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Synthesis, Characterization and Evaluation of Analgesic, Anti-Inflammatory, Ulcerogenic Potential of Some 2-Pyrazoline Derivatives

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ABSTRACT

The discovery of cyclooxygenase-2, expressed in response to inflammatory stimuli, present in the central nervous system, not in the gastric mucosa has provided a unique opportunity in the development of NSAIDs that lack the ulcerogenic effect. Thus it has led to the hypothesis that selective inhibitor of COX-2 over COX-1 may be better anti-inflammatory agent with less adverse effects than the classical NSAIDs. As classical NSAIDs like aspirin, indomethacin are nonselective with respect two both isoforms. The preferential binding affinity of classical NSAIDs to COX-1 leads to mechanism based gastrointestinal, renal, hepatic side effects. Two varieties of acetophenones were condensed with three varieties of substituted benzaldehyde derivatives to get six chalcone derivatives which undergo condensation followed by cyclisation with isoniazid and 1-(2-napthyloxy acetate) hydrazine two get the final twelve 2-pyrazoline derivatives. The synthesized compounds were characterized by IR, ^{1}H-NMR and mass spectral studies. The synthesized compounds were evaluated for their analgesic and anti-inflammatory activity. Two compounds which exhibited comparatively better analgesic and anti-inflammatory activity among the synthesized derivatives were screened for their ulcerogenic potential and were found to be less ulcerogenic than the standard diclofenac sodium.

Keywords: Chalcone, 2-Pyrazoline, Analgesic, Anti-inflammatory, Ulcerogenic potential.

INTRODUCTION

Cyclooxygenases (COX) are the principal enzymes involved in the biosynthetic pathway of prostaglandins (PGs), thromboxanes and prostacyclins. The cyclooxygenase enzyme exists in two distinct isoforms: Cyclooxygenase-1 and Cyclooxygenase-2 [1]. Comparison of COX-1 and COX-2 leads to 60% identity of the sequence. Single peptide of COX-2 is seven residues shorter than that of COX-1. The C-terminus of COX-2 has 18-residue insertion while the N-terminus of COX-1 has 8-residue insertion [2]. COX-1 is constitutive and is important for cytoprotection of gastrointestinal tract, platelet aggregation and renal blood flow. But COX-2 is inducible and expressed during inflammation, pain and oncogenesis [3]. They are induced by pro-inflammatory molecules like lipopolysaccharide, tumor necrosis factor, interleukin, carrageenan etc [4]. Non-steroidal anti-inflammatory drugs find the most clinical

importance in the management of inflammation, pain and fever [5]. These drugs exert anti-inflammatory activity and relieve inflammation associated pain by the interacting and inhibiting the enzymatic activity [6] leading to the inhibition of prostaglandins. But the classical NSAIDs like aspirin, indomethacin are nonselective with respect two both isoforms. Aspirin inhibits COX-1 strongly than COX-2 and inhibition of COX-1 reduces production of PGE2 and PGI2 which results in ulcerogenic effect [7].The preferential binding affinity of classical NSAIDs to COX-1 leads to mechanism based side effects like gastrointestinal ulcerations, dyspepsia and nephrotoxicity [8]. The discovery of COX-2, expressed in response to inflammatory stimuli, present in the central nervous system, not in the gastric mucosa has provided a unique opportunity to develop NSAIDs that lack the ulcerogenic effect [9]. Thus it has led to the hypothesis that selective inhibitor of COX-2 over COX-1 may be better anti-inflammatory agent with less adverse effects than the classical NSAIDs. Selective COX-2 inhibitors having better safety profile are now marketed as new generation NSAIDs [9-10]. But these selective COX-2 inhibitors (coxibs) are found to have some unexpected cardiovascular adverse effects [11], increased systemic blood pressure and hypersensitivity [12] and some of them are already withdrawn from market [3]. So, design and development of NSAIDs with enhanced safety profile is still a necessity and challenge for the pharmaceutical industry. Moreover NSAIDs are getting lot of attraction because of their utility in early phases of many serious disorders like Alzheimer's dementia, cancer, heart vascular disease etc.

Literature survey reveals that the 2-pyrazoline derivatives have engrossed substantial attention from medicinal chemists. 2-pyrazoline is an important scaffold since several 2-pyrazoline derivatives are found to be associated with wide range of biological activities like antimicrobial [13-14], anti-amoebic [15], antimycobacterial [16], antiviral [17], analgesic, anti-inflammatory [18-22], antinociceptive [23], anticancer [24], antioxidant [25], hypotensive [26], anti-convulsant [27], antidepressant [28], CB1 receptor antagonist [29] etc. Many pyrazoline derivatives have gained their clinical application as NSAIDs. Antipyrine (2,3-dimethyl-1-phenyl-3-pyrazolin-5-one) was the first pyrazoline derivative used in the management inflammation and pain. Phenylbutazone and its metabolite oxyphenbutazone are pyrazolinedione derivatives having potent anti-inflammatory activity. However they became restricted due to their GI side effects. Several pyrazolidine-3,5- diones, pyrazolin-5-ones and pyrazolin-3-ones are available as NSAIDs e.g. felcobuzone, famprofazone, morazone, mefobutazone and ramifenazone. Selective COX2 inhibitors are lack of carboxylate group unlike the classical NSAIDs. Besides these many pyrazoline derivatives are reported in the literature as potent anti-inflammatory and analgesic agent.

