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SYNTHESIS, CHARACTERIZATION AND BIOLOGICAL EVALUATION OF SUBSTITUTED NOVEL PYRAZOLONE AND PYRAZOLE DERIVATIVES

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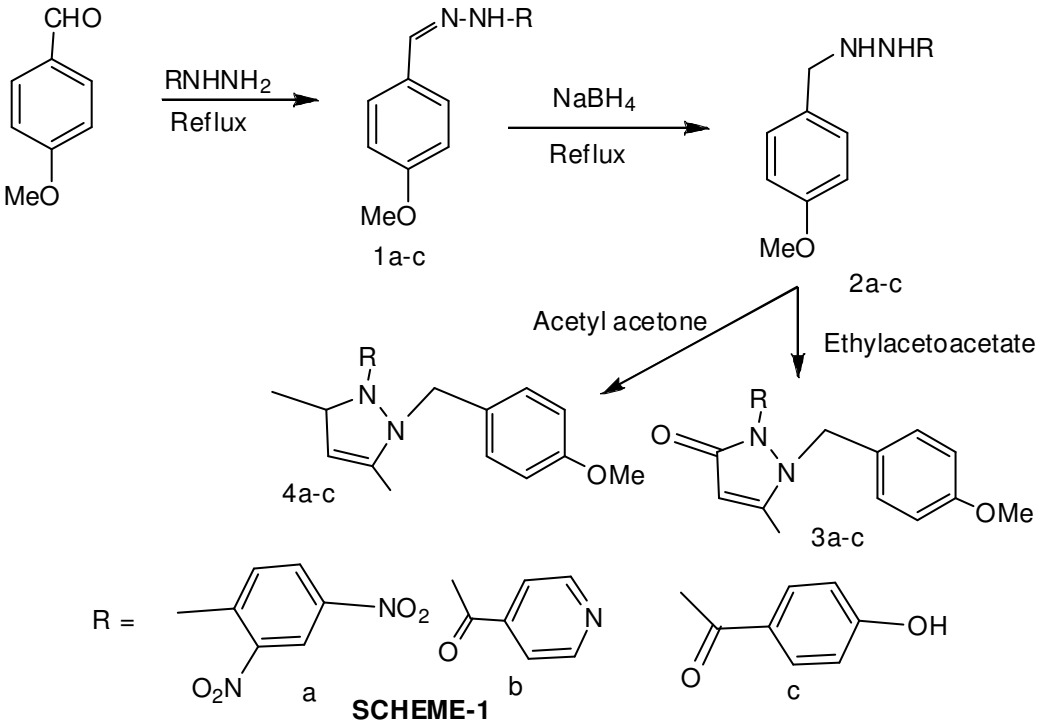
In the present investigation, series of 2-(4-methoxybenzyl) substituted-3-methyl-1,2-dihydropyrazol-5-one and 2-(4-methoxybenzyl)-substituted-3,5-dimethyl-2,5-dihydro-1*H*-pyrazole have been synthesized. All synthesized compounds were characterized and tested for anti-inflammatory and analgesic activity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

The pyrazolone ring is an important structural moiety found in numerous pharmaceutically active compounds. This is mainly due to the easy preparation and the important versatile biological activity. When pyrazolones were discovered, they were mostly useful as a anti-inflammatory and analgesic[1] but in recent times, they are known to exhibit antioxidant[2], anticancer[3], antibacterial[4] and several other pharmacological actions like antifungal[5], protein-kinase inhibitor[6], antipyretic[7], anticancer[3], anticonvulsant[8], antidiabetic[9], 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 agents. 2-(4-Methoxybenzyl) substituted-3-methyl-1,2-dihydropyrazol-5-one and 2-(4-methoxybenzyl)-substituted-3,5-dimethyl-2,5-dihydro-1*H*-pyrazole were synthesized by cyclocondensation reaction with ethyl acetoacetate and acetyl acetone. The synthesis of compounds was confirmed by TLC, IR and NMR



SCHEME-1

Table-1
Physiochemical data of all synthesized compounds

Compd	M.P. (^{0}C)	Yield (%)	R_f	Solvent system
1a	132-134	82	0.97	A
1b	108-110	80	0.66	B
1c	112-114	79	0.67	B
2a	136-138	73	0.90	A
2b	111-114	78	0.57	B
2c	120-122	75	0.56	B
3a	156-158	92	0.88	A
3b	136-138	84	0.67	B
3c	144-146	83	0.72	B
4a	128-129	86	0.92	A
4b	139-140	80	0.78	B
4c	141-143	91	0.79	B

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

spectrocopy. The synthesized compounds were screened for anti-inflammatory activity. The compounds with pyrazole and pyrazolone ring were found active but pyrazole nucleus showed optimal activity.

