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Research paper

Design and synthesis of some new pyrazolyl-pyrazolines as potential anti-inflammatory, analgesic and antibacterial agents

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ABSTRACT

In the present study, an efficient synthesis of some new substituted pyrazoline derivatives linked to a substituted pyrazole scaffold was performed by a multistep reaction sequences and compounds were screened for their anti-inflammatory, analgesic and antibacterial activities. The preliminary results revealed that the *N*-acylated (**5e**, **5h**) and nitro substituted *N*-phenyl (**6f**) pyrazolyl-pyrazolines derivatives exhibited a very promising anti-inflammatory activity whereas **5h**, **6f** were interesting analgesic agents. The compounds with halo substituted phenyl group at C-3 of the pyrazoline ring (**4a**, **5g**, **5h**, **6a** and **6b**) were found to be active against clinical bacterial pathogens with MIC in the range of 0.2–0.4 mg/mL. Compound containing *N*-propionyl pyrazolyl-pyrazoline (**5h**) could be identified as the most active member within this study with a dual anti-inflammatory and antibacterial profile. Taken together, this study has led to the development of promising compounds.

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1. Introduction

Nonsteroidal anti-inflammatory drugs (NSAIDs) are of huge therapeutic benefit in the treatment of various types of inflammatory conditions. The pharmacological activity of NSAIDs relates to the suppression of prostaglandin biosynthesis from arachidonic acid by inhibiting the enzyme cyclooxygenases (COXs) and thromboxane synthase with a different degree of selectivity [1]. COX enzymes exist in two isoforms: COX-1 and COX-2 [2]. The COX-1enzyme is constitutively expressed in most tissues and the COX-2 enzyme is induced at sites of inflammation, pain and oncogenesis [3,4]. The therapeutic use of NSAIDs includes major side effects at the gastrointestinal (GI) and renal level [5]. The search for safer NSAIDs continues with the failure of anticipated 'Ideal' anti-inflammatory agents, the coxibs, on long term usage [6].

In addition to the inflammatory drugs, there is an urgent need to develop new antibiotic agents with novel targets for the augmentation of bacterial invasions in recent years [7]. To overcome the emerging drug resistance, the discovery of novel antibiotic chemical scaffolds with new mode of action is vital for bacterial survival. In the same way, the discovery of new antibacterial entities with inexpensive economic inputs has always a prolific option to cope with this state of affairs.

The remedy of co-administration of a variety of drugs for treatment of inflammatory conditions, a part of some microbial infections may add adverse health problems, especially in patients with exacerbated liver or kidney functions [8]. A mono therapy of an anti-inflammatory drug with antimicrobial properties will probably become more advantageous from the pharmaco-economic point of view. Some of the reported pyrazole derivatives, **A**, **B** [8,9] and **C** [23] (Fig. 3) showed pronounced dual anti-inflammatory and antimicrobial activities. Enthused by the aforementioned investigations, as an integrated part with this trend we are now endeavouring to design, synthesis of a unique series of pyrazolyl-pyrazoline compounds which exhibit anti-inflammatory and antibacterial properties.

The literature survey reveals that pyrazoline shows an integral architectural concept in heterocyclic chemistry. Antipyrine is the first pyrazoline derivative used as an anti-inflammatory agent. Several analogues of pyrazoline for example Phenylbutazone, Celecoxib, Ramifenazone, Morazone and Famprofazone (Fig. 1) are potent anti-inflammatory and analgesic agents [10]. The pyrazole

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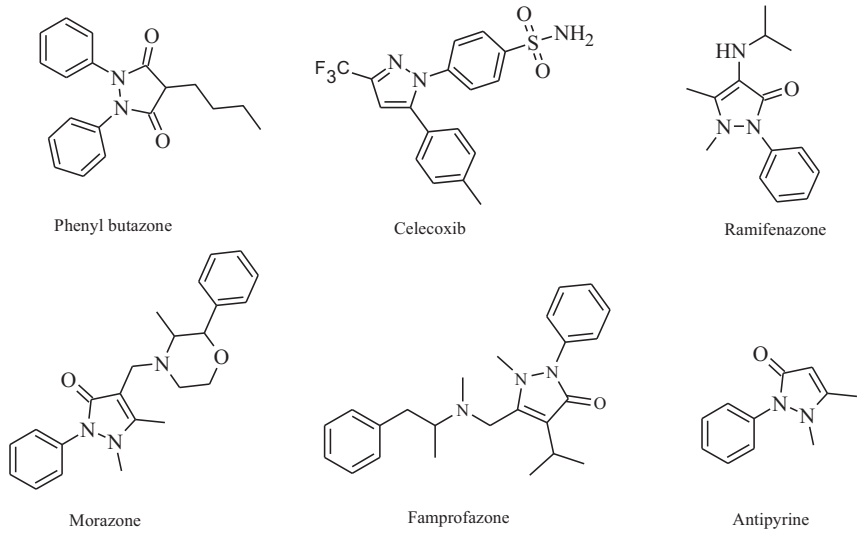


Fig. 1. Structures of some known pyrazole NSAIDs.

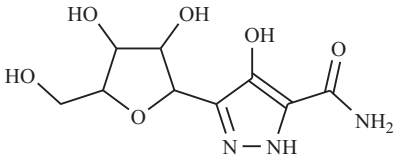


Fig. 2. Structures of antibiotic pyrazofurin.

scaffold represents a common motif in many pharmaceutical active compounds and demonstrating a wide range of activities such as anti-inflammatory, analgesic, antimicrobial, anticonvulsant and anticancer etc [11–14]. The antibiotic pyrazofurin (Fig. 2) has provided a basis for more rationale design and synthesis of new pyrazoles as potential antimicrobial [8]. Also, pyrazole incorporated with other biologically active heterocycles like oxazole, oxadiazole, pyrazoline, triazole, thiazole, thiadiazole etc., are created in an endeavour to acquire synergistic chemotherapeutic activity with higher selectivity and less toxicity [15,16].

Against this background, the efforts are concentrated on establishing pyrazole scaffold integrated with pyrazoline framework to describe the relevance pharmacological activity. Based on these interesting biological activity profiles of pyrazoles and pyrazolines analogues, we are inspired and made an effort to synthesize some new number of pyrazole integrated pyrazolines analogues as potent anti-inflammatory, analgesic and antibacterial agents.

2. Results and discussion

2.1. Chemistry

The synthesis of target pyrazolyl-pyrazolines derivatives was carried out as outlined in Scheme 1. In the present investigation the starting material 3-(3,4-dichlorophenyl)-1-phenyl-1*H*-pyrazole-4-carbaldehyde (**2**) was prepared by Vilsmeier–Haack reaction of the phenyl hydrazone (**1**), which was previously prepared from 3,4-dichloroacetophenone and phenyl hydrazine according to the previous reports of our research group [17,18]. The pyrazole aldehyde was then subjected to base catalysed Claisen–Schmidt condensation reaction with appropriate 4-substituted acetophenones resulting in the formation of required α, β-unsaturated carbonyl compounds **3a**–**c** [19]. For all the chalcones **3a**–**c**, the *J* values were in the range of 14–16 Hz, indicating that they were stereoselective and attained trans (*E*) configuration. With the objective of synthesizing pyrazolyl-pyrazoline derivatives, the heterochalcones **3a**–**c** was treated with hydrazine hydrate and its derivatives by nucleophilic cycloaddition reaction by conventional

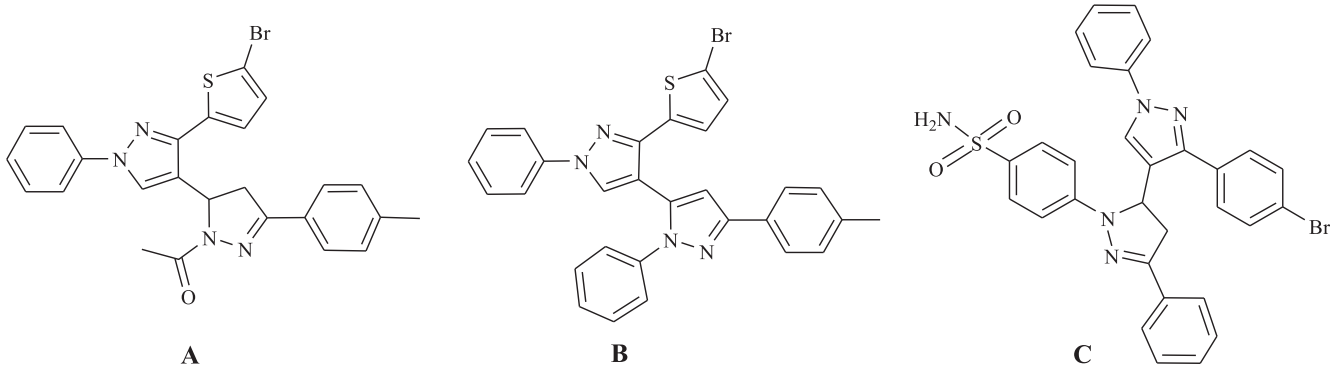
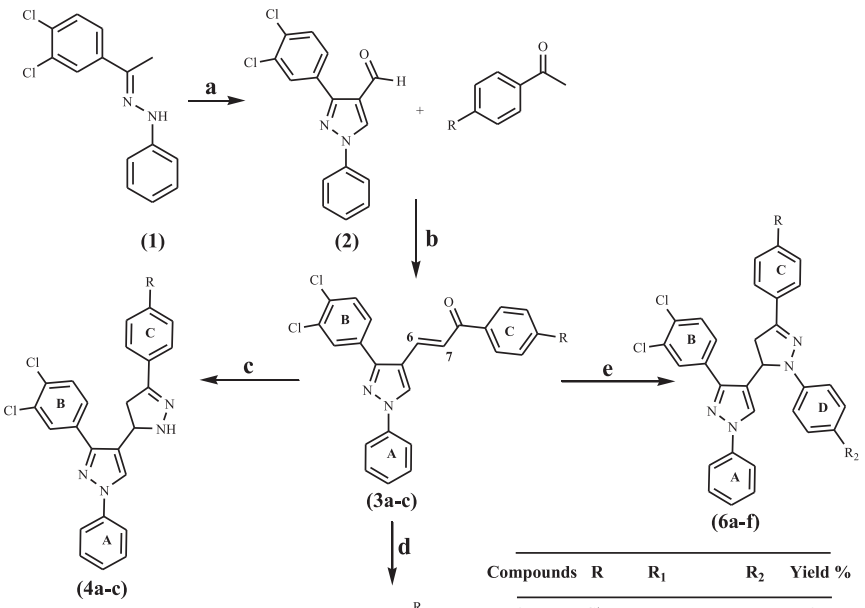


Fig. 3. Structures of pyrazole derivatives **A**, **B** and **C** with dual anti-inflammatory-antimicrobial activity.