As per literature review the pyridine, naphthalene, furan, thiophene derivatives also possess analgesic [30-33] and anti-inflammatory [34-36] activities. Encouraged by these facts it was planned to synthesize 2-pyrazoline derivatives containing pyridyl/napthyl, furyl/thienyl/4-nitrophenyl and 4-chlorophenyl/4-hydroxyphenyl groups as substituents without the presence of carboxylate group because incorporation of an acidic group results in the poor selectivity towards COX2. The synthesized twelve 2-pyrazoline derivatives were characterized by IR, ^{1}H-NMR, Mass spectroscopy and were found to possess an interesting profile of anti-inflammatory, analgesic activity with significant reduction in their ulcerogenic potential when compared to the standard drug.

MATERIALS AND METHODS

The all reagents used in the present study were of analytical grade and were obtained from the store of C.L. Baid Metha College of Pharmacy. The melting points of the synthesized compounds were determined by open capillary tube method and are uncorrected. The ^{1}H-NMR spectra were recorded at 400 MHz at BRUKER NMR spectrophotometer in DMSO and chemical shifts are expressed in parts per million (δ) relative to tetramethylsilane. The IR spectra were recorded on Shimadzu FTIR 8400S using potassium bromide pellet technique. The completions of the reaction were monitored by Thin Layer Chromatographic technique (TLC) on pre-coated silica gel (HF254-200 mesh) aluminium plates from E-merk using ethyl acetate: n-hexane (4:1) as the mobile phase. Detection of the spots was done under UV chamber. The test animals were obtained under the IAEC Reference No: IAEC/XXXIV/11/CLBMCP/2011 dated 07.12.2011.

Synthesis:

Scheme 1: Preparation of Chalcones (A_1-A_6) [37]

A mixture of acetophenone (0.01mol) and aryl aldehyde (0.01 mol) was stirred in ethanol (30ml) and then an aqueous solution of KOH (40%, 15ml) added to it. The mixture was kept overnight at room temperature and then it was poured into crushed ice and acidified with HCl. The solid separated was filtered and crystallized from ethanol.

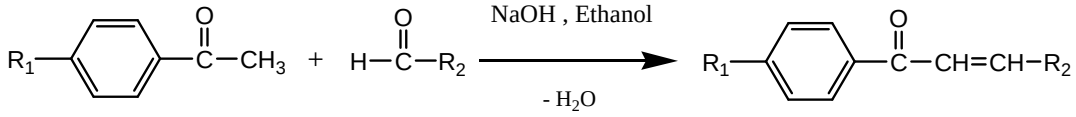
Scheme 2: Preparation of 1-(2-napthyloxyacetyl) hydrazine (B) [36]

2-napthol (1.44g, 0.01mol), anhydrous K_2CO_3 and ethyl bromoacetate (1.67g, 0.01mol) in 50 ml anhydrous acetone were refluxed on an oil bath for 6 hr. After that the reaction mixture was filtered, excess of solvent was removed by distillation under reduced pressure. The residue and 1.00 g hydrazine monohydrate (0.02mol) were dissolved in 50 ml absolute ethanol and refluxed on steam bath for 1 hour. The solid mass obtained was filtered, dried and recrystallised from ethanol.

Scheme 3: Preparation of 2-Pyrazoline derivatives [19, 38]

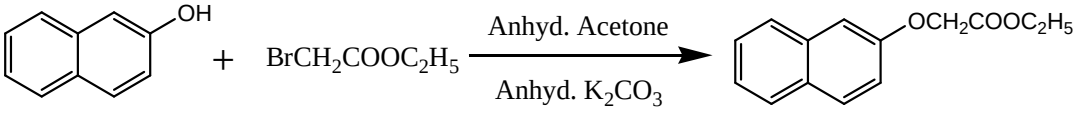
To a solution of compounds A_1-A_6 (0.01mol) in absolute ethanol (30 ml), hydrazine derivatives (0.01mol) and few drops of glacial acetic acid were added. The reaction mixtures were refluxed for 8 hr. The excess of solvent was distilled off and crude products were poured into ice water. The separated solids were filtered and recrystallised from ethanol.

Scheme 1:

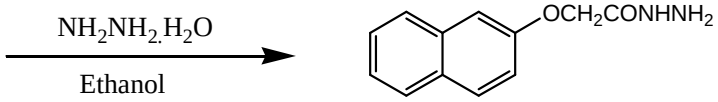


Substituted acetophenone — Substituted benzaldehyde — Chalcone **(A_1-A_6)**

Scheme 2:

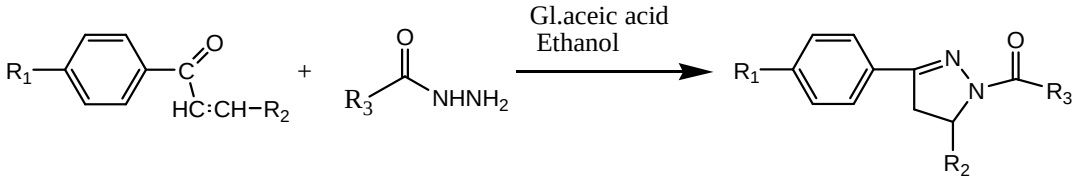


2-Napthol — ethyl bromo acetate — 2-Napthyloxy acetate



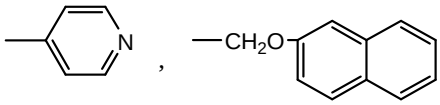
1-(2-Naptyloxyacetyl)hydrazine **(B)**

Scheme 3:



