# Microwave assisted synthesis and anti-Inflammatory activity evaluation of Pyrazole derivatives

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**Abstract. Microwave promoted easy, efficient and environment friendly procedure has been devised for the synthesis of a series of pyrazole derivatives in high yields by the reaction of chalcones with phenyl hydrazine hydrochloride under catalyst free, solvent free conditions. The newly synthesized compounds were characterized by NMR ( $^{1}H$ and $^{13}C$), Mass, IR and C, H, N analyses. *In vitro* anti-inflammatory activity of the synthesized compounds was investigated against inhibition of Albumin Denaturation and Membrane Stabilization test methods. Majority of the compounds showed good activity when compared with the standard drugs.**

***Keywords — microwave irradiation, pyrazole derivatives, chalcones, anti-inflammatory activity***

## I. INTRODUCTION

Pyrazole refer to both to class of simple aromatic ring organic compound of heterocyclic diazole series characterized by a 5- member ring structure composed of three carbon atom and two nitrogen atom in the adjacent position and to unsaturated parent compound. These are important organic compounds for pharmaceutical [1] and agrochemical industry [2]. Numerous compounds containing pyrazole moiety are known to exhibit anti-hyperglycemic [3], analgesic [4], anti-inflammatory [5], antipyretic [6], antibacterial [7], antimicrobial [8], antihypertensive [9] and antidepressant [10] activities. They are also used as herbicides [11] and dyestuffs [12]. Pyrazole is a multipurpose lead compound developed by chemical architecture for effective molecules which are biologically active. Several synthetic routes are accorded to the development of pyrazole containing reactions to afford a novel molecule which is an enormous opportunity in the field of medicinal chemistry.[13a-l]

In recent years, microwave assisted organic syntheses have gained enormous attention of the chemists due to their advantages such as shorter reaction times, cleaner products, operational simplicity, higher yields and being a potential alternative to accomplish the effective synthesis of heterocyclic bioactive compounds. [14] Solvent-free reaction condition has been demonstrated to be an efficient technique for various organic reactions. It often leads to a remarkable decrease in reaction time, increased yields, easier workup, enhancement of regio and stereo selectivity of reaction matches with the green chemistry protocol.[15]

Paracetamol is a relatively COX-2 selective, non-steroidal anti-inflammatory drug (NSAID) with analgesic and antipyretic properties. But the continuous use of Paracetamol can cause diarrhea, vomiting, skin rash, dizziness and bitterness in the mouth. Hence there is a need for developing superior anti-inflammatory with better safety profile.

Motivated by the afore-mentioned literature and in continuation of our studies towards developing new methods for the synthesis of pyrazole derivatives having good anti-inflammatory activity[16], we envision our approach towards the design and synthesis of novel structurally diverse series of pyrazole derivatives for their anti-inflammatory activity.

## II. EXPERIMENTAL DETAILS

The reactions were carried out in a round bottom 50 mL flask fitted with reflux condenser, a dropping funnel and in Nitrogen atmosphere. A magnetic stirrer cum hot plate was used for stirring and heating the reaction mixtures. Rota evaporator was used for removing the solvent from the reaction mixture. All the solvents and reagents were dried and purified before use by adopting the standard procedures and techniques. Progress of the reaction and purity of the compounds were monitored by thin layer chromatography (TLC) on aluminium sheet of silica gel 60F254, E-Merck, Germany using iodine as visualizing agent. Melting points (mp) were determined in open capillary tubes using a calibrated thermometer by Guna Digital Melting Point apparatus, expressed in degrees centigrade (°C) and are

uncorrected. $^1$H NMR spectra was recorded as solutions in $CDCl_3$ on a Bruker AMX/ VARIAN 400 MHz spectrometer operating at 400 MHz. The $^1$H chemical shifts were expressed in parts per million (ppm) with reference to tetramethylsilane (TMS). The following abbreviations were used while presenting the NMR data: **s** = singlet, **d** = doublet, **t** = triplet, **q** = quartet and **m** = multiplet. All the chemicals used in the present work were obtained from Sd. Fine Chem. Ltd., Boisar, India and Qualigens, Mumbai and were used after purifying them by following the established procedures.

