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Synthesis and characterization of some substituted pyrazoles as analgesics and anti inflammatory agents

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ABSTRACT

*A convenient synthesis of some new substituted 3,5- dimethyl pyrazole **(4a-c)**, 3-methyl pyrazol-5-one derivatives **(5a-c),** 3-Methyl-1-(substituted phenyl) pyrazol-5-ones **(7a-b)** and 2,3-dimethyl-1-(substituted phenyl)pyrazol-5-ones **(8a-b)** have been synthesized. All the newly synthesized compounds were tested for their in vivo anti-inflammatory and analgesic activity by bioassays namely: Carrageenan-induced paw edema method and acetic acid induced writhing method respectively. All the synthesized compounds have been characterized by their melting point, elemental analysis, IR and ^{1}H-NMR. Compound **8$_b$** exhibited promising and significant inhibitory activity against COX-2 enzyme. Therefore, such compounds would represent a fruitful matrix for the development of anti-inflammatory and analgesic agents.*

Keywords: pyrazoles, Analgesic, anti-inflammatory.

INTRODUCTION

Pyrazoles played a crucial role in the development of theoretical studies in heterocyclic chemistry and also useful building blocks in organic chemistry [1,2], with wide application as dyestuff, analytical reagents and agrochemicals [3]. The pyrazole ring system is a useful structural moiety found in numerous biologically active compounds. Pyrazole is useful structural unit in the field of the medicinal chemistry [4-6] and has been reported to exhibit a variety of biological activities such as, analgesic [7], anti-inflammatory [8], antianxiety [9], antibacterial [10,11], antifungal [12], antitumour [13,15], antitubercular [14], and antiparasitic [16] etc. Motivated by the afore-mentioned findings and as a continuation of our research to develop novel series of pyrazole derivatives that would act as better and potent anti-inflammatory-analgesic agents.

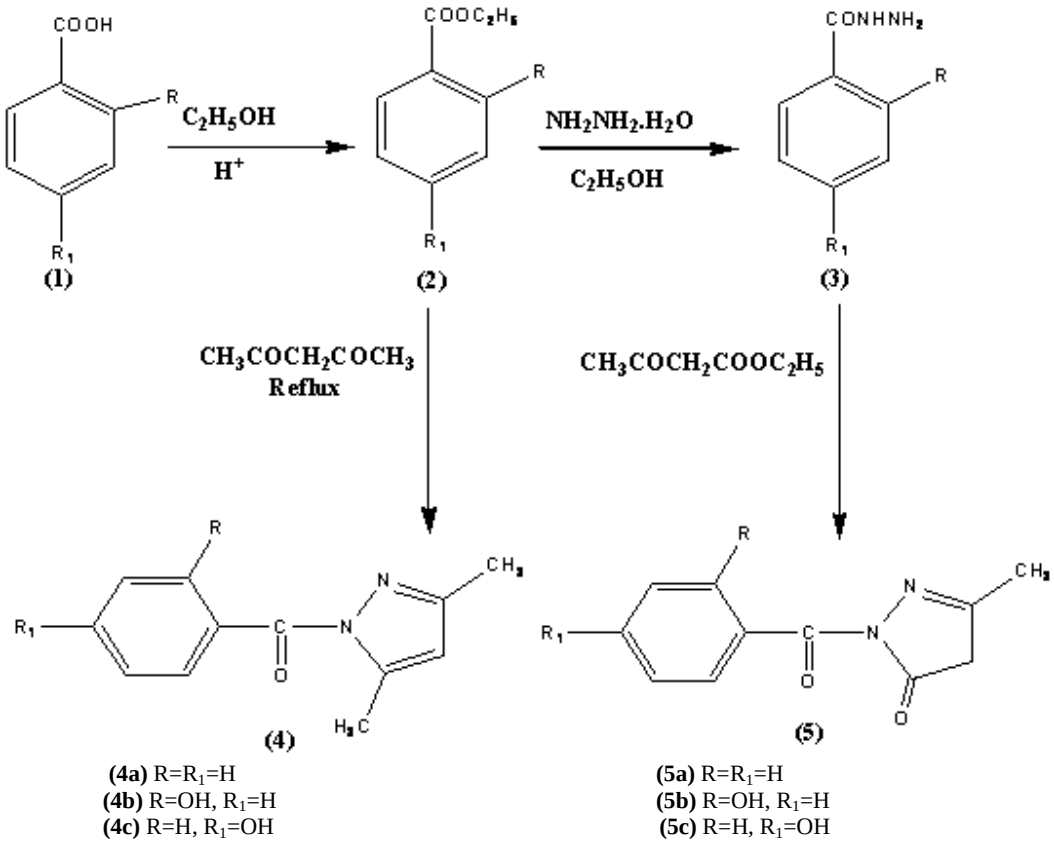
In the present work, substituted 1-Benzoyl-3, 5-dimethyl pyrazoles **(4a-c)** were synthesized by treatment of substituted ethyl benzoate **(2)** and acetylacetone and substituted 1-Benzoyl-3-methyl