A_1- A_6 — Title compounds D1-D12

R_1 = -Cl , -OH ; R_2 = 4-$NO_2C_6H_5$- , 2-furyl , 2-thienyl

R_3 = 

Compound D1: (3-(4-chlorophenyl)-4,5-dihydro-5-(4-nitrophenyl)pyrazol-1-yl) pyridin-4-yl) methanone. Mf-$C_{21}H_{15}ClN_4O_3$, Mp-135^0 -137^0 C, R_f- 0.62, yield- 62.15%, I R (KBr) ν_{max} (cm^{-1}) : 3087.19 (C-H aromatic str.), 1658.41 (C=O str.), 1616.02 (C=N str. Pyrazoline), 1592.12 (C=C str.), 1528.36 (NO_2 assym. str.), 1345.03 (NO_2 sym. str.), 710.93 (C-Cl str.); ^{1}H NMR (DMSO) δ ppm: 7.57-8.53(m,6H, ArH), 5.19-5.26 (dd,1H,CH Pyrazoline), 3.46- 3.52(dd,1H, CH_2 Pyrazoline), 3.05-3.12(dd,1H, CH_2 Pyrazoline)MS(m/e): 406.8 ($M^{+)}$, 408.8(M+2)

Compound D2: (3-(4-hydroxyphenyl)-4,5-dihydro-5-(4-nitrophenyl)pyrazol-1-yl)(pyridin-4-yl) methanone. Mf- $C_{21}H_{16}N_4O_4$, Mp-214-216^{0}C, R_f- 0.45, yield- 54.24%, IR (KBr) ν_{max} (cm^{-1}) : 3616.92 (O-H aromatic str.) 3118.89 (C-H aromatic str.), 1645.58 (C=O str.), 1602.41 (C=N str. Pyrazoline), 1593.84 (C=C str.),1526.37 (NO_2 assym. str.), 1345.02 (NO_2 sym.str.); ^{1}H NMR (DMSO) δ ppm: 9.89(s,1H,ArOH), 7.46-8.40(m,6H, ArH), 5.13-5.18(dd,1H,CH Pyrazoline), 3.52-3.58(dd,1H, CH_2 Pyrazoline), 3.10-3.18(dd,1H, CH_2 Pyrazoline); MS(m/e): 388.4($M^{+)}$

Compound D3: (3-(4-chlorophenyl)-5-(furan-2-yl)-4,5-dihydropyrazol-1-yl)(pyridin-4-yl) methanone, Mf- $C_{19}H_{14}ClN_3O_2$, Mp-102-104^{0}C, R_f- 0.57, yield- 65.5%, IR (KBr) ν_{max} (cm^{-1}) : 3141.84 (C-H aromatic str. furan), 3101.30 (C-H aromatic str. substituted benzene), 1651.09 (C=O str.), 1605.93 (C=N str. Pyrazoline), 1587.06 (C=C str.), 702.30 (C-Cl str.); ^{1}H NMR (DMSO) δ ppm: 6.75-8.31(m,6H, ArH), 5.23-5.29(dd,1H,CH Pyrazoline), 3.43-3.49(dd,1H, CH_2 Pyrazoline), 3.17-3.22(dd,1H, CH_2 Pyrazoline); MS(m/e): 351.8(M^+), 353.8(M+2)

Compound D4: (5-(furan-2-yl)-4,5-dihydro-3-(4-hydroxyphenyl)pyrazol-1-yl)(pyridin-4-yl) methanone. Mf- $C_{19}H_{15}N_3O_3$, Mp-128-130^{0}C, R_f- 0.52, yield- 47.74%, IR (KBr) ν_{max} (cm^{-1}) : 3611.37 (O-H aromatic str.), 3141.82 (C-H aromatic str. furan), 3101.65 (C-H aromatic str. substituted benzene), 1659.58 (C=O str.), 1612.05 (C=N str. Pyrazoline), 1594.06 (C=C str.); ^{1}H NMR (DMSO) δ ppm: 9.88(s,1H,ArOH), 6.93-8.30(m,6H, ArH), 5.20-5.25(dd,1H,CH Pyrazoline), 3.49-3.55(dd,1H, CH_2 Pyrazoline), 3.16-3.21(dd,1H, CH_2 Pyrazoline); MS(m/e): 333.3(M^+)

Compound D5: (3-(4-chlorophenyl)-4,5-dihydro-5-(thiophen-2-yl)pyrazol-1-yl)(pyridin-4-yl) methanone. Mf- $C_{19}H_{14}ClN_3OS$, Mp-124-126^{0}C, R_f- 0.60, yield- 57.85%, IR (KBr) ν_{max} (cm^{-1}) :3128.91 (C-H aromatic str. thiophene), 3091.68(C-H aromatic str. substituted benzene), 1657.70 (C=O str.), 1610.17 (C=N str. Pyrazoline), 1599.12 (C=C str.), 705.03 (C-Cl str.); ^{1}H NMR (DMSO) δ ppm: 6.94-8.38(m,6H, ArH), 5.42-5.47(dd,1H,CH Pyrazoline), 3.33-3.39(dd,1H, CH_2 Pyrazoline), 3.09-3.16(dd,1H, CH_2 Pyrazoline); MS(m/e): 367.8(M^+), 369.8(M+2)

