-1*H*-pyrazole (3i): Yellow coloured solid, m.p. 100 °C, Yield: 80.29%, 3057.17 (C-H aromatic), 2943.37 (C-H aliphatic), 1598.99 (C=N), 1571.99 (C=C), 779.24 (C-Cl), λ_{max}: 226.5.

1- (dichloromethyl)- 5- (4-methoxyphenyl)- 3-phenyl- 4, 5-dihydro-1*H*-pyrazole (3j): Yellow coloured solid, m.p. 104 °C, Yield: 80.42%, 3057.17 (C-H aromatic), 2900.94 (C-H aliphatic), 1598.99 (C=N), 1568.13 (C=C), 779.24 (C-Cl), λ_{max}: 328.

RESULTS AND DISCUSSION:

Biological Evaluation:

In vivo Anti-inflammatory Activity:

Carrageenan-Induced Rat Paw Edema Method:

Animal ethical committee approval number: PES RTBCOP/ IAEC. Clear 2016-17/ R- 22.

In vivo anti-inflammatory activity was evaluated by carrageenan-induced rat paw edema method on the synthesized compounds using Diclofenac sodium as reference standard. Albino rats (180 - 250 g) of either sex were used. The animals were divided into six in each group. One group was taken as normal control, second group as positive control, third group was administered with a dose of 10 mg/kg of the suspension of diclofenac sodium and other groups were administered with a dose of 50 mg/kg of test compounds.

All the synthesized compounds were suspended in 0.2 ml of 2% w/v carboxy methyl cellulose with 2.0% Tween 80. After 30 min; a sub-plantar injection of 0.1 ml carrageenan (100µg/rat) in 0.9% NaCl was administered to the sub-plantar region of right hind paw.

The paw edema volume was measured with a plethysmograph at 30 min, 1 h, 2 h and 3 h after carrageenan injection. The amount of edema in the groups treated with reference standard and groups treated with synthesized compounds were compared in relation to the positive control group with the corresponding time intervals [4]. The results are represented in **Table 2**.

TABLE 2: EFFECTS OF SYNTHESIZED COMPOUNDS ON CARRAGEENAN INDUCED RAT PAW EDEMA MODEL

Sr. o.	Groups (n = 6)	30 min (ml)	1st h (ml)	2nd h (ml)	3rd h (ml)
1	Normal control	0.85 ± 0.02	0.83 ± 0.01	0.83 ± 0.03	0.84 ± 0.02
2	Positive control	1.20 ± 0.03	1.27 ± 0.06	1.85 ± 0.07	2.03 ± 0.04
3	Sample 3a (50 mg/kg)	1.10 ± 0.07	1.13 ± 0.05	1.23 ± 0.05	1.30 ± 0.03
4	Sample 3b (50 mg/kg)	0.90 ± 0.06	0.99 ± 0.04	1.09 ± 0.07	1.11 ± 0.05
5	Sample 3c (50 mg/kg)	0.82 ± 0.03	1.33 ± 0.19	1.13 ± 0.14	1.03 ± 0.05
6	Sample 3d (50 mg/kg)	0.80 ± 0.02	0.84 ± 0.01	0.89 ± 0.02	0.91 ± 0.01
7	Sample 3e (50 mg/kg)	0.93 ± 0.03	0.98 ± 0.03	0.99 ± 0.06	1.02 ± 0.07
8	Diclofenac sodium (10 mg/kg)	1.02 ± 0.09	0.90 ± 0.04	0.86 ± 0.11	0.79 ± 0.02

CONCLUSION: A series of novel 1, 3, 5-trisubstituted-2-pyrazoline derivatives were synthesized. These compounds were confirmed by various spectroscopic techniques and were screened for *in-vivo* anti-inflammatory activity by using Carrageenan induced rat paw edema method. Compound 3d with acetyl substituent was found to be the most active compound.

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CONFLICT OF INTEREST: The author(s) confirm this article content has no conflict of interest.

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