pyrazol-5-ones **(5a-c)** were synthesized by the condensation of substituted phenyl carbamide **(3)** and ethyl acetoacetate **(Scheme-1)**. 2, 3-dimethyl-1- (substituted phenyl) pyrazol- 5-ones **(8a-b)** were synthesized by the reaction of 3-methyl-(1-substituted phenyl) - pyrazol-5-ones **(7a-b)** with dimethyl sulphate and compound **(7a-b)** were synthesized by the mixing of ethyl acetoacetate and substituted phenylhydrazine **(6')**. **(Scheme-2).**

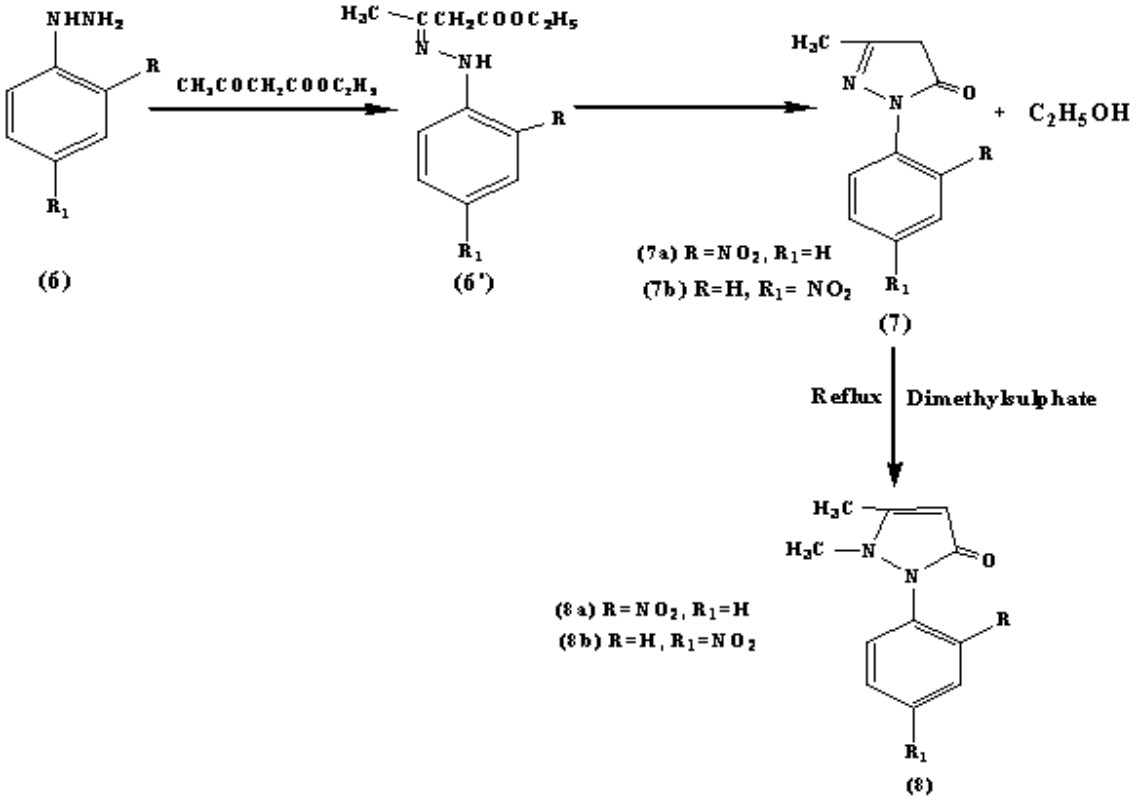
MATERIALS AND METHODS

Experimental

The melting points were determined in open capillary tubes and were uncorrected. The homogeneity of all the newly synthesized compounds were checked by TLC on silica gel-protected aluminum sheets (Type 60 F_{254}, Merck) and the spots were detected by exposure to UV-lamp at λ 254 nm for few seconds. The infrared (IR) spectra were recorded on 470-Shimadzu infrared spectrophotometer using the KBr disc technique and expressed in cm^{-1}. ^1H NMR spectra were recorded on Bruker DRX-300 in DMSO-d_6 as solvent. The chemical shift was given in δ (ppm) downfield from tetramethylsilane (TMS) as an internal standard. Splitting patterns were designated as follows: s: singlet; d: doublet; m: multiplet. Elemental analysis was carried on Elemental Vario EL III Carlo Erba 1108 and the values were within ±0.4% of the theoretical values.