Compound D6: (4,5-dihydro-3-(4-hydroxyphenyl)-5-(thiophen-2-yl)pyrazol-1-yl)(pyridin4-yl)methanone. Mf- $C_{19}H_{15}N_3O_2S$, Mp-163-165^{0}C, R_f- 0.48, yield- 66.33; IR (KBr) ν_{max} (cm^{-1}) : 3621.43 (O-H aromatic str.), 3127.14 (C-H aromatic str. thiophene), 3089.12 (C-H aromatic str. substituted benzene), 1640.57 (C=O str.), 1604.57 (C=N str. Pyrazoline), 1600.47 (C=C str.)^{1}H NMR (DMSO) δ ppm: 9.98(s,1H,ArOH), 7.12-8.28(m,6H,ArH), 5.38-5.43(dd,1H,CHPyrazoline), 3.58-3.63(dd,1H, CH_2 Pyrazoline), 3.17-3.22(dd,1H,CH_2 Pyrazoline); MS(m/e): 349.4(M^+)

Compound D7: 1-(3-(4-chlorophenyl)-4,5-dihydro-5-(4-nitrophenyl)pyrazol-1-yl)-2-(naphthae n-2-yloxy)ethanone. Mf- $C_{27}H_{20}ClN_3O_4$, Mp-140-142^{0}C, R_f- 0.90, yield- 48.80%, IR (KBr) ν_{max} (cm^{-1}) : 3109.57 (C-H aromatic str.), 3026.16 (C-H aliphatic str.) 1650.93 (C=O str.), 1616.19 (C=N str. Pyrazoline), 1592.30 (C=C str.), 1528.36 (NO_2 assym. str.), 1345.31 (NO_2 sym. str.), 710.93 (C-Cl str.); ^{1}H NMR (DMSO) δ ppm: 6.82-8.42(m,10H, ArH), 5.30-5.35(dd,1H,CH Pyrazoline), 4.76 (s,2H, $COCH_2$), 3.33-3.38(1H, CH_2 Pyrazoline), 3.13-.19(dd,1H, CH_2 Pyrazoline); MS(m/e): 485.9(M^+), 487.9(M+2)

Compound D8: 1-(4,5-dihydro-3-(4-hydroxyphenyl)-5-(4-nitrophenyl) pyrazol-1-yl)-2-(naphtha len -2-yloxy)ethanone . Mf- $C_{27}H_{21}N_3O_5$, Mp-191-192^{0}C, R_f- 0.87, yield- 54.42%, IR(KBr) ν_{max}(cm^{-1}) : 3604.52 (O-H phenolic str.), 3112.09 (C-H aromatic str.), 3026.86 (C-H aliphatic str.) 1654.28 (C=O str.), 1610.26 (C=N str. Pyrazoline), 1597.41 (C=C str.), 1528.36 (NO_2 assym. str.), 1345.19 (NO_2 sym. Str); ^{1}H NMR (DMSO) δ ppm: 9.75(s,1H,ArOH), 6.87-8.30(m,10H, ArH), 5.29-5.34(dd,1H,CH Pyrazoline), 4.82 (s,2H, $COCH_2$), 3.38-3.44(dd,1H, CH_2 Pyrazoline), 3.10-3.15(dd,1H, CH_2 Pyrazoline); MS(m/e): 467.4(M^+)

Compound D9: 1-(3-(4-chlorophenyl)- 4,5-dihydro-5-(furan-2-yl)-pyrazol-1-yl)-2-(naphthalen-2-yloxy)ethanone. Mf- $C_{25}H_{19}ClN_2O_3$,Mp-118-120^{0}C, R_f- 0. 0.72, yield- 45.55%, IR (KBr) ν_{max}(cm^{-1}) : 3158.12 (C-H aromatic str. furan), 3091.30 (C-H aromatic str. Substituted benzene), 3012.34 (C-H aliphatic str.) 1652.79 (C=O str.), 1605.93 (C=N str. Pyrazoline), 1591.31 (C=C str.) ^{1}H NMR (DMSO) δ ppm: 6.87-8.08(m,10H, ArH), 5.39-5.45(dd,1H,CH Pyrazoline), 4.78 (s,2H, $COCH_2$), 3.38-3.44(dd,1H, CH_2 Pyrazoline), 3.05-5.10(dd,1H, CH_2 Pyrazoline); MS(m/e): 430.8(M^+), 432.8(M+2)

