



Synthesis, Characterization and Biological Evaluation of Substituted Novel Pyrazolone and Pyrazole Derivatives

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Abstract In the present investigation, series of 2-(4-methoxybenzyl) substituted-3-methyl-1,2 dihydropyrazol-5-one and 2-(4-methoxybenzyl)-substituted-3,5-dimethyl-2,5-dihydro-1H-pyrazole have been synthesized. All synthesized compound were characterized and were tested for anti-inflammatory and analgesic activity by caraggenan induced rat paw edema method and hot plate method viz. It was interestingly found that pyrazolone derivatives have shown lesser activity than that of the pyrazole derivatives. Among all synthesized compounds compound **4a** showed good activity.

Keywords: Pyrazolones, Pyrazoles, Anti-inflammatory and Analgesics

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INTRODUCTION

The pyrazolone ring is an important structural moiety found in numerous pharmaceutically active compounds. This is mainly due to the ease preparation and the important versatile biological activity. When pyrazolones were discovered, they were mostly useful as a anti-inflammatory and analgesic¹ but in recent times, they are known to exhibit antioxidant², anticancer³, antibacterial⁴ and several other pharmacological actions like antifungal⁵, protein-kinase inhibitor⁶, antipyretic⁷, anticancer³, anticonvulsant⁸, anti diabetic⁹, plant growth regulator, herbicidal and as an azodyes.¹⁰⁻¹⁴ The present study involves synthesis of substituted pyrazolone and pyrazole derivatives as an anti-inflammatory activity. 2-(4-Methoxybenzyl) substituted-3-methyl-1,2 dihydropyrazol-5-one and 2-(4-methoxybenzyl)-substituted-3,5-dimethyl-2,5-dihydro-1H-pyrazole were synthesized by cyclocondensation reaction with ethyl acetoacetate and acetyl acetate viz. The synthesis of compounds was confirmed by TLC, IR, NMR and Mass

pyrazole and pyrazolone both were found active but pyrazole nucleus show optimal activity.

MATERIALS AND METHODS

EXPERIMENTAL

Melting points were determined using a VEEGO make microprocessor based melting point apparatus having silicone oil bath and are uncorrected. IR spectra (wave numbers in cm^{-1}) were recorded on a BRUKER ALPHA T FT-IR spectrophotometer using potassium bromide discs. NMR spectra were recorded on BRUKER AVANCE II 400 MHz instrument in CDCl_3 with TMS as internal standard for ^1H NMR. Chemical shift values are mentioned in δ , ppm. Chromatographic separations were performed on columns using silica gel 100–200 mesh. The progress of all reactions was monitored by TLC on 2 cm X 5 cm pre-coated silica gel 60 F254 (Merck) plates of thickness of 0.25 mm. The chromatograms were visualized under UV (254 nm) and/or exposure to iodine vapours. The term “dried” refers to the use of anhydrous sodium sulfate. All reagents used were of analytical reagent grade, obtained from s. d. fine chemicals, Spectrochem and Qualigens. Chemicals and solvents were purified by general laboratory techniques before use. All moisture free operations were performed in oven dried glassware.

