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Syntheses, characterization and evaluation of some 1,3,4-tri substituted-5-pyrazolone derivatives as dual anti-inflammatory and antimicrobial agents

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ABSTRACT

*A series of 4-arylidene-3-methyl-1-phenyl-5-pyrazolone derivatives (**2a–j**) were synthesized by reacting various substituted aromatic aldehydes with 3-methyl-1-phenyl-5-pyrazolone through Knoevenagel condensation by conventional as well as by exposure to microwave irradiations. Structures of all new synthesized compounds were characterized on the basis of spectral data. The title compounds were screened for in vivo anti-inflammatory activity and in vitro antimicrobial activity. Among the synthesized derivatives, compounds **2b**, **2f** and **2h** exhibited significant anti-inflammatory activity whereas compounds **2b**, **2c**, **2h** and **2j** emerged as the most active antibacterial agents. Compound **2b** was identified as the most active member of the series with an interesting dual anti-inflammatory and antibacterial profile.*

Keywords: Pyrazolones, Microwave irradiations, Knoevenagel condensation, Anti-inflammatory, Antimicrobial activity.

INTRODUCTION

Pyrazolone is a biologically important scaffold associated with multiple pharmacological activities such as antimicrobial [1-5], anti-inflammatory [6-8], analgesic [9-10], antidepressant [11], anticonvulsant [12], antidiabetic [13], anti hyperlipidemic [14-15], antiviral [16-17], anti tubercular [18-19], antioxidant [20-21], anticancer [22-23] etc. The synthesis of pyrazolone and its derivatives have engrossed substantial attention from organic and medicinal chemists for many years as they belong to a class of compounds with proven utility in medicinal chemistry. After the discovery of the natural pyrazole C-glycoside pyrazofurin; 4-hydroxy-3-β-D-ribofuranosyl-1H-pyrazole-5-carboxamide as an antibiotic with broad spectrum of antimicrobial and antiviral activities in addition to being active against several tumor cell lines [24], there has been a renewed interest in pyrazoles.

Multi drug resistance is widespread with specific relevance to Gram positive bacteria. Infections caused by these organisms create a serious challenge to the community. The therapeutic problem is more pronounced in patients with immuno-compromised system or those undergoing anticancer therapy substantiating the need for design and development of novel less toxic potent antimicrobial agents. Inflammation is a non specific immune response in which the body reacts to infection, localized irritation, free radicals, other injury or disease [25].

In addition, inflammation is not only known as a symptom of prevalent diseases but also as an early phase of some life-threatening diseases such as cancer, cardiovascular diseases and Alzheimer's dementia. Numerous pyrazolone derivatives have found their clinical application as NSAIDs. Antipyrine, 2,3-dimethyl-1-phenyl-3-pyrazolin-5-one, was the first pyrazolone derivative used in the management of pain and inflammation. Several analogues of pyrazolidin-3,5-diones, pyrazolin-3-ones and pyrazolin-5-ones are also available as NSAIDs; examples are felcobuzone, mefobutazone, morazone, famprofazone and ramifenazone [26]. Simultaneous administration of several drugs to treat inflammatory conditions that might be associated with some microbial infections may cause serious health problems, especially in patients with compromised liver or kidney functions. So the discovery of a dual anti-inflammatory-antimicrobial agent with potential activity and fewer adverse effects not only results in a pharmacoeconomic agent but also leads to better patient compliance. This study was aimed at the synthesis, characterization and screening of some new 5-pyrazolone derivatives for anti-inflammatory and antimicrobial activities.

MATERIALS AND METHODS

2.1 General

All research chemicals were purchased from Sigma-Aldrich and S.D. Fine Chemicals India Pvt. Ltd. and used as such for the reactions. Reactions were monitored by thin-layer chromatography (TLC) on pre-coated silica gel plates.

Melting points of the synthesized compounds were determined by open capillary method and are uncorrected. UV spectra were recorded on Shimadzu 1700 UV–Visible spectrophotometer and IR spectra were recorded on Shimadzu 8400S FTIR spectrometer using KBr pellets. High-Resolution Mass spectra were recorded on JEOL-Accu TOF JMS-T100LC spectrometer. The ^{1}H NMR were recorded on Bruker WM-300 (at 300 MHz) using $CDCl_3$ as solvent. Chemical shifts are reported in δ ppm units with respect to TMS as internal standard. Purity of the compounds was checked on precoated TLC plates using silica gel G plates and iodine vapors as visualizing agent. The anti-inflammatory activity was carried out using plethysmometer and antimicrobial activity was evaluated using cup plate method.