Scheme: 1- Synthesis of substituted 1-Benzoyl-3, 5-dimethyl pyrazoles (4a-c) and substituted 1-Benzoyl-3-methyl pyrazole-5-ones (5a-c).



Scheme 2: Synthesis of 3-Methyl-1-(substituted phenyl) pyrazol-5-ones (7a-b) and 2,3-Dimethyl-1-substituted phenyl-pyrazol-5-ones (8a-b).

Synthesis of Substituted 1-Benzoyl-3, 5-dimethyl pyrazoles (4a-c):

A mixture of substituted phenyl carbamide (1.3g, 10.0 mmol) and acetylacetone (1g, 10.0 mmol) was refluxed in methanol (25 ml) containing concentrated hydrochloric acid (1 ml) for 10 to 12 hours on a water bath. The resulting solution was then concentrated and cooled at room temperature. The solid thus separated was washed with methanol and recrystallized from ethanol.

Derivatives synthesized: (Table-1)

1Benzoyl-3, 5-dimethyl pyrazole (4a): IR (KBr) *v*: 3116.71(Ar C-H), 2941.24 (asymmetric CH_3;C-H), 2883.38 (symmetric CH_3;C-H), 1682.24 (C=O), 1528.86 (C=N), 1446.51 (C=C) and 842.83 cm^{-1} (HC=N); ^{1}H- NMR (DMSO-d_6): δ 2.42 (s, 6H, CH_3), 6.15 (s, 1H, CH-pyrazole), 7.44-7.48 (m, 2H, $H_{3,5}$ 1-benzoyl), 7.57 (m, 1H, H_4 1-benzoyl), 8.02-8.04 ppm (d, 2H, $H_{2,6}$ 1-benzoyl); Elemental analysis: Calcd. for $C_{12}H_{12}N_2O$: C, 72.00; H, 6.00; N, 14.00%; Found: C, 71.97; H, 5.98; N, 13.97%.

