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ORIGINAL ARTICLE

Microwave-assisted synthesis, characterization and biological activity of novel pyrazole derivatives

Theivendren Panneer Selvam [a,*], Palanirajan Vijayaraj Kumar [b], Govindaraj Saravanan [c], Chinnasamy Rajaram Prakash [d]

[a] *Department of Pharmaceutical Chemistry, PES's Rajaram and Tarabai Bandekar College of Pharmacy, Faramagudi, Ponda 403 401, Goa, India*
[b] *School of Pharmacy, UCSI (University College Sadaya International) University, Jalan Menara Gading, 56000 Cheras, Kuala Lumpur, Malaysia*
[c] *Medicinal Chemistry Research Laboratory, Bapatla College of Pharmacy, Bapatla 522 101, Andhra Pradesh, India*
[d] *Department of Medicinal Chemistry, DCRM Pharmacy College, Inkollu 523 167, Andhra Pradesh, India*

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Abstract A series of 1-(4-substitutedphenyl)-3-phenyl-1*H*-pyrazole-4-carbaldehydes **4a–l** have been synthesized and tested for their biological activities. Formation of the pyrazole derivatives was achieved by treating with Vilsmeier-Haack reagent. The newly synthesized compounds were evaluated for their anti-inflammatory and analgesic activities compared to Diclofenac sodium as standard drug. Compounds **4g**, **4i** and **4k** exhibited the maximum anti-inflammatory and analgesic activities. The detailed synthesis, spectroscopic and toxicity data are reported.

1. Introduction

Pain is an unpleasant sensation and it is widely accepted to be one of the most important determinants of quality of life. A study reported by the World Health Organization (WHO) demonstrated that individuals who live with persistent pain suffer fourfold more from depression (or) anxiety compared to healthy subjects (Gureje et al., 1998). The identification of compounds able to treat both acute and chronic pain with limited side effects is one of the prominent goals in biomedical research. Non-steroidal anti-inflammatory drugs (NSAIDs) exert their effects by inhibition of prostaglandin production. The pharmacological target of NSAIDs is cyclooxygenase (COX), which catalyzes the first committed step in arachidonic acid metabolism. Two isoforms of the membrane protein COX are known: COX-1, which is constitutively expressed in most tissues, is responsible for the physiological production of prostaglandins; and COX-2, which is induced by cytokines, mitogens and endotoxins in inflammatory cells, is responsible for the elevated production of prostaglandins during inflammation (Kurumbail et al., 1996). The widely prescribed anti-inflammatory pyrazole derivatives such as, Celecoxib, Deracoxib,

* Corresponding author. Mobile: +91 9145360006.
E-mail address: tpsphc@gmail.com (T.P. Selvam).
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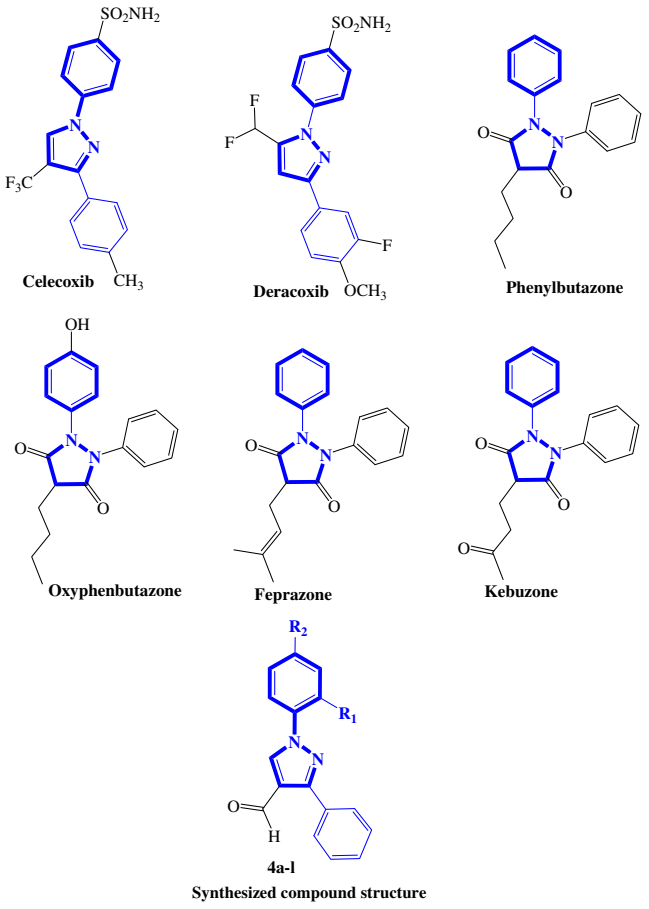


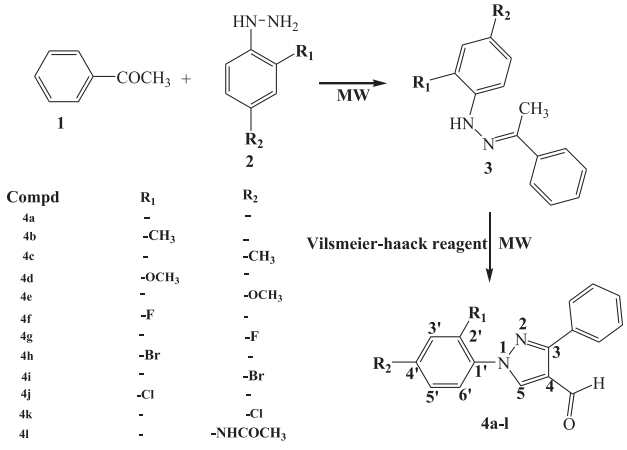
Figure 1 Structures of well established NSAIDs and title compounds with its common pharmacophore features.