2.2 Synthesis

2.2.1 General procedure for the synthesis of 3-methyl-1-phenyl-5-pyrazolone (1n)

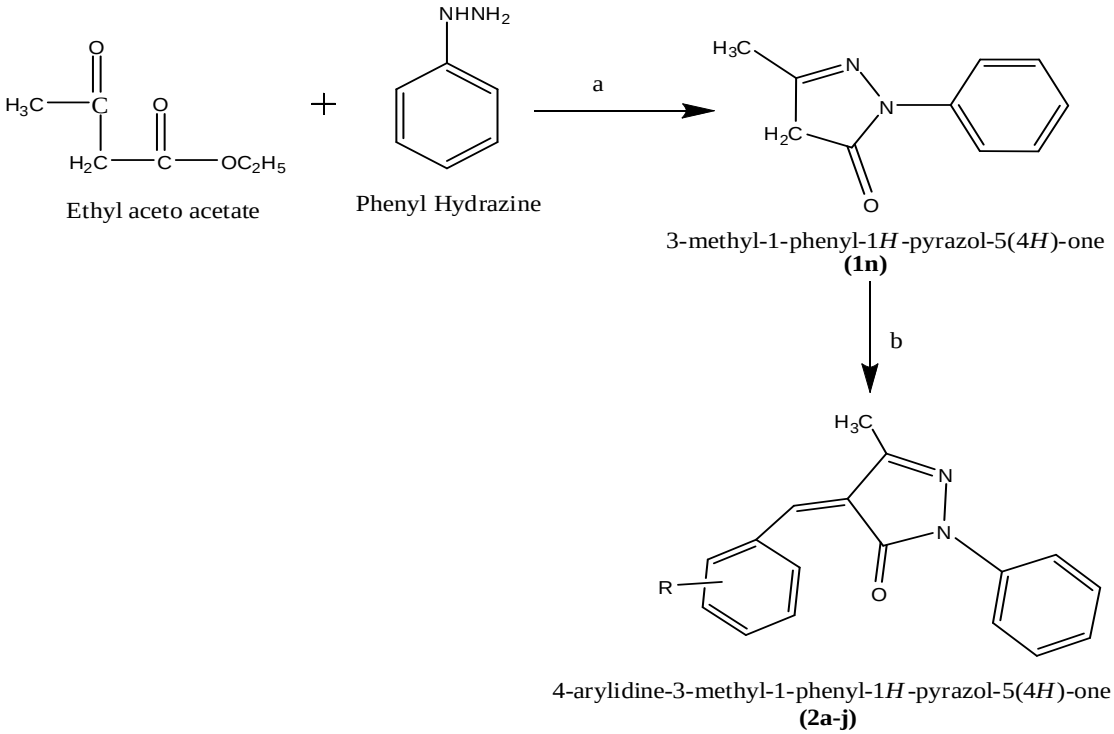
Pure ethyl acetoacetate (0.05 mol, 6.2 ml) was mixed with pure phenyl hydrazine (0.05 mol, 5 ml), followed by addition of 0.5 ml of acetic acid and then heated on a boiling water bath in a fume cupboard for one hour with occasional stirring. The heavy syrup was allowed to cool and 30-40 ml of ether was added and stirred the mixture vigorously to get crystalline pyrazolone. The product was filtered at the pump and the solid material washed thoroughly with ether and then recrystallised from a small quantity of a mixture of equal volume of water and ethanol. The methyl phenyl pyrazolone was obtained as colourless crystals, melting point 127ºC and yield was 83.6% [27].

2.2.2 General procedure of preparation of 4-arylidene-3-methyl-1-phenyl-5-pyrazolone derivatives (2a-j).

A mixture of 1-aryl-3-methyl-5-pyrazolone (0.01 mol, 1.74 g) and substituted aromatic aldehydes (0.012 mol) was heated on oil bath at 150-160^{o}C for 2-4hrs. The progress of the reaction was monitored by TLC using ethyl acetate: hexane (9:1) as solvent system. The mixture was cooled, triturated with ether (20 ml) and filtered off. The coloured residue was recrystallised from ethanol to give the corresponding 4-arylidene-3-methyl-1-phenyl-5-pyrazolone (**2a-2j**) respectively, as coloured products [28].