1-(2-Hydroxybenzoyl)-3, 5-dimethyl pyrazole (4b): IR (KBr) *v*: 3595.45 (O-H), 3392.13 (Ar C-H), 2941.24 (asymmetric CH_3;C-H), 2885.31(symmetric CH_3;C-H), 1718.46 (CO-N-CO), 1680.74 (C=O), 1532.88 (C=N), 1521.73 cm^{-1} (C=C); ^{1}H-NMR (DMSO-d_6): δ 2.49 (s, 6H, CH_3), 6.08 (s, 1H, CH- pyrazole), 6.87-6.96 (m, 2H, $H_{4,5}$ 2-hydroxybenzoyl), 7.41-7.43 (d, 1H, $H_6$2-hydroxybenzoyl), 10.93 ppm (s, 1H, OH, D_2O exchangeable); Elemental analysis: Calcd. for $C_{11}H_{10}N_2O_3$: C, 66.67; H, 5.56; N, 12.96 %; Found: C, 66.64; H, 5.54; N, 12.94%.

1-(4-Hydroxybenzoyl)-3, 5-dimethyl pyrazole (4c): IR(KBr) *v:* 3609.01 (O-H), 3304.43, 3040.63 (Ar C-H), 2960 (asymmetric CH_3;C-H), 1683.64 (C=O), 1587.12 (C=N),1431 (C=C) and 1190.07 cm^{-1} (C-O) ; ^{1}H-NMR (DMSO-d_6): δ 2.17 (s, 3H, CH_3), 2.49 (s, 3H, CH_3), 6.06(s, 1H, CH- pyrazole), 6.84-6.88(d, 2H, $H_{2,6}$ 1-benzoyl), 7.86-7.89 (d, 2H, $H_{3,5}$ 1-benzoyl), 9.63 ppm (s, 1H, CHO); Elemental analysis: Calcd. for $C_{12}H_{12}N_2O_2$: C, 66.67; H, 5.56; N, 13.00%; Found: C, 66.64; H, 5.54; N, 12.97%.

Synthesis of Substituted 1-Benzoyl-3-methyl pyrazol-5-ones (5a-c):

A mixture of substituted phenyl carbamide (1.3 g, 10.0 mmol) and ethyl acetoacetate (0.13 g, 10.0 mmol) was refluxed in methanol (25 ml), containing 1.0 ml of concentrated hydrochloric acid for 8 to 10 hours on a water bath. The resulting solution was then concentrated and cooled at room temperature. The solid thus separated was washed with methanol, dried and recrystallized with acetone.

1-Benzoyl-3-methyl pyrazol-5-one (5a): IR (KBr) *v*: 3033(Ar C-H), 2941.47(asymmetric CH_3; C-H), 2883.33 (symmetric CH_3;C-H), 1655.24 (C=O), 1530.41 (C=N), 1431.21 (C=C) and 842.02 cm^{-1} (C-N) ; ^{1}H-NMR (DMSO-d_6): δ 2.57 (s, 3H, CH_3), 5.26 (s, 2H CH_2-pyrazole), 6.84-6.87 (m, 3H, $H_{3,4,5}$ 1-benzoyl), 7.85-7.88 ppm (d, 2H, $H_{2,6}$ 1-benzoyl); Elemental anaysis: Calcd. for $C_{11}H_{10}N_2O_2$: C, 65.34; H, 4.95; N, 13.86%; Found: C, 65.30; H, 4.92; N, 13.84%.

1-(2-Hydroxybenzoyl)-3-methyl pyrazol-5-one (5b): IR (KBr) *v*: 3608.46 (O-H), 3024.24 (Ar C-H), 2933.13 (asymmetric CH_3;C-H), 1657.27(C=O), 1531.08, 1488.13 (C=N), 1436.56, 1448.18, 1458.56 (C=C ring stretching) and 1240.09 cm^{-1} (C-O); ^{1}H-NMR (DMSO-d_6): δ 2.49 (s, 3H, CH_3), 5.47 (s, 2H CH_2-pyrazole), 6.87-6.96 (m, 2H, $H_{4,5}$ 2-hydroxybenzoyl), 7.41-7.43(d, 1H, H_6 2-hydroxy benzoyl), 7.98-8.00 (d, 1H, H_3 2-hydroxybenzoyl), 10.34 ppm (s, 1H, OH, D_2O exchangeable); Elemental anaysis: Calcd. for $C_{11}H_{10}N_2O_3$: C, 60.56; H, 4.59; N, 12.84%; Found: C, 60.52; H, 4.56; N, 12.80%.