Phenylbutazone, Oxyphenbutazone, Feprazone and Kebuzone Fig. 1 are COX inhibitors with reduced ulcerogenic side effects. Due to the importance of pyrazole derivatives considerable efforts have been made by several investigators, to prepare new compounds bearing a single substituent or more complicated systems, including the heterocyclic rings mainly at 1-, 3- and 4-positions. Also, the literature survey reveals excellent anti-inflammatory, analgesic (Farghaly et al., 2001; Balsamo et al., 2003; Youssef et al., 2007; Souza et al., 2001; Godoy et al., 2004; Souza et al., 2002; Prokopp et al., 2006), anti-microbial (Pimerova et al., 2001; Bekhit et al., 2008), anti-viral, anti-tumor (Park et al., 2005), anti-convulsant (Michon et al., 1995), anti-histaminic (Yildirim et al., 2005) and anti-depressant (Bailey et al., 1985) activities with some compounds containing the heterocyclic ring such as pyrazole. Based on the above mentioned research results, the objective of this study aimed to synthesize some novel derivatives of 1-(4-substitutedphenyl)-3-phenyl-1*H*-pyrazole-4-carbaldehyde **4a–l** in order to screen them for anti-inflammatory and analgesic activities.

2. Experimental

2.1. Materials

Synthetic starting material, reagents and solvents were of analytical grade or of the highest quality commercially available.



Compd	R_1	R_2
4a	-	-
4b	$-CH_3$	-
4c	-	$-CH_3$
4d	$-OCH_3$	-
4e	-	$-OCH_3$
4f	-F	-
4g	-	-F
4h	-Br	-
4i	-	-Br
4j	-Cl	-
4k	-	-Cl
4l	-	$-NHCOCH_3$

Scheme 1 Pyrazole-4-carbaldehyde derivatives.

The chemicals were purchased from Aldrich Chemical Co., and Merck Chemical Co., respectively, these solvents used were of analytical grade and purified before their use.

2.2. Instrumentation

The silica gel G used for analytical chromatography (TLC) was obtained from E. Merck India Ltd. Solvent systems used were $CHCl_3$–MeOH (7:3). All the melting points were taken in an open glass capillary and are uncorrected. ^1H NMR spectra were taken on a Bruker ultra shield (300 MHz) NMR spectrometer in $CDCl_3$ using tetramethylsilane [$(CH_3)_4Si$] as an internal standard. Chemical shifts (δ) are expressed in ppm. Mass spectra were obtained on a JEOL-SX-102 instrument using electron impact ionization. All the IR spectra were recorded in KBr pellets on a Jasco FT-IR 410 spectrometer. Elemental analyses were performed on a Perkin Elmer model 240c analyzer and were within $\pm 0.4\%$ of the theoretical values.

2.3. General procedure

The synthetic strategy of the target compounds is illustrated in Scheme 1. The acetophenone (0.01 mol), substituted phenyl hydrazine (0.01 mol) and DMF (0.5 mL) were exposed to microwave at 200 W intermittently at 10 s intervals. The specified reaction time of 3 min was observed of compound 1-substituted phenyl-2-(1-phenylethylidene)hydrazine **3**. The reaction mixture was cooled with cold water. The precipitate thus obtained was filtered, washed with water and purified by recrystallization from ethanol to furnish **3**. The compound **3** (0.01 mol) was added portion wise with Vilsmeier-Haack reagent ($POCl_3$–DMF/SiO_2) (0.03 mol). After the addition was complete, the reaction flask was kept at room temperature for 5 min and silica gel 3 g was added and properly mixed with the help of a glass rod, till free flowing powder was obtained. The powder is then irradiated in a microwave oven at 400 W intermittently at 30 s intervals. The specified reaction time of 5 min was observed of compound **4a–l**. The reaction mixture was cooled and treated with cold water. The solid obtained by the neutralization of the filtrate with $NaHCO_3$ was filtered, washed with water and purified by recrystallization from methanol to afford **4a–l**. The completion of reaction is monitored by TLC method [eluent: $CHCl_3$–MeOH (7:3)].

*2.3.1. 1-Phenyl-2-(1-phenylethylidene)hydrazine (**3**)*

Yield: 79%; mp. 173–175 °C, IR (KBr) cm^{-1}: 3342 (N–H stretching), 3012 (Ar-CH), 1541 (C═N), 1481 (C═C), 1331 (N–H bending); ^1H NMR (300 MHz, $CDCl_3$, δ ppm): 7.98 (s, 1H, N–H), 6.12–7.22 (m, 10H, Ar-H), 2.27 (m, 3H, $-CH_3$); MS (EI) *m/z*: $[M]^+$ 210; (Calcd for $C_{14}H_{14}N_2$; 210.27). Anal. Calcd for $C_{14}H_{14}N_2$; C, 79.97; H, 6.71; N, 13.32; Found: C, 80.03; H, 6.72; N, 13.34.

*2.3.2. 1,3-Diphenyl-1H-pyrazole-4-carbaldehyde (**4a**)*

Yield: 82%; mp. 189–191 °C, IR (KBr) cm^{-1}: 3036 (Ar-CH), 2756 (C–H in CHO), 1706 (C═O), 1552 (C═N), 1481 (C═C); ^1H NMR (300 MHz, $CDCl_3$, δ ppm): 8.79 (s, 1H, –CHO), 7.49 (s, 1H, Pyrazole-CH), 6.29–7.19 (m, 10H, Ar-H); MS (EI) *m/z*: $[M]^+$ 248; (Calcd for $C_{16}H_{12}N_2O$; 248.28). Anal. Calcd for $C_{16}H_{12}N_2O$; C, 77.40; H, 4.87; N, 11.28; Found: C, 77.62; H, 4.88; N, 11.26.

