(ESI, m/z) (M+H)+: Calcd. For $C_{23}H_{19}FN_2O$: 267.3258; found: 268.1441

5-(4-fluoro-phenyl)-1-phenyl-3-p-tolyl-4,5-dihydro-1H-pyrazole (3d): Dark brown powder, mol. formula-$C_{22}H_{19}FN_2$, mol. w–330.40 ;IR cm^{-1} Sp^2 C-H stretch-3037.89, Sp^3 C-H stretch-2920.23, Ter C-N stretch-1219, C-NO_2 stretch-1315.17, C=C stretch-1598.99, C-Cl stretch-696.30; ^{1}HNMR7.4 (d, ^{1}H, J=8.6 Hz), 7.3 (t, ^{1}H, J=8.6 Hz), 7.2 (t, ^{1}H, J=7.6 Hz), 7.2 (d, ^{1}H, J=7.7 Hz), 7.2 (d, ^{1}H, J=6.7 Hz), 6.9 (d, ^{1}H, J=8.6 Hz), 6.7 (d, ^{1}H, J=7.4 Hz), 6.7 (d, ^{1}H, J=7.5 Hz,), 4.8 (dd, ^{1}H, J=10..3/8.5 Hz), 3.9 (s, 3H), 3.75 (dd, ^{1}H, J=7.9/5.8 Hz), 3.15 (dd, 1H, J=16.1/8.4 Hz), for ^{13}CNMR 159.1, 153.9, 146.2, 134.9, 130.0, 129.4, 127.6, 127.6, 116.4, 115.7, 114.1, 114.1, 113.9, 62.2, 55.4, 42.7; HRMS: MS (ESI, m/z) (M+H)+ : Calcd. For $C_{23}H_{19}FN_2O$: 267.3258; found: 268.1441

3-(4-Chloro-phenyl)-1-phenyl-5-(3.4.5-trimethoxy-phenyl)-4,5-dihydro-1H-pyrazole (3e): Light brown powder, mol. formula-$C_{24}H_{23}ClN_2O_3$, mol. wt–422.90 ;IR cm^{-1} Sp^2 C-H stretch-2958.80, Sp^3 C-H stretch-2835.36, C-N stretch-1244.09, C-O stretch-1282.66, C=C stretch-1581.63, C-Cl stretch-779.24; ^{1}HNMR7.4 (d, ^{1}H, J=8.6 Hz), 7.3 (t, ^{1}H, J=8.6 Hz), 7.2 (t, ^{1}H, J=7.6 Hz), 7.2 (d, ^{1}H, J=7.7 Hz), 7.2 (d, ^{1}H, J=6.7 Hz), 6.9 (d, ^{1}H, J=8.6 Hz), 6.7 (d, ^{1}H, J=7.4 Hz), 6.7 (d, ^{1}H, J=7.5 Hz,), 4.8 (dd, ^{1}H, J=10.3/8.5 Hz), 3.9 (s, ^{3}H), 3.75 (dd, ^{1}H, J=7.9/5.8 Hz), 3.15 (dd, ^{1}H, J=16.1/8.4 Hz), for ^{13}CNMR 159.1, 153.9, 146.2, 134.9, 130.0, 129.4, 127.6, 127.6, 116.4, 115.7, 114.1, 114.1, 113.9, 62.2, 55.4, 42.7; HRMS: MS (ESI, m/z) (M+H)+ : Calcd. For $C_{23}H_{19}FN_2O$: 267.3258; found: 268.1441.