1-(4-Hydroxy benzoyl)-3-methyl pyrazol-5-one (5c): IR (KBr) *v*: 3610.22 (O-H), 3053.11(Ar C-H), 3006.82 (C-H stretching of CH_3), 1718.46 (CO-N-CO), 1659.31(C=O), 1535.67 (C=N), 1579.59, 1535.23, 1487.01, 1434.94 (C=C), 1238.21 (C-O) and 869.84 cm^{-1} (C-N); ^{1}H-NMR (DMSO-d_6): δ 2.57 (s, 3H, CH_3), 5.47 (s, 2H CH_2-pyrazole), 7.43-7.47 (d, 2H, $H_{2,6}$ 1-benzoyl), 7.96-8.01 (d, 2H, $H_{3,5}$ 1-benzoyl), 10.34 ppm (s, 1H, OH, D_2O exchangeable); Elemental anaysis: Calcd. for $C_{11}H_{10}N_2O_3$: C, 60.56; H, 4.59; N, 12.84%; Found: C, 60.53; H, 4.56; N, 12.81%.

Synthesis of 3-Methyl-1-(substituted phenyl) pyrazol-5-ones (7a-b):

Ethyl acetoacetate (3.10 g, 6.52 mmol) and 1.0 g (6.52 mmol) of substituted phenylhydrazine were mixed together in an evaporating dish. The mixture was heated on a boiling water bath in a fume cupboard for 2 to 2.5 hours and stirred from time to time with a glass rod. The heavy reddish syrup was allowed to cool, 10.0 ml of ether was added and the mixture was stirred vigorously. The syrup, which was insoluble in ether, was solidified within 15 minutes. The solid was filtered at pump and washed thoroughly with ether to remove coloured impurities. Recrystallised from equal volume of ethanol and water.

3-Methyl- 1-(4-nitrophenyl) pyrazol-5-one (7a): IR (KBr) *v*: 3316.61 (N-H), 3316.11(Ar C-H), 2977.12 (C-H stretching of CH_3), 1684.30 (C=O), 1596.22 (C=C), 1495.31 {asymmetric

($ArNO_2$) $(N=O)_2$ stretching}, 1394.11 {symmetric $(ArNO_2)_2$ stretching} and 838.22 cm^{-1} (C-N); ^{1}H-NMR (DMSO-d_6): δ 3.10 (s, 3H, CH_3), 5.28 (s, 1H, CH), 7.04-7.07 (d, 2H, $H_{2,6}$ 1-nitrophenyl), 8.12-8.14 (d, 2H, $H_{3,5}$ 1-nitrophenyl), 10.29 ppm (s, broad, 1H, NH, D_2O exchangeable); Elemental anaysis : Calcd. for $C_{10}H_9N_3O_3$: C, 54.79; H, 4.10; N, 19.17%; Found: C, 54.75; H, 4.07; N, 19.14%.

3-Methyl- 1-(2, 4-dinitrophenyl) - pyrazol-5-one (7b): IR (KBr) *v*: 3309.08 (N-H), 3100.09(C-H), 2977.27 (C-H stretching of CH_3), 1681.12 (C=O),1698.07, 1594.04(C=C), 1512.21 {asymmetric ($ArNO_2$) $(N=O)_2$ stretching}, 1423.31 {symmetric ($ArNO_2$) $(N=O)_2$ stretching} and 833.97 cm^{-1} (C-N); ^{1}H-NMR (DMSO-d_6): δ 1.29 (s, 3H, CH_3), 5.26 (s, 1H, CH), 8.12-8.14 (d, 2H, $H_{5,6}$ 2,4-dinitrophenyl), 8.52 (s, 1H, H_3, 2,4-dinitrophenyl), 10.29 ppm (s,broad,1H, NH, D_2O exchangeable); Elemental anaysis: Calcd. for $C_{10}H_8N_4O_5$: C, 45.45; H, 3.03; N, 21.21%; Found: C, 45.42; H, 3.06; N, 21.18%.