***Preparation of pyrazole derivatives (5a-j)***

In the first step, to a cold solution (below 10°C) of paracetamol (**1)** (0.01 mol), 4-methoxybenzaldehyde (**2b**) (0.01 mol) in ethanol and a chilled solution of NaOH (10 mL of 30% solution) were added. Then the reaction mixture was allowed to stir for 12h. It was diluted with water (100 mL) and acidified with Conc. HCl. The product obtained was filtered, washed with water and recrystallized from ethanol to get pure chalcone (**3a**).

In the second step, a mixture of the chalcone (**3a**) (0.01 mol) and phenyl hydrazine hydrochloride (**4**) (0.01 mol) were taken in flat-bottomed flask and irradiated with microwave radiations in a microwave oven at 490 Watts. The reaction mixture was heated consecutively two times for 2-3 min period each time followed by a 1 min cooling interval between irradiations. To avoid the continuous overheating of the reactants, this method was proposed. In order to maintain the homogeneity of the irradiating field throughout the reaction, the reaction mixture was kept under stirring. The progress of the reaction was monitored by TLC on silica gel using ethyl acetate-hexane (8:2 v/v). After complection of the reaction, the solvent was removed under reduced pressure to get the crude product. The resulting crude product was purified by column chromatography on silica gel (100-200 mesh) using ethyl acetate-hexane (1:1) as eluent to get pure 4-(5-(4-methoxyphenyl)-1-phenyl-4,5-dihydro-1H-pyrazol-3-ylamino)phenol (**5a**). The remaining compounds **5b-j** were prepared by adapting to the above described procedure. The synthetic protocol for the title compounds is presented in **Scheme 1**.

***Physical, analytical and spectral data for the compounds (5a-j)***

4-(5-(4-methoxyphenyl)-1-phenyl-4,5-dihydro-1H-pyrazol-3-ylamino)phenol (**5a**): Yield: 87%; solid. M.P. 80-81 °C; $^1$H NMR (400 MHz, $CDCl_3$**)**: $\delta$ 9.29 (s, 1H, -OH), 8.88 (s, 1H, Ar-NH), 7.46-6.74 (m, 9H, Ar-H), 3.89 (t, 1H, pyrazole-C-H), 3.48 (s, 3H, $OCH_3$), 2.14 (m, 1H, pyrazole-$CH_2$), 0.88 (m, 1H, pyrazole-$CH_2$); $^{13}$C NMR spectrum (100 MHz, $CDCl_3$): $\delta$ 157.4 (C-22), 146.5 (C-10), 143.6 (C-3), 142.5 (C-13), 134.8 (C-19), 131.5 (C-7), 129.8 (C-15, C-17), 125.2 (C-20, C-24), 121.2 (C-16), 116.7 (C-8, C-12), 115.7 (C-9, C-11), 114.6 (C-14, C-18), 114.0 (C-21, C-23), 54.5 (C-25), 50.3 (C-5), 38.7 (C-4); IR (KBr) ($\nu_{max}$ cm$^{-1}$): 3336 (OH), 3217 (NH), 1585 (C=N); LCMS (m/z, %): 360 (M+H$^+$, 100); Anal. Calcd for $C_{22}H_{21}N_3O_2$: C, 73.52; H, 5.89; N, 11.69; %; found: C, 73.60; H, 5.82; N, 11.76%.