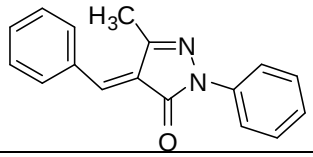
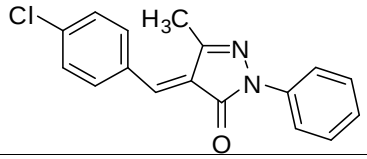
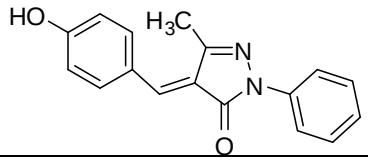
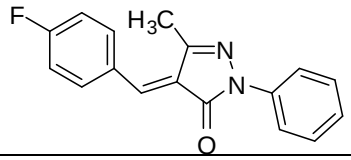
2.2.3 Microwave assisted synthesis 4-arylidene-3-methyl-1-phenyl-5-pyrazolone derivatives (2a-j)

1-aryl-3-methyl-5-pyrazolone (0.01 mol, 1.74 g) and substituted aromatic aldehydes (0.012 mol) were placed in a microwave oven and irradiated at a power of 480 W for 2-4 min. The solid obtained after cooling was triturated with ether and collected, to afford product **2a-2j** (yield 77-84%) as coloured solid (Table 2) [28].



Scheme-1 Synthesis of 4-arylidene-3-methyl-1-phenyl-5-pyrazolone derivatives (2a–j). Reagents and conditions: (a) acetic acid, heat, stir, 45 min; (b) Ar-CHO, condensation, 150-160°C, 3- 4 h/MW 2-4 min.

Table 1 Structures of synthetic compounds (2a-2j)

Compd. Code	R	Structure	IUPAC Name	Mol. formula
2a	4-H		4-benzylidine-3-methyl-1-phenyl-1H-pyrazol-5(4H)-one	$C_{17}H_{14}N_2O$
2b	4-Cl		4-(4-chloro benzylidene)-3-methyl-1-phenyl-1H-pyrazol-5(4H)-one	$C_{17}H_{13}ClN_2O$
2c	4-OH		4-(4-hydroxy benzylidene)-3-methyl-1-phenyl-1H-pyrazol-5(4H)-one	$C_{17}H_{14}N_2O_2$
2d	4-F		4-(4-fluoro benzylidene)-3-methyl-1-phenyl-1H-pyrazol-5(4H)-one	$C_{17}H_{13}FN_2O$

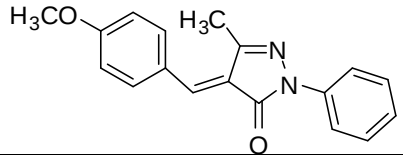
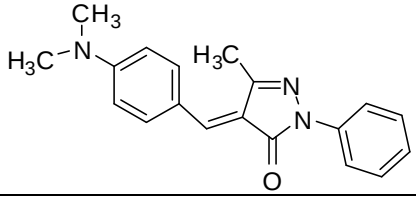
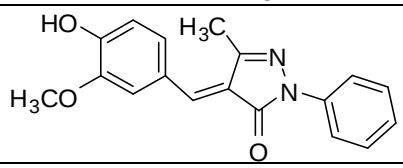
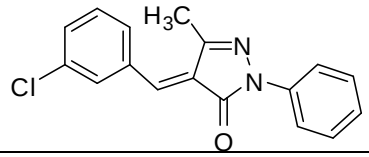
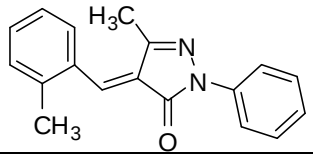
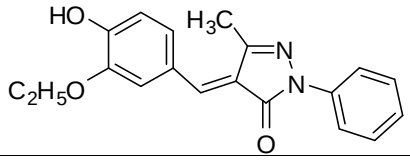
2e	4-OCH_3		4-(4-methoxy benzylidene)-3-methyl-1-phenyl-1H-pyrazol-5(4H)-one	$C_{18}H_{16}N_2O_2$
2f	$N(CH_3)_2$		4-(4-dimethylamino benzylidene)-3-methyl-1-phenyl-1H-pyrazol-5(4H)-one	$C_{19}H_{19}N_3O$
2g	4-OH, 3-OCH_3		4-(4-hydroxy-3-methoxybenzylidene)-3-methyl-1-phenyl-1H-pyrazol-5(4H)-one	$C_{18}H_{16}N_2O_3$
2h	3-Cl		4-(3-chloro benzylidene)-3-methyl-1-phenyl-1H-pyrazol-5(4H)-one	$C_{17}H_{13}ClN_2O$
2i	2-CH_3		3-methyl-4-(2-methyl benzylidene)-1-phenyl-1H-pyrazol-5(4H)-one	$C_{18}H_{16}N_2O_2$
2j	4-OH, 3-OC_2H_5		4-(3-ethoxy-4-hydroxy benzylidene)-3-methyl-1-phenyl-1H-pyrazol-5(4H)-one	$C_{19}H_{18}N_2O_3$