Synthesis of 2, 3-Dimethyl- 1-substituted phenyl - pyrazol-5-ones (8a-b)

In a 50 ml three necked flask, equipped with a dropping funnel, a sealed stirrer unit and a double surface condenser was set up in a fume cupboard. A solution of 0.5 g of sodium hydroxide in small volume of water was added to a solution of 1.40 g (5.73 mmol) of 3-methyl-1-substituted phenyl- pyrazol-5-one **(7)** in 1.0 ml of methanol. The mixture was warmed on a water bath and 0.72 g (0.54 ml, 5.73 mmol) of dimethyl sulphate was added. The mixture was refluxed for 1 hour and allowed to cool, with continuous stirring. Methanol was distilled off, hot water was added to the residue, filtered the impurities, 2, 3-dimethyl-1-substituted phenyl-pyrazol-5-one was extracted with benzene and solvent was evaporated. The crude product was recrystallised from benzene.

2, 3-Dimethyl- 1-(4-nitrophenyl) - pyrazol-5-one (8a): IR (KBr) *v*: 3467.12(C-H), 2977.31(C-H stretching of CH_3), 1650.90 (C=O), 1498.65 (C=C), 1447.13 {asymmetric ($ArNO_2$) $(N=O)_2$ stretching}, 1322{symmetric ($ArNO_2$) $(N=O)_2$ stretching} and 849.04 cm^{-1} (C-N); ^{1}H-NMR (DMSO-d_6): δ 1.27 (s, 3H, CH_3), 2.01 (s, 3H, CH_3), 3.39 (s, 1H, CH), 7.04-7.08 (d, 2H, $H_{2,6}$ 1-nitrophenyl), 8.12-8.14 ppm (d, 2H, $H_{3,5}$ 1-nitrophenyl); Elemental anaysis: Calcd. for $C_{11}H_{11}N_3O_3$: C, 56.65; H, 4.72; N, 18.03 %; Found: C, 56.63; H, 4.70; N, 18.06%.

Table-1 Physical data of the compounds prepared

Compound No.	R	R_1	Yield(%)	R_f value	mp(^oC)
4a	**H**	**H**	71.9	0.90	177-178
4b	**OH**	**H**	74.0	0.86	189-190
4c	**H**	**OH**	60.8	0.67	182-183
5a	**H**	**H**	81.0	0.56	183-184
5b	**OH**	**H**	72.0	0.78	181-182
5c	**H**	**OH**	82.0	0.89	128-129
7a	**NO_2**	**H**	83.0	0.67	83-84
7b	**H**	**NO_2**	78.2	0.76	66-67
8a	**NO_2**	**H**	69.7	0.81	224-225
8b	**H**	**NO_2**	65.0	0.89	240-241

2, 3-Dimethyl- 1-(2, 4-dinitrophenyl) - pyrazol-5-one (8b) : IR (KBr) *v*: 3100.87(C-H), 2977.32 (C-H stretching of CH_3), 1651.54 (C=O), 1594.31(C=C), 1512.21 {asymmetric ($ArNO_2$)

$(N=O)_2$ stretching}, 1423.42 {symmetric ($ArNO_2$) $(N=O)_2$ stretching} and 833.82 cm^{-1} (C-N); ^{1}H-NMR (DMSO-d_6): δ 1.31(s, 3H, CH_3), 2.57(s, 3H, CH_3), 5.26(s, 1H, CH), 8.12-8.14(d, 2H, $H_{5,6}$ 2,4-dinitrophenyl), 8.52 ppm (s, 1H, H_3 2,4-dinitrophenyl); Elemental anaysis: Calcd. for $C_{10}H_8N_4O_5$: C, 47.48; H, 3.60;N, 20.14% ; Found: C, 47.45; H, 3.62; N, 20.11%.