Compounds	R	R_1	R_2	Yield %
4a	Cl	-	-	65
4b	F	-	-	69
4c	CH_3	-	-	73
5a	Cl	H	-	90
5b	F	H	-	85
5c	CH_3	H	-	87
5d	Cl	$-CH_3$	-	77
5e	F	$-CH_3$	-	59
5f	CH_3	$-CH_3$	-	70
5g	Cl	$-CH_2CH_3$	-	68
5h	F	$-CH_2CH_3$	-	92
5i	CH_3	$-CH_2CH_3$	-	69
6a	Cl	-	H	83
6b	F	-	H	88
6c	CH_3	-	H	73
6d	Cl	-	NO_2	67
6e	F	-	NO_2	74
6f	CH_3	-	NO_2	88

Scheme 1. Synthetic route for the preparation of compounds **4a**–**c**, **5a**–**i** and **6a**–**f**.

heating method. According to the Baldwin's rule, the formation of pyrazoline ring, rationalized on the basis of reaction pathways, involves a Michael addition of hydrazine on chalcone, followed by a 5-exo-trig cyclization and dehydration [20]. In the present study, we were intended to investigate three series of compounds which differ in their *N* substitution on the pyrazoline ring. Simple pyrazoline derivatives **4a**–**c** was synthesized by the reaction of hydrazine hydrate with the chalcones in ethanol used as a solvent [21]. Furthermore, hydrazine hydrate reacted with α,β-unsaturated ketones in the presence of different aliphatic acid, namely formic, acetic and propionic acid resulted in the formation of pyrazoline moiety containing *N*-acyl chain **5a**–**i** [22]. The incorporation of substituted aromatic group on *N1* of pyrazoline ring **6a**–**f** was achieved by the reaction of chalcones with simple and substituted phenylhydrazines in glacial acetic acid under reflux condition [23]. The completion of the reaction was monitored by TLC. All the synthesized compounds were characterized by IR, ^{1}H NMR, ^{13}C NMR and Mass spectroscopy and elemental analysis.

The IR spectra of **4a**–**c** showed varied absorption bands in the range of 3207–3242 cm^{-1} assigned to NH functionality. In the **5a**–**i** series, carbonyl stretching frequency of *N*-formyl group **5a**–**c** occurs within the range 1687–1692 cm^{-1}. In the case of *N*-acetyl derivatives **5d**–**f** and *N*-propionyl derivatives **5g**–**i**, because of the electron-releasing effect of the additional alkyl group, the C=O bond stretching is shifted at 1643–1670 cm^{-1}. Stretching frequency of all other functional groups are found in the expected region.

The formation of pyrazoline ring in the target series **4a**–**c**, **5a**–**i** and **6a**–**f** was confirmed by the appearance of interesting ABX pattern in their ^{1}H NMR spectrum [24]. In the ^{1}H NMR spectrum for compound **6a**, the protons on the diastereotopic centre C-4 of the pyrazoline ring appears as two double-doublets at $\delta = 3.20$ and 3.93 ppm with $^2J_{ab} = 17.4$, $^3J_{ax} = 7.6$ and $^3J_{bx} = 12.8$ Hz, while the H-5 proton is observed as a double-doublet at 5.61 ppm with $^3J_{bx} = 12.8$ and $^3J_{ax} = 7.6$ Hz. Protons bound to aromatic ring were observed within the expected chemical shift region and exhibit the expected integral values. Further, the ^{13}C NMR spectra of **6a** confirmed the presence of pyrazoline ring by exhibiting signals at 42.7 and 56.7 ppm due to the sp^3 carbons C-4 and C-5, respectively. The aromatic carbon atoms appeared in the region 113.6–147.4 ppm. The mass spectrum and elemental analysis supported the structure of various synthesized pyrazolyl-pyrazolines.

2.2. Pharmacological screening

We investigated all the newly synthesized compounds for their analgesic, anti-inflammatory and antimicrobial activities. The close analogy in the chemical structures of the synthesized compounds **4a**–**c**, **5a**–**i** and **6a**–**f** with anti-inflammatory drugs prompted us to test them for evaluating the presence of anti-inflammatory and analgesic property. The tested compounds were administered in the form of a suspension (1% carboxy methyl cellulose as a vehicle). Anti-inflammatory and analgesic activities of the tested

compounds were measured with respect to the control and compared with the standard drugs Diclofenac sodium and Pentazocin respectively. The anti-inflammatory activity data indicated that all the compounds exhibited significant activity by decreasing the paw volume that was produced by carrageenan. All the pharmacological data were expressed as mean ± SEM; statistical analysis was applied to determine the significance of the difference between the control group and a group of animals tested with the tested compounds. The percentage inhibition for the synthesized compounds as well as standard drug was determined [25].

All the synthesized compounds were tested *in vitro* for their antimicrobial activity with 15 bacterial and 7 fungal pathogens. Among the tested compounds none of the compounds exhibited significant growth inhibition zones with the fungal strains used, but revealed good antibacterial activity. Some of the tested compounds showed inhibitory activity against *Staphylococcus citreus, Staphylococcus aureus*, (Gram positive bacteria) and *Aeromonas hydrophila, Escherichia coli* (Gram negative bacteria) respectively which was detected by disc diffusion assay based on the zones (in mm).

In the present study we were decided to compare the anti-inflammatory, analgesic and antibacterial activity values in terms of their chemical structure. As a result interesting structure–activity relationships can be deduced.

2.2.1. Anti-inflammatory activity

According to the anti-inflammatory profile of the synthesized compounds, variable activity values were observed depending on the substituents of the target compound. The results were summarized in Table 1. The unsubstituted pyrazoline ring in the target series (compounds **4a**–**c**) afforded a moderate anti-inflammatory activity. Compounds **4a**, **4c** exhibited 48.4% while **4b** showed 46.1% oedema inhibition, which revealed the indispensability of the unsubstituted pyrazoline ring. Literature reveals that some *N*-acyl substituents are well known for giving potent anti-inflammatory and analgesic activities [26]. According to our results, by replacement of pyrazoline NH with acyl (**5a**–**i**) and substituted phenyl (**6a**–**f**) groups, a remarkably increased potency was observed. Anti-inflammatory results of **5a**–**i**, amongst all the compounds show good anti-inflammatory activity. Compounds **5e**, **5f**, **5h** and **5i** are found to be potent molecule compare to standard. Among compounds **5a**–**i**, the *N*-propionyl derivatives **5g**, **5h**, **5i** showed maximum percentage of inhibition of oedema (68.75%, 75.56% and 70.31%, respectively), followed by *N*-acetyl derivatives **5d**–**f** [**5d** (66.40%), **5e** (72.65%), **5f** (70.31%)]. Besides, compounds **5a** (57.81%), **5b** (60.93%) and **5c** (60.93%), bearing the *N*-formyl substituent, were comparably less active than compounds **5d**–**i**. From the activity results it was clear that there is a gradual increase in the activity with introduction of alkyl group. Electron releasing effect of these additional alkyl substituents may increase the activity. From results of **6a**–**f**, it is observed that electronic nature of *N*-phenyl substituent on pyrazoline ring had profound effect on the activity. Anti-inflammatory profile of **6d** and **6f** was comparable with that of the standard drug. In compounds **6a**–**f**, remarkable increased potency was observed for the *p*-nitro phenyl substituted **6d**–**f** over **6a**–**c**. The high electron withdrawing ability of nitro substituent at 4th position on aromatic ring displayed enhanced activity. The SAR of compounds with the electron withdrawing (Cl, F) and electron donating (CH_3) substituents on *N3* substituted phenyl ring was less clear-cut.

2.2.2. Analgesic activity

Tested compounds exhibited moderate analgesic activity at 30 min of reaction time; the activity increased and reached to peak level at 60 min and declining in activity was observed after 90 min (Table 2). Among compounds **4a**–**c**, the 4-methyl substituted phenyl group (**4c**) at C-3 of pyrazoline ring afforded maximum activity compared with those having a 4-Cl-phenyl (**4a**) and 4-F-phenyl (**4b**) as substituents. In contrast, the analgesic activity of compounds **5a**–**i** was affected by their *N*-acyl substituents. The SAR study revealed that results of analgesic activity of compounds **5a**–**i** followed the same trend that was observed in the anti-inflammatory activity i.e., *N*-propionyl derivatives > *N*-acetyl derivatives > *N*-formyl derivatives. Out of these compounds, **5h** is equipotent to the standard one. In a series of *N*-arylated products **6a**–**f**, **6c**, **6d** and **6f** shows good activity. Moreover compounds which have shown best anti-inflammatory activity are also exhibited significant analgesic activity.

2.2.3. Acute toxicity

Test compounds used in the pharmacologic study did not show any acute toxicity. Common side effects such as mild diarrhoea and

Table 1
Anti-inflammatory activity data of compounds (**4a**–**c**), (**5a**–**i**) and (**6a**–**f**).