3-(4-Chloro-phenyl)-5-(3-nitro- phenyl)-1-phenyl-4,5-dihydro-1H-pyrazole (3f): Dark brown powder, mol. formula- $C_{21}H_{16}$ ClN_3O_2, mol. wt–377.82; IR cm^{-1} Sp2 C-H stretch-3051.39, Sp^3 C-H stretch-2927.94, Ter C-N stretch-1014.56, C-F stretch-1126.43, C=C stretch-1598. ^{1}HNMR7.4 (d, 1H, J=8.6 Hz), 7.3 (t, ^{1}H, J=8.6 Hz), 7.2 (t, ^{1}H, J=7.6 Hz), 7.2 (d, ^{1}H, J=7.7 Hz), 7.2 (d, ^{1}H, J=6.7 Hz), 6.9 (d, ^{1}H, J=8.6 Hz), 6.7 (d, ^{1}H, J=7.4 Hz), 6.7 (d, ^{1}H, J=7.5 Hz,), 4.8 (dd, ^{1}H, J=10.3/8.5 Hz), 3.9 (s, 3H), 3.75 (dd, ^{1}H, J=7.9/5.8 Hz), 3.15 (dd, ^{1}H, J=16.1/8.4 Hz), for ^{13}CNMR 159.1, 153.9, 146.2, 134.9, 130.0, 129.4, 127.6, 127.6, 116.4, 115.7, 114.1, 114.1, 113.9, 62.2, 55.4, 42.7; HRMS: MS (ESI, m/z) (M+H)+ : Calcd. For $C_{23}H_{19}FN_2O$: 267.3258; found: 268.1441

3-(5-(4-Chloro-phenyl)-2-phenyl-3,4-dihydro-2H-pyrazol-3-yl)-phenol (3g): Yellowish brown powder, mol. formula-$C_{21}H_{17}ClN_2O$, mol. wt–348.83 ;IR cm^{-1} OH stretch-3333.06, Ar-CH stretch-3060, Ali CH stretch-2967, C=O stretch-1674, C=N stretch-1588, C-O stretch-1125, C-Cl stretch-825.47, ^{1}HNMR 9.84 (1H, J=8.6 Hz), 7.85 (1H, J=8.6 Hz), 7.79 (^{1}H, J=7.6 Hz), 7.63 (^{1}H, J=7.7 Hz), 7.5 (^{1}H, J=6.7 Hz), 7.31 (d, ^{1}H, J=8.6 Hz), 6.87 (^{1}H, J=7.4 Hz), 6.79 (^{1}H, J=7.5 Hz,), 5.18 (^{1}H, J=10.3/8.5 Hz), 3.9 (s, 3H), for ^{13}CNMR 167.2, 151.6 159.1, 153.9, 146.2, 134.9, 130.0, 129.4, 127.6, 127.6, 116.4, 115.7, 114.1, 114.1, 113.9, 62.2, 55.4, 42.7,38.3 ; HRMS: MS (ESI, m/z) (M+H)+ : Calcd. For $C_{21}H_{17}ClN_2O$: 348.83; found: 268.1441.

3-(4-Chloro-phenyl)-5-(4-fluro-phenyl)-1-phenyl-4,5-dihydro-1H-pyrazole (3h): Brown powder, Mol. Formula-$C_{21}H_{16}$ $ClFN_2$, Mol.Wt–350.85 ;IR cm^{-1} OH stretch-3369, Ar-CH stretch-3066, Ali CH stretch-2938, C=O stretch-1737, C=N stretch-1601, C-O stretch-1123, ^{1}HNMR 8.96 (1H, J=8.6 Hz), 7.84 (^{1}H, J=8.6 Hz), 7.82 (1H, J=7.6 Hz), 7.71 (^{1}H, J=7.7 Hz), 7.043 (^{1}H, J=6.7 Hz), 6.84 (^{1}H, J=8.6 Hz), 6.7 (^{1}H, J=7.4 Hz), 6.711 (^{1}H, J=7.5 Hz,), 5.65 (^{1}H, J=10.3/8.5 Hz), 3.85 (s, 3H), 3.75 (1H, J=7.9/5.8 Hz), 3.15 (s, 1H, J=16.1/8.4 H-+z), for ^{13}CNMR 168, 162.1, 151.1, 137.6, 134.8, 133.4, 131.6, 127.6, 116.4, 115.7, 114.1, 114.1, 113.9, 67.2, 55.4, 42.7; HRMS: MS (ESI, m/z) (M+H)+ : Calcd. For $C_{21}H_{16}FN_2O$: 350.85; found: 349.5.