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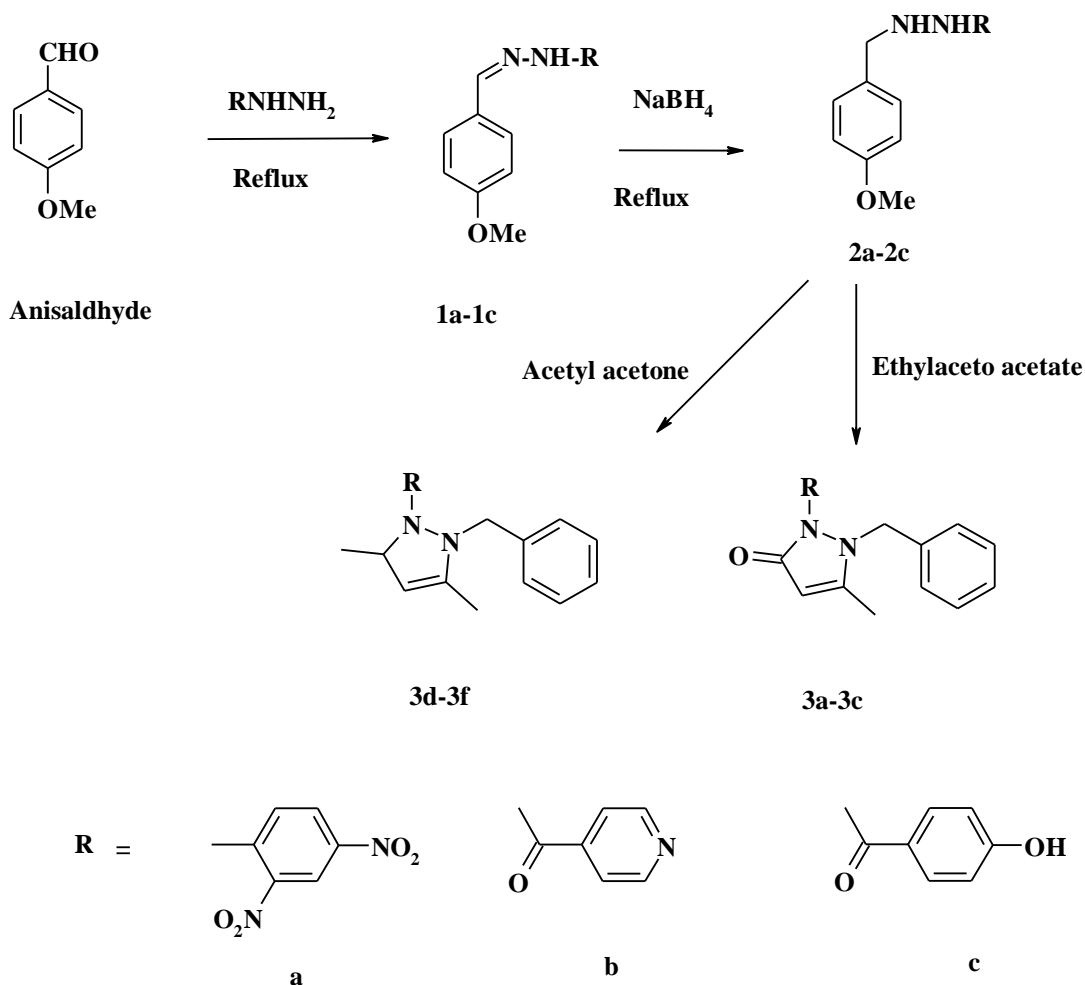


Fig.1 Synthesis of 2-(4-methoxybenzyl) substituted-3-methyl-1,2 dihydropyrazol-5-one (3a-3c) and 2-(4-methoxybenzyl)-substituted-3,5-dimethyl-2,5-dihydro-1H-pyrazole (4a-4c)

1-(4-Methoxybenzylidene)-2-(2,4-dinitrophenyl)hydrazine (1a)

Anisaldehyde (0.01 mole) and 2,4-dinitrophenyl hydrazine (0.01 mole) was dissolve in methanol. A few drops of sulphuric acid were added to reaction mixture, and solution was warmed. The orange yellow precipitate was formed. The crude product was fillterd off, wash with methanol and dried. The compound (1-(4-methoxybenzylidene)-2-(2,4-dinitrophenyl) hydrazine **1a** was recrystalized using ethanol.

N'-(4-Methoxybenzylidene) isonicotinohydrazide (1b)

Mixture of isoniazide (0.01 mole) and anisaldehyde (0.01 mole) was dissolved in 25 ml of ethanol. Few drops of glacial acetic acid were added to reaction mixture. Reaction mixture was further heated for 4-5 hrs on water bath. Progress of reaction was monitored by TLC. Reaction mixture was allowed to stand at room temperature for 24

hrs. Crude product so formed was filtered, dried under vacuum. The compound N'-(4-Methoxybenzylidene) isonicotinohydrazide **1b** was recrystalized by using ethanol.