Table 2 Physicochemical data of 4-arylidene-3-methyl-1-phenyl-5-pyrazolone derivatives (2a-2j).

Comp. Code	Time		Colour	Solubility	% yield		Melting Range ˚C	R_f value
	CM	MW			CM	MW		
2a	3h	3min	Red	Methanol	80.90	79.84	88-92	0.84
2b	4h	3min	Red	Methanol	82.62	81.80	80-84	0.82
2c	3.5h	4min	Yellow	Methanol	78.69	77.24	208-212	0.64
2d	3h	3min	Yellow	Methanol	79.84	80.56	98-102	0.83
2e	3h	3min	Orange	Methanol	81.95	82.06	100-106	0.78
2f	3h	2min	Orange	Methanol	78.82	78.90	188-192	0.80
2g	4h	3min	Orange	Methanol	82.62	81.42	160-164	0.79
2h	3.5h	4min	Red	Methanol	76.29	76.82	120-122	0.74
2i	4h	3min	Yellow	Methanol	79.62	76.63	100-104	0.81
2j	4h	3min	Red	Methanol	77.21	78.46	90-94	0.76

2.3 Chemistry

Synthetic method for the preparation of 4-arylidene-3-methyl-1-phenyl-5-pyrazolone derivatives (**2a–j**) are summarized in Scheme 1. It is clear from the scheme that the new heterocyclic compounds possess 5-pyrazolone unit. Reaction of 3-methyl-1-phenyl-5-pyrazolone with aromatic aldehydes proved to be a convenient route to fulfill this aim. Synthesis of 3-methyl-1-phenyl-5-pyrazolone was carried out by reacting phenyl hydrazine with ethylacetoacetate in the presence of acetic acid and stirring with the aid of heat [27]. The reaction involved in final step is an example of the Knoevenagel condensation, in which the active methylene group at position-4 of 3-methyl-1-phenyl-5-pyrazolone reacts with aromatic aldehydes to form 4-arylidene-3-methyl-1-phenyl-5-pyrazolone derivatives. The reaction was carried out in the presence of mixture of piperidine and glacial acetic acid. The final

compounds (**2a-j**) were obtained in good yields. The synthesis of compounds was also carried out by exposing to microwave irradiations. The reaction time was reduced from hours to just few minutes. The yields were comparable to those obtained by conventional method. The progress of the reactions was monitored by TLC (ethylacetate: hexane 90: 10) using precoated silica gel G plates.

Table 3 Spectral analyses data of 4-arylidene-3-methyl-1-phenyl-5-pyrazolone derivatives (2a-j).