(E)-4-(1-phenyl-5-styryl-4,5-dihydro-1H-pyrazol-3-ylamino)phenol (**5b**): Yield: 91%; solid. M.P. 142-146 °C; $^1$H NMR (400 MHz, $CDCl_3$**)**: $\delta$ 9.29 (s, 1H, -OH), 8.88 (s, 1H, Ar-NH), 7.57-6.70 (m, 14H, Ar-H), 6.25 (d, 1H, Ar-**CH**=CH), 6.12 (d, 1H, Py-**CH**=CH), 3.83 (t, 1H, pyrazole-C-H), 2.14 (m, 1H, pyrazole-$CH_2$), 0.88 (m, 1H, pyrazole-$CH_2$); $^{13}$C NMR spectrum (100 MHz, $CDCl_3$): $\delta$ 148.5 (C-10), 145.6 (C-3), 143.8 (C-13), 136.4 (C-21), 134.4 (C-20), 132.7 (C-7), 129.5 (C-15, C-17), 128.8 (C-19), 128.6 (C-23, C-25), 128.5 (C-22, C-26), 127.9 (C-24), 120.8 (C-16), 117.7 (C-8, C-12), 116.7 (C-9, C-11), 114.3 (C-14, C-18), 50.8 (C-5), 37.3 (C-4); IR (KBr) ($\nu_{max}$ cm$^{-1}$): 3339 (OH), 3227 (NH), 1593 (C=N); LCMS (m/z, %): 356 (M+H$^+$, 100); Anal. Calcd for $C_{23}H_{21}N_3O$: C, 77.72; H, 5.96; N, 11.82%; found: C, 77.78; H, 5.85; N, 11.89%.

4-(1,5-diphenyl-4,5-dihydro-1H-pyrazol-3-ylamino)phenol (**5c**): Yield: 93%; solid. M.P.270-272 °C; $^1$H NMR (400 MHz, $CDCl_3$**)**: $\delta$ 9.29 (s, 1H, -OH), 8.88 (s, 1H, Ar-NH), 7.53-6.68 (m, 14H, Ar-H), 3.89 (t, 1H, pyrazole-C-H), 2.14 (m, 1H, pyrazole-$CH_2$), 0.88 (m, 1H, pyrazole-$CH_2$); $^{13}$C NMR spectrum (100 MHz, $CDCl_3$): $\delta$ 147.5 (C-10), 143.6 (C-3), 142.8 (C-13), 135.4 (C-19), 131.7 (C-7), 129.8 (C-15, C-17), 127.6 (C-21, C-23), 127.5 (C-20, C-24), 126.2 (C-22), 121.5 (C-16), 117.0 (C-8, C-12), 116.2 (C-9, C-11), 114.3 (C-14, C-18), 54.8 (C-25), 50.7 (C-5), 38.6 (C-4); IR (KBr) ($\nu_{max}$ cm$^{-1}$): 3344 (OH), 3216 (NH), 1592 (C=N); LCMS (m/z, %): 330 (M+H$^+$, 100); Anal. Calcd for $C_{21}H_{19}N_3O$: C, 76.57; H, 5.81; N, 12.76%; found: C, 76.64; H, 5.78; N, 12.83%.

4-(5-(4-nitrophenyl)-1-phenyl-4,5-dihydro-1H-pyrazol-3-ylamino)phenol (**5d**): Yield: 85%; solid. M.P.207-208 °C; $^1$H NMR (400 MHz, $CDCl_3$**)**: $\delta$ 9.29 (s, 1H, -OH), 8.88 (s, 1H, Ar-NH), 8.29-6.70 (m, 13H, Ar-H), 3.89 (t, 1H, pyrazole-C-H), 2.14 (m, 1H, pyrazole-$CH_2$), 0.88 (m, 1H, pyrazole-$CH_2$); $^{13}$C NMR spectrum (100 MHz, $CDCl_3$): $\delta$ 150.6 (C-19), 147.5 (C-10), 144.6 (C-22), 143.5 (C-3), 142.9 (C-13), 131.8 (C-7), 128.5 (C-15, C-17), 122.8 (C-21, C-23), 121.2 (C-20, C-24), 120.6 (C-16), 117.9 (C-8, C-12), 116.0 (C-9, C-11), 114.1 (C-14, C-18), 50.5 (C-5), 38.6 (C-4); IR (KBr) ($\nu_{max}$ cm$^{-1}$): 3351 (OH), 3220 (NH), 1593 (C=N); LCMS (m/z, %): 375 (M+H$^+$, 100); Anal. Calcd for $C_{21}H_{18}N_4O_3$: C, 67.37; H, 4.85; N, 14.96%; found: C, 67.44; H, 4.92; N, 14.90%.