Compound D10: 1-(3-(4-hydroxyphenyl)-4,5-dihydro-5-(furan-2-yl)pyrazol-1-yl)-2-(naphthale n-2-yloxy) ethanone. Mf- $C_{25}H_{20}N_2O_4$, Mp-132-134^{0}C, R_f- 0.79, yield- 57.72%, IR(KBr) ν_{max}(cm^{-1}) : 3615.93 (O-H aromatic str.), 3158.65 (C-H aromatic str. furan), 3084.14 (C-H aromatic str. Substituted benzene), 3012.61 (C-H aliphatic

str.) 1652.84 (C=O str.), 1604.11 (C=N str. Pyrazoline), 1598.52 (C=C str.) ^{1}H NMR (DMSO) δ ppm: 9.68(s,1H,ArOH), 6.88-8.04(m,10H, ArH), 5.35-5.40(dd,1H,CH Pyrazoline), 4.78 (s,2H, $COCH_2$), 3.38-3.44(dd,1H, CH_2 Pyrazoline), 3.04-3.10(dd,1H, CH_2 Pyrazoline); MS(m/e): 412.4(M^+)

Compound D11: 1-(3-(4-chlorophenyl)-4,5-dihydro-5-(thiophen-2-yl)pyrazol-1-yl)-2-(naphtha en- 2-yloxy) ethanone. Mf- $C_{25}H_{19}ClN_2O_2S$, Mp-144-146^{0}C, R_f- 0.83, yield- 60.28%, IR (KBr) ν_{max} (cm^{-1}): 3154.46 (C-H aromatic str. thiophene), 3135.83 (C-H aromatic str. Substituted benzene), 3009.30 (C-H aliphatic str.) 1657.70 (C=O str.), 1614.95 (C=N str. Pyrazoline), 1589.32 (C=C str.); ^{1}H NMR (DMSO) δ ppm: 6.90-8.05(m,10H, ArH), 5.35-5.41(dd,1H,CH Pyrazoline), 4.77 (s,2H, $COCH_2$), 3.31-3.38(dd,1H, CH_2 Pyrazoline), 3.04-3.10(dd,1H, CH_2 Pyrazoline); MS(m/e): 446.9(M^+), 448.9(M+2)

Compund D12: 1-(3-(4-hydroxyphenyl)-4,5-dihydro-5-(thiophen-2-yl)pyrazol-1-yl)-2-(naphtha en- 2-yloxy) ethanone. Mf- $C_{25}H_{20}N_2O_3S$, Mp-171-173^{0}C, R_f- 0.8, yield- 46.60%, IR (KBr) ν_{max} (cm^{-1}):3087.19 (C-H aromatic str.), 1658.41 (C=O str.), 1616.02 (C=N str. Pyrazoline), 1592.12 (C=C str.), 1528.36 (NO_2 assym. str.), 1345.03 (NO_2 sym. str.), 710.93 (C-Cl str.); ^{1}H NMR (DMSO) δ ppm: 9.75(s,1H,ArOH), 6.87-8.05(m,10H,ArH), 5.37-5.42(dd,1H,CH Pyrazoline), 4.79 (s,2H, $COCH_2$), 3.38-3.42(dd,1H, CH_2 Pyrazoline), 3.07-3.12(dd,1H, CH_2 Pyrazoline); MS(m/e): 428.5(M^+)

Evaluation of Acute Oral Toxicity [39]
The synthesized compounds were evaluated for acute toxicity by acute toxic class method. No sign of toxicity and mortality were observed for all the synthesized compounds at 2000 mg/kg b.w., the LD_{50} value of the synthesized compounds (D1-D12) expected to exceed 2000 mg/kg b.w. and represented GHS category 5 or unclassified (LD_{50} > 2000 mg/kg-5000 mg/kg). Thus two dose levels 100 mg/kg b.w. and 200 mg/kg b.w. were selected for the evaluation of analgesic and anti-inflammatory activities.

Evaluation of analgesic activity [40]
The analgesic activity of the synthesized compounds was evaluated by tail flick method. Wistar rats (n=6) were grouped by random sampling technique for the study. Diclofenac sodium at the dose of 10 mg/kg (p.o.) ** was administered as standard drug for comparison. The test compounds were administered by the oral routes at the dose level of 200 mg/kg b.w. The rats were hold in position by a proper restrainer with the tail extending out and the tail (up to 6 cm) was taken and dipped in a beaker. In that beaker water should be maintained at 56 ± 4^{0}C. The time in sec taken by the rats to withdraw their tail completely out of water was taken these are the reaction time. The observation was carried out at 0, 90, 120,180 min after the administration of our synthesized compounds. A cut off point of 15 sec was observed to avoid the tail damage. The percentage analgesic activity was easily calculated by the below mentioned formula.

PAA = [(B-A)/B] X 100%

B - Reaction time in sec after treatment
A -Reaction time in sec before treatment
PAA - Percentage analgesic activity.

Evaluation of Anti-inflammatory activity [41]
Evaluation of anti-inflammatory activity was carried out by Carrageenan induced hind paw edema in rats by the method of C.A. Winter et.al.[103] The animals were randomly divided into groups of 6 animals and were fasted for 24 hours before the experiment. The control group received only 0.5% carboxy methyl cellulose solution. Diclofenac sodium (13.5 mg/kg) was administered intra peritoneally as standard drug for comparison. The synthesized compounds were administered at two dose levels (100 and 200 mg/kg). Carrageenan solution 0.1 ml (1% in sterile 0.9% NaCl solution) were injected subcutaneously into the sub planter region of the right hind paw of each rat,1 hour before administration of the standard and test drugs. The right hind paw volumes were measured before and after 120, 180 and 240 min after administration with the aid of plethysmometer. The percentage of edema inhibition was calculated from the mean effect in the control and treated animals according the following equation.