The title compounds 2-(4-methoxybenzyl)-substituted-3-methyl-1,2-dihydropyrazol-5-ones (**3**a-c) and 2-(4-methoxybenzyl)-substituted-3,5-dimethyl-2,5-dihydro-1*H*-pyrazoles (**4**a-c) were synthesized as per Scheme-1. The starting materials, 1-(4-methoxybenzylidene)-2-substituted hydrazines (**1**a-c) were obtained in good yields by the treatment of anisaldehyde and substituted hydrazines in acidic condition as per the reported method. The 1-(4-methoxybenzylidene)-2-substituted hydrazines (**1**a-c) were reduced with sodium borohydride to corresponding 1-(4-methoxybenzyl)-2-substituted hydrazine[8] (**2**a-c). The title compounds (**3**a-c & **4**a-c) were prepared by refluxing 1-(4-methoxybenzyl)-2-substituted hydrazines (**2**a-c) with ethyl acetoacetate and acetyl acetone respectively in ethanol.

The synthesized compounds were confirmed on the basis of TLC, melting point, FT-IR and ^{1}H NMR spectral data. The FTIR spectra of the title compounds (**3**a-c & **4**a-c) exhibited very similar features and showed the expected bands for the characteristic groups which are present in the compounds, such as C=O, C=C and C-N stretching vibrations. In the proton ^{1}H NMR spectral data, all protons were seen according to the expected chemical shift and integral values. The aromatic protons appeared as multiplet peaks within the range 6.93-7.27 δ ppm.

Biological activity

The title compounds (**3**a-c & **4**a-c) were screened for anti-inflammatory activity using carrageenan-induced rat paw edema method compared with indomethacin as a standard drug and analgesic activity using tail flick method with Pentazocin as a standard drug.

Antiinflammatory activity

Carrageenan-induced rat paw edema method

Animals were weighed and marked on both the hind paw (right and left) just beyond tibo-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 for 6 animals. To one group normal saline as a control, second group indomethacin (20 mg/kg) and third group test drug (20mg/kg) was injected 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 given. 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 drug[15].

Percentage inhibition = (1-Vt/Vc) x 100

Where, Vt= mean increase in paw volume for test

Table-2
(% Inhibition of edema) by compounds

Compd	Inhibition of inflammation (mm)					Percentage inhibition ± 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±0.81	32±0.42	54±1.116	69±0.61
3b	0.00	0.152	0.18	0.24	0.23	10±0.84	25±0.67	34±0.42	53±0.55
3c	0.00	0.148	0.175	0.19	0.182	12±0.6	28±0.66	50±0.84	67±0.42
4a	0.00	0.14	0.17	0.15	0.145	17±0.91	30±0.7	62±0.6	74±0.68
4b	0.00	0.152	0.178	0.192	0.19	10±0.84	26±0.42	49±0.47	60±0.3
4c	0.00	0.148	0.16	0.165	0.149	12±0.69	32±0.84	54±0.89	69±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±0.89	66±0.63	80±1.11	96±0.3

Antiinflammatory activity of test compounds were compared w.r.t. standard.

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

Vc = mean increase in paw volume of control.

The compound **4**a shows highest anti-inflammatory activity as compared to the standard drug among all synthesized compounds. The compounds **3**a, **3**c and **4**c exhibited optimal activity, while compound **3**b and **4**b show moderate anti-inflammatory activity as compared to the standard drug as shown in Table-3.

Analgesic activity

Mice were numbered 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 (20 mg/kg) was injected and reaction time was noted at 5,10,15 and 30 min. Percentage increase in reaction time was calculated, pentazocin was used as a standard drug (20mg/kg).

The analgesic effects of compounds were studied in terms of % analgesia in 15 & 30 min. The compounds **3**a, **3**c, **4**a and **4**c showed 49, 47, 50 & 48% analgesia respectively, found to be comparable with pentazocin 64% analgesia as produced in 30 min shown in Table-3.