4-(5-(4-chlorophenyl)-1-phenyl-4,5-dihydro-1H-pyrazol-3-ylamino)phenol (**5e**): Yield: 90%; solid. M.P.179-180 °C; $^1$H NMR (400 MHz, $CDCl_3$**)**: $\delta$ 9.29 (s, 1H, -OH), 8.88 (s, 1H, Ar-NH), 7.56-6.65 (m, 13H, Ar-H), 3.89 (t, 1H, pyrazole-C-H), 2.14 (m, 1H, pyrazole-$CH_2$), 0.88 (m, 1H, pyrazole-$CH_2$); $^{13}$C NMR spectrum (100 MHz, $CDCl_3$): $\delta$ 147.5 (C-10), 143.6 (C-3), 142.6 (C-13), 140.2 (C-19), 133.7 (C-7), 131.6 (C-22), 129.8 (C-15, C-17), 127.3 (C-21, C-23), 126.8 (C-20, C-24), 121.9 (C-16), 117.2 (C-8, C-12),

116.1 (C-9, C-11), 114.0 (C-14, C-18), 50.2 (C-5), 38.6 (C-4); IR (KBr) ($\nu_{max}$ $cm^{-1}$): 3349 (OH), 3225 (NH), 1596 (C=N); LCMS (m/z, %): 364 ($M+H^+$, 100); Anal. Calcd for $C_{21}H_{18}ClN_3O$: C, 69.32; H, 4.99; N, 11.55%; found: C, 69.42; H, 4.93; N, 11.60%.

4-(5-(4-fluorophenyl)-1-phenyl-4,5-dihydro-1H-pyrazol-3-ylamino)phenol (**5f**): Yield: 94%; solid. M.P.215-217 °C; $^1H$ NMR (400 MHz, $CDCl_3$): $\delta$ 9.29 (s, 1H, -OH), 8.88 (s, 1H, Ar-NH), 7.30-6.56 (m, 13H, Ar-H), 3.89 (t, 1H, pyrazole-C-H), 2.14 (m, 1H, pyrazole-$CH_2$), 0.88 (m, 1H, pyrazole-$CH_2$); $^{13}C$ NMR spectrum (100 MHz, $CDCl_3$): $\delta$ 167.6 (C-22), 147.4 (C-10), 144.6 (C-3), 142.7 (C-13), 137.1 (C-19), 133.5 (C-7), 129.9 (C-15, C-17), 128.0 (C-20, C-24), 121.2 (C-16), 117.5 (C-8, C-12), 116.2 (C-9, C-11), 115.6 (C-21, C-23), 114.1 (C-14, C-18), 50.3 (C-5), 37.8 (C-4); IR (KBr) ($\nu_{max}$ $cm^{-1}$): 3363 (OH), 3234 (NH), 1597 (C=N); LCMS (m/z, %): 348 ($M+H^+$, 100); Anal. Calcd for $C_{21}H_{18}FN_3O$: C, 72.61; H, 5.22; N, 12.10%; found: C, 72.68; H, 5.15; N, 12.16%.

4-(5-(3,5-dichlorophenyl)-1-phenyl-4,5-dihydro-1H-pyrazol-3-ylamino)phenol (**5g**): Yield: 83%; solid. M.P.167-168 °C; $^1H$ NMR (400 MHz, $CDCl_3$): $\delta$ 9.29 (s, 1H, -OH), 8.88 (s, 1H, Ar-NH), 7.60-6.57 (m, 12H, Ar-H), 3.89 (t, 1H, pyrazole-C-H), 2.14 (m, 1H, pyrazole-$CH_2$), 0.88 (m, 1H, pyrazole-$CH_2$); $^{13}C$ NMR spectrum (100 MHz, $CDCl_3$): $\delta$ 147.6 (C-10), 145.4 (C-19), 143.6 (C-3), 142.8 (C-13), 136.3 (C-21, C-23), 131.8 (C-7), 129.9 (C-15, C-17), 127.8 (C-22), 125.9 (C-20, C-24), 121.2 (C-16), 117.2 (C-8, C-12), 116.1 (C-9, C-11), 114.3 (C-14, C-18), 49.2 (C-5), 38.6 (C-4); IR (KBr) ($\nu_{max}$ $cm^{-1}$): 3350 (OH), 3222 (NH), 1590 (C=N); LCMS (m/z, %): 398 ($M+H^+$, 100); Anal. Calcd for $C_{21}H_{17}Cl_2N_3O$: C, 63.33; H, 4.30; N, 10.55%; found: C, 63.38; H, 4.25; N, 10.61%.