Percent edema inhibition = $\mathbf{(V_C - V_t / V_c) \times 100}$

$\mathbf{V_t}$ = Mean increase in the paw volume in rats tested with test compound.

V_C = Mean increase in the paw volume in control group of rats.

Evaluation of ulcerogenic potential [1, 20]
The extent of ulcerogenic effect was evaluated for the two synthesized compounds D7 and D8 which had shown comparatively better anti-inflammatory and analgesic activity among the synthesized compounds. Albino rats of either sex were divided into control, standard and test groups of six animals each group. The animals were starved for 48 hr (water *ad libitum*) prior to drug administration. The control group received only 0.5% CMC solution. Standard group was orally administered with diclofenac sodium and test compounds were administered orally at the dose of 100 mg/kg b.w.in CMC solution. Diclofenac sodium is a nonselective inhibitor of COX which is known to cause ulcerogenicity. Animals were sacrificed after 7 hr of the drug administration by decapitation. The stomachs were removed, collected, opened along the greater curvature. Then they were washed with distilled water and were cleaned gently by dipping in saline. The mucosal damage of each stomach was examined with the aid of magnifying lens for the presence of macroscopically visible lesions. Number of the lesions in each stomach, if present, were counted and recorded.

Ulcers were classified into the following levels-
Level-I: in which ulcer area is less than $1mm^2$
Level-II: in which ulcer area is in the range from 1-3 mm^2
Level-III: in which ulcer area is more than $3mm^2$
Ulcer index was calculated according formula-
1x number of ulcers of level I + 2x number of ulcers of level II + 3x number of ulcers of level III

RESULTS

Analgesic activity
Analgesic activity was observed as graded dose response was observed for the synthesized compounds. Most of the synthesized compounds had shown higher analgesic activity at 120 min. At 120 min, when compared with standard drug diclofenac sodium the compounds D8 and D7 showed comparable analgesic activity at tested dose levels. Compound D5 exhibited lowest analgesic activity at tested dose levels at 120 min. The decreasing order of analgesic activity (at 120 min) of the synthesized compounds was found to be

At 100 mg/kg b.w. : D8 >D7 >D2 >D10 >D9 >D1 >D11 >D3 >D6 >D4 >D12 >D5
At 200 mg/ kg b.w. : D8 >D7 >D2 >D10 >D9 >D1 >D6 >D3 >D4 >D12 >D11 >D

Table I: Analgesic activity of the synthesized compounds at 100 mg/kg b.w.

Compounds	Dose (mg/kg)	0 min	90 min		120 min		180 min	
		MEAN ±SEM	MEAN ±SEM	%	MEN ± SEM	%	MEAN ± SEM	%
D1	**100**	2.33±0.21	4.00±0.44 ^{ns}	41.47	4.50±0.42^{ns}	48.22	3.00±0.44^{ns}	22.33
D2	**100**	2.66±0.21	5.16±0.30**	48.44	5.83±0.60***	54.37	4.83±0.60***	44.92
D3	**100**	2.15±0.30	3.16±0.47 ^{ns}	31.96	3.66±0.33^{ns}	41.25	4.83±0.47*	48.03
D4	**100**	2.50±0.22	3.50±0.56 ^{ns}	28.57	3.66±0.55 ^{ns}	31.69	4.50±0.42^{ns}	44.44
D5	**100**	2.33±0.21	2.66±0.31 ^{ns}	12.40	3.16±0.47 ^{ns}	26.26	3.00±0.25^{ns}	22.33
D6	**100**	2.50±0.22	3.30±0.33 ^{ns}	24.24	4.00±0.36 ^{ns}	37.50	3.16±0.47 ^{ns}	20.88
D7	**100**	2.86±0.16	5.50±0.56***	48.00	7.16±0.79***	60.05	6.66±0.66***	57.05
D8	**100**	2.86±0.16	5.83±0.70***	50.94	7.26±0.63***	60.60	7.00±0.44***	59.14
D9	**100**	2.33±0.21	3.66±0.49 ^{ns}	36.33	4.83±0.60*	51.75	3.83±0.47*	39.16
D10	**100**	2.50±0.22	4.33±0.61*	42.26	5.33±0.49**	53.09	4.83±0.60**	48.24
D11	**100**	2.86±0.16	3.66±0.33 ^{ns}	21.85	5.16±0.47^{ns}	44.57	4.00±0.51^{ns}	28.50
D12	**100**	2.33±0.21	3.00±0.44 ^{ns}	22.33	3.33±0.42 ^{ns}	30.03	3.33±0.42^{ns}	30.03
Control	-	2.15±0.30	2.33±.21	-	2.66±0.21	-	2.83±0.30	-
Diclofenac sodium	**10**	2.66±0.21	7.50±0.42***	64.53	10.33±0.98***	74.24	9.50±0.42***	72.00

Table II: Analgesic activity of the synthesized compounds at 200 mg/kg b.w.