*2.3.3. 1-(2-Methylphenyl)-3-diphenyl-1H-pyrazole-4-carbaldehyde (**4b**)*

Yield 76%, mp. 172–174 °C; IR (KBr) cm^{-1}: 3031 (Ar-CH), 2774 (C–H in CHO), 1713 (C═O), 1543 (C═N), 1484 (C═C); ^1H NMR (300 MHz, $CDCl_3$, δ ppm): 8.34 (s, 1H, –CHO), 7.44 (s, 1H, Pyrazole-CH), 6.33–7.14 (m, 9H, Ar-H), 2.71 (m, 3H, $-CH_3$); MS (EI) *m/z*: $[M]^+$ 262; (Calcd for $C_{17}H_{14}N_2O$; 262.31). Anal. Calcd for $C_{17}H_{14}N_2O$; C, 77.84; H, 5.38; N, 10.68; Found: C, 77.56; H, 5.40; N, 10.69.

*2.3.4. 1-(4-Methylphenyl)-3-diphenyl-1H-pyrazole-4-carbaldehyde (**4c**)*

Yield 72%, mp. 167–169 °C; IR (KBr) cm^{-1}: 3012 (Ar-CH), 2748 (C–H in CHO), 1719 (C═O), 1512 (C═N), 1474 (C═C); ^1H NMR (300 MHz, $CDCl_3$, δ ppm): 8.19 (s, 1H, –CHO), 7.46 (s, 1H, Pyrazole-CH), 6.61–7.21 (m, 9H, Ar-H), 2.19 (m, 3H, $-CH_3$); MS (EI) *m/z*: $[M]^+$ 262; (Calcd for $C_{17}H_{14}N_2O$; 262.31). Anal. Calcd for $C_{17}H_{14}N_2O$; C, 77.84; H, 5.38; N, 10.68; Found: C, 77.62; H, 5.39; N, 10.71.

*2.3.5. 1-(2-Hydroxyphenyl)-3-diphenyl-1H-pyrazole-4-carbaldehyde (**4d**)*

Yield 81%, mp. 171–173 °C; IR (KBr) cm^{-1}: 3413 (Ar-OH), 3016 (Ar-CH), 2762 (C–H in CHO), 1718 (C═O), 1572 (C═N), 1472 (C═C); ^1H NMR (300 MHz, $CDCl_3$, δ ppm): 9.91 (s, 1H; Ar-OH), 8.74 (s, 1H, –CHO), 7.79 (s, 1H, Pyrazole-CH), 6.51–7.22 (m, 9H, Ar-H); MS (EI) *m/z*: $[M]^+$ 264; (Calcd for $C_{16}H_{12}N_2O_2$; 264.28). Anal. Calcd for $C_{16}H_{12}N_2O_2$; C, 72.72; H, 4.58; N, 10.60; Found: C, 72.92; H, 4.60; N, 10.62.

*2.3.6. 1-(4-Hydroxyphenyl)-3-diphenyl-1H-pyrazole-4-carbaldehyde (**4e**)*

Yield 84%, mp. 161–163 °C; IR (KBr) cm^{-1}: 3402 (Ar-OH), 3031 (Ar-CH), 2788 (C–H in CHO), 1712 (C═O), 1522 (C═N), 1483 (C═C), ^1H NMR (300 MHz, $CDCl_3$, δ ppm): 9.93 (s, 1H; Ar-OH), 8.42 (s, 1H, –CHO), 7.31 (s, 1H, Pyrazole-CH), 6.16–6.98 (m, 9H, Ar-H); MS (EI) *m/z*: $[M]^+$ 264; (Calcd for $C_{16}H_{12}N_2O_2$; 264.28). Anal. Calcd for $C_{16}H_{12}N_2O_2$; C, 72.72; H, 4.58; N, 10.60; Found: C, 72.81; H, 4.60; N, 10.61.

*2.3.7. 1-(2-Flurophenyl)-3-phenyl-1H-pyrazole-4-carbaldehyde (**4f**)*

Yield: 73%; mp. 182–184 °C, IR (KBr) cm^{-1}: 3044 (Ar-CH), 2770 (C–H in CHO), 1712 (C═O), 1532 (C═N), 1471 (C═C), 1029 (C–F); ^1H NMR (300 MHz, $CDCl_3$, δ ppm): 8.71 (s, 1H, –CHO), 7.59 (s, 1H, Pyrazole-CH), 6.54–7.17 (m, 9H, Ar-H), MS (EI) *m/z*: $[M]^+$ 266; (Calcd for $C_{16}H_{11}FN_2O$; 266.27). Anal. Calcd for $C_{16}H_{11}FN_2O$; C, 72.17; H, 4.16; N, 10.52; Found: C, 72.26; H, 4.18; N, 10.56.

*2.3.8. 1-(4-Flurophenyl)-3-phenyl-1H-pyrazole-4-carbaldehyde (**4g**)*

Yield: 76%; mp. 174–176 °C, IR (KBr) cm^{-1}: 3061 (Ar-CH), 2758 (C–H in CHO), 1697 (C═O), 1511 (C═N), 1501 (C═C), 1032 (C–F); ^1H NMR (300 MHz, $CDCl_3$, δ ppm): 8.31 (s, 1H, –CHO), 7.49 (s, 1H, Pyrazole-CH), 6.24–7.01 (m, 9H, Ar-H); MS (EI) *m/z*: $[M]^+$ 266; (Calcd for $C_{16}H_{11}FN_2O$; 266.27). Anal. Calcd for $C_{16}H_{11}FN_2O$; C, 72.17; H, 4.16; N, 10.52; Found: C, 72.42; H, 4.18; N, 10.55.