Anti-inflammatory activity:

Anti-inflammatory activity of the newly synthesized compounds was screened using the carrageenan induced rat paw edema method by Winter et al. [17] on rat (Wistar strain) of either sex weighing 100-200 g. The rats were randomly divided into groups (6 rats each). Acute oedema in the hind paws of rats was induced by the subplantar injection of freshly prepared 1% solution of carrageenan in distilled water. The anti-inflammatory activity of the newly synthesized compounds was compared with the standard anti-inflammatory drug indomethacin. The synthesized compounds were suspended in 2% Tween 80. Different doses of test compounds 5mg/kg, 10mg/kg and 20mg/kg respectively and the standard drug 20mg/kg were administered 1 hour before the carageenan injection. The results are expressed as percent inhibition of the edema as compared to the control. The reduction in paw edema at 3 hr in comparison to the standard is given in **Table 2**

Analgesic activity:

The analgesic activity of representative ten compounds of pyrazole (4a-c, 5a-c, 7a-b and 8a-b) series were screened using the acetic acid induced writhing method in Swiss mice by Koster et al [18]. The mice (20-30 g) of either sex were used. The animals were randomly divided into groups (6 mice each). The analgesic activity of the newly synthesized compounds was compared with the standard analgesic drug pentazocin. The synthesized compounds were suspended in 2% Tween 80. After 1 hour of drug administration, 0.10 ml of acetic acid solution was given to the mice intraperitoneally. Stretching movements consisting of arching of the back, elongation of body and extension of hind limbs were counted for 5-15 minutes after acetic acid injection. The analgesic activity was expressed in terms of percent inhibition in comparison to the standard is given in **Table2**.

Table-2 Biological data of the compounds

Compound No.	Anti-inflammatory (%inhibition±SEM)	ED_{50} (mg/kg)	Analgesic (%inhibition±SEM)	ED_{50} (mg/kg)
4a	82.30 ± 0.01*****	3.72	58.5 ± 0.55*****	10.66
4b	81.43 ± 0.001*****	3.92	54.8 ± 0.51*****	11.56
4c	86.20 ± 0.004*****	3.30	67.4 ± 0.51*****	9.90
5a	75.80 ± 0.02*****	6.07	43.7 ± 0.51*****	17.76
5b	74.28 ± 0.01*****	6.44	40.7 ± 0.51*****	31.45
5c	75.80 ± 0.02*****	6.08	48.3 ± 0.1*****	17.27
7a	76.52 ± 0.02*****	5.83	29.5 ± 0.0*****	58.03
7b	77.2± 0.004*****	5.08	46.9 ± 0.21*****	13.14
8a	85.21 ± 0.002*****	3.05	66.9 ± 0.21*****	9.96
8b	96.5 ± 0.02*****	2.45	70.0 ± 0.05*****	8.87
Control	-	-	-	-
Indomethacin	**96.50 ± 0.1***	**3.00**	-	-
Pentazocin	-	-	**70.0 ± 0.01***	**12.05**

Anti-inflammatory and analgesic data of the test compounds were compared w.r.t standard.
**P< 0.01; Data were analyzed by Dennett's test for n=6*

RESULTS AND DISCUSSION

The novel pyrazole derivatives were synthesized successfully in moderate to good yields. The newly synthesized compounds were identified on the basis of R_f values, melting point range, solubility in different solvents, IR, ^{1}H-NMR and elemental analysis. The ^{1}H-NMR spectrum showed the presence of nitro phenyl protons and hydroxy benzoyl protons due to linkage of (=HC-NO) and (=C-OH) between δ: 7.04-8.52 ppm and 6.84-8.04 ppm respectively. The IR spectrum showed the presence of characterstic C=O peak between 1650-1685 cm^{-1} and C=N peak at 1538 cm^{-1} with (Ar NO_2) stretching vibration at 1394 cm^{-1} and peak of OH at 3608 cm^{-1} confirmed the presence of pyrazole nucleus with substitution of nitro and hydroxyl groups at various positions.