3-(4-Chloro-phenyl)-5-(4-nitro-phenyl)-1-phenyl-4,5-dihydro-1H-pyrazole (3i): Reddish brown powder, mol. formula-$C_{21}H_{16}$ ClN_3O_2, mol. wt–377.8; OH stretch-3326, CH stretch-3059, Ali CH stretch-2948, C=O stretch-1744, Ar-CH stretch-3059, C=N stretch-1625 , C-O stretch-1122; ^{1}HNMR 10.16 (^{1}H, J=8.6 Hz), 8.98 (^{1}H, J=8.6 Hz), 7.93 (^{1}H, J=7.6 Hz), 7.65 (^{1}H, J=7.7 Hz), 7.63 (^{1}H, J=6.7 Hz), 6.8 (^{1}H, J=8.6 Hz), 6.73 (^{1}H, J=7.4 Hz), 5.65 (^{1}H, J=7.5 Hz,), 4.8 (d, ^{1}H, J=10.3/8.5 Hz), 3.4 (s, 3H), 3.75 (^{1}H, J=7.9/5.8 Hz), 3.15 (^{1}H, J=16.1/8.4 Hz), for ^{13}CNMR 170.9, 167.6, 149.2, 148.9, 143.5, 137.2, 135.6, 132.9,112.5, 115.7, 114.1, 114.1, 112.9, 62.6, 57.9, 41.2; HRMS: MS (ESI, m/z) (M+H)+ : Calcd. For $C_{21}H_{16}ClN_3O_2$: 377.8; found: 376.5.

Biological activity

Antioxidant activity: DPPH radical scavenging method DPPH is a stable free radical widely used to assess the radical scavenging activity of antioxidant components. DPPH free radical has a stable violet colour in methanol and becomes colourless or yellow colour when paired with antioxidant or reducing agents. These radicals can accept the odd electron or hydrogen from the antioxidant and converted to a stable diamagnetic molecule (yellow). To identify the antioxidant activity, the solution of synthesised compounds has been prepared by dissolving in methanol (100 µg/ml). Simultaneously, a solution of DPPH was also prepared in another container had been shown maximum absorbance at 517 nm due to the presence of stable 1,1-diphenyl 2-picryl hydrazyl stable free radical (violet colour). Each test compound (4 ml) was added to 4 ml of DPPH solution and kept aside for 30 min at room temperature. Absorbance was measured at 517 nm using a Shimadzu ultraviolet spectrometer. Blank and standard absorbance were also calibrated. Antioxidant activity of ascorbic acid derivatives was determined through directly substituting the absorbance in the mentioned formula.

$$\textbf{Percentage inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of sample} \times 10}{\text{Absorbance of control}}$$

DPPH method: DPPH is a perfect method to evaluate the antioxidant activity of newly synthesised compounds due to the short time required for the analysis and high reliability. The result reveals that decreases the absorbance due to the antioxidant molecule may supply the electron to the DPPH free radical. Most of the compounds were shown excellent antioxidant activity. The anti-oxidant activity of synthesised compounds was shown in Table 2 (Figures 4-6).

Table 2: Antioxidant activity of pyrazoline derivatives (IC_{50} in µg/mL).

Sr.no.	Comp. code	% Inhibition				
		5 µg/ml	10 µg/ml	15 µg/ml	20 µg/ml	IC50 µg/ml
1	3a	20.51	35.61	79.74	86.04	10.37
2	3b	35.89	42.67	70.25	85.44	10.78
3	3c	19.54	38.93	62.65	75.82	12.72
4	3d	28.61	39.67	59.75	86.70	11.72
5	3e	24.45	44.76	71.51	77.84	11.56
6	3f	30.65	43.62	79.11	87.97	10.38
7	3g	28.64	39.67	73.41	90.05	10.82
Std.	Ascorbic acid	38.99	55.78	72.51	93.15	9.52