*2.3.9. 1-(2-Bromophenyl)-3-phenyl-1H-pyrazole-4-carbaldehyde (**4h**)*

Yield: 78%; mp. 177–179 °C, IR (KBr) cm^{-1}: 3039 (Ar-CH), 2766 (C–H in CHO), 1712 (C═O), 1561 (C═N), 1489 (C═C), 606 (C–Br); ^1H NMR (300 MHz, $CDCl_3$, δ ppm): 8.12 (s, 1H, –CHO), 7.62 (s, 1H, Pyrazole-CH), 7.12–7.49 (m, 9H, Ar-H); MS (EI) *m/z*: [M + 2] 329; (Calcd for $C_{16}H_{11}BrN_2O$; 327.18). Anal. Calcd for $C_{16}H_{11}BrN_2O$; C, 58.74; H, 3.39; N, 8.56; Found: C, 58.91; H, 3.40; N, 8.54.

*2.3.10. 1-(4-Bromophenyl)-3-phenyl-1H-pyrazole-4-carbaldehyde (**4i**)*

Yield: 72%; mp. 172–174 °C, IR (KBr) cm^{-1}: 3018 (Ar-CH), 2781 (C–H in CHO), 1722 (C═O), 1543 (C═N), 1493 (C═C), 662 (C–Br); ^1H NMR (300 MHz, $CDCl_3$, δ ppm): 8.23 (s, 1H, –CHO), 7.81 (s, 1H, Pyrazole-CH), 6.32–7.49 (m, 9H, Ar-H); MS (EI) *m/z*: [M + 2] 329; (Calcd for $C_{16}H_{11}BrN_2O$; 327.18). Anal. Calcd for $C_{16}H_{11}BrN_2O$; C, 58.74; H, 3.39; N, 8.56; Found: C, 58.82; H, 3.40; N, 8.57.

*2.3.11. 1-(2-Chlorophenyl)-3-phenyl-1H-pyrazole-4-carbaldehyde (**4j**)*

Yield: 83%; mp. 163–165 °C, IR (KBr) cm^{-1}: 3052 (Ar-CH), 2743 (C–H in CHO), 1716 (C═O), 1564 (C═N), 1492 (C═C), 794 (C–Cl); ^1H NMR (300 MHz, $CDCl_3$, δ ppm): 8.96 (s, 1H, –CHO), 7.97 (s, 1H, Pyrazole-CH), 6.52–7.72 (m, 9H, Ar-H); MS (EI) *m/z*: [M + 2] 284; (Calcd for $C_{16}H_{11}ClN_2O$; 282.72). Anal. Calcd for $C_{16}H_{11}ClN_2O$; C, 67.97; H, 3.92; N, 9.91; Found: C, 68.02; H, 3.93; N, 9.93.

*2.3.12. 1-(4-Chlorophenyl)-3-phenyl-1H-pyrazole-4-carbaldehyde (**4k**)*

Yield: 84%; mp. 180–182 °C, IR (KBr) cm^{-1}: 3061 (Ar-CH), 2778 (C–H in CHO), 1712 (C═O), 1523 (C═N), 1477 (C═C), 791 (C–Cl); ^1H NMR (300 MHz, $CDCl_3$, δ ppm): 8.91 (s, 1H, –CHO), 7.89 (s, 1H, Pyrazole-CH), 6.12–7.42 (m, 9H, Ar-H); MS (EI) *m/z*: [M + 2] 284; (Calcd for $C_{16}H_{11}ClN_2O$; 282.72). Anal. Calcd for $C_{16}H_{11}ClN_2O$; C, 67.97; H, 3.92; N, 9.91; Found: C, 68.21; H, 3.93; N, 9.92.

*2.3.13. 1-(4-Acetamidophenyl)-3-phenyl-1H-pyrazole-4-carbaldehyde (**4l**)*

Yield: 82%; mp. 182–184 °C, IR (KBr) cm^{-1}: 3329 (N–H stretching), 3064 (Ar-CH), 2792 (C–H in CHO), 1717 (C═O), 1552 (C═N), 1471 (C═C), 1326 (N–H bending); ^1H NMR (300 MHz, $CDCl_3$, δ ppm): 8.82 (s, 1H, –CHO), 7.91 (s, 1H, N–H), 7.44 (s, 1H, Pyrazole-CH), 6.17–7.10 (m, 9H, Ar-H), 2.78 (s, 3H, $–CH_3$); MS (EI) *m/z*: $[M]^+$ 305; (Calcd for $C_{18}H_{15}N_3O_2$; 305.33). Anal. Calcd for $C_{18}H_{15}N_3O_2$; C, 70.81; H, 4.95; N, 13.76; Found: C, 70.94; H, 4.96; N, 13.78.

2.4. Pharmacology

The synthesized compounds were evaluated for anti-inflammatory, analgesic and ulcerogenic activities. One-way analysis of variance (ANOVA) was performed to ascertain the significance of all the exhibited activities. The test compounds and the standard drugs were administered in the form of a suspension (1% carboxy methyl cellulose as a vehicle) by oral route of administration for anti-inflammatory and analgesic but for ulcerogenicity studies by intraperitoneally as suspension in 10% v/v Tween-80. Each group consisted of six animals. The animals were maintained in colony cages at 25 ± 2 °C, relative humidity of 45–55%, under a 12 h light and dark cycle; were fed standard animal feed (Olfert et al., 1993). All the animals were acclimatized for a week before use.