Compound	Dose mg/kg	Oedema volume in mL (Mean ± SEM)			Percentage inhibition (%)		
		30 min	60 min	120 min	30 min	60 min	120 min
4a	50	0.63 ± 0.07***	0.71 ± 0.1*	0.66 ± 0.8***	32.97	45.80	48.43
4b	50	0.51 ± 0.03*	0.78 ± 0.02*	0.69 ± 0.02*	45.74	40.45	46.09
4c	50	0.58 ± 0.5	0.57 ± 0.9	0.66 ± 0.3	38.29	56.48	48.43
5a	50	0.61 ± 0.07*	0.64 ± 0.1*	0.54 ± 0.08*	35.10	51.14	57.81
5b	50	0.59 ± 0.5	0.57 ± 0.1	0.5 ± 0.9	37.23	56.48	60.93
5c	50	0.61 ± 0.9	0.61 ± 0.3	0.5 ± 0.6	35.10	53.43	60.93
5d	50	0.51 ± 0.03***	0.56 ± 0.02*	0.43 ± 0.03*	45.74	57.25	66.40
5e	50	0.51 ± 0.8	0.53 ± 0.5	0.35 ± 0.5	45.74	59.54	72.65
5f	50	0.68 ± 0.2	0.53 ± 0.4	0.38 ± 0.9	27.65	59.54	70.31
5g	50	0.49 ± 0.04***	0.51 ± 0.02***	0.4 ± 0.03*	47.87	61.06	68.75
5h	50	0.46 ± 0.1*	0.48 ± 0.8***	0.32 ± 0.3***	51.06	63.35	75.56
5i	50	0.66 ± 0.5*	0.51 ± 0.5	0.38 ± 0.3	29.78	61.06	70.31
6a	50	0.51 ± 0.03***	0.61 ± 0.02**	0.49 ± 0.03**	45.74	53.43	61.71
6b	50	0.52 ± 0.02**	0.58 ± 0.03**	0.51 ± 0.03**	44.68	55.72	60.15
6c	50	0.5 ± 0.8	0.54 ± 0.4	0.48 ± 0.35	46.80	58.77	62.5
6d	50	0.48 ± 0.03**	0.56 ± 0.04**	0.39 ± 0.06**	48.93	57.25	69.53
6e	50	0.51 ± 0.02**	0.52 ± 0.1***	0.44 ± 0.4**	45.74	60.30	65.62
6f	50	0.4 ± 0.5*	0.36 ± 0.6***	0.36 ± 0.4**	57.44	72.51	71.87
Diclofenac sodium	20	0.50 ± 0.02***	0.38 ± 0.03***	0.30 ± 0.08***	46.80	70.99	76.56
Control	–	0.94 ± 0.21	1.31 ± 0.1	1.28 ± 0.02	–	–	–

ANOVA analysis followed by Dunnett's-t-test, (n = 6) significance levels *P < 0.05, **P < 0.01, ***P < 0.001 as compared with the respective control.

Table 2
Analgesic activity of compounds (**4a**–**c**), (**5a**–**i**) and (**6a**–**f**).

Compound	Dose mg/kg	Tail flick latency in secs			
		0 min	30 min	60 min	90 min
4a	50	3.6 ± 0.3	5.4 ± 0.1	5.7 ± 0.1	4.6 ± 0.09
4b	50	3.1 ± 0.4	4.5 ± 0.1	4.8 ± 0.1	4.2 ± 0.5
4c	50	3.6 ± 0.1	5.9 ± 0.2***	6.3 ± 0.1***	6.5 ± 0.1***
5a	50	3.0 ± 0.11	4.5 ± 0.1	4.7 ± 0.1	4.3 ± 0.07
5b	50	3.5 ± 0.1	6.3 ± 0.1	6.4 ± 0.1***	6.6 ± 0.3***
5c	50	3.4 ± 0.1	4.7 ± 0.9	4.6 ± 0.1	4.4 ± 0.7
5d	50	3.5 ± 0.2	5.5 ± 0.1**	5.9 ± 0.1**	5.1 ± 0.17*
5e	50	3.5 ± 0.4	6.8 ± 0.1***	6.5 ± 0.1***	6.6 ± 0.2***
5f	50	3.5 ± 0.2	5.3 ± 0.9	5.5 ± 0.1***	5.5 ± 0.4***
5g	50	3.3 ± 0.8	6.1 ± 0.1	6.8 ± 0.2***	5.4 ± 0.2***
5h	50	3.4 ± 0.2	7.9 ± 0.2***	7.7 ± 0.1***	7.2 ± 0.1***
5i	50	3.3 ± 0.8	5.3 ± 0.9***	6.4 ± 0.1**	5.9 ± 0.1***
6a	50	4.3 ± 0.4	6.1 ± 0.1**	6.1 ± 0.1***	4.9 ± 0.1
6b	50	3.1 ± 0.1	4.6 ± 0.1	5.2 ± 0.1	4.8 ± 0.1
6c	50	3.3 ± 0.4	6.7 ± 0.5***	7.1 ± 0.8***	6.6 ± 0.8***
6d	50	3.4 ± 0.7	6.9 ± 0.1***	7.0 ± 0.1***	6.6 ± 0.14***
6e	50	3.8 ± 0.2	5.1 ± 0.12	5.7 ± 0.1**	5.2 ± 0.1**
6f	50	3.4 ± 0.7	7.0 ± 0.8***	7.4 ± 0.1***	6.6 ± 0.7***
Pentazocin	20	3.6 ± 0.5	7.4 ± 0.2***	7.7 ± 0.1***	7.9 ± 0.2***
Control	–	3.5 ± 0.8	3.46 ± 0.1	3.47 ± 0.1	3.70 ± 0.1

ANOVA analysis followed by Dunnett's-t-test, (n = 6) significance levels *P < 0.05, **P < 0.01, ***P < 0.001 as compared with the respective control.

depression were not recorded. Median lethal dose (LD_{50}) in male Swiss albino mice was determined by employing the standard methods [27]. From the experiment, oral LD_{50} of the test compound was found to be 500 mg/kg body weight at 48 h duration. So that 50 mg/kg i.e., 1/10 of cut off value was taken as screening dose for the evaluation of anti-inflammatory and analgesic activity.

2.2.4. Antibacterial activity

Based on the initial screening results, it was seen that the compounds **4a**, **5g**, **5h**, **6a** and **6b** showed potent antibacterial activities (Table 3). These compounds are characterized by either a 4-Cl or 4-F substituent on the phenyl ring at pyrazoline C-3, showing that the presence of suitable substituents at this terminal benzene ring is a favourable feature for high antibacterial activity. The minimum inhibitory concentrations (MICs) for the five compounds showing maximum activity were determined by agar dilution method (as described by Standard procedure of M07-A9 with minor modifications) (Table 4) [28]. For MICs, the compounds were diluted with DMSO from a highest (1 mg/mL) to lowest (0.1 mg/mL) concentration. Each of the dilution were added to the sterile Muller-Hinton agar (Hi-Media) at a temperature of 45 °C and then poured into sterile petriplates. Plates were allowed to solidify and pathogens were spot (4 μL) inoculated from liquid culture media having a cell density of individual bacterial cultures at 10^4 CFU/mL. A similar assay was repeated with the standard antibiotics *viz.* Vancomycin, Ampicillin, Tetracycline and Cefuroxime. DMSO was used as a negative control. No visible bacterial growth was observed from 0.2 to 0.4 mg/mL concentrations of the compounds as well of the antibiotics, and thus was considered as minimum inhibitory concentration (MICs).

The results of MICs indicated that among the tested compounds and the standard drugs, **4a** showed excellent activity against *E. coli* at concentrations of 0.2 mg/mL and it was found to be more active than Cefuroxime and Tetracycline with MIC of 0.4 mg/mL and 0.5 mg/mL respectively. The compound **5g** against *S. aureus* strain tested which was resistant to Cefuroxime, was found to be significantly active with a MIC of 0.3 mg/mL and was prominent as compared to Vancomycin (0.5 mg/mL). This activity was comparable to Ampicillin (0.3 mg/mL) and Tetracycline (0.3 mg/mL). *A. hydrophila* bacterial stain thus was found to be inhibited by the compounds **5h** and **6a** at 0.4 mg/mL as compared to the standards

Table 3
Antibacterial activity of compounds (**4a**–**c**), (**5a**–**i**) and (**6a**–**f**). Bold indicates the significant activity of compounds against the respective bacterial strains.

Compound	Zone of inhibition (in mm)			
	Gram positive bacteria		Gram negative bacteria	
	S. citreus	*S. aureus*	*A. hydrophila*	*E. coli*
4a	18	21	15	**34**
4b	23	14	16	–
4c	–	19	11	15
5a	21	18	–	–
5b	18	13	10	12
5c	16	–	–	20
5d	13	21	19	–
5e	–	–	19	12
5f	–	19	–	18
5g	18	**30**	23	19
5h	15	23	**28**	21
5i	–	24	–	22
6a	19	15	**28**	–
6b	**37**	**31**	–	21
6c	–	15	18	20
6d	18	22	21	31
6e	21	20	–	–
6f	16	21	19	16

Disc size was 6 mm and 20 μL of 1 mg/mL stock concentration of the compound; (–) stands for no activity.

Table 4
Minimum inhibitory concentrations (MICs) of the selected compounds.

Compound/drug	Minimum inhibitory concentrations (MICs) in mg/mL			
	S. citreus	*S. aureus*	*A. hydrophila*	*E. coli*
4a	–	–	–	0.2
5g	–	0.3	–	–
5h	–	–	0.4	–
6a	–	–	0.4	–
6b	0.2	0.3	–	–
Vancomycin	0.5	0.5	–	–
Ampicillin	0.2	0.3	0.2	0.1
Tetracycline	0.3	0.3	0.4	0.4
Cefuroxime	0.6	–	0.5	0.5

viz Tetracycline (0.4 mg/mL) and Cefuroxime (0.5 mg/mL). However, the *N*-phenyl substituted pyrazoline derivative **6b** exhibited additional activity with Gram positive bacteria *S. citreus* as well as *S. aureus* at 0.2 mg/mL and 0.3 mg/mL respectively which were more significant compared to the standard antibiotics where MIC ranged from 0.3 mg/mL to 0.6 mg/mL for both the pathogens. Whereas the other compounds exhibited less activity against all the tested microorganisms as compared to the standard antibiotics tested.

3. Conclusion

In conclusion, a new series of pyrazolyl-pyrazolines derivatives have been prepared and fully assigned by analytical and spectroscopic methods. From the result of pharmacological activity, we can conclude that integration of *p*-nitro phenyl substituted and *N*-acylated pyrazoline into the pyrazole moiety is fruitful as the compounds **5e**, **5f**, **5h**, **5i**, **6d** and **6f** are found to show potent anti-inflammatory activity and **5g**, **5h**, **6f**, **6c** and **6d** as analgesic agents. In addition to this, it is clear from our SAR results that there is a gradual increase in the anti-inflammatory activity with the increase the length of *N*-acylated substituent groups. Most importantly, substitution with a 4-halo group on aryl ring at the C-3 position of the pyrazoline ring (**4a**, **5g**, **5h**, **6a** and **6b**) enhances the potency effectively in some compounds. Finally compound **5h** is identified as an efficient and biologically active member within this study with an interesting dual anti-inflammatory and antibacterial profile.