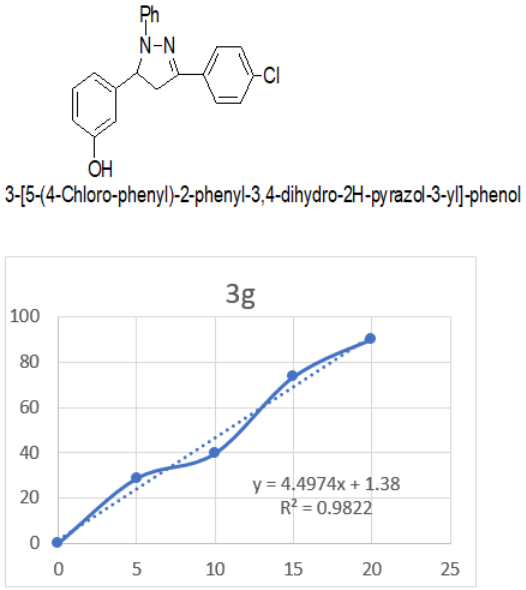

Figure 4: Antioxidant activity of comp 3 g (DPPH) by conc. *Vs.* abs.

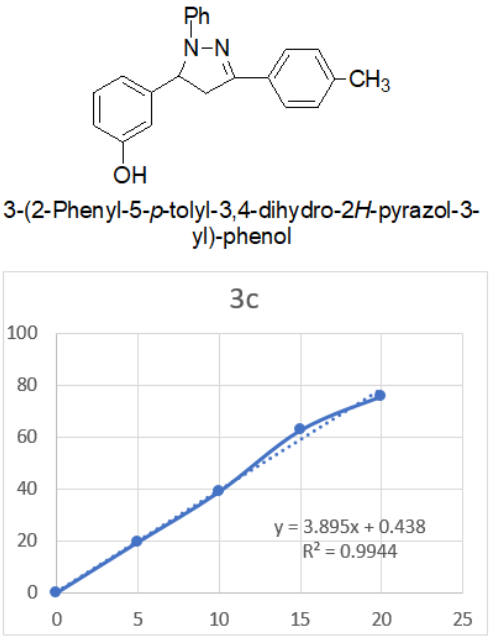

Figure 5: Antioxidant activity of comp 3 c (DPPH) by conc. *Vs.* abs.

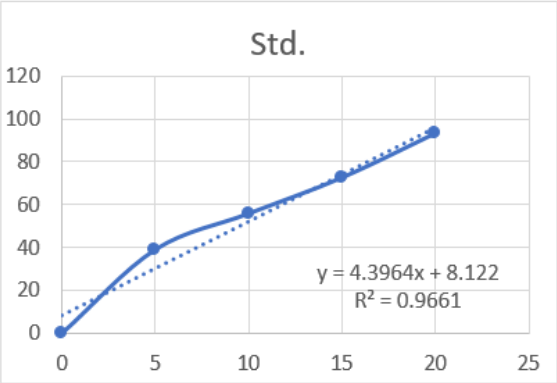

Figure 6: Antioxidant activity of Std. comp (DPPH) by conc. *Vs.* abs.

Anti-inflammatory activity

Anti-inflammatory agents inhibit the cyclooxygenase enzymes which are responsible for the conversion of arachidonic acid to prostaglandins. Because Human Red Blood Cell (HRBC) membranes are similar to these lysosomal membrane components, the prevention of hypotonicity induced HRBC membrane lysis was taken as a measure in estimating anti-inflammatory activity. Thus, HRBC membrane stabilization method was used to estimate anti-inflammatory activity of all synthesized compounds.

According to this result, the newly synthesized pyrazolines had more promising anti-inflammatory activity compared with β- diketones and flavones. Compound 3c showed the most promising activity (EC_{50}=11.00 ± 1.26 µg/mL). Among the series. Compounds 3d and 3e showed good anti-inflammatory activity. Compared with standard Aspirin (EC_{50}=11.70 ± 0.98 µg/mL). Fresh whole human blood was collected and it was mixed with equal volumes of sterilized Alsever's solution (dextrose 2%, sodium citrate 0.8%, citric acid 0.05%, sodium chloride 0.42% and distilled water 100 mL). this blood solution was centrifuged at 3000 rpm for 10 min and was washed three times with equal volume of normal saline. The volume of the blood is measured and reconstituted as 10% v/v suspension with normal saline. The reaction mixture consists of 1.0 mL of test sample of different concentrations in normal saline and 0.5 mL of 10% HRBC suspension, 1 mL of 0.2 M phosphate buffer, 1 mL hyposaline were incubated at 37°C for 30 min and centrifuged at 3000 rpm for 30 mints, the haemoglobin content of the supernatant solution was estimated spectrophotometric ally at 560 nm. Each experiment was performed in triplicate. Dichlorofenac sodium was used as standard and distilled water as control in this study. Where the blood control represents 100% lysis or zero percent stability, the percentage of HRBC haemolysis calculated by formula (Table 3).