2.4.1. Anti-inflammatory activity

Anti-inflammatory activity was evaluated by carrageenan-induced paw edema test in rats (Winter et al., 1962). Diclofenac sodium 10 and 20 mg/kg was administered as standard drug for comparison. The test compounds were administered at two dose levels (10 and 20 mg/kg). The paw volumes were measured using the mercury displacement technique with the help of plethysmograph immediately before and 30 min, 1, 2 and 3 h after carrageenan injection. The percent inhibition of paw edema was calculated according to the following formula:

$$\%\text{Inhibition } I = 100[1 - (a - x)/(b - y)],$$

where x is the mean paw volume of rats before the administration of carrageenan and test compounds or reference compounds (test group), a is the mean paw volume of rats after the administration of carrageenan in the test group (drug treated), b is the mean paw volume of rats after the administration of carrageenan in the control group, y is the mean paw volume of rats before the administration of carrageenan in the control group.

2.4.2. Analgesic activity

The analgesic activity was performed by tail-flick technique using Wistar albino mice (25–35 g) of either sex selected by random sampling technique (Kulkarni, 1980; Amour and Smith, 1941). Diclofenac sodium at a dose level of 10 and 20 mg/kg was administered orally as the reference drug for comparison. The test compounds at two dose levels (10 and 20 mg/kg) were administered orally. The reaction times were recorded at 30 min, 1, 2 and 3 h after the treatment and the cut-off time was 10 s. The percent analgesic activity (PAA) was calculated by the following formula:

$$\text{PAA} = [T_2 - T_1/10 - T_1] \times 100,$$

where T_1 is the reaction time (s) before treatment and T_2 is the reaction time (s) after treatment.

2.4.3. Ulcerogenicity

Ulceration in rats was induced as reported by (Goyal et al., 1985). Albino rats of Wistar strain weighing 150–200 g of either sex were divided into various groups each of six animals. Control group of animals were administered only with 10% v/v Tween-80 suspension intraperitoneally. One group was administered with Aspirin intraperitoneally at a dose of 200 mg/kg once daily for three days. Diclofenac was also administered as the standard drug at 20 mg/kg once daily for 3 days to another group of animals in the same route. The remaining group of animals were administered with test compounds intraperitoneally at a dose of 20 mg/kg. On the fourth day, pylorus was ligated as per the method of (Shay et al., 1945). Animals were fasted for 36 h before the pylorus ligation procedure. Four hours after the ligation, animals were sacrificed. The stomach was removed and opened along the greater curvature. Ulcer index was determined by the method of Ganguly and Bhatnagar (1973).

3. Results and discussion

3.1. Chemistry

The series of heterocycles, 1-(4-substitutedphenyl)-3-phenyl-1*H*-pyrazole-4-carbaldehyde **4a–l** was synthesized by the reaction of acetophenone with substituted phenyl hydrazine in the presence of Vilsmeier-Haack reagent. The IR, ^1H NMR, mass spectroscopy and elemental analysis for the new compound is in accordance with the assigned structures. The IR spectra of the prepared 1-phenyl-2-(1-phenylethylidene)hydrazine **3** and pyrazole-4-carbaldehyde **4a–l** revealed the disappearance of respective N–H stretching and bending absorption (3342 and 1331 cm^{-1}) and the appearance of the characteristic carbonyl (C═O) band ranging from 1697 to 1722 cm^{-1}. It is noteworthy to focus on the change in the carbonyl group of pyrazole-4-carbaldehyde **4a–l**. The ^1H NMR spectra of the prepared 1-phenyl-2-(1-phenylethylidene)hydrazine **3** showed N–H signal in the range of δ 7.98 (s) and pyrazole-4-carbaldehyde (–CHO) proton of synthesized compounds appeared as a singlet in the range of δ 8.12–8.96. The disappearance of the respective N–H signal and appearance of the –CHO singlet confirm the presence of a pyrazole moiety. All these observed facts clearly demonstrate that the intermediate **3** is converted into 1-(4-substitutedphenyl)-3-phenyl-1*H*-pyrazole-4-carbaldehyde **4a–l**.

3.2. Pharmacology

Twelve derivatives were synthesized and their structures were confirmed by IR, NMR, mass spectroscopy and elemental analysis. All the 12 derivatives were tested for their anti-inflammatory activity by carrageenan-induced paw edema test and analgesic activity by tail-flick test. The results of anti-inflammatory and analgesic testing indicate that the test compounds exhibited moderate activity at 30 min of reaction time and an increase in activity at 1 h, which reached a peak level at 2 h. Decline in activity, was observed at 3 h. The results of the anti-inflammatory and analgesic evaluation of the tested compounds are presented in Table 1, Figs. 2 and 3.

Table 1 Anti-inflammatory, analgesic activities and ulcer index of the synthesized compounds (**4a–l**).