Compounds	Dose (mg/kg)	0 min	90 min		120 min		180 min	
		MEAN ± SEM	MEAN ± SEM	%	MEN ± SEM	%	MEAN ± SEM	%
D1	**100**	2.33±0.21	4.16±0.47^{ns}	43.99	5.16±0.47*	54.84	3.83±0.47^{ns}	39.16
D2	**100**	2.66±0.21	5.33±0.55***	50.09	6.33±0.55***	57.97	5.00±0.44*	46.80
D3	**100**	2.15±0.30	3.66±0.33^{ns}	41.25	4.00±0.44^{ns}	46.25	4.50±0.67^{ns}	52.22
D4	**100**	2.50±0.22	4.16±0.54^{ns}	39.90	4.33±0.47^{ns}	42.26	4.66±0.49^{ns}	46.35
D5	**100**	2.33±0.21	3.00±0.25^{ns}	22.33	3.66±0.49^{ns}	36.33	3.16±0.40^{ns}	26.26
D6	**100**	2.50±0.22	3.83±0.54^{ns}	34.72	4.83±0.54^{ns}	48.24	3.66±0.61^{ns}	31.69
D7	**100**	2.86±0.16	6.00±0.73***	52.33	7.66±0.66***	62.66	6.83±0.70***	58.12
D8	**100**	2.86±0.16	6.33±0.71***	54.81	7.83±0.65***	63.47	7.33±0.66***	60.98
D9	**100**	2.33±0.21	4.33±0.55*	46.18	5.33±0.49^{ns}	56.28	4.00±0.51^{ns}	41.75
D10	**100**	2.50±0.22	5.00±0.51**	50.00	5.83±0.47**	57.11	5.16±0.47*	51.55
D11	**100**	2.86±0.16	4.16±0.40^{ns}	31.25	4.66±0.66^{ns}	38.62	4.16±0.47^{ns}	31.25
D12	**100**	2.33±0.21	3.50±0.56^{ns}	33.42	3.83±0.30^{ns}	39.16	3.50±0.42^{ns}	33.42
Control	-	2.15±0.30	2.33±.21	-	2.66±0.21	-	2.83±0.30	-
Diclofenac sodium	**10**	2.66±0.21	7.50±0.42***	64.53	10.33±0.98***	74.24	9.50±0.42***	72.00

*Significant differences with respect to control was evaluated by (ANOVA), Dunnet's t test * P<0.05, **P<0.01, ***P<0.001, ns (non significant), % (analgesic activity)*

Anti-inflammatory activity

Graded dose response was observed for the synthesized compounds. Most of the synthesized compounds exhibited highest anti-inflammatory activity at 180min, so it is considered as the basis of discussion. When compared with standard drug diclofenac sodium the compound D1 showed comparable anti-inflammatory activity at tested dose levels. Compound D6 exhibited lowest anti-inflammatory activity at 100 mg/kg b.w. and D3exhibited lowest activity 200 mg/kg b.w. The decreasing order of analgesic activity of the synthesized compounds was found to be

At 100 mg/kg b.w. : D1 > D7 > D10 > D8 > D2 >D4 >D12 >D9 =D11 >D5 >D3>D6
At 200 mg/kg b.w. : D1 > D10 > D7 > D8 > D2 >D12 >D6 >D9 >D4 >D5 >D11 >D3

Table III: Anti-Inflammatory Activity of Synthesized Compounds (100mg/kg b.w.)

Compounds	Dose (mg/kg)	120 min		180min		240min	
		MEAN ± SEM	%	MEN ± SEM	%	MEAN ± SEM	%
D1	**100**	**0.520 ± 0.011*****	**21.92**	**0.311 ± 0.017*****	**54.12**	**0.413 ± 0.016*****	**39.08**
D2	**100**	0.605 ± 0.018*	09.15	0.516 ± 0.016***	23.98	0.556 ± 0.016***	17.99
D3	**100**	0.645 ± 0.014^{ns}	03.15	0.631 ± 0.012^{ns}	06.93	0.633 ± 0.014^{ns}	06.63
D4	**100**	0.611 ± 0.018^{ns}	08.25	0.600 ± 0.018**	11.50	0.571 ± 0.015***	15.78
D5	**100**	0.656 ± 0.011^{ns}	01.50	0.626 ± 0.011^{ns}	07.66	0.635 ± 0.012^{ns}	06.34
D6	**100**	0.645 ± 0.008^{ns}	03.15	0.633 ± 0.014^{ns}	06.63	0.616 ± 0.009^{ns}	09.14
D7	**100**	0.595 ± 0.017*	10.66	0.456 ± 0.016***	32.74	0.536 ± 0.031***	20.94
D8	**100**	0.608 ± 0.013^{ns}	08.70	0.508 ± 0.018***	25.07	0.576 ± 0.015**	15.04
D9	**100**	0.631 ± 0.012^{ns}	05.25	0.621 ± 0.014^{ns}	08.40	0.608 ± 0.015^{ns}	10.32
D10	**100**	0.571 ± 0.020***	14.20	0.458 ± 0.016***	32.44	0.493 ± 0.025***	27.28
D11	**100**	0.648 ± 0.009^{ns}	02.70	0.621 ± 0.010^{ns}	08.40	0.654 ± 0.018^{ns}	03.53
D12	**100**	0.653 ± 0.014^{ns}	01.95	0.606 ± 0.014*	10.61	0.620 ± 0.019^{ns}	08.55
Control	-	0.666 ± 0.007	-	0.678 ± 0.009	-	0.678 ± 0.009	-
Diclofenac sodium	**13.5**	0.325 ± 0.024***	51.12	0.220 ± 0.013***	67.55	0.260 ± 0.015***	61.65

Table IV: Anti-Inflammatory Activity of Synthesized Compounds (200mg/kg b.w.)