Anti-inflammatory and analgesic activity

Anti-inflammatory activity of the newly synthesized compounds was screened using the carrageenan induced rat paw edema method by Winter et al [17]. Compounds **4_a**, **4_b**, **4_c**, **8_a** and **8_b** were found to exhibit significant anti-inflammatory activity. Analgesic activity of the same compounds were screened using the writhing method by Koster et al [18] and the compounds **4_a**, **4_b**, **4_c**, **8_a** and **8_b** were found to be effective analgesics. It was found that the presence of two methyl groups (electron donating) at position 3 and 5 increase the activities. The presence of substituent like hydroxyl or nitro at *p*-position retains the activities due to increase in the electron density of the compounds. When the methyl group present at 5th position of pyrazole nucleus was replaced by an oxo group it was observed that there was decrease in the activity of the compounds.

CONCLUSION

A series of 10 compounds were synthesized and screened for anti-inflammatory and analgesic activities. From the anti-inflammatory and analgesic screening data, it was concluded that the compounds possessing substituents like methyl, nitro and hydroxy *viz.* **8_a**, **8_b**, **4_c**, **4_a** and **4_b** exhibited highest degree of inhibition, compound **8_b** was found to be the most potent anti-inflammatory and analgesic agents, with 96.5% inhibition of edema and 2.45 mg/kg of ED_{50}, which was comparable with standard drug indomethacin and showed significant 70% inhibition of writhing in mice and 8.87 mg/kg of ED_{50} which was comparable with standard drug pentazocin.

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REFERENCES

[1] M. Amir, S. Sahani, *J. Ind. Heterocycl.*, **1998**, 8, 107.
[2] M. Amir, A. Oberoi, S. Alam, *Indian J Chem.*, **1999**, 38B, 96.
[3] R. Mulder, K. Wellinga, J.J. Van Daalen, *Naturwissenschaften*, **1975**, 437.
[4] S. Sharma, V.K. Srivastava, A. Kumar, *J. Ind. Chem.*, **2002**, 41(B), 2647.

[5] S.N. Sawhney, S. Bhutani, D. Vir, *J. Ind. Chem.*, **1989**, 28(B), 667.
[6] A. Dandia, V. Sehgal, P. Singh, *J. Ind. Chem.*, **1993**, 32(B), 1288.
[7] M. Amir, S. Kumar, *J. Ind. Chem*., **2005**, 44(B), 2533.
[8] K. Satyanarayana, M.N.A. Rao, *J. Eur. Med. Chem*., **1995**, 33, 642.
[9] A. Camacho, J. Leon, A. Entrena, *J. Med. Chem*., **2004**, 47, 5647.
[10] K.M. Mahadevan, K.M. Basavaraj, V.P. Vaida, *J. Ind. Chem*., **2003**, 45B, 483.
[11] M. Amir, S.A. Khan, S. Drabu, *J. Ind. Heterocycl. Chem*., **2001**, 11, 57.
[12] K.Y. Lee, M.J. Kim, J.N. Kim, *Eur. J. Med. Chem*., **2003**, 50, 694.
[13] H. Naito, S. Ohsuki, M. Sugimori, R. Artsumi, A. Ejima, *Chem. Pharm. Bull*., **2002**, 50(4),451
[14] N.P. Shetgiri, A.D. Chitre, S.M. Ghate, S.S. Patil, *J. Ind.Chem*., **2006**, 45(B), 1310.
[15] B.A. Bhat, K.L. Dhar, S.C. Puri, *Bioorg. Med. Chem. Lett*., **2005**, 11, 96.
[16] P. Rathelot, N. Azas, H. El-Kashef, F. Delmas, C.D. Giorgio, P. Vanelle, *J. Eur. Med. Chem*., **2002**, 37, 671.
[17] C.A. Winter, E.A. Risley, W. Nuss, *Proc. Soc. Exp. Bio. Med*., **1962**, 111, 544.
[18] R. Koster, M. Anderson, E.J. De Beer, *Fed Proc*., **1959**, 18, 412.