Compounds	Dose (mg/kg)	Percentage analgesic activity[a]				Percentage protection in paw edema[a]				Ulcer index[a]
		30 min	1 h	2 h	3 h	30 min	1 h	2 h	3 h	
4a	10	22 ± 1.36*	26 ± 1.54*	29 ± 1.02**	25 ± 1.43*	15 ± 1.56*	23 ± 1.07*	30 ± 1.51*	17 ± 1.50*	0.62 ± 0.45*
	20	29 ± 1.41*	46 ± 1.90**	42 ± 1.24**	29 ± 1.47*	37 ± 1.51**	42 ± 1.91**	50 ± 1.57**	34 ± 1.27*	
4b	10	15 ± 1.70NS	19 ± 1.27*	24 ± 1.90*	18 ± 1.44*	12 ± 1.23*	14 ± 1.93*	21 ± 1.37*	11 ± 1.90*	0.78 ± 0.34
	20	30 ± 1.01**	37 ± 1.57**	45 ± 1.01**	20 ± 1.32*	30 ± 1.26*	34 ± 1.71**	38 ± 1.87*	26 ± 1.50*	
4c	10	24 ± 1.37**	29 ± 1.70***	33 ± 1.60***	30 ± 1.07*	19 ± 1.67**	24 ± 1.69**	37 ± 1.34***	20 ± 1.15*	0.51 ± 0.29*
	20	42 ± 1.61***	52 ± 1.43***	56 ± 1.86***	35 ± 1.43*	40 ± 1.87**	48 ± 1.47***	55 ± 1.87***	38 ± 1.60*	
4d	10	12 ± 1.51*	17 ± 1.48*	20 ± 1.02*	15 ± 1.81*	09 ± 1.71*	11 ± 1.51*	18 ± 1.92*	11 ± 1.04*	0.73 ± 0.35*
	20	29 ± 1.91*	35 ± 1.50*	33 ± 1.00**	18 ± 1.44*	28 ± 1.91*	30 ± 1.50**	35 ± 1.16**	25 ± 1.92*	
4e	10	25 ± 1.72**	28 ± 1.72***	31 ± 1.57***	27 ± 1.45*	18 ± 1.43**	24 ± 1.58*	35 ± 1.91**	19 ± 1.16*	0.56 ± 0.36*
	20	40 ± 1.36**	49 ± 1.53***	52 ± 1.82***	33 ± 1.36*	39 ± 1.26***	45 ± 1.62***	53 ± 1.51***	38 ± 1.26*	
4f	10	20 ± 1.12**	25 ± 1.27***	18 ± 1.43***	23 ± 1.36*	14 ± 1.24**	20 ± 1.15*	25 ± 1.42**	14 ± 1.29*	0.76 ± 0.65*
	20	35 ± 1.50***	41 ± 1.77*	40 ± 1.01***	25 ± 1.46*	34 ± 1.91*	38 ± 1.27**	42 ± 1.90**	31 ± 1.23*	
4g	10	29 ± 1.37**	36 ± 1.70***	40 ± 1.60***	35 ± 1.07*	24 ± 1.42**	30 ± 1.91***	46 ± 1.01**	25 ± 1.41*	0.40 ± 0.43*
	20	49 ± 1.61***	59 ± 1.43***	68 ± 1.83***	43 ± 1.43*	47 ± 1.82***	56 ± 1.76***	65 ± 1.47***	44 ± 1.15*	
4h	10	20 ± 1.72**	23 ± 1.72*	27 ± 1.57***	21 ± 1.45*	13 ± 1.67**	17 ± 1.69**	28 ± 1.34***	12 ± 1.15*	0.71 ± 0.21*
	20	33 ± 1.36**	40 ± 1.53*	39 ± 1.82***	24 ± 1.36*	33 ± 1.87**	35 ± 1.47***	40 ± 1.87***	31 ± 1.60*	
4i	10	27 ± 1.70NS	33 ± 1.27*	38 ± 1.90*	34 ± 1.44*	23 ± 1.42**	27 ± 1.91***	40 ± 1.01**	23 ± 1.41*	0.44 ± 0.26*
	20	46 ± 1.01**	57 ± 1.57**	62 ± 1.01**	40 ± 1.32*	46 ± 1.57*	52 ± 1.01**	61 ± 1.54***	43 ± 1.92*	
4j	10	17 ± 1.37**	22 ± 1.70*	22 ± 1.60*	20 ± 1.07*	13 ± 1.47*	15 ± 1.05*	24 ± 1.33*	11 ± 1.91*	0.81 ± 0.35*
	20	34 ± 1.61***	38 ± 1.43*	36 ± 1.61NS	22 ± 1.43*	31 ± 1.91*	36 ± 1.27*	39 ± 1.90*	28 ± 1.23*	
4k	10	26 ± 1.37**	31 ± 1.70***	35 ± 1.60***	32 ± 1.07*	21 ± 1.47*	26 ± 1.05*	39 ± 1.33*	22 ± 1.91*	0.47 ± 0.26*
	20	45 ± 1.61***	55 ± 1.43***	59 ± 1.61NS	38 ± 1.43*	44 ± 1.91***	51 ± 1.27***	58 ± 1.90***	41 ± 1.23*	
4l	10	23 ± 1.37**	27 ± 1.70***	32 ± 1.60***	28 ± 1.07*	16 ± 1.47*	25 ± 1.05*	34 ± 1.33*	17 ± 1.91*	0.49 ± 0.23*
	20	39 ± 1.61***	47 ± 1.43***	57 ± 1.61***	30 ± 1.43*	38 ± 1.91***	43 ± 1.27***	50 ± 1.90***	36 ± 1.23*	
Control	–	2 ± 0.33NS	6 ± 0.47NS	4 ± 0.57NS	4 ± 0.89NS	5 ± 0.27NS	6 ± 0.25 NS	5 ± 0.30 NS	3 ± 0.91 NS	0.18 ± 0.10*
Diclofenac	10	25 ± 1.67*	31 ± 1.40*	42 ± 0.90*	31 ± 0.96*	20 ± 0.61*	26 ± 1.56*	49 ± 1.95*	21 ± 0.91*	1.49 ± 0.78**
	20	44 ± 0.93*	53 ± 1.14**	71 ± 1.47***	37 ± 1.11*	43 ± 0.59**	50 ± 0.90***	78 ± 1.50***	40 ± 1.34**	
Aspirin	20	–	–	–	–	–	–	–	–	1.21 ± 0.59**

Each value represents the mean ± SEM ($n = 6$); NS: non-significant.