However, further structural modification is planned to increase the analgesic and anti-inflammatory activities. These results further confirm that the suitable incorporation of the different structural elements into a new single chemical entity enables an achievement of higher inhibitory potency and selectivity. It was concluded that, there are ample scopes for further study in developing these structures as efficient lead compounds.

4. Experimental section

4.1. Materials and methods

All the reagents and solvents were purchased from commercial suppliers Sigma–Aldrich, Spectrochem India and used without further purification. All the solvents were dried and distilled before use. Melting points were determined in open capillary tubes and are uncorrected. The progress of each reaction was monitored by ascending thin layer chromatography (TLC) on silica gel G (Merck 1.05570.0001), visualized by UV light. The IR spectra (in KBr pellets) were recorded on a Shimadzu-FTIR spectrometer and the wave numbers were given in cm^{-1}. The ^1H NMR, ^{13}C NMR spectra were recorded in $CDCl_3$/DMSO-d_6 solvent on a Bruker AMX 400 NMR spectrometer with 5 mm PABBO BB-1H TUBES with TMS as internal standard. LC-MS was obtained using Agilent 1200 series LC and Micromass zQ spectrometer. Mass spectra of some compounds were recorded on a Jeol SX-102 (FAB) mass spectrometer. Elemental analyses were carried out using VARIO EL-III (Elementar Analysensysteme GmBH).

4.1.1. Preparation of (3-(3,4-dichlorophenyl)-1-phenyl-1H-pyrazole-4-carbaldehyde (2)

(3,4-dichlorophenyl)ethanone phenylhydrazone (**1**) (2.79 g, 0.01 mol) was added in a mixture of the Vilsmeier-Haack reagent (prepared by dropwise addition of $POCl_3$ (1.2 mL) in ice cooled in dimethyl formamide (10 mL)) and refluxed for 6 h. The reaction mixture was poured into crushed ice followed by neutralization using sodium bicarbonate. The solid product obtained was filtered off and recrystallized from 30 mL ethanol to give (**2**), Pale brown amorphous solid, yield (2.63 g, 83%); FT-IR (KBr) ν_{max} (cm^{-1}): 3041 (Ar C–H), 1678 (C=O), 1571 (C=N), 1489 (C=C), 813 (C–Cl); ^1H NMR (DMSO-d_6, 400 MHz, δ ppm): 7.42–7.59 (m, 5H, Ring A–H), 7.74 (d, 1H, Ring B–H, J = 8.4 Hz), 7.97 (dd, 1H, Ring B–H, J = 8.4, 2.0 Hz), 8.27 (d, 1H, Ring B–H, J = 2.0 Hz), 9.38 (s, 1H, pyrazole-H), 9.97 (s, 1H, CHO); ^{13}C NMR (DMSO-d_6, 100 MHz, δ ppm): 119.7, 122.7, 127.4, 128.4, 129.1, 130.2, 130.6, 131.2, 131.7, 132.3, 137.1, 138.8, 149.9, 184.8 (C=O); MS (m/z, %): 317.0 ($C_{16}H_{10}^{35}Cl_2N_2O$ + H, 99%), 319.0 ($C_{16}H_{10}^{35}Cl^{37}ClN_2O$ + H, 60%), 321.2 ($C_{16}H_{10}^{37}Cl_2N_2O$ + H, 11%); Anal. Calcd. for $C_{16}H_{10}Cl_2N_2O$: C, 60.59; H, 3.18; N, 8.83. Found: C, 60.56; H, 3.13; N, 8.86.

4.1.2. General procedure for the synthesis of chalcones (3a–c)

To a stirred solution of 3-(3,4-dichlorophenyl)-1-phenyl-1H-pyrazole-4-carbaldehyde (**2**) (3.17 g, 0.01 mol) and 4-substituted acetophenones (0.01 mol) in 30 mL ethanol was added an aqueous solution of 15 mL 40% KOH. The mixture was kept overnight at room temperature. It was poured into crushed ice and acidified with concentrated HCl. The product obtained was filtered, washed with water and recrystallized from proper solvent.

*4.1.2.1. (2E)-1-(4-chlorophenyl)-3-[3-(3,4-dichlorophenyl)-1-phenyl-1H-pyrazol-4-yl]prop-2-en-1-one (**3a**).* Pale brown amorphous solid, Yield (3.31 g, 73%); FT-IR (KBr) ν_{max} (cm^{-1}): 2960 (Ar C–H), 1670 (C=O), 1602 (C=N), 1533 (C=C), 839 (C–Cl); ^1H NMR (DMSO-d_6, 400 MHz, δ ppm): 7.38 (d, 1H, H_7, J = 14.4 Hz), 7.53–7.62 (m, 6H, Ring A–H, H_6), 7.77 (d, 1H, Ring B–H, J = 8.4 Hz), 7.84 (dd, 1H, Ring B–H, J = 8.4, 1.6 Hz), 8.02 (d, 2H, Ring C–H, J = 8.0 Hz), 8.11 (d, 1H, Ring B–H, J = 1.6 Hz), 8.28 (d, 2H, Ring C–H, J = 8.0 Hz), 9.06 (s, 1H, pyrazole-H); ^{13}C NMR ($CDCl_3$, 100 MHz, δ ppm): 118.7, 121.2, 126.8, 127.2, 127.6, 128.5, 128.9, 129.8, 130.3, 130.7, 131.2, 131.6, 132.4, 133.2, 134.0, 134.3, 138.0, 138.8, 151.6, 187.4 (C=O); MS (m/z, %): 453.2 ($C_{24}H_{15}^{35}Cl_3N_2O$ + H, 100%), 455.2 ($C_{24}H_{15}^{35}Cl_2^{37}ClN_2O$ + H, 98%), 457.2 ($C_{24}H_{15}^{35}Cl^{37}Cl_2N_2O$ + H, 37%), 459.2 ($C_{24}H_{15}^{37}Cl_3N_2O$ + H, 4%); Anal. Calcd. for $C_{24}H_{15}Cl_3N_2O$: C, 63.53; H, 3.33; N, 6.17. Found: C, 63.56; H, 3.30; N, 6.16.

*4.1.2.2. (2E)-3-[3-(3,4-dichlorophenyl)-1-phenyl-1H-pyrazol-4-yl]-1-(4-fluorophenyl)prop-2-en-1-one (**3b**).* White crystalline solid, Yield (3.49 g, 80%); m.p. 133–136 °C (DMF); FT-IR (KBr) ν_{max} (cm^{-1}): 2987 (Ar C–H), 1665 (C=O), 1600 (C=N), 1523 (C=C), 823 (C–Cl); ^1H NMR ($CDCl_3$, 400 MHz, δ ppm): 7.16 (t, 2H, Ring C–H, J = 8.6 Hz), 7.33 (d, 1H, H_7, J = 15.6 Hz), 7.50–7.57 (m, 6H, Ring A–H, H_6), 7.78–7.84 (m, 2H, Ring B–H), 7.86 (d, 1H, Ring B–H, J = 1.6 Hz), 7.99–8.03 (m, 2H, Ring C–H), 8.35 (s, 1H, pyrazole-H); ^{13}C NMR ($CDCl_3$, 100 MHz, δ ppm): 115.9, 119.4, 121.8, 127.1, 127.6, 127.9, 129.7, 130.4, 130.7, 130.9, 131.0, 132.3, 133.0, 133.1, 134.3, 134.6, 139.2, 151.2, 168.2 (C–F), 188.2 (C=O); MS (m/z, %): 437.0 ($C_{24}H_{15}^{35}Cl_2FN_2O$ + H, 95%), 439.0 ($C_{24}H_{15}^{35}Cl^{37}ClFN_2O$ + H, 64%), 441.0 ($C_{24}H_{15}^{37}Cl_2FN_2O$ + H, 10%). Anal. Calcd. for $C_{24}H_{15}Cl_2FN_2O$: C, 65.92; H, 3.46; N, 6.41. Found: C, 65.90; H, 3.49; N, 6.47.

*4.1.2.3. (2E)-3-[3-(3,4-dichlorophenyl)-1-phenyl-1H-pyrazol-4-yl]-1-(4-methylphenyl)prop-2-en-1-one (**3c**).* Light cream amorphous solid, Yield (3.81 g, 88%); FT-IR (KBr) ν_{max} (cm^{-1}): 2987 (Ar C–H), 1650 (C=O),1599 (C=N), 1511 (C=C), 831 (C–Cl); ^1H NMR (DMSO-d_6, 400 MHz, δ ppm): 2.41 (s, 3H, Ring C–CH_3), 7.39 (d, 2H, Ring C–H, J = 8.3 Hz), 7.45 (d, 1H, H_7, J = 14.8 Hz), 7.63 (d, 1H, H_6, J = 14.8 Hz), 7.72–7.87 (m, 5H, Ring A–H), 7.92 (d, 1H, Ring B–H, J = 8.6 Hz), 8.01 (dd, 1H, Ring B–H, J = 8.6, 1.8 Hz), 8.16 (d, 2H, Ring C–H, J = 8.2 Hz), 8.25 (d, 1H, Ring B–H, J = 1.8 Hz), 9.18 (s, 1H, pyrazole-H); ^{13}C NMR ($CDCl_3$, 100 MHz, δ ppm): 21.1 (CH_3), 119.0, 122.4, 126.8, 127.3, 128.0, 129.2, 129.5, 129.8, 130.4, 130.8, 131.1, 131.4, 132.6, 133.5, 133.8, 134.6, 138.9, 139.5, 151.6, 188.0 (C=O); MS

(m/z, %): 433.2 ($C_{25}H_{18}^{35}Cl_2N_2O$ + H, 99%), 435.2 ($C_{25}H_{18}^{35}Cl^{37}ClN_2O$ + H, 62%), 437.2 ($C_{25}H_{18}^{37}Cl_2N_2O$ + H, 11%); Anal. Calcd. for $C_{25}H_{18}Cl_2N_2O$: C, 69.29; H, 4.19; N, 6.46. Found: C, 69.25; H, 4.16; N, 6.40.