N'-(4-Methoxybenzylidene)-4-hydroxybenzohydrazide (1c)

Mixture of benzhydrazide (0.01 mole) and anisaldehyde (0.01 mole) was dissolved in sufficient amount of ethanol. Few drops of glacial acetic acid were added to reaction mixture. Reaction mixture was further refluxed for 5-6 hrs on water bath. Progress of reaction was monitored by TLC. Reaction mixture was allowed to stand at room temperature for overt night. Crude product so formed was filtered, dried under vacuum. The crude Product N'-(4-methoxybenzylidene)-4-hydroxybenzohydrazide **1c** was recrystalized from ethanol.

1-(4-Methoxybenzyl)-2-(2,4-dinitrophenyl)hydrazine (2a)

2,4-Dinitrophenylhydrazine **1a** (0.044 moles) was dissolved in mixture of 30 ml methanol and 3 ml of water taken in two necks round bottom flask with constant stirring on oil bath. The reaction mixture was heated up to 40-50 °C; sodium borohydride (0.044 moles) was added in fraction over a period of 1-2 hrs and further stirred for 1-2 hrs. After stirring reaction mixture was refluxed for 1 hrs and poured into ice water to obtain precipitate of 1-(4-methoxybenzyl)-2-(2,4-dinitrophenyl)hydrazine **2a**. It was filtered off, washed with water and dried. Precipitate was recrystallized by using methanol.

N'-(4-Methoxybenzyl)isonicotinohydrazide (2b)

Isonicotinyl hydrazine **1b** (0.005 moles) was dissolved in mixture of 30 ml methanol and 3 ml of water taken in two necks round bottom flask with constant stirring on oil bath. The reaction mixture was heated up to 60 °C; sodium borohydride (0.015 moles) was added in fraction over a period of 1-2 hrs and further stirred for 7-8 hrs. After stirring, reaction mixture was refluxed for 1 hrs and poured into ice water to obtain precipitate. It was filtered off, washed with water and dried. The compound N'-(4-methoxybenzyl)isonicotinohydrazide **2b** was recrystallized by using ethanol.

N'-(4-Methoxybenzyl)-4-hydroxybenzohydrazide (2c)

Benzhydrazide hydrazine **1c** (0.005 moles) was dissolved in mixture of 30 ml Methanol and 3 ml of water taken in two necks round bottom flask with constant stirring on oil bath. The reaction mixture was heated up to 60 °C; sodium borohydride (0.015 moles) was added in fraction over a period of 1-2 hrs and further stirred for 7-8 hrs. After stirring reaction mixture was refluxed for 1-2 hrs. Progress of reaction was monitored by TLC. Reaction mixture was poured into ice water to give precipitate of N'-(4-Methoxybenzyl)-4-hydroxybenzohydrazide **2c**. It was filtered off, washed with water and dried. Precipitate was recrystallized by using ethanol.

2-(4-Methoxybenzyl)-1-(2,4-dinitrophenyl)-3-methyl-1,2-dihydropyrazol-5-one (3a)

1-(4-Methoxybenzyl)-2-(2,4-dinitrophenyl)hydrazine (0.1 mole) **2a** was refluxed with ethyl acetoacetate (0.1 mole) in ethanol for 8-10 hrs. Reaction was monitored by TLC. Reaction mixture was cooled and poured into the crushed ice to obtain yellow colored precipitate. Dark yellow colored precipitate was refluxed in methanol and charcoal for 30 min to remove colored impurities. Charcoal was separated out by filtration and filtrate was concentrated to obtain light yellow precipitate of 2-(4-methoxybenzyl)-1-(2,4-dinitrophenyl)-3-methyl-1,2-dihydropyrazol-5-one **3a**, recrystallized from ethanol.

2-(4-Methoxybenzyl)-1-isonicotinoyl-3-methyl-1,2-dihydropyrazol-5-one (3b)

N'-(4-methoxybenzyl) isonicotinohydrazide (0.1 mole) **2b** was refluxed with ethyl acetoacetate (0.1 mole) in ethanol for 10 hrs. Progress of reaction was monitored by TLC. Reaction mixture was cooled and poured into the crushed ice to yield 2-(4-Methoxybenzyl)-1-isonicotinoyl-3-methyl-1,2-dihydropyrazol-5-one **3b**. The crude product was filtered off, dried and passed through a column of silica gel (100-200 mesh) using Chloroform: Methanol 8: 2 as an eluent.