Compound Code	UV λ_{max}(nm)	I.R.(KBr,cm^{-}1)	Mass M^{+}+1	^{1}HNMR($CDCl_3$) (δ in ppm)
1n	237	1592(C=N),1680(C=O), 2919, 3058(CH)	175.0123	2.321 (s, 3H, CH_3), 2.013 (s, 2H, CH_2), 7.413-7.956 (m, 5H)
2a	279	1508(C=C), 1600(C=O), 2924, 3129(CH)	263.1152	2.365 (s, 3H, CH_3), 7.214-8.510 (m, 11H).
2b	282	1586(C=C), 1677(C=O), 2918, 3073,(CH), 1093(Ar-Cl)	297.0236	2.355 (s, 3H, CH_3), 7.170-8.489 (m, 10H).
2c	299	1589(C=C), 1655(C=O), 2915 (CH), 3201(OH)	279.4589	2.378 (s, 3H, CH_3), 6.949-8.552 (m, 10H), 10.150 (s, 1H, OH).
2d	280	1591(C=C), 1688(C=O), 2922, 3060(CH), 1312(Ar-F)	281.0129	2.358 (s, 3H, CH_3), 7.170-8.489 (m, 10H).
2e	294	1590(C=C), 1678(C=O), 2924, 3069(CH)	293.0659	2.341 (s, 3H, CH_3), 3.903 (s, 3H, CH_3), 6.989-8.604 (m, 10H).
2f	392	1553(C=C), 1666(C=O), 2915 (CH)	306.0231	2.459 (s, 3H, CH_3), 3.128 (s, 6H, CH_3), 6.723-8.590 (m, 10H).
2g	305	1577(C=C), 1654(C=O), 3083 (CH), 3413(OH)	309.1132	2.377 (s, 3H, CH_3), 3.958 (s, 3H, CH_3), 6.357-7.959 (m, 9H), 9.197 (s, 1H, OH).
2h	281	1583(C=C), 1676(C=O), 2918, 3072(CH), 1095(Ar-Cl)	297.0459	2.355 (s, 3H, CH_3), 7.170-8.489 (m, 10H).
2i	290	1585(C=C), 1674(C=O), 2920, 3060(CH)	277.0236	2.035 (s, 3H, CH_3), 2.477 (s, 3H, CH_3), 7.139-8.637 (m, 10H).
2j	312	1595(C=C), 1676(C=O), 2929, 2979(CH), 3423(OH)	323.1187	2.377 (s, 3H, CH_3), 3.958-4.377 (m, 5H), 6.357-7.959 (m, 9H), 9.200 (s, 1H, OH).

3. Pharmacological evaluation

3.1 Animals

Healthy male albino adult rats weighing 130–200 g were used for various pharmacological screenings. Animals were procured from Central Drug Research Institute Lucknow, India. The ethical clearance was obtained from Institutional Animal Ethics Committee (IAEC) duly approved by CPCSEA (Reg. no. BBDNITM/IAEC/30/2010). The animals were housed individually in polypropylene cages, maintained under standard conditions of alternating 12 h light and dark cycles at a constant temperature (25±2ºC and 35–60% relative humidity) and were fed with standard rat pellet diet and water *ad libitum.*

3.2 Acute toxicity

The acute toxicity test was carried out according to the Organization for Economic Co-operation and Development (OECD) guidelines to establish the effective dose of test compounds after obtaining ethical clearance from Institutional Animal Ethics Committee (IAEC) duly approved by CPCSEA (Reg. no. BBDNITM/IAEC/30/2010). The acute toxicity of synthesized compounds were determined using albino rats of either sex (130-200g) those maintained under standard husbandry conditions. The animals were fasted overnight prior to the experiment and fixed dose of 50 mg/kg body weight was administered (oral). The animals were monitored for the next 14 days and no behavioral change was observed. A dose of 20 mg/kg b.w. was chosen for the pharmacological evaluations.

3.3 Anti-inflammatory activity

In vivo acute anti-inflammatory activity was evaluated using carrageenan-induced rat paw edema assay model of inflammation [29] for the compounds. Male albino rats (130–200 g) were fasted with free access to water at least 12 h prior to experiments and were divided randomly into 12 groups of five each. Control group received 1 ml of 0.5% carboxymethyl cellulose (CMC), standard group received 10 mg/kg of diclofenac sodium and test groups received 20 mg/kg of synthesized compounds (**2a-j**). The rats were dosed orally, 30 minute prior to administration of a subplantar injection of 0.05 ml of 1% solution of carrageenan in sterile distilled water to the left hind footpad of each animal. The paw edema volume was measured with a plethysmometer at 0, 1, 2, 3, 4 h after carrageenan injection. Paw edema volume was compared with vehicle control group and percent reduction was calculated as 1-(edema volume in the drug treated group/edema volume in the control group) ×100.