% Haemolysis=100-absorbance of sample/absorbance of control × 100

Table 3: Anti-inflammatory activity of pyrazoline derivatives (IC_{50} in µg/mL).

Compounds	Anti-inflammatory activity in µg/mL (EC_{50} ± SD)
2a	58.23 ± 2.36
2b	54.00 ± 2.13

2c	52.23 ± 2.19
2d	36.00 ± 2.03
3a	50.13 ± 2.03
3b	49.99 ± 1.33
3c	11.23 ± 1.26
3d	14.03 ± 2.15
3e	15.98 ± 0.57
Aspirin	11.70 ± 0.98

Antimicrobial activity: All the synthesized compounds were screened for their antibacterial *Staphylococcus aureus* and *Escherichia coli* organisms using a modified agar diffusion assay method streptomycin was used as positive control. Incubation time and period for antibacterial activity was at 37℃ for 24 hrs results of antimicrobial activities are expressed in terms of ZOI. The result of activity of the synthesized compounds is shown in Table 3 respectively. Based upon the value of ZOI in mm, compounds are classified as, significantly active (ZOI ≥ 15), moderately active (ZOI 13 to 14), poor active (ZOI 10 to 12) and least active (ZOI less than 10). The investigation of antibacterial screening shows that compound 3b exhibited highest activity against *E.coli*, 3c have maximum zone of inhibition against *S. aureus* respectively. Overall compound 3a and 3e had good activity against both gram negative and gram positive bacteria (Table 4).

Table 4: Antimicrobial activity of pyrazoline derivatives.

Microorganisms →	**Zone of inhibition (mm) of compounds**	
Comp. code	Gram negative *E. Coli*	Gram positive *S. aureus*
3a	13 mm	12 mm
3b	18 mm	15 mm
3c	14.5 mm	9 mm
3d	10.5 mm	10.5 mm
3e	16.5 mm	11.7 mm
Streptomycin	21 mm	24 mm

RESULTS AND DISCUSSION

Microwave assisted synthesis, in general, has a large impact on synthetic organic chemistry compared to traditional processing of organic synthesis. Microwave enhanced organic synthesis saves significant time and very often improves yields. In order to synthesize the chalcones, benzaldehyde was treated with different heteroaryl active methyl compounds under conventional heating method by using ethanol as a solvent in basic condition. It was found that the synthesis of chalcones attempted by conventional method took a longer time (36 h) for completion of reaction with moderate yield (65%-70%). In order to find the best reaction conditions and to obtain chalcones in a short span of time with improved yield, subsequent studies were carried out using microwave irradiation technique under solvent free condition. As visualized, the reaction proceeded smoothly and the chalcones were obtained in excellent yields (82%-92%) within a few minutes (3-5 min). The assigned molecular structures of all pyrazoline were based on spectroscopic analysis (IR, ^{1}H, ^{13}C NMR, and MS) and elemental analysis data. Regarding the structure of pyrazoline from chalcone,

the assignment of 3a, was described. The IR spectra of 3a showed characteristic absorption band at 3251 cm^1 and 3384 cm^1 due to the presence NH and OH groups.