[a] Data expressed as mean ± SD.

* Significance levels $p < 0.05$ as compared with the respective control.

** Significance levels $p < 0.01$ as compared with the respective control.

*** Significance levels $p < 0.001$ as compared with the respective control.

3.2.1. Anti-inflammatory activity

Results revealed that all of the tested compounds showed a variant significant anti-inflammatory activity. It is obvious that most of the tested pyrazole-4-carbaldehydes **4c**, **4e**, **4g**, **4i**, **4k** and **4l** revealed relatively higher activities. The results also revealed that in some cases, the presence of the para position halogen moieties **4g**, **4i** and **4k** enhances the anti-inflammatory activity in comparison with their corresponding para position derivatives such as in compounds **4c**, **4e** and **4l**. It is obvious that this is a direct correlation between enhancement of the anti-inflammatory activity and the presence of para position halogen moieties. Data in Table 1 revealed that the compounds **4c**, **4e**, **4g**, **4i**, **4k** and **4l** showed higher anti-inflammatory activities ranging from 53% to 65% at a dose of 20 mg/kg and 35 to 46% at a dose of 10 mg/kg respectively. The most potent compound was 4 g with anti-inflammatory activity of 65% at a dose of 20 mg/kg and 46% at a dose of 10 mg/kg, whereas compounds with unsubstituted and ortho derivatives such as **4a**, **4b**, **4d**, **4f**, **4h** and **4j** exhibited lower anti-inflammatory activities ranging from 35% to 50% at a dose of 20 mg/kg and from 18% to 30% at a dose of 10 mg/kg, respectively.

3.2.2. Analgesic activity

The results showed that most of the tested compounds with para substitutions have a significant analgesic effect. Compounds **4g**, **4i** and **4k** containing –F, –Br and –Cl substitutions at the para position showed significant activity while, compounds **4c**, **4e** and **4l** containing $–CH_3$, $–OCH_3$, and $–NHCOCH_3$ substitutions at the para position exhibited little lesser significant activity. Moreover, compound 4 g showed a highest analgesic activity of 68% at a dose of 20 mg/kg and 40% at a dose of 10 mg/kg. Results revealed that the compounds **4c**, **4e**, **4g**, **4i**, **4k** and **4l** showed higher activities ranging from 52% to 68% at a dose of 20 mg/kg and 31% to 40% at a dose of 10 mg/kg, respectively, whereas compounds with unsubstituted and ortho derivatives such as **4a**, **4b**, **4d**, **4f**, **4h** and **4j** exhibited lowest analgesic activities ranging from 33% to 45% at a dose of 20 mg/kg and from 18% to 29% at a dose of 10 mg/kg, respectively.

On correlating the structures of the synthesized compounds with their anti-inflammatory activity, it has been observed that, out of the various phenyl substituted derivatives (**4a–l**), six compounds (**4g** > **4i** > **4k** > **4l** > **4c** > **4e**) exhibited significant anti-inflammatory and analgesic activities. All the above mentioned six compounds were *p*-substituted.

3.2.3. Ulcer index

The ulcer index of the test compounds revealed that the compounds with para substituents (**4g**, **4i**, **4k**, **4l**, **4c** and **4e**) showed a negligible ulcer index, whereas those with unsubstituted compound (**4a**) (ulcer index 0.62 ± 0.45) exhibited little increase in ulcer index and the ortho substituted derivatives (**4b**, **4d**, **4f**, **4h** and **4j**) exhibited higher ulcer index over other test compounds. When compared with the reference standard Diclofenac sodium (1.49 ± 0.78) and Aspirin (1.21 ± 0.59) ulcer index the test compounds exhibited only 0.40 ± 0.43% to 0.81 ± 0.35% of the ulcer index. Among the title compounds, 1-(2-hydroxyphenyl)-3-diphenyl-1*H*-pyrazole-4-carbaldehyde (**4j**) showed the highest (ulcer index 0.81 ± 0.65). The results of the tested compounds are given in Table 1.

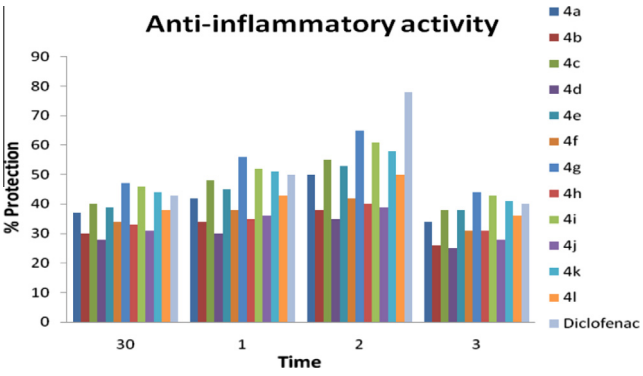


Figure 2 Anti-inflammatory activity of the synthesized compounds (**4a–l**).