4-(5-(2-nitrophenyl)-1-phenyl-4,5-dihydro-1H-pyrazol-3-ylamino)phenol (**5h**): Yield: 92%; solid. M.P.183-184 °C; $^1H$ NMR (400 MHz, $CDCl_3$): $\delta$ 9.29 (s, 1H, -OH), 8.88 (s, 1H, Ar-NH), 8.17-6.72 (m, 13H, Ar-H), 3.89 (t, 1H, pyrazole-C-H), 2.14 (m, 1H, pyrazole-$CH_2$), 0.88 (m, 1H, pyrazole-$CH_2$); $^{13}C$ NMR spectrum (100 MHz, $CDCl_3$): $\delta$ 166.4 (C-22), 147.5 (C-10), 144.2 (C-24), 143.5 (C-3), 142.9 (C-13), 136.5 (C-19), 134.1 (C-21), 133.7 (C-7), 129.9 (C-15, C-17), 125.7 (C-20), 122.5 (C-23), 120.9 (C-16), 117.2 (C-8, C-12), 116.3 (C-9, C-11), 114.0 (C-14, C-18), 45.2 (C-5), 37.5 (C-4); IR (KBr) ($\nu_{max}$ $cm^{-1}$): 3342 (OH), 3219 (NH), 1590 (C=N); LCMS (m/z, %): 375 ($M+H^+$, 100); Anal. Calcd for $C_{21}H_{18}N_4O_3$: C, 67.37; H, 4.85; N, 14.96%; found: C, 67.42; H, 4.90; N, 14.90%.

4-(1-phenyl-5-(4-(trifluoromethyl)phenyl)-4,5-dihydro-1H-pyrazol-3-ylamino)phenol (**5i**): Yield: 86%; solid. M.P.177-179 °C; $^1H$ NMR (400 MHz, $CDCl_3$): $\delta$ 9.29 (s, 1H, -OH), 8.88 (s, 1H, Ar-NH), 7.65-6.68 (m, 13H, Ar-H), 3.89 (t, 1H, pyrazole-C-H), 2.14 (m, 1H, pyrazole-$CH_2$), 0.88 (m, 1H, pyrazole-$CH_2$); $^{13}C$ NMR spectrum (100 MHz, $CDCl_3$): $\delta$ 147.7 (C-10), 144.5 (C-19), 143.6 (C-3), 142.4 (C-13), 133.7 (C-7), 129.9 (C-15, C-17), 129.0 (C-22), 124.9 (C-25), 123.6 (C-20, C-24), 122.8 (C-21, C-23), 120.8 (C-16), 117.3 (C-8, C-12), 116.4 (C-9, C-11), 114.0 (C-14, C-18), 50.5 (C-5), 38.6 (C-4); IR (KBr) ($\nu_{max}$ $cm^{-1}$): 3345 (OH), 3224 (NH), 1587 (C=N); LCMS (m/z, %): 398 ($M+H^+$, 100); Anal. Calcd for $C_{22}H_{18}F_3N_3O$: C, 66.49; H, 4.57; N, 10.57; found: C, 66.55; H, 4.51; N, 10.62%.