2-(4-Methoxybenzyl)-1-(4-hydroxybenzoyl)-3-methyl-1,2-dihydropyrazol-5-one (3c)

A mixture N'-(4-methoxybenzyl)-4-hydroxybenzohydrazide (0.1 mole) **2c** was refluxed with ethyl acetoacetate (0.1 mole) in Ethanol for 7-8 hrs. Reaction was monitored by TLC. Reaction mixture was cooled and poured into the crushed ice to give precipitate of 2-(4-Methoxybenzyl)-1-(4-hydroxybenzoyl)-3-methyl-1,2-dihydropyrazol-5-one **3c**. Precipitate was filtered off, dried and recrystallized by using ethanol.

2-(4-Methoxybenzyl)-1-(2,4-dinitrophenyl)-3,5-dimethyl-2,5-dihydro-1H-pyrazole (4a)

(4-Methoxybenzyl)-2-(2,4-dinitrophenyl)hydrazine (0.1 mole) **2a** was refluxed with acetyl acetone (0.1 mole) in 15 ml ethanol for 10-12 hrs on water bath. Reaction was monitored by TLC. Excess of ethanol was removed under reduced pressure and crude sticky product was washed with hexane. The solid product was passed through a column of silica gel (100-200 mesh) by using Chloroform: Methanol 8: 2 as an eluent.

2-(4-Methoxybenzyl)-3,5-dimethyl-2H-pyrazol-1(5H)-yl(pyridin-4-yl)methanone (4b)

N'-(4-methoxybenzyl)isonicotinohydrazide (0.1 mole) **2b** was refluxed with Acetyl acetone (0.1 mole) in ethanol for 10-12 hrs. Reaction was monitored by TLC. Reaction mixture was cooled and poured into the crushed ice to afford precipitate of 2-(4-Methoxybenzyl)-3,5-dimethyl-2H-pyrazol-1(5H)-yl(pyridin-4-yl)methanone **4b**. The crude product was filtered off, washed with hexane, dried and crystallized from ethanol.

2-(4-Methoxybenzyl)-1-(4-hydroxybenzoyl)-3-methyl-1,2-dihydropyrazol (4c)

N'-(4-Methoxybenzyl)-4-hydroxybenzohydrazide (0.1 mole) **2c** was refluxed with Acetyl acetone (0.1 mole) in ethanol for 10-12 hrs. Reaction was monitored by TLC. Reaction mixture was cooled and poured into the crushed ice to give crude product. The crude product was filtered off, dried and passed through a column of silica gel (100-200 mesh) using Chloroform: Methanol 8: 2 as an eluent.

Table-1**Physiochemical data of all synthesized compounds**

Compound	Mol. Formula	Mol. gm	Wt.	Yield %	R _f	Solvent System
1a	C ₁₄ H ₁₄ O ₅ N ₄	318		82	0.97	A
1b	C ₁₅ H ₁₇ O ₂ N ₃	268		80	0.66	B
1c	C ₁₅ H ₁₇ O ₃ N ₂	288		79	0.67	B
2a	C ₁₄ H ₁₄ O ₅ N ₄	320		73	0.90	A
2b	C ₁₅ H ₁₉ O ₂ N ₃	270		78	0.57	B
2c	C ₁₅ H ₁₇ O ₃ N ₂	290		75	0.56	B
3a	C ₁₈ H ₁₆ O ₆ N ₄	385		92	0.88	A
3b	C ₁₈ H ₁₇ O ₃ N ₃	323		84	0.67	B
3c	C ₁₉ H ₁₈ O ₄ N ₂	338		83	0.72	B
4a	C ₁₉ H ₂₀ O ₅ N ₄	384		86	0.92	A
4b	C ₁₉ H ₂₁ O ₂ N ₃	323		80	0.78	B
4c	C ₁₉ H ₁₈ O ₄ N ₂	338		91	0.79	B