*4.1.3. General procedures for the synthesis of 3-(3,4-dichlorophenyl)-4-(3-(4-substituted phenyl)-4,5-dihydro-1H-pyrazol-5-yl)-1-phenyl-1H-pyrazoles (**4a**–**c**)*

A mixture of chalcones **3a**–**c** (0.01 mol) and hydrazine hydrate (99%) (0.7 mL, 0.02 mol) were placed in a round bottom flask in 30 mL ethanol and 2–3 drops of glacial acetic acid were added drop wise in ethanol after which the mixture was heated under reflux for 1–2 h. The reaction mixture was cooled to room temperature and the separated solid was collected by filtration, washed with water and recrystallized from 25 mL ethanol.

*4.1.3.1. 4-(3-(4-Chlorophenyl)-4,5-dihydro-1H-pyrazol-5-yl)-3-(3,4-dichlorophenyl)-1-phenyl-1H-pyrazole (**4a**).* White crystalline solid, Yield (3.04 g, 65%); m.p. 100–104 °C (ethanol); FT-IR (KBr) ν_{max} (cm^{-1}): 3207 (NH), 3057 (Ar C–H), 1591 (C=N), 1496 (C=C), 827 (C–Cl); ^{1}H NMR (DMSO-d_6, 400 MHz, δ ppm): 2.92 (dd, 1H, H_a, J_{ab} = 16.4 Hz, J_{ax} = 10.8 Hz), 3.41 (dd, 1H, H_b, J_{ba} = 16.4 Hz, J_{bx} = 7.6 Hz), 5.00 (dd, 1H, H_x, J_{xa} = 10.8 Hz, J_{xb} = 7.6 Hz), 7.32 (t, 1H, Ring A–H, J = 7.2 Hz), 7.41 (d, 2H, Ring C–H, J = 8.4 Hz), 7.50 (t, 2H, Ring A–H, J = 8.0 Hz), 7.61 (d, 2H, Ring C–H, J = 8.4 Hz), 7.68 (d, 1H, Ring B–H, J = 8.6 Hz), 7.71 (s, 1H, NH, D_2O-exchangeable), 7.74 (dd, 1H, Ring B–H, J = 8.4, 2.0 Hz), 7.88 (d, 2H, Ring A–H, J = 7.6 Hz), 8.00 (d, 1H, Ring B–H, J = 2.0 Hz), 8.60 (s, 1H, pyrazole-H); ^{13}C NMR ($CDCl_3$, 100 MHz, δ ppm): 39.8, 54.9 (C-4, C-5 pyrazoline), 118.1, 122.0, 125.5, 126.2, 126.4, 127.9, 128.6, 129.0, 129.8, 130.1, 131.4, 132.1, 132.3, 132.8, 134.0, 138.7, 147.6, 149.1; MS (m/z, %): 467.2 ($C_{24}H_{17}^{35}Cl_3N_4$+H, 100%), 469.2 ($C_{24}H_{17}^{35}Cl_2^{37}ClN_4$+H, 98%), 471.2 ($C_{24}H_{17}^{35}Cl^{37}Cl_2N_4$+H, 24%), 473.2 ($C_{24}H_{17}^{37}Cl_3N_4$+H, 4%); Anal. Calcd. for $C_{24}H_{17}Cl_3N_4$: C, 61.62; H, 3.66; N, 11.98. Found: C, 61.60; H, 3.63; N, 11.96.

*4.1.3.2. 3-(3,4-Dichlorophenyl)-4-(3-(4-fluorophenyl)-4,5-dihydro-1H-pyrazol-5-yl)-1-phenyl-1H-pyrazole (**4b**).* Light brown amorphous solid, Yield (3.11 g, 69%); FT-IR (KBr) ν_{max} (cm^{-1}): 3242 (NH), 2962 (Ar C–H), 1583 (C=N), 1485 (C=C), 1056 (C–F), 842 (C–Cl); ^{1}H NMR (DMSO-d_6, 400 MHz, δ ppm): 2.87 (dd, 1H, H_a, J_{ab} = 16.6 Hz, J_{ax} = 10.6 Hz), 3.42 (dd, 1H, H_b, J_{ba} = 16.6 Hz, J_{bx} = 7.4 Hz), 5.04 (dd, 1H, H_x, J_{xa} = 10.6 Hz, J_{xb} = 7.4 Hz), 7.13 (t, 2H, Ring C–H, J = 8.5 Hz), 7.34–7.50 (m, 5H, Ring A–H), 7.63 (s, 1H, NH, D_2O-exchangeable), 7.70–7.82 (m, 2H, Ring B–H), 7.88 (d, 1H, J = 1.8 Hz, Ring B–H), 7.97–8.04 (m, 2H, Ring C–H), 8.72 (s, 1H, pyrazole-H); ^{13}C NMR ($CDCl_3$, 100 MHz, δ ppm): 39.7, 54.5 (C-4, C-5 pyrazoline), 114.5, 118.2, 122.8, 124.9, 126.0, 127.3, 128.8, 129.7, 130.0, 130.9, 131.9, 132.4, 132.8, 134.3, 139.7, 148.2, 152.8, 166.3 (C–F); MS (m/z, %): 451.2 ($C_{24}H_{17}^{35}Cl_2FN_4$+H, 98%), 453.2 ($C_{24}H_{17}^{35}Cl^{37}ClFN_4$+H, 64%), 455.2 ($C_{24}H_{17}^{37}Cl_2FN_4$+H, 12%); Anal. Calcd. for $C_{24}H_{17}Cl_2FN_4$: C, 63.87; H, 3.80; N, 12.41. Found: C, 63.84; H, 3.79; N, 12.37.

*4.1.3.3. 3-(3,4-Dichlorophenyl)-1-phenyl-4-(3-p-tolyl-4,5-dihydro-1H-pyrazol-5-yl)-1H-pyrazole (**4c**).* Light brown crystalline solid, Yield (3.26 g, 73%); m.p. 129–131 °C (ethanol); FT-IR (KBr) ν_{max} (cm^{-1}): 3213 (NH), 2998 (Ar C–H), 1576 (C=N), 1468 (C=C), 860 (C–Cl); ^{1}H NMR (DMSO-d_6, 400 MHz, δ ppm): 2.32 (s, 3H, Ring C–CH_3), 2.74 (dd, 1H, H_a, J_{ab} = 16.2 Hz, J_{ax} = 10.4 Hz), 3.52 (dd, 1H, H_b, J_{ba} = 16.2 Hz, J_{bx} = 7.6 Hz), 5.08 (dd, 1H, H_x, J_{xa} = 10.4 Hz, J_{xb} = 7.6 Hz), 7.17 (d, 2H, Ring C–H, J = 8.0 Hz), 7.35 (t, 1H, Ring A–H, J = 7.3 Hz), 7.53 (t, 2H, Ring A–H, J = 7.8 Hz), 7.58 (d, 2H, Ring C–H, J = 8.0 Hz), 7.66 (d, 1H, Ring B–H, J = 8.6 Hz), 7.75 (s, 1H, NH, D_2O-exchangeable), 7.80 (dd, 1H, Ring B–H, J = 8.6, 1.8 Hz), 7.92 (d, 2H, Ring A–H, J = 7.8 Hz), 8.10 (d, 1H, Ring B–H, J = 1.8 Hz), 8.63 (s, 1H, pyrazole-H); ^{13}C NMR ($CDCl_3$, 100 MHz, δ ppm): 19.9 (CH_3), 39.8, 54.4 (C-4, C-5 pyrazoline), 118.2, 122.9, 124.8, 126.5, 127.2, 128.1, 128.4, 128.7, 129.0, 130.0, 130.7, 132.5, 132.8, 133.4, 140.1, 141.3, 149.0, 152.3; MS (m/z, %): 447.2 ($C_{25}H_{20}^{35}Cl_2N_4$+H, 95%), 449.2 ($C_{25}H_{20}^{35}Cl^{37}ClN_4$+H, 65%), 451.2 ($C_{25}H_{20}^{37}Cl_2N_4$+H, 10%); Anal. Calcd. for $C_{25}H_{20}Cl_2N_4$: C, 67.12; H, 4.51; N, 12.52. Found: C, 67.07; H, 4.53; N, 12.51.

*4.1.4. General procedures for the synthesis of 3-(3,4-dichlorophenyl)-4-(3-(4-substitutedphenyl)-1-acyl-4,5-dihydro-1H-pyrazol-5-yl)-1-phenyl-1H-pyrazoles (**5a**–**i**)*

A mixture of chalcones **3a**–**c** (0.01 mol) and hydrazine hydrate (99%) (0.4 mL, 0.012 mol) in 20 mL formic acid/acetic acid/propionic acid was heated under reflux for 1 h for formic acid, 3 h for acetic acid and 2 h for propionic acid. The reaction mixture was cooled and poured into 50 mL ice-cold water. The precipitate was collected by filtration and purified by recrystallization from the proper solvent.

*4.1.4.1. 3-(4-Chlorophenyl)-5-(3-(3,4-dichlorophenyl)-1-phenyl-1H-pyrazol-4-yl)-4,5dihydropyrazole -1-carbaldehyde (**5a**).* Cream amorphous solid, Yield (4.46 g, 90%); FT-IR (KBr) ν_{max} (cm^{-1}): 3078 (Ar C–H), 1687 (CHO), 1539 (C=N), 1440 (C=C), 759 (C–Cl); ^{1}H NMR (DMSO-d_6, 400 MHz, δ ppm): 3.08 (dd, 1H, H_a, J_{ab} = 17.6 Hz, J_{ax} = 4.8 Hz), 3.67 (dd, 1H, H_b, J_{ba} = 17.6 Hz, J_{bx} = 11.6 Hz), 5.76 (dd, 1H, H_x, J_{xa} = 4.8 Hz, J_{xb} = 11.6 Hz), 7.31 (t, 1H, Ring A–H, J = 7.6 Hz), 7.40–7.45 (m, 2H, Ring A–H), 7.51 (d, 2H, Ring C–H, J = 8.2 Hz), 7.58 (d, 2H, Ring C–H, J = 8.2 Hz), 7.66 (d, 1H, Ring B–H, J = 8.4 Hz), 7.77 (dd, 1H, Ring B–H, J = 8.1, 2.0 Hz), 7.84 (d, 2H, Ring A–H, J = 8.0 Hz), 7.92 (d, 1H, Ring B–H, J = 2.0 Hz), 8.34 (s, 1H, pyrazole-H), 8.97 (s, 1H, –CHO); ^{13}C NMR ($CDCl_3$, 100 MHz, δ ppm): 39.5, 50.6 (C-4, C-5 pyrazoline), 117.8, 121.6, 124.8, 126.0, 126.8, 127.6, 128.9, 129.4, 129.7, 130.2, 131.7, 132.7, 133.2, 133.6, 134.0, 139.2, 153.1, 158.4, 187.5 (C=O); MS (m/z, %): 495.2 ($C_{25}H_{17}^{35}Cl_3N_4O$ + H, 100%), 497.2 ($C_{25}H_{17}^{35}Cl_2^{37}ClN_4O$ + H, 98%), 499.2 ($C_{25}H_{17}^{35}Cl^{37}Cl_2N_4O$ + H, 36%), 501.2 ($C_{25}H_{17}^{37}Cl_3N_4O$ + H, 4%); Anal. Calcd. for $C_{25}H_{17}Cl_3N_4O$: C, 60.56; H, 3.46; N, 11.30. Found: C, 60.52; H, 3.45; N, 11.27.

*4.1.4.2. 5-(3-(3,4-Dichlorophenyl)-1-phenyl-1H-pyrazol-4-yl)-3-(4-fluorophenyl)-4,5dihydropyrazole -1-carbaldehyde (**5b**).* White crystalline solid, Yield (4.07 g, 85%); m.p. 99–101 °C (acetic acid); FT-IR (KBr) ν_{max} (cm^{-1}): 3053 (Ar C–H), 1692 (CHO), 1567 (C=N), 1486 (C=C), 1158 (C–F), 772 (C–Cl); ^{1}H NMR (DMSO-d_6, 400 MHz, δ ppm): 3.13 (dd, 1H, H_a, J_{ab} = 17.2 Hz, J_{ax} = 4.6 Hz), 3.53 (dd, 1H, H_b, J_{ba} = 17.2 Hz, J_{bx} = 11.4 Hz), 5.45 (dd, 1H, H_x, J_{xa} = 4.6 Hz, J_{xb} = 11.4 Hz), 7.12 (t, 2H, Ring C–H, J = 8.4 Hz), 7.37 (t, 1H, Ring A–H, J = 7.4 Hz), 7.45 (t, 2H, Ring A–H, J = 8.2 Hz), 7.56–7.72 (m, 2H, Ring B–H), 7.80 (d, 2H, Ring A–H, J = 8.2 Hz), 7.89 (d, 1H, Ring B–H, J = 1.8 Hz), 8.04–8.25 (m, 2H, Ring C–H), 8.31 (s, 1H, pyrazole-H), 8.97 (s, 1H, –CHO); ^{13}C NMR ($CDCl_3$, 100 MHz, δ ppm): 40.6, 51.2 (C-4, C-5 pyrazoline), 114.8, 118.3, 121.9, 125.3, 126.7, 127.6, 128.7, 129.8, 130.0, 130.3, 131.8, 132.0, 132.5, 135.1, 139.4, 150.3, 157.6, 166.9 (C–F), 187.0 (C=O); MS (m/z, %): 479.2 ($C_{25}H_{17}^{35}Cl_2FN_4O$ + H, 98%), 481.2 ($C_{25}H_{17}^{35}Cl^{37}ClFN_4O$ + H, 65%), 483.2 ($C_{25}H_{17}^{37}Cl_2FN_4O$ + H, 11%); Anal. Calcd. for $C_{25}H_{17}Cl_2FN_4O$: C, 62.64; H, 3.57; N, 11.69. Found: C, 62.64; H, 3.59; N, 11.68.

*4.1.4.3. 5-(3-(3,4-Dichlorophenyl)-1-phenyl-1H-pyrazol-4-yl)-3-p-tolyl-4,5-dihydropyrazole-1-carbal dehyde (**5c**).* White amorphous solid, Yield (4.13 g, 87%); FT-IR (KBr) ν_{max} (cm^{-1}): 3034 (Ar C–H), 1687 (CHO), 1594 (C=N), 1482 (C=C), 782 (C–Cl); ^{1}H NMR (DMSO-d_6, 400 MHz, δ ppm): 2.35 (s, 3H, Ring C–CH_3), 2.98 (dd, 1H, H_a, J_{ab} = 17.4 Hz, J_{ax} = 4.8 Hz), 3.48 (dd, 1H, H_b, J_{ba} = 17.4 Hz, J_{bx} = 11.3 Hz), 5.73 (dd, 1H, H_x, J_{xa} = 4.8 Hz, J_{xb} = 11.3 Hz), 7.28 (d, 2H, Ring C–H, J = 8.6 Hz), 7.31 (t, 1H, Ring A–H, J = 7.6 Hz), 7.46 (t, 2H,

Ring A–H, J = 8.2 Hz), 7.53 (d, 2H, Ring C–H, J = 8.6 Hz), 7.69 (d, 1H, Ring B–H, J = 8.3 Hz), 7.79–7.83 (m, 1H, Ring B–H), 7.89 (d, 2H, Ring A–H, J = 8.1 Hz), 8.10 (d, 1H, Ring B–H, J = 1.8 Hz), 8.42 (s, 1H, pyrazole-H), 8.97 (s, 1H, –CHO); ^{13}C NMR ($CDCl_3$, 100 MHz, δ ppm): 20.3 (CH_3), 39.7, 50.9 (C-4, C-5 pyrazoline), 120.1, 122.5, 124.3, 126.5, 126.8, 127.3, 128.7, 129.2, 129.4, 130.9, 131.7, 132.8, 133.4, 134.0, 143.5, 145.3, 152.9, 158.2, 188.6 (C=O); MS (m/z, %): 475.2 ($C_{26}H_{20}^{35}Cl_2N_4O$ + H, 96%), 477.2 ($C_{26}H_{20}^{35}Cl\ ^{37}ClN_4O$ + H, 61%), 479.2 ($C_{26}H_{20}^{37}Cl_2N_4O$ + H, 10%); Anal. Calcd. for $C_{26}H_{20}Cl_2N_4O$: C, 65.69; H, 4.24; N, 11.79. Found: C, 65.62; H, 4.23; N, 11.70.

*4.1.4.4. 1-(3-(4-Chlorophenyl)-5-(3-(3,4-dichlorophenyl)-1-phenyl-1H-pyrazol-4-yl)-4,5dihydropyraz ol-1-yl)ethanone (**5d**).* White crystalline solid, Yield (3.92 g, 77%); m.p. 215–218 °C (acetic acid); FT-IR (KBr) ν_{max} (cm^{-1}): 3062 (Ar C–H), 1658 (CO–CH_3), 1568 (C=N), 1492 (C=C), 773 (C–Cl); ^{1}H NMR (DMSO-d_6, 400 MHz, δ ppm): 2.00 (s, 3H, –CO$\underline{CH_3}$), 3.11 (dd, 1H, H_a, J_{ab} = 17.2 Hz, J_{ax} = 4.6 Hz), 3.78 (dd, 1H, H_b, J_{ba} = 17.2 Hz, J_{bx} = 11.2 Hz), 5.74 (dd, 1H, H_x, J_{xa} = 4.6 Hz, J_{xb} = 11.2 Hz), 7.27 (t, 1H, J = 7.6 Hz, H_p Ar-ring A), 7.40–7.43 (m, 2H, Ring A–H), 7.48 (d, 2H, Ring C–H, J = 8.0 Hz), 7.60 (d, 2H, Ring C–H, J = 8.2 Hz), 7.65 (d, 1H, Ring B–H, J = 8.6 Hz), 7.72 (dd, 1H, Ring B–H, J = 8.4, 2.0 Hz), 7.81 (d, 2H, Ring A–H, J = 8.7 Hz), 7.97 (d, 1H, Ring B–H, J = 2.0 Hz), 8.30 (s, 1H, pyrazole-H); ^{13}C NMR ($CDCl_3$, 100 MHz, δ ppm): 20.9 (–CO$\underline{CH_3}$), 41.4, 51.6 (C-4, C-5 pyrazoline), 118.0, 122.6, 126.1, 126.3, 126.6, 128.0, 128.8, 129.2, 129.4, 129.8, 131.3, 132.4, 132.7, 133.0, 134.3, 138.9, 148.1, 149.8, 167.1 (C=O); MS (m/z, %): 509.2 ($C_{26}H_{19}^{35}Cl_3N_4O$ + H, 100%), 511.2 ($C_{26}H_{19}^{35}Cl_2^{37}ClN_4O$ + H, 98%), 513.2 ($C_{26}H_{19}^{35}Cl^{37}Cl_2N_4O$ + H, 38%), 515.2 ($C_{26}H_{19}^{37}Cl_3N_4O$ + H, 4%); Anal. Calcd. for $C_{26}H_{19}Cl_3N_4O$: C, 61.25; H, 3.76; N, 10.99. Found: C, 61.21; H, 3.71; N, 10.96.

*4.1.4.5. 1-(5-(3-(3,4-Dichlorophenyl)-1-phenyl-1H-pyrazol-4-yl)-3-(4-fluorophenyl)-4,5-dihydropyra zol-1-yl)ethanone (**5e**).* White crystalline solid, Yield (2.92 g, 59%); m.p. 190–192 °C (acetic acid); FT-IR (KBr) ν_{max} (cm^{-1}): 3047 (Ar C–H), 1652 (CO–CH_3), 1589 (C=N), 1498 (C=C), 1141 (C–F), 765 (C–Cl); ^{1}H NMR (DMSO-d_6, 400 MHz, δ ppm): 2.08 (s, 3H, –CO$\underline{CH_3}$), 3.01 (dd, 1H, H_a, J_{ab} = 17.4 Hz, J_{ax} = 4.2 Hz), 3.62 (dd, 1H, H_b, J_{ba} = 17.4 Hz, J_{bx} = 11.4 Hz), 5.65 (dd, 1H, H_x, J_{xa} = 4.2 Hz, J_{xb} = 11.4 Hz), 7.12 (t, 2H, Ring C–H, J = 8.6 Hz), 7.30 (t, 1H, Ring A–H, J = 7.4 Hz), 7.46 (t, 2H, Ring A–H, J = 8.4 Hz), 7.53–7.66 (m, 2H, Ring B–H), 7.78 (d, 2H, Ring A–H, J = 8.4 Hz), 7.87 (d, 1H, Ring B–H, J = 1.6 Hz), 8.02–8.30 (m, 2H, Ring C–H), 8.43 (s, 1H, pyrazole-H); ^{13}C NMR ($CDCl_3$, 100 MHz, δ ppm): 21.9 (–CO$\underline{CH_3}$), 42.2, 51.6 (C-4, C-5 pyrazoline), 115.7, 118.2, 122.7, 125.1, 126.8, 127.5, 128.5, 129.4, 129.7, 130.1, 131.6, 132.0, 132.7, 134.8, 139.2, 148.4, 153.0, 164.4 (C–F), 167.6 (C=O); MS (m/z, %): 493.2 ($C_{26}H_{19}^{35}Cl_2FN_4O$ + H, 97%), 495.2 ($C_{26}H_{19}^{35}Cl^{37}ClFN_4O$ + H, 64%), 497.2 ($C_{26}H_{19}^{37}Cl_2FN_4O$ + H, 12%); Anal. Calcd. for $C_{26}H_{19}Cl_2FN_4O$: C, 63.30; H, 3.88; N, 11.36. Found: C, 63.34; H, 3.86; N, 11.33.