It has been reported that a, b-unsaturated ketone which are attractive due to their electrophilic properties can react with nitrogenous bases to give the corresponding pyrazolines. Thus, a series of pyrazoline derivatives were obtained by refluxing of chalcones (1a-i) with hydrazine hydrate (2a-i) and INH (3a-i) in ethanol in the presence of catalytic amount of glacial acetic acid (scheme 1), but due to partial solubility of it took longer time for completion of reaction (36 h) and afforded the products with low percentage yield (57%-68%). To accelerate the reaction and to improve the yield of products, the microwave irradiation technique was applied. The microwave-assisted reaction in solvent and catalyst-free environment proceeded efficiently and completed within 3-5 min with excellent yield of products (76%-89%). Formation of pyrazolines (3a, 2d, 2h) established on the basis of IR, 1H NMR, and mass spectral analysis. The IR spectrum of 5a showed two broad absorption bands at 3681 cm^1 and 3270 cm^1 due to the presence of C=O groups of pyrazoline respectively. The absorption band for the carbonyl group was discernible at 1680 cm^1. The 1H NMR spectrum showed a broad singlet at δ 8.7 due to the presence of 1H proton. The presence of pyrazoline unit was clearly indicated by the presence of two double doublets at δ 4.01 (2H), 7.16 (H). Further confirmation of the structure was provided by mass spectrometry, which showed molecular ion peak at m/z 346. During the course of reaction for the synthesis of compounds 2a-i and 3a-i, we examined the reaction time by conventional heating method and microwave irradiation method. It was clear that the microwave assisted synthesis of compounds 2a-i and 3a-i were found to be time specific as compared to conventional method, led to the formation of compounds in short time, and overall yield much higher than the conventional route of synthesis (Table 1).

All the synthesized pyrazoline derivatives were screened for antioxidant activity by using DPPH free radical scavenging assay. Ascorbic acid was used as standard for the activity. The results of antioxidant activity showed that few compounds showed considerable antioxidant activity while the 3c and 3g showed good to excellent antioxidant activity (IC_{50}=48.13 and 46.03 µg/ml), remaining synthesized compounds showed poor activity. All the synthesized pyrazoline derivatives were screened for anti-microbial activity shows that compound 2b and 2e exhibited highest activity against *E. coli* and *S. aureus*, 2c have maximum zone of inhibition against S. aureus remaining compound had good activity against both gram negative and gram-positive bacteria. According to this result, the newly synthesized pyrazolines had more promising anti-inflammatory activity compared with β-diketones and flavones. Compound 3c showed the most promising activity (EC_{50}=11.00 ± 1.26 µg/mL) among the series. Compounds 3d and 3e showed good anti-inflammatory activity. Compared with standard aspirin (EC_{50}=11.70 ± 0.98 µg/mL).

CONCLUSION

A series of novel heterocyclic derivatives containing pyrazoline ring were derived from chalcones and based on the study activities have been voted for the anti-inflammatory and as biological screening. From the analytical study such as IR, ^{1}H NMR, ^{13}C NMR and mass spectrometry we got to know determination and identification of the chemical composition of synthesized drugs. As synthesized compounds are found to show moderate to good anti-inflammatory activity in appraisal with standards, further modification in the structure of compounds may lead to development of potent anti-inflammatory moiety. Among the series 3b and 3f found to be most potent anti-inflammatory drugs. It can be concluded that presence of electron withdrawing groups may be responsible for showing anti-inflammatory activity.

From the synthesized derivatives it can be resolved that the results of anti-oxidant activity showed moderate to good activity in appraisal with standards. Among all the synthesized compounds 3c and 3g were found to show most potent activity at 30 ppm and 40 ppm whereas compounds 3d and 3f showed moderate activity among tested compounds. Remaining synthesized derivatives showed very poor activity comparing with standard. Antimicrobial activity was performed by using broth dilution method using streptomycin as standard. Compound 2b and 2e were found to be most active compound and remaining compounds 2a, 2c and 2d showed poor activity among tested compounds. Hence, newly synthesized pyrazoline derivatives possess considerable anti-inflammatory, antioxidant and antimicrobial activity. Structure and biological activity relationship of title compound showed that the electron withdrawing group enhanced the activity.

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COMPETING INTEREST

The authors declare that they have no competing interests.

CONSENT FOR PUBLICATION

Not applicable.

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AVAILABILITY OF DATA AND MATERIALS

The dataset used and analysed during current study available from the corresponding author on reasonable request.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

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