A = Chloroform: Methanol, 9:1 B = Chloroform: Methanol, 8:2

Table-2**Spectral characterization of final compounds**

Compound	IR (cm ⁻¹)	NMR (ppm)
3a	1680(C=O),1416(C=C),1505(asymmetric NO ₂),1310 (symmetric),1209 (C-N)	1.56 (s, 3H, CH ₃), 3.12 (s, 2H, CH ₂), 3.88 (s, 3H, OCH ₃), 5.25 (S, 1H, C=C-H), 6.9-7.27 (d, 4H, Ar-H) 7.5-9.15 (d, 3H, Ar-NO ₂).
3b	1704(C=O stretching),1453(C=C stretching), 1252 (C-N)	1.91 (s, 3H, CH ₃), 3.88 (s, 2H, CH ₂), 3.17 (s, 3H, OCH ₃), 4.97 (S, 1H, C=C-H), 6.9-7.3 (d, 4H, Ar-H) 8-9.16 (d, 4H, Ar-H of pyridine).
3c	1733(C=O), 1505 (C=C), 1254 (C-N)	1.26 (s, 3H, CH ₃), 3.23 (s, 2H, CH ₂), 3.83 (s, 3H, OCH ₃), 5.20 (S, 1H, C=C-H), 5.96 (S, 1H, OH), 6.86-6.92 (d, 4H, Ar-H) 7.6-7.8 (d, 4H, Ar-OH)
4a	1416(C=C), 1505 (asym., NO ₂), 1309(symm. NO ₂), 1336(C-N), 2848 (CH ₃)	1.6 (d, 3H, CH ₃), 1.9 (d, 3H, CH ₃), 3.32 (s, 2H, CH ₂), 3.75 (s, 3H, OCH ₃), 5.01 (S, 1H, C=C-H), 2.9 (S, 1H, C=C-C-H), 6.8-7 (d, 4H, Ar-H) 7.4-9 (d, 3H, Ar-NO ₂).
4b	1712 (C=O), 1484(C=C), 1278(C-N), 2876 1(CH ₃)	1.55 (d, 3H, CH ₃), 1.16 (d, 3H, CH ₃), 3.23 (s, 2H, CH ₂), 3.87(s, 3H, OCH ₃), 5.28 (S, 1H, C=C-H), 3.88 (S, 1H, C=C-C-H), 6.8-6.9 (d, 4H, Ar-H) 7.6-9.01 (d, 3H, Pyridine)
4c	C=O(1698), 1400(C=C), 1250 (C-N), 2926 cm ⁻¹ (CH ₃)	1.25 (d, 3H, CH ₃), 1.56 (d, 3H, CH ₃), 3.75 (s, 2H, CH ₂), 3.88 (s, 3H, OCH ₃), 5.01 (S, 1H, C=C-H), 3.91 (S, 1H, C=C-C-H), 6.82-6.92 (d, 4H, Ar-H) 7.4-7.6 (d, 3H, Ar-OH)

BIOLOGICAL ACTIVITY**Anti-inflammatory activity**Carrageenan-induced rat paw edema

Animal was weighed the, mark was made on both the hind paw (right and left) just beyond tibio-tarsal junction, so that every time the paw could dipped in the mercury column up to fixed mark to ensure constant paw volume. Initial paw

volume (both right and left) was noted of each rat by mercury displacement method. Animals were divided into three groups each comprised of 6 animals. To one group normal saline, second group indomethacin (20mg/kg) and third group test drug (20mg/kg) was injected by subcutaneously. After 30 min Carrageenan (0.1 ml, 1 %) was injected in the planter region of the left paw of each group. So, right paw was served as reference non-inflamed

paw for comparison. Paw volume of both legs of control, test and standard group was noted at 0, 30, 60, 90 min after Carrageenan challenge. Percentage difference was calculated in the both right and left paw volumes of each animal of control, test and indomethacin. The mean percentage change in paw volume in control, test and standard was compared which expressed as percentage edema inhibition by the drug¹⁵.