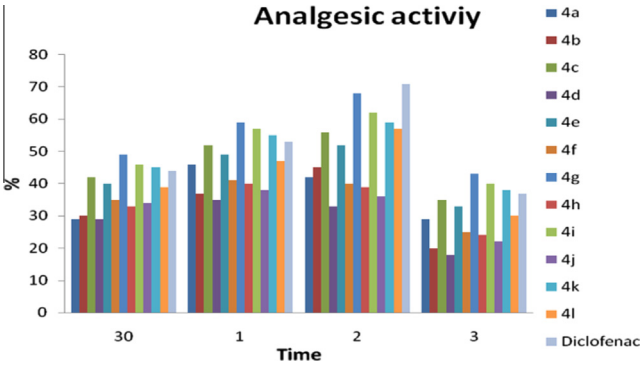


Figure 3 Analgesic activity of the synthesized compounds (**4a–l**).

3.3. Structure–activity relationship studies

Structure–activity relationship studies revealed that different substitutions on the pyrazole nucleus exerted remarkable biological activity. The electronic nature of the substituent groups at para positions in the phenyl ring played a significant role in anti-inflammatory and analgesic activities. The observed data showed that the para substituted derivatives exhibited better activity than other ortho and unsubstituted derivatives. Among the different substitutions at para position halogen analogs showed better activity in order (**4g** > **4i** > **4k**), respectively, than methyl, hydroxy and nitro substituted compounds. The studies revealed that the position of the substituted group on the phenyl ring appeared to greatly influence the pharmacological activity.

4. Conclusion

In summary, a new series of pyrazole derivatives have been prepared and fully assigned by analytical and spectral data. The results of the analgesic and anti-inflammatory activities of the synthesized compounds showed moderate enhancement of the activity. The compounds **4g**, **4i** and **4k** exhibited good anti-inflammatory and analgesic activities. Interestingly this compound showed one-third of the ulcer index of the reference i.e. Diclofenac and Aspirin. Among all the synthesized compounds, *p*-substituted derivatives showed excellent activities. Hence this analog could be developed as a new class of anti-inflammatory and analgesic agents. However, further struc-

tural modification is planned to enhance the anti-inflammatory and analgesic activities with the decreased ulcerogenic index.

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References

Amour, R.E., Smith, D.L.J., 1941. Pharmacol. Exp. Ther. 72, 74.
Bailey, D.M., Hansen, P.E., Hlavac, A.G., Baizman, E.R., Pearl, J., Defelice, A.F., Feigenson, M.E., 1985. J. Med. Chem. 28, 256.
Balsamo, A., Coletta, I., Guglielmotti, A., Landolfi, C., Mancini, F., Martinelli, A., Milanese, C., Minutolo, F., Nencetti, S., Orlandini, E., Pinza, M., Rapposelli, S., Rossello, A., 2003. Eur. J. Med. Chem. 38, 157.
Bekhit, A.A., Ashour, A.M.H., Abdel Ghany, S.Y., Bekhit, A.A.E.D., Baraka, A., 2008. Eur. J. Med. Chem. 43, 456.
Farghaly, M.A., Soliman, G.S.F., El-Semary, M.M., Rostom, F.A.S.H., 2001. Pharmazie 56, 28.
Ganguly, A.K., Bhatnagar, O.P., 1973. Can. J. Physiol. Pharmacol. 51, 148.
Godoy, M.C.M., Fighera, M.R., Souza, F.R., Flores, A.E., Rubin, M.A., Oliveira, M.R., Zanatta, N., 2004. Eur. J. Pharmacol. 496, 93.
Goyal, R.K., Chakrabarti, A., Sanyal, A.K., 1985. Planta Med. 29, 85.
Gureje, O., Von Knoff, M., Simon, G.E., Gater, R., 1998. J. Am. Med. Assoc. 280, 147.
Kulkarni, S.K., 1980. Life Sci. 27, 185.
Kurumbail, G.R., Stevens, M.A., Gierse, K.J., McDonald, J.J., Stegeman, A.R., Pak, Y.J., Gildehaus, D., Miyashiro, M.J., Penning, D.T., Seibert, K., Isakson, C.P., Stallings, C.W., 1996. Nature 384, 644.
Michon, V., Du Penhoat, C.H., Tombret, F., Gillardin, J.M., Lepagez, F., Berthon, L., 1995. Eur. J. Med. Chem. 1, 47.
Olfert, E.D., Cross, B.M., McWilliam, A.A., 1993. Guide to the Care and Use of Experimental Animals, second ed., vol.1. Canadian Council of Animal Care, Ottawa.
Park, H.J., Lee, K., Park, S., Ahn, B., Lee, J.C., Cho, H.Y., Lee, K.I., 2005. Bioorg. Med.Chem. Lett. 15, 3307.
Pimerova, E.V., Voronina, E.V.J., 2001. Pharm. Chem. 35, 18.
Prokopp, C.R., Rubin, M.A., Sauzem, P.D., de Souza, A.H., Berlese, D.B., Lourega, R.V., 2006. Braz. J. Med. Biol. Res. 39, 795.
Shay, M., Komarov, S.A., Fels, D., Meranze, D., Grunstein, H., Siplet, H., 1945. Gastroenterology 5, 43.
Souza, F.R., Fighera, M.R., Lima, T.T.F., Bastiani, J., Barcellos, I.B., Almeida, C.E., 2001. Pharmacol. Biochem. Behav. 68, 525.
Souza, F.R., Ratzlaff, V.T., Borges, L.P., Oliveira, M.R., Bonacorso, H.G., Zanatta, N., 2002. Eur. J. Pharmacol. 451, 141.
Winter, C.A., Risley, E.A., Nuss, G.W., 1962. Exp. Biol. Med. 111, 544.
Yildirim, I., Özdemir, N., Akçamur, Y., Dinçer, M., Andaç, O., 2005. Acta Crystallogr. 61, 256.
Youssef, M.A., Saudi, S.N.M., Omar, G.M., Baraka, M.A., 2007. J. Pharm. Sci. 21, 103.