3.4 Statistical analysis

Statistical analysis was performed by one-way analysis of variance (ANOVA) followed by Dunnett's t-test for multiple comparisons of all compounds in various pharmacological assays. Data are expressed as mean ±SEM.

3.5 In vitro antimicrobial activity

The test organisms were inoculated in nutrient broth and were incubated for 18-48 hrs depending upon the strains. A definite volume of this suspension was added in agar media and poured on to sterile petri dishes. The surface of agar plates was pierced using a sterile cork borer. The prepared wells were filled with equal volume of respective solution of compounds (**2a-j**) in different concentrations (100, 200, 300 and 400µg/ml). Miconazole and Ciprofloxacin at a concentration of 100µg/ml were used as standard drugs for fungal and bacterial strains respectively. After a period of pre-incubation diffusion, the plates were incubated face up for a definite time and at the specified temperature. The diameters of zone of inhibition were measured to the nearest millimeter (including the cup size) [30] and are reported in Table 5.

RESULTS AND DISCUSSION

4.1 Synthetic and spectral studies

A series of 4-arylidene-3-methyl-1-phenyl-5-pyrazolone derivatives have been synthesized by condensing 3-methyl-1-phenyl-5-pyrazolone with substituted aromatic aldehydes as illustrated in Scheme 1. Structure of the synthesized compounds (**2a-j**) was established on the basis of physicochemical and spectral data (IR, HRMS and ^{1}HNMR), which are summarized in Tables 2 and 3 respectively.

4.2 Acute toxicity study

The acute toxicity of synthesized compounds was determined using albino rats of either sex (130-200g), maintained under standard husbandry conditions. The animals were fasted overnight prior to the experiment and a dose of 50 mg/kg body weight was administered (oral) as per OECD guideline No. 420.

Table 4 *In vivo* anti-inflammatory activity of 4-arylidene-3-methyl-1-phenyl-5-pyrazolone derivatives (2a-j) in carrageenan-induced paw edema.

Compounds	Normal paw Volume	Mean paw volume ± SEM(ml) and % Inhibition				
		Time after Carrageenan injection				
		0 hr	1 hr	2 hr	3 hr	4 hr
Control	1.080±0.037	1.960±0.024	3.240± 0.024	4.240± 0.024	5.140± 0.024	5.940± 0.024
Std	1.040±0.024	2.046±0.024	2.840±0.024 (12.34%)*	2.540±0.024 (40.09%)*	2.240±0.024 (56.42%)*	2.020±0.037 (65.99%)*
2a	1.160±0.051	1.920±0.037	3.040±0.024 (6.17%)*	2.840±0.024 (33.01%)*	2.540±0.024 (50.58%)*	2.340±0.024 (60.60%)*
2b	1.120±0.037	2.080±0.037	2.960±0.024 (8.64%)*	2.640±0.024 (37.73%)*	2.340±0.024 (54.47%)*	2.240±0.024 (62.96%)*
2c	1.100±0.071	2.060±0.051	3.000±0.032 (7.40%)*	2.740±0.024 (35.37%)*	2.440±0.024 (52.52%)*	2.280±0.037 (61.61%)*
2d	1.280±0.086	2.000±0.071	3.060±0.024 (5.55%)*	2.880±0.024 (32.07%)*	2.320±0.037 (54.86%)*	2.300±0.045 (61.27%)*
2e	1.080±0.037	2.100±0.045	2.140±0.169 (3.08%)*	2.820±0.037 (33.49%)*	2.380±0.037 (53.69%)*	2.360±0.051 (60.26%)*
2f	1.180± 0.058	2.060±0.051	3.080±0.037 (4.93%)*	2.720±0.037 (35.84%)*	2.480±0.037 (51.75%)*	2.220±0.037 (62.62%)*
2g	1.000±0.000	2.080±0.086	2.086±0.049 (4.75%)*	2.860±0.037 (32.54%)*	2.460±0.051 (52.14%)*	2.320±0.037 (60.94%)*
2h	1.160±0.060	2.100±0.071	3.068±0.051 (5.30%)*	2.700±0.037 (36.32%)*	2.360±0.024 (54.08%)*	2.240±0.058 (62.28%)*
2i	1.220±0.066	2.130±0.044	2.092±0.037 (4.56%)*	2.780±0.037 (34.43%)*	2.520±0.037 (50.97%)*	2.380±0.051 (59.93%)*
2j	1.160±0.051	2.160±0.081	2.980±0.037 (8.02%)*	2.840±0.051 (33.96%)*	2.420±0.051 (52.91%)*	2.260±0.058 (61.95%)*