From the observation, we found that compounds **3**a-c & **4**a-c posses minimum to moderate anti-inflammatory and analgesic activity. It is interestingly to discuss the fact regarding the comparison pyrazolone and pyrazole; the pyrazolone drug **3**a has shown lesser activity than that of the pyrazole drug **4**a. The presence of methyl group (pyrazole) in place of keto group (pyrazolone) may increases the activity in compound **4**a. 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 nito at *p*-position retains the activities due to increase in the electron density of the compounds.

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 FT-IR spectrophotometer using potassium bromide discs. ^1H NMR spectra were recorded on

Table-3
(% analgesia) of compounds

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

Basal reaction time - 3.2 sec.

BRUKER AVANCE –II 400 MHz instrument in $CDCl_3$ with TMS as internal standard. 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 F_{254} (Merck) plates of thickness of 0.25 mm. The chromatograms were visualized under UV (254 nm) and/ or exposure to iodine vapours.

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

Anisaldehyde (0.01 mol) and 2,4-dinitrophenyl hydrazine (0.01 mol) was dissolved in 25 ml of methanol with constant stirring; few drops of sulphuric acid were added to reaction mixture. The reaction mixture was warmed for 30 min, orange yellow precipitate was formed. The crude product was filtered off, wash with methanol and dried. The compound (1-(4-methoxybenzylidene))-2-(2,4-dinitrophenyl) hydrazine **1**a was recrystallized using ethanol.

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

A mixture of isoniazide (0.01 mol) and anisaldehyde (0.01 mol) was dissolved in 25 ml of ethanol. Then few drops of gl acetic acid were added to reaction mixture. Reaction mixture was further heated for 4-5 hr on water bath. Progress of reaction was monitored by TLC. Reaction mixture was allowed to stand at room temp for 24 hr. Crude product so formed was filtered and dried under vacuum. The compound N′-(4-methoxybenzylidene) isonicotinohydrazide **1**b was recrystallized by using ethanol.

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

A mixture of 4-hydrory benzhydrazide (0.01 mol) and anisaldehyde (0.01 mol) 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 hr on water bath. Progress of reaction was monitored by TLC. Reaction mixture was allowed to stand at room temp for over night. Crude product so formed was filtered and dried under vacuum. The crude product N′-(4-methoxybenzylidene)-4-hydroxybenzohydrazide (**1**c) was recrystallized from ethanol.

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

2,4-Dinitrophenyl hydrazone (**1**a) (0.044 mol) was dissolved in mixture of 30 ml methanol and 3 ml of water into two neck round bottom flask with constant stirring on oil bath. The reaction mixture was heated up to $40\text{-}50^0$; sodium borohydride (0.044 mol) was added in fraction over a period of 1-2 hr and further

stirred for 1-2 hr. After stirring, reaction mixture was refluxed for 1 hr and poured into ice water to obtain precipitate of 1-(4-methoxybenzyl)-2-(2,4-dinitrophenyl) hydrazine (**2**a). It was filtered off, washed with water and dried. Precipitate was recrystallized by using methanol.

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

Isonicotinyl hydrazone (**1**b) (0.05 mol) was dissolved in mixture of 30 ml methanol and 3 ml of water into two neck round bottom flask with constant stirring on oil bath. The reaqction mixture was heated up to 60^0; sodium borohydride (0.015 mol) was added in fraction over a period of 1-2 hr and further stirred for 7-8 hr. After stirring, reaction mixture was refluxed for 1 hr and poured into ice water to obtain precipitate. It was filtered off, washed with water and dried. The compound N′-(4-methoxybenzyl) isonicontinohydrazide (**2**b) was recrystallized by using ethanol.

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

4-Hydrory benzhydrazide hydrazone (**1**c) (0.05 mol) was dissolve in mixture of 30 ml methanol and 3 ml of water into two neck round bottom flask with constant stirring on oil bath. The reaction mixture was heated up to 60^0; sodium borohydride (0.015 mol) was added in fraction over a period of 1-2 hr and further stirred for 7-8 hr. After stirring reaction mixture was refluxed for 1-2 hr. Progress of reaction was monitored by TLC. Reaction mixture was poured into ice water to give precipitate of N′-(4-methoxybenzyl)-4-hydroxybenzohydrazide (**2**c). 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 (0.01 mol) (**2**a) was refluxed with ethyl acetoacetate (0.01 mol) in ethanol for 8-10 hr. Reaction mixture was cooled and poured into the crushed ice to obtained dark yellow colored precipitate. Dark yellow colored precipitate was refluxed with charcoal for 30 min to remove color impurities. Charcoal was separated out by filtration and filtrate was concentrated to obtained light yellow precipitate. Precipitate was filtered off, dried and recrystallized by using ethanol to obtain 2-(4-methoxybenzyl)-1-(2,4-dinitrophenyl)-3-methyl-1,2-dihydropyrazol-5-one (**3**a).