*4.1.4.6. 1-(5-(3-(3,4-Dichlorophenyl)-1-phenyl-1H-pyrazol-4-yl)-3-p-tolyl-4,5-dihydropyrazol-1-yl) ethanone (**5f**).* Colourless amorphous solid, Yield (3.42 g, 70%); FT-IR (KBr) ν_{max} (cm^{-1}): 3057 (Ar C–H), 1643 (CO–CH_3), 1562 (C=N), 1508 (C=C), 825 (C–Cl); ^{1}H NMR ($CDCl_3$, 400 MHz, δ ppm): 2.05 (s, 3H, –CO$\underline{CH_3}$), 2.44 (s, 3H, Ring C–CH_3), 3.04 (dd, 1H, H_a, J_{ab} = 17.2 Hz, J_{ax} = 4.4 Hz), 3.62 (dd, 1H, H_b, J_{ba} = 17.2 Hz, J_{bx} = 11.6 Hz), 5.79 (dd, 1H, H_x, J_{xa} = 4.4 Hz, J_{xb} = 11.6 Hz), 7.20 (d, 2H, Ring C–H, J = 7.6 Hz), 7.29 (t, 1H, Ring A–H, J = 7.4 Hz), 7.42 (t, 2H, Ring A–H, J = 8.0 Hz), 7.47 (d, 1H, Ring B–H, J = 8.0 Hz), 7.56 (d, 2H, Ring C–H, J = 8.0 Hz), 7.64–7.67 (m, 3H, Ring A–H, Ring B–H), 7.89 (d, 1H, Ring B–H, J = 2.0 Hz), 8.38 (s, 1H, pyrazole-H); ^{13}C NMR ($CDCl_3$, 100 MHz, δ ppm): 20.7 (CH_3), 22.0 (–CO$\underline{CH_3}$), 42.2, 51.9 (C-4, C-5 pyrazoline), 119.2, 122.9, 126.1, 126.5, 126.8, 127.6, 128.3, 129.4, 129.6, 130.2, 130.5, 132.2, 132.7, 133.2, 139.6, 140.9, 148.0, 154.3, 169.0 (C=O); MS (m/z, %): 489.2 ($C_{27}H_{22}^{35}Cl_2N_4O$ + H, 98%), 491.2 ($C_{27}H_{22}^{35}Cl^{37}ClN_4O$ + H, 62%), 493.2 ($C_{27}H_{22}^{37}Cl_2N_4O$ + H, 11%); Anal. Calcd. for $C_{27}H_{22}Cl_2N_4O$: C, 66.26; H, 4.53; N, 11.45. Found: C, 66.29; H, 4.52; N, 11.45.

*4.1.4.7. 1-(3-(4-Chlorophenyl)-5-(3-(3,4-dichlorophenyl)-1-phenyl-1H-pyrazol-4-yl)-4,5dihydropyraz ol-1-yl)propan-1-one (**5g**).* White amorphous solid, Yield (3.56 g, 68%); FT-IR (KBr) ν_{max} (cm^{-1}): 2971 (Ar C–H), 1653 (CO– CH_2–CH_3), 1576 (C=N), 1499 (C=C), 762 (C–Cl); ^{1}H NMR (DMSO-d_6, 400 MHz, δ ppm): 1.16 (t, 3H, –$COCH_2\underline{CH_3}$, J = 7.6 Hz), 2.13 (q, 2H, –$CO\underline{CH_2}CH_3$, J = 7.6 Hz), 3.12 (dd, 1H, H_a, J_{ab} = 17.4 Hz, J_{ax} = 4.4 Hz), 3.65 (dd, 1H, H_b, J_{ba} = 17.4 Hz, J_{bx} = 11.8 Hz), 5.56 (dd, 1H, H_x, J_{xa} = 4.4 Hz, J_{xb} = 11.8 Hz), 7.26 (t, 1H, Ring A–H, J = 7.8 Hz), 7.42–7.45 (m, 2H, Ring A–H), 7.54 (d, 2H, Ring C–H, J = 8.3 Hz), 7.61 (d, 2H, Ring C–H, J = 8.3 Hz), 7.69 (d, 1H, Ring B–H, J = 8.4 Hz), 7.75 (dd, 1H, Ring B–H, J = 8.0, 2.0 Hz), 7.86 (d, 2H, Ring A–H, J = 8.0 Hz), 7.98 (d, 1H, Ring B–H, J = 2.0 Hz), 8.30 (s, 1H, pyrazole-H); ^{13}C NMR ($CDCl_3$, 100 MHz, δ ppm): 8.8 (–$COCH_2\underline{CH_3}$), 27.3 (–$CO\underline{CH_2}CH_3$), 41.6, 51.3 (C-4, C-5 pyrazoline), 117.9, 121.7, 125.0, 126.6, 126.8, 127.7, 128.9, 129.2, 129.7, 130.2, 131.5, 132.4, 132.7, 133.3, 133.9, 138.8, 147.1, 148.6, 166.5 (C=O); MS (m/z, %): 523.2 ($C_{27}H_{21}^{35}Cl_3N_4O$ + H, 100%), 525.2 ($C_{27}H_{21}^{35}Cl_2^{37}ClN_4O$ + H, 98%), 527.2 ($C_{27}H_{21}^{35}Cl^{37}Cl_2N_4O$ + H, 36%), 529.2 ($C_{27}H_{21}^{37}Cl_3N_4O$ + H, 4%); Anal. Calcd. for $C_{27}H_{21}Cl_3N_4O$: C, 61.91; H, 4.04; N, 10.70. Found: C, 61.92; H, 4.06; N, 10.69.

*4.1.4.8. 1-(5-(3-(3,4-Dichlorophenyl)-1-phenyl-1H-pyrazol-4-yl)-3-(4-fluorophenyl)-4,5-dihydropyraz ol-1-yl)propan-1-one (**5h**).* White amorphous solid, Yield (4.67 g, 92%); FT-IR (KBr) ν_{max} (cm^{-1}): 2960 (Ar C–H), 1670 (CO–CH_2–CH_3), 1581 (C=N), 1481 (C=C), 1178 (C–F), 756 (C–Cl); ^{1}H NMR ($CDCl_3$, 400 MHz, δ ppm): 1.18 (t, 3H, –$COCH_2\underline{CH_3}$, J = 7.4 Hz), 2.79 (q, 2H, –$CO\underline{CH_2}CH_3$, J = 7.4 Hz), 3.01 (dd, 1H, H_a, J_{ab} = 17.4 Hz, J_{ax} = 4.8 Hz), 3.59 (dd, 1H, H_b, J_{ba} = 17.4 Hz, J_{bx} = 11.6 Hz), 5.79 (dd, 1H, H_x, J_{xa} = 4.8 Hz, J_{xb} = 11.6 Hz), 7.07 (t, 2H, Ring C–H, J = 8.6 Hz), 7.30 (t, 1H, Ring A–H, J = 7.6 Hz), 7.42 (t, 2H, Ring A–H, J = 8.0 Hz), 7.46 (d, 1H, Ring B–H, J = 8.4 Hz), 7.62–7.68 (m, 3H, Ring B–H, Ring C–H), 7.79 (d, 2H, Ring A–H, J = 8.0 Hz), 7.83 (d, 1H, Ring B–H, J = 2.0 Hz), 8.29 (s, 1H, pyrazole-H); ^{13}C NMR ($CDCl_3$, 100 MHz, δ ppm): 9.0 (–$COCH_2\underline{CH_3}$), 27.7 (–$CO\underline{CH_2}CH_3$), 41.8, 52.1 (C-4, C-5 pyrazoline), 115.8, 119.2, 122.9, 126.2, 126.8, 127.7, 128.6, 129.4, 130.3, 130.5, 132.3, 132.8, 133.2, 134.0, 139.6, 148.0, 151.8, 168.5 (C–F), 170.2 (C=O); MS (m/z, %): 507.2 ($C_{27}H_{21}^{35}Cl_2FN_4O$ + H, 96%), 509.2 ($C_{27}H_{21}^{35}Cl^{37}ClFN_4O$ + H, 63%), 511.2 ($C_{27}H_{21}^{37}Cl_2FN_4O$ + H, 12%); Anal. Calcd. for $C_{27}H_{21}Cl_2FN_4O$: C, 63.91; H, 4.17; N, 11.04. Found: C, 63.84; H, 4.15; N, 11.01.

*4.1.4.9. 1-(5-(3-(3,4-Dichlorophenyl)-1-phenyl-1H-pyrazol-4-yl)-3-p-tolyl-4,5-dihydropyrazol-1-yl) propan-1-one (**5i**).* White amorphous solid, Yield (3.47 g, 69%); FT-IR (KBr) ν_{max} (cm^{-1}): 2958 (Ar C–H), 1655 (CO–CH_2–CH_3), 1538 (C=N), 1492 (C=C), 738 (C–Cl); ^{1}H NMR (DMSO-d_6, 400 MHz, δ ppm): 1.05 (t, 3H, –$COCH_2\underline{CH_3}$, J = 7.6 Hz), 2.07 (q, 2H, –$CO\underline{CH_2}CH_3$, J = 7.6 Hz), 2.70 (s, 3H, Ring C–CH_3), 3.20 (dd, 1H, H_a, J_{ab} = 18.0 Hz, J_{ax} = 5.4 Hz), 3.81 (dd, 1H, H_b, J_{ba} = 18.0 Hz, J_{bx} = 12.2 Hz), 5.64 (dd, 1H, H_x, J_{xa} = 5.4 Hz, J_{xb} = 12.2 Hz), 7.17 (d, 2H, Ring C–H, J = 8.8 Hz), 7.30 (t, 1H, Ring A–H, J = 7.4 Hz), 7.47 (t, 2H, Ring A–H, J = 8.0 Hz), 7.51 (d, 2H, Ring C–H, J = 8.8 Hz), 7.70 (d, 1H, Ring B–H, J = 8.4 Hz), 7.74–7.76 (m, 1H, Ring B–H), 7.86 (d, 2H, Ring A–H, J = 7.6 Hz), 8.00 (d, 1H, Ring B–H, J = 1.6 Hz), 8.46 (s, 1H, pyrazole-H); ^{13}C NMR ($CDCl_3$, 100 MHz, δ ppm): 8.9 (–$COCH_2\underline{CH_3}$), 21.5 (CH_3), 27.5 (–$CO\underline{CH_2}CH_3$), 41.7, 52.0 (C-4, C-5 pyrazoline), 119.6, 123.2, 125.9, 126.2, 126.8, 127.5, 128.6, 129.3, 129.7, 130.6, 131.0, 132.4, 133.1, 133.5, 140.0, 141.3, 149.2, 153.9, 168.3 (C=O); MS (m/z, %): 503.2 ($C_{28}H_{24}^{35}Cl_2N_4O$ + H, 97%), 505.2 ($C_{28}H_{24}^{35}Cl^{37}ClN_4O$ + H, 63%), 507.2 ($C_{28}H_{24}^{37}Cl_2N_4O$ + H, 11%);

Anal. Calcd. for $C_{28}H_{24}Cl_2N_4O$: C, 66.80; H, 4.81; N, 11.13. Found: C, 66.75; H, 4.79; N, 11.09.

*4.1.5. General procedures for the synthesis of 3-(3,4-dichlorophenyl)-4-(3-(4-substitutedphenyl)-1-aryl-4,5-dihydro-1H-pyrazol-5-yl)-1-phenyl-1H-pyrazoles (**6a**–**f**)*

A mixture of chalcones **3a**–**c** (0.01 mol) and phenyl hydrazine (or *p*-nitrophenyl hydrazine) (0.01 mol) in 20 mL glacial acetic acid was refluxed for 8 h. The reaction mixture was cooled and poured into ice-cold water. The precipitate was collected by filtration and purified by recrystallization from the proper solvent.

*4.1.5.1. 4-(3-(4-Chlorophenyl)-1-phenyl-4,5-dihydro-1H-pyrazol-5-yl)-3-(3,4-dichlorophenyl)-1-phenyl-1H-pyrazole (**6a**).* Light brown crystalline solid, Yield (4.51 g, 83%); m.p. 153–155 °C (ethanol); FT-IR (KBr) ν_{max} (cm^{-1}): 3064 (Ar C–H), 1598 (C=N), 1477 (C=C), 765 (C–Cl); ^{1}H NMR (DMSO-d_6, 400 MHz, δ ppm): 3.20 (dd, 1H, H_a, J_{ab} = 17.4 Hz, J_{ax} = 7.6 Hz), 3.93 (dd, 1H, H_b, J_{ba} = 17.4 Hz, J_{bx} = 12.8 Hz), 5.61 (dd, 1H, H_x, J_{xa} = 7.6 Hz, J_{xb} = 12.8 Hz), 6.74 (t, 1H, Ring D–H, J = 7.2 Hz), 7.06 (d, 2H, Ring D–H, J = 8.0 Hz), 7.15 (d, 2H, Ring D–H, J = 7.8 Hz), 7.30 (t, 1H, Ring A–H, J = 7.2 Hz), 7.46–7.48 (m, 4H, Ring C–H, Ring A–H), 7.68–7.72 (m, 4H, Ring C–H, Ring B–H), 7.83 (d, 2H, Ring A–H, J = 8.0 Hz), 7.88 (d, 1H, Ring B–H, J = 2.0 Hz), 8.45 (s, 1H, pyrazole-H); ^{13}C NMR ($CDCl_3$, 100 MHz, δ ppm): 42.7, 56.7 (C-4, C-5 pyrazoline), 113.6, 119.0, 119.9, 122.9, 126.9, 127.0, 128.8, 129.0, 129.1, 129.4, 129.7, 129.8, 130.7, 130.9, 132.4, 133.0, 133.1, 134.6, 139.4, 144.4, 146.0, 147.4; MS (m/z, %): 543.2 ($C_{30}H^{35}_{21}Cl_3N_4$+H, 100%), 545.2 ($C_{30}H^{35}_{21}Cl^{37}_2ClN_4$+H, 85%), 547.2 ($C_{30}H^{35}_{21}Cl^{37}Cl_2N_4$+H, 26%), 549.2 ($C_{30}H^{37}_{21}Cl_3N_4$+H, 4%); Anal. Calcd. for $C_{30}H_{21}Cl_3N_4$: C, 66.25; H, 3.89; N, 10.30. Found: C, 66.20; H, 3.91; N, 10.28.

*4.1.5.2. 3-(3,4-Dichlorophenyl)-4-(3-(4-fluorophenyl)-1-phenyl-4,5-dihydro-1H-pyrazol-5-yl)-1-phenyl-1H-pyrazole (**6b**).* Light brown crystalline solid, Yield (4.64 g, 88%); m.p. 98–100 °C (ethanol); FT-IR (KBr) ν_{max} (cm^{-1}): 3042 (Ar C–H), 1569 (C=N), 1492 (C=C), 1053 (C–F), 762 (C–Cl); ^{1}H NMR (DMSO-d_6, 400 MHz, δ ppm): 3.28 (dd, 1H, H_a, J_{ab} = 17.3 Hz, J_{ax} = 7.7 Hz), 3.93 (dd, 1H, H_b, J_{ba} = 17.3 Hz, J_{bx} = 12.0 Hz), 5.63 (dd, 1H, H_x, J_{xa} = 7.7 Hz, J_{xb} = 12.0 Hz), 6.92 (t, 1H, Ring D–H, J = 7.4 Hz), 7.03 (d, 2H, Ring D–H, J = 8.2 Hz), 7.10 (t, 2H, Ring C–H, J = 8.6 Hz), 7.18 (d, 2H, Ring D–H, J = 8.2 Hz), 7.28 (t, 1H, Ring A–H, J = 7.4 Hz), 7.49 (t, 2H, Ring A–H, J = 8.2 Hz), 7.56–7.72 (m, 2H, Ring B–H), 7.80 (d, 2H, Ring A–H, J = 8.2 Hz), 7.87 (d, 1H, Ring B–H, J = 2.0 Hz), 8.12–8.33 (m, 2H, Ring C–H), 8.48 (s, 1H, pyrazole-H); ^{13}C NMR ($CDCl_3$, 100 MHz, δ ppm): 41.3, 55.6 (C-4, C-5 pyrazoline), 113.4, 115.6, 119.5, 120.2, 122.7, 125.1, 126.3, 127.2, 128.5, 129.3, 129.7, 129.9, 130.0, 131.2, 131.6, 133.0, 134.8, 139.7, 143.8, 150.0, 152.2, 165.2 (C–F); MS (m/z, %): 527.2 ($C_{30}H^{35}_{21}Cl_2FN_4$+H, 98%), 529.2 ($C_{30}H^{35}_{21}Cl^{37}ClFN_4$+H, 61%), 531.2 ($C_{30}H^{37}_{21}Cl_2FN_4$+H, 12%); Anal. Calcd. for $C_{30}H_{21}Cl_2FN_4$: C, 68.32; H, 4.01; N, 10.62. Found: C, 68.33; H, 3.99; N, 10.61.

*4.1.5.3. 3-(3,4-Dichlorophenyl)-1-phenyl-4-(1-phenyl-3-p-tolyl-4,5-dihydro-1H-pyrazol-5-yl)-1H-pyrazole (**6c**).* Light brown crystalline solid, Yield (3.82 g, 73%); m.p. 166–168 °C (ethanol); FT-IR (KBr) ν_{max} (cm^{-1}): 3039 (Ar C–H), 1598 (C=N), 1496 (C=C), 769 (C–Cl); ^{1}H NMR ($CDCl_3$, 400 MHz, δ ppm): 2.37 (s, 3H, Ring C–CH_3), 3.16 (dd, 1H, H_a, J_{ab} = 16.4 Hz, J_{ax} = 6.6 Hz), 3.81 (dd, 1H, H_b, J_{ba} = 16.4 Hz, J_{bx} = 11.8 Hz), 5.43 (dd, 1H, H_x, J_{xa} = 6.6 Hz, J_{xb} = 11.8 Hz), 6.83 (t, 1H, Ring D–H, J = 7.4 Hz), 7.07 (d, 2H, Ring D–H, J = 8.0 Hz), 7.20 (d, 2H, Ring D–H, J = 8.0 Hz), 7.23 (d, 2H, Ring C–H, J = 8.4 Hz), 7.39 (t, 1H, Ring A–H, J = 7.6 Hz), 7.55 (d, 2H, Ring A–H, J = 8.4 Hz), 7.60–7.65 (m, 4H, Ring C–H, Ring B–H), 7.81 (d, 2H, Ring A–H, J = 8.4 Hz), 7.93 (d, 1H, Ring B–H, J = 2.0 Hz), 8.58 (s, 1H, pyrazole-H); ^{13}C NMR ($CDCl_3$, 100 MHz, δ ppm): 21.4 (CH_3), 43.0, 56.6 (C-4, C-5 pyrazoline), 113.5, 119.0, 119.5, 123.2, 125.8, 126.8, 126.9, 129.0, 129.3, 129.4, 129.6, 129.7, 130.7, 131.0, 132.3, 133.1, 133.2, 139.0, 139.5, 144.9, 147.4, 152.4; MS (m/z, %): 523.2 ($C_{31}H^{35}_{24}Cl_2N_4$+H, 93%), 525.2 ($C_{31}H^{35}_{24}Cl^{37}ClN_4$+H, 