4-(1-phenyl-5-(pyridin-4-yl)-4,5-dihydro-1H-pyrazol-3-ylamino)phenol (**5j**): Yield: 91%; solid. M.P.156-157 °C; $^1H$ NMR (400 MHz, $CDCl_3$): $\delta$ 9.25 (s, 1H, -OH), 8.86 (s, 1H, Ar-NH), 8.35-6.88 (m, 13H, Ar-H), 3.89 (t, 1H, pyrazole-C-H), 2.14 (m, 1H, pyrazole-$CH_2$), 0.88 (m, 1H, pyrazole-$CH_2$); $^{13}C$ NMR spectrum (100 MHz, $CDCl_3$): $\delta$ 148.2 (C-19), 147.5 (C-10), 147.0 (C-21, C-23), 143.4 (C-3), 140.7 (C-13), 132.6 (C-7), 129.9 (C-15, C-17), 123.5 (C-20, C-24), 120.4 (C-16), 117.2 (C-8, C-12), 116.1 (C-9, C-11), 114.0 (C-14, C-18), 51.0 (C-5), 38.2 (C-4); IR (KBr) ($\nu_{max}$ $cm^{-1}$): 3325 (OH), 3223 (NH), 1595 (C=N); LCMS (m/z, %): 331 ($M+H^+$, 100); Anal. Calcd for $C_{20}H_{18}N_4O$: C, 72.71; H, 5.49; N, 16.96; found: C, 72.75; H, 5.43; N, 16.99%.

### *In vitro* Anti-inflammatory activity

*In vitro* anti-inflammatory activity of the synthesized compounds **5a-j** was investigated against inhibition of Albumin Denaturation [17] and Membrane Stabilization test methods [18].

### (a) Inhibition of Albumin Denaturation

All the synthesized compounds **5a-j** were evaluated for their anti-inflammatory activity with respect to inhibition of Albumin Denaturation method. The reaction mixture consisting of test compounds at different concentrations and 1% aqueous solution of bovine albumin fraction. $p^H$ of the reaction mixture was adjusted using small amount of 1N HCl to 7. The samples were incubated at 37° C for 20 min and then heated at 57° C for 20 min. After cooling the samples, the turbidity was measured spectrophotometrically at 660 nm. The experiment was performed in triplicate. Percent inhibition of protein denaturation was calculated as follows.

Percentage inhibition=($Abs_{control}$–$Abs_{sample}$)X 100/ $Abs_{control}$

Denaturation of protein is a well documented cause of inflammation. Phenyl butazone and flufenamic acids (anti-inflammatory drugs) have shown a dose dependent ability to thermally induced protein denaturation.

### (b) Membrane Stabilization test

#### *Preparation of red Blood cells (RBCs) Suspension*

Fresh human blood (10 mL) was collected and transferred to the heparinized centrifuged tubes. The tubes were centrifuged at 3000 rpm for 10 min and were washed three times with equal volume of normal saline. The volume of the blood was measured and reconstituted as10% v/v suspension with saline.

#### *Heat Induced Hemolysis*

The reaction mixture (2 mL) consisting of 1mL of test drug solution and 1 mL of 10% RBCs suspension, instead of the drug only, saline was added to the control test tube [16]. Salicylic acid was taken as a standard drug. All the centrifuge tubes containing reaction mixture were incubated in a water bath at 56° C for 30 min. At the end of the incubation, the tubes were cooled under running tap water. The reaction mixture was centrifuged at 2500 rpm for 5 min and the absorbance of the supernatants was taken at 560 nm. The experiment was performed in triplicates. Per cent membrane stabilization activity was calculated by the formula.