Percentage inhibition = $(1 - V_t/V_c) \times 100$

Where,

V_t = mean increase in paw volume of test,

V_c = mean increase in paw volume of control.

Analgesic activity

Mice were number and weighed. Basal reaction time was noted to radiant heat source by placing the tip of tail on radiant heat source. Flicking response was taken as the endpoint. Normally mouse withdraws its tail within 3-5 sec.

a cut off period of 10-12 sec was observed to prevent damage to the tail. 3-5 basal reaction times for each mouse were taken to check the normal behavior of the animals. Drug (20mg/kg) was injected and reaction time was noted at 5, 10, 15 and 30. Percentage increase in reaction time was calculated¹⁶. pentazocin was used as a standard drug (20mg/kg).

RESULTS AND DISCUSSION

Novel pyrazolone and pyrazole derivatives were synthesized in moderate to good yield. The compounds were identified by R_f value, melting point, NMR and by mass spectroscopy. The compound **4a** and **4c** has shown optimal Anti-inflammatory activity, while compound **3a** and **3b** shows moderate Anti-inflammatory activity. The compound **4a** shows highest anti-inflammatory activity as well as anti-inflammatory activity among all synthesized compounds.

Table-3

(% inhibition of edema) by compounds

Compound	Inhibition of inflammation (mm)					% inhibition \pm SEM			
	0 Min.	30 Min.	60 Min.	90 Min.	120 Min.	30 Min.	60 Min.	90 Min.	120 Min.
3a	0.00	0.15	0.16	0.175	0.18	11 \pm 0.81	32 \pm 0.42	54 \pm 1.116	69 \pm 0.61
3b	0.00	0.152	0.18	0.24	0.23	10 \pm 0.84	25 \pm 0.67	34 \pm 0.42	53 \pm 0.55
3c	0.00	0.148	0.175	0.19	0.182	12 \pm 0.6	28 \pm 0.66	50 \pm 0.84	67 \pm 0.42
4a	0.00	0.14	0.17	0.15	0.145	17 \pm 0.91	30 \pm 0.7	62 \pm 0.6	74 \pm 0.68
4b	0.00	0.152	0.178	0.192	0.19	10 \pm 0.84	26 \pm 0.42	49 \pm 0.47	60 \pm 0.3
4c	0.00	0.148	0.16	0.165	0.149	12 \pm 0.69	32 \pm 0.84	54 \pm 0.89	69 \pm 0.44
Control	0.00	0.17	0.24	0.36	0.48	-	-	-	-
Indomethacin	0.00	0.09	0.08	0.07	0.06	42 \pm 0.89	66 \pm 0.63	80 \pm 1.11	96 \pm 0.3

Anti-inflammatory activity of test compounds were compared w.r.t standard.

* $P < 0.01$; Data were analyzed by Dennett's test for $n=6$

Table-4

(% analgesia) of compounds

Compound	Increase in reaction time in (sec.)		Percentage analgesia \pm SEM	
	15 min.	30 min.	15 min.	30 min.
3a	4.5	6.2	28 \pm 0.28	49 \pm 0.48
3b	4.3	5.1	25 \pm 0.44	37 \pm 0.25
3c	4.7	6.1	30 \pm 0.25	47 \pm 0.43
4a	4.6	6.3	31 \pm 0.3	50 \pm 0.32
4b	4.1	5.3	20 \pm 0.46	39 \pm 0.64
4c	4.2	6.2	24 \pm 0.8	48 \pm 0.75
Pentazocin	5.8	9	44 \pm 0.3	64 \pm 0.15

Basal reaction time – 3.2 sec.

CONCLUSION

From the observation, we found that compounds **3a-4c** posses both anti-inflammatory and analgesic activity. It is interestingly to discuss the fact regarding the comparison pyrazolone and pyrazole. The pyrazolone drug **3a** has shown lesser activity than that of the pyrazole drug **4a**. The presence of methyl group (pyrazole) in place of keto group (pyrazolone) increases the activity in compound **4a**. 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.

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