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

N′-(4-Methoxybenzyl) isonicotinohydrazide (0.1 mol) (**2**b) was refluxed with ethyl acetoacetate (0.1 mol) in ethanol for 10 hr. Reaction mixture was cooled and poured into the crushed ice to obtain crude product. The crude product was filtered off, dried and passed through a column of silica gel (100-200 mesh) using Chloroform: Metahnol (8:2) as an eluent to afford 2-(4-methoxybenzyl)-1-isonicotinoyl-3-methyl-1,2-dihydropyrazol-5-one (**3**b).

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

A mixture N′-(4-methoxybenzyl)-4-hydroxybenzohydrazide (0.1 mol) (**2**c) and ethyl acetoacetate (0.1 mol) was refluxed in ethanol for 7-8 hr. After completion of reaction, reaction mixture was cooled and poured into the crushed ice to obtain precipitate. Precipitate was filtered off, dried and recrystallized by using ethanol to afford 2-(4-methoxybenzyl)-1-(4-hydroxybenzoyl)-3-methyl-1,2-dihydropyrazol-5-one (**3**c).

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

N′-(4-Methoxybenzyl)-2-(2,4-dinitrophenyl) hydrazine (0.01 mol) (**2**a) was refluxed with acetyl acetone (0.1 mol) in 15 ml ethanol for 10-12 hr on water bath. After completion of the reaction excess of ethanol was removed under reduced pressure and crude sticky product was washed with hexane. The crude product was passed through a column of silica gel (100-200 mesh) by using Chloroform: Methanol 8:2 as an eluent to get compound **4**a.

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

N′-(4-Methoxybenzyl) isonicotinohydrazide (0.1 mol) (**2**b) was refluxed with acetyl acetone (0.1 mol) in ethanol for 10-12 hr. Reaction mixture was cooled and poured into the crushed ice to afford precipitate of 2-(4-Methoxybenzyl)-3,5-dimethyl-2*H*-pyrazol-1(5*H*-yl) (pyridine-4-yl) methanone (**4**b). 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 mol) (**2**c) was refluxed with acetyl acetone (0.1 mol) in ethanol for 10-12 hr. 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.

Spectral data

3a : IR (cm^{-1}), 1680 (C=O), 1416 (C=C), 1505 (asymmetric NO_2), 1310 (symmetric), 1209 (C-N), NMR (ppm) : 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), 6.93-7.27 (d, 4H, ArH), 7.5-9.15 (d, 3H, Ar-NO_2).

3b : IR : 1704 (C=O str), 1453 (C=C str), 1252 (C-N), NMR : 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), 6.91-7.33 (d, 4H, ArH), 8-9.16 (d, 4H, ArH of pyridine).

3c: IR : 1733 (C=O), 1505 (C=C), 1254 (C-N): NMR : 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, ArH), 7.62-7.83 (d, 4H, Ar-OH).

4a : IR : 1416 (C=C), 1505 (asym., NO_2), 1309 (asym., NO_2), 1309 (symm. NO_2), 1336 (C-N), 1336 (C-N), 2848 (CH_3). NMR : 1.62 (d, 3H, CH_3), 1.95 (d, 3H, CH_3), 3.32 (s, 2H, CH_2), 3.75 (s, 3H, OCH_3), 5.01 (s, 1H, C=C-H), 2.91 (s, 1H, C=C- C-H), 6.82-7.22 (d, 4H, ArH), 7.41-7.86 (d, 3H, Ar-NO_2).

4b : IR: 1712 (C=O), 1484 (C=C), 1278 (C-N), 2876 (CH_3). NMR : 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), 6.81-6.98 (d, 4H, ArH), 7.61-8.01 (d, 3H, pyridine).

4c: IR : C=O (1698), 1400 (C-N), 1250 (C-N), 2926 cm^{-1} (CH_3). NMR : 1.25 (d, 3H, CH_3), 1.56 (d, 3H, CH_3), 3.75 (d, 2H, CH_2), 3.88 (d, 3H, OCH_3), 5.01 (s, 1H, C=C-H), 3.91 (s, 1H, C=C- C-H), 6.82-6.92 (d, 4H, ArH), 7.41-7.69 (d, 3H, Ar-OH).

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