Compounds	Dose	120 min	180min	240min

	(mg/kg)	MEAN + SEM	%	MEN + SEM	%	MEAN + SEM	%
D1	**200**	**0.480 ± 0.009*****	**27.92**	**0.286 ± 0.012*****	**57.81**	**0.390 ± 0.018*****	**42.47**
D2	**200**	0.596 ± 0.019*	10.51	0.485 ± 0.020***	28.46	0.528 ± 0.023***	22.12
D3	**200**	0.626 ± 0.015^{ns}	06.00	0.620 ± 0.012^{ns}	08.55	0.600 ± 0.017*	11.50
D4	**200**	0.593 ± 0.018*	10.96	0.601 ± 0.020**	11.35	0.601 ± 0.019*	11.35
D5	**200**	0.630 ± 0.012^{ns}	05.40	0.610 ± 0.014^{ns}	10.02	0.620 ± 0.01^{ns}	08.55
D6	**200**	0.600 ± 0.008*	09.90	0.588 ± 0.018*	13.27	0.593 ± 0.016**	12.53
D7	**200**	0.521 ± 0.018***	21.77	0.423 ± 0.022***	37.61	0.480 ± 0.018***	29.20
D8	**200**	0.586 ± 0.015***	12.01	0.450 ± 0.023***	33.62	0.510 ± 0.011***	24.77
D9	**200**	0.610 ± 0.019^{ns}	08.40	0.600 ± 0.014*	11.50	0.591 ± 0.014**	12.83
D10	**200**	0.580 ± 0.019**	12.91	0.396 ± 0.015***	41.59	0.486 ± 0.015***	28.31
D11	**200**	0.635 ± 0.016^{ns}	04.65	0.613 ± 0.013^{ns}	09.58	0.626 ± 0.015^{ns}	07.66
D12	**200**	0.623 ± 0.014^{ns}	06.45	0.585 ±0.011**	13.71	0.616 ± 0.014^{ns}	09.14
Control	**-**	0.666± 0.007	-	0.678 ± 0.009	-	0.678 ± 0.009	-
Diclofenac sodium	**13.5**	0.325 ± 0.024***	51.12	0.220 ± 0.013***	67.55	0.260 ± 0.015***	61.65

*Significant differences with respect to control was evaluated by (ANOVA), Dunnet's t test * P<0.05, **P<0.01, ***P<0.001, ns (non significant), % (percentage reduction of edema)*

Ulcerogenic Potential

The synthesized compoundsD7 and D8 were evaluated for their ulcerogenicity in rats. The ulcerogenicity of these two synthesized compounds were evaluated at 100 mg/kg b.w.(p.o.). Diclofenac sodium was used as the reference standard 100 mg/kg b.w.(p.o.). Both the synthesized compounds were found to be less ulcerogenic than the standard drug Diclofenac. Among the two test compounds **D8** is found to be less ulcerogenic than the compound **D7.**

Table VI: Ulcer Index of the Selected Compounds

Compounds	Dose (mg/kg)	Ulcer index MEAN ± SEM
Control	-	1.83 ± 0.30
Standard	100	43.00 ± 2.20
D7	100	20.50 ± 1.94
D8	100	17.17 ± 1.49

DISCUSSION

From the study it can be reported that the presence of p-nitro phenyl at 5th position along with p-chloro phenyl / p-hydroxy phenyl group at 3rd position and napthyloxyacetate group at 2nd position of the pyrazoline nucleus exhibited better analgesic activity when compared to other synthesized pyrazoline derivatives. Thus it can be suggested that the electron withdrawing effect of the nitro group at the phenyl group at 5th position of the pyrazoline moiety may have resulted in the enhancement of the analgesic activity than the presence of heterocyclic moiety like furan or thiophene at 5th position of the pyrazoline ring. Presence of bulky groups might have resulted in increased analgesic activity.

Regarding anti-inflammatory activity it can be reported that the presence of p-nitro phenyl at 5thposition, p-chloro phenyl group at 3rd position and pyridinyl group at 2nd position of the pyrazoline moiety exhibited better anti-inflammatory activity when compared to other synthesized pyrazoline derivatives. It can be suggested that the presence the electron withdrawing effect of –Cl and $–NO_2$ at the phenyl groups at the 3rd and 5th position of the pyrazoline moiety might have resulted in the enhancement of the anti-inflammatory activity than the presence of heterocyclic moiety at 5th position.

Both the synthesized D7 and D8 compounds were found to be less ulcerogenic than the standard drug Diclofenac. Due to the presence of nitro group on these compounds both may have nitric oxide donating ability. Tested compounds might have donated nitric oxide (NO) which modulates various physiological functions at the digestive system and have resulted in gastro protective action[18].

Thus finally it can be concluded from this study that D8 was found to be the most effective compound among all the synthesized 2-pyrazoline derivatives with respect to their analgesic and anti-inflammatory activity as well as it is less ulcerogenic than the standard drug diclofenac. Further optimization of these compounds may result in more effective agents.

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