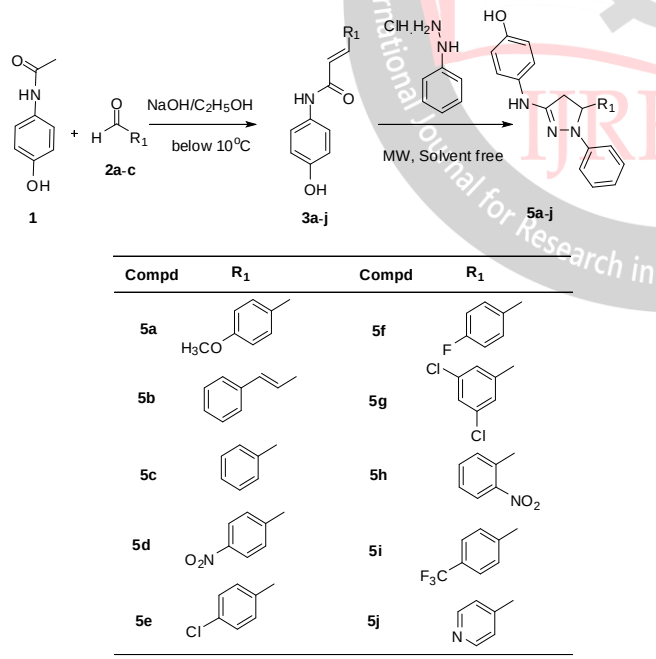
Percentage inhibition= ($Abs_{control}$–$Abs_{sample}$) X 100/ $Abs_{control}$

***Statistical Analysis***

The anti-inflammatory activity were screened by Carregeenan rat paw Oedema, Cotton pellet (*in vivo*) and Albumin Denaturation , Membrane Stabilization (*in vitro*) was compared *versus* negative control group by one way Analysis of Variance (ANOVA) with Dunnets post-test a value of $p< 0.05$ was considered significant.

## III. RESULTS AND DISCUSSION

In this letter, we report an efficient and environmentally benign protocol for the synthesis of pyrazole derivatives (**5a-j**) by the reaction of chalcones (**3a-j**) (0.01 mol) with phenyl hydrazine hydrochloride (**4**) (0.01 mol) under solvent-free conditions at room temperature using microwave radiation at 490W (**Scheme 1**).




**Scheme 1. Synthesis of biologically active pyrazole derivatives**

The chemical structure of the title compounds **5a-j** were characterized by spectral data ($^1$H and $^{13}$C NMR, IR and LC-MS), elemental analysis and the results were presented in experimental section. The proton signals at 8.88-8.86 and 9.29-9.25 are due to Ar-NH and OH protons for the compound (**5a-j**). The $^1$H NMR spectra of the compounds (**5a-j**) gave signals due to Ar-H in the range of 8.29-6.56 ppm. The proton signals at 3.89 is due to C-H proton of pyrazole ring for the compounds (**5a-j**). The methylene protons of pyrazole ring gave two multiplets at $\delta$ 2.14 and $\delta$ 0.88 respectively for the compounds (**5a-j**). $^{13}$C NMR chemical shift for C-H and $CH_2$ carbon of pyrazole ring were observed at 38,6-37.3 and 51.0-45.2 for the compounds **5a-j.** IR absorptions in the regions 3363-3325, 3234-3216 and 1597-1585 $cm^{-1}$ were assigned to OH, NH and C=N stretching vibrations respectively for the compounds **5a-j**. In their mass spectra, $M^{+\cdot}$ ions were observed in the expected m/z values.

***In vitro anti inflammatory activity***

*In vitro* anti-inflammatory activity of the tested compounds (**5a-j**) were evaluated with Albumin denaturation method and Membrane test methods. Phenyl butazone, flufenamic acid and salicylic acid are used as standard reference drugs for the study. The experiment was performed in triplicate and the results were presented in **Table 1** and **Table 2**. The title compounds **(5a-j)** showed moderate to good anti-inflammatory activity ranging from 39-75 % (Albumin denaturation method) and 41-79% (Membrane test methods), where as flufenamic acid and starting compound exhibited 79% and 57% respectively in Albumin denaturation method and salicylic acid and starting compound exhibited 85% and 62% respectively in Membrane test method. Especially the compounds compounds **5d**, bearing with 4-nitrophenyl moiety; **5f**, incorporated with 4-fluorophenyl moiety and **5j**, bearing with pyridinyl group showed potent anti-inflammatory activity compared to other derivatives.

It was observed that the position of the substituent on terminal benzene ring of pyrazole moiety has profound effect on the activity. The para position on the terminal benzene ring is the favorable site for the higher potency. Evidently, the compound **5d** with $NO_2$ and **5f** with **F** at para position, exhibiting highest inhibitory activity in the two methods. The presence of pyridine as substituent also exhibits high inhibitory activity in the two methods when compared with standard drugs. The remaining compounds were found to have moderate to good activity.