*Values are expressed as mean ±SEM; n=5; *P<0.01 compared with vehicle treated group using one way ANOVA followed by Dunnett's test.*

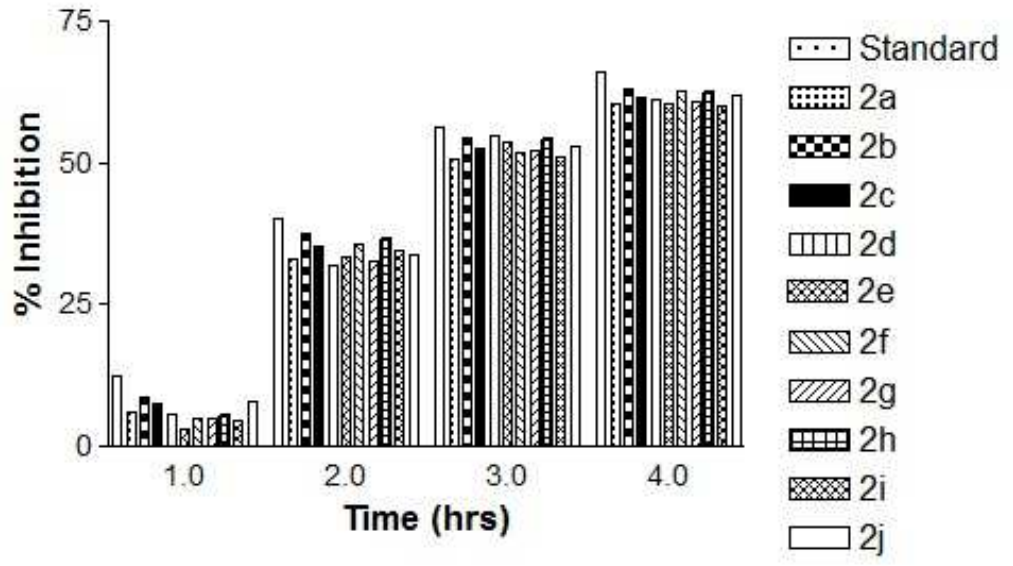


Figure 1 Anti-inflammatory activity of synthesized compounds

4.3 Anti-inflammatory activity

The *in vivo* acute anti-inflammatory activity of the series of 4-arylidene-3-methyl-1-phenyl-5-pyrazolone derivatives (**2a-j**) at a dose of 20 mg/kg in carrageenan-induced paw edema method is depicted in Table-4. Carrageenan-induced edema is a non-specific inflammation resulting from a complex of diverse mediators. Since edema of this type is highly sensitive to NSAIDs, carrageenan has been accepted as a useful agent for studying new anti-inflammatory agents. This model reliably predicts the anti-inflammatory efficacy of the NSAIDs, and during the second phase it detects compounds that are anti-inflammatory agents as a result of inhibition of prostaglandin amplification. As shown in Table 4, the entire series of investigated compounds exhibited moderate to good anti-inflammatory activity when compared to reference drug diclofenac sodium (10 mg/kg). Compounds **2b**, **2c**, **2d**, **2f**, **2h** showed significant anti-inflammatory activity at fourth hour being 62.98%, 61.61%, 61.27%, 62.62%, 62.28% and 61.95% respectively, which was quite closer to that of the standard drug (65.99%) (Figure 1). The order of anti-inflammatory activity of the compounds was **2d** < **2c** < **2i** < **2h** < **2f** < **2b**.

4.4 In vitro antimicrobial activity

All of the newly synthesized compounds were evaluated for their *in vitro* antibacterial activity against *Staphylococcus aureus* (MTCC 96) and *Bacillus subtilis* (MTCC 121) as Gram positive bacteria and *Escherichia coli* (MTCC 739) and *Pseudomonas aeruginosa* (MTCC 2453) as Gram negative bacteria. They were also evaluated for their *in vitro* antifungal potential against recultured *Candida albicans* (MTCC 227) fungal strain at 100, 200, 300 and 400µg/ml. Cup plate method was used for the determination of the preliminary antibacterial and antifungal activity [30]. Ciprofloxacin and Miconazole were used as reference drugs. The results were recorded for each tested compound as the average diameter of inhibition zones of bacterial or fungal growth around the disks in mm. The results are shown in Table-5.

All the synthesized compounds were found to be active against both the Gram positive and Gram negative organisms at different concentrations**.** Among the synthesized compounds, **2b**, **2f** and **2h** were found to be active against *Staphylococcus aureus, Pseudomonas aeruginosa and Bacillus subtilis* at a minimum concentration of 100µg/ml whereas all other compounds were active at 200, 300 and 400µg/ml. On the other hand, all the compounds showed good activity against *Escherichia coli* at 100, 200, 300 and 400µg/ml. However, none of the compounds showed any activity against *Candida albicans*.

Table 5 *In vitro* antimicrobial Activity of synthesized compounds (2a-j)

Compound code	Conc. (ug/ml)	Zone of Inhibition diameter (mm)				
		Gram+ve bacteria		Gram-ve bacteria		Fungus
		SA	BS	EC	PA	CA
Ciprofloxacin	100	20	20	18	20	-
Miconazole	100	-	-	-	-	20
2a	100	-	-	-	-	-
	200	6	7	6	7	-
	300	9	9	9	9	-
	400	12	13	11	13	-
2b	100	6	6	-	6	-
	200	6	7	7	7	-
	300	8	9	9	8	-
	400	11	12	11	11	-
2c	100	-	-	-	-	-
	200	7	7	8	7	-
	300	9	9	10	9	-
	400	12	11	13	12	-
2d	100	-	-	-	-	-
	200	6	7	7	7	-
	300	8	9	10	8	-
	400	11	11	12	12	-
2e	100	-	-	-	-	-
	200	6	7	7	7	-
	300	8	10	9	8	-
	400	11	12	11	11	-
2f	100	6	6	-	6	-
	200	7	8	8	7	-
	300	9	10	10	8	-
	400	12	12	12	10	-
2g	100	-	-	-	-	-
	200	7	6	7	7	-
	300	9	8	9	10	-
	400	12	11	11	13	-
2h	100	6	6	-	6	-
	200	7	7	8	6	-
	300	9	9	10	8	-
	400	12	11	13	10	-
2i	100	-	6	-	-	-
	200	7	9	6	7	-
	300	9	11	8	9	-
	400	12	13	10	12	-
2j	100	-	-	-	-	-
	200	7	7	6	7	-
	300	10	9	8	9	-
	400	13	12	11	12	-
Control (DMSO)	10% v/v	-	-	-	-	-

SA - *Staphylococcus aureus*, BS - *Bacillus subtilis*, EC- *Escherichia coli*, PA- *Pseudomonas aeruginosa*, CA- *Candida albicans*

CONCLUSION

In the present study, the synthesis, spectral studies, and pharmacological evaluation of a novel series of 4-arylidene-3-methyl-1-phenyl-5-pyrazolone derivatives (**2a-j**) are being reported. These heterocyclic compounds containing pyrazolone ring systems were prepared by the Knoevenagel condensation between 3-methyl-1-phenyl-5-pyrazolone and aromatic aldehydes. The compounds were synthesized by microwave assisted method also and the time for synthesis was reduced from hours to just a few minutes. The examined compounds did not show toxic effects at doses upto 50 mg/kg body weight in acute toxicity experiments.

Among the ten prepared compounds, compounds **2b**, **2f** and **2h** exhibited significant anti-inflammatory activity in model of acute inflammation such as carrageenan-induced rat paw edema at a dose of 20mg/kg body weight. The presence of chlorine, fluorine and methoxy groups in the aromatic ring of 4-position of the pyrazolone nucleus gave

rise to an increased anti-inflammatory activity. Thus, these compounds constitute an interesting template for the evaluation of new inflammatory inhibitors and may be helpful for the design of new therapeutic tools against inflammation.

All the synthesized compounds have been found to be active against Gram-positive (*Staphylococcus aureus & Bacillus subtilis)* and Gram-negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa).* Electron donating groups (OH, CH_3) at para positions (**2c**, **2i** and **2j**) increased activity. Meta and para directing chloro group (**2b** and **2h**) showed good activity. The synthesized compounds were found to be inactive towards the used fungus (*Candida albicans*).

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