**Table 1: *In vitro* Anti-inflammatory activity of the title compounds (5a-j) by Inhibition of Albumin Denaturation method**

| Compounds | Dose (mg/mL) | Mean OD | SD | %Inhibition |
|---|---|---|---|---|
| **NC** | RO Water | 2.385 | 0.043 | - |
| **PC1** | 100 | 0.954[b] | 0.010 | 60.432 |
| **PC2** | 200 | 0.504[b] | 0.005 | 79.363 |
| **SC** | 0.2 | 1.045[b] | 0.022 | 57.676 |
| **5a** | 0.2 | 1.521[b] | 0.009 | 39.235 |
| **5b** | 0.2 | 1.236[b] | 0.005 | 43.242 |
| **5c** | 0.2 | 1.151[b] | 0.006 | 54.474 |

| | | | | |
|---|---|---|---|---|
| **5d** | 0.2 | 0.624 [b] | 0.025 | 75.328 |
| **5e** | 0.2 | 1.014 [b] | 0.008 | 62.743 |
| **5f** | 0.2 | 0.737 [b] | 0.010 | 74.137 |
| **5g** | 0.2 | 1.114 [b] | 0.009 | 56.948 |
| **5h** | 0.2 | 1.197 [b] | 0.019 | 53.754 |
| **5i** | 0.2 | 0.823 [b] | 0.007 | 68.203 |
| **5j** | 0.2 | 0.687 [b] | 0.015 | 74.272 |

NC = Phenyl Butazone; PC = Flufenamic acid; [b] $p<0.05$

**Table 2: *In vitro* Anti-inflammatory activity of the title compounds (5a-j) by Membrane Stabilization test method**

| Compounds | Dose (mg/mL) | Mean OD | SD | % Inhibition |
|---|---|---|---|---|
| **NC** | RO Water | 2.430 | 0.133 | - |
| **PC1** | 100 | 0.912 [b] | 0.025 | 62.713 |
| **PC2** | 200 | 0.373 [b] | 0.042 | 85.253 |
| **SC** | 0.2 | 0.962 [b] | 0.076 | 61.921 |
| **5a** | 0.2 | 1.395 [b] | 0.008 | 41.045 |
| **5b** | 0.2 | 1.203 [b] | 0.011 | 45.933 |
| **5c** | 0.2 | 1.333 [b] | 0.324 | 42.152 |
| **5d** | 0.2 | 0.730 [b] | 0.015 | 76.625 |
| **5e** | 0.2 | 0.706 [b] | 0.016 | 70.964 |
| **5f** | 0.2 | 0.656 [b] | 0.026 | 79.720 |
| **5g** | 0.2 | 1.153 [b] | 0.010 | 52.898 |
| **5h** | 0.2 | 1.295 [b] | 0.007 | 47.701 |
| **5i** | 0.2 | 0.814 [b] | 0.026 | 66.545 |
| **5j** | 0.2 | 1.687 [b] | 0.040 | 77.042 |

PC = Salicylic acid; [b] $p<0.05$

## IV. CONCLUSION

In conclusion, we demonstrated here an efficient, environmentally benign protocol for the formation of *pyrazole derivatives* in high yields through an intermediate chalcone under solvent free condition in the presence of microwave irradiation at room temperature. *In vitro* anti-inflammatory screening results of **5a-j** showed that most of the derivatives exhibits good inhibitory activity in the two methods. **5d**, bearing with 4-nitrophenyl moiety; **5f**, incorporated with 4-fluorophenyl moiety and **5j**, bearing with pyridinyl group showed potent anti-inflammatory activity compared to other derivatives.

## ACKNOWLEDGMENTS

The authors acknowledge department of biochemistry, Sri Venkateswara University, Tirupathi, India, for providing biological activity. Also, the authors express their thanks to Hyderabad Central University for providing instrumentation facilities for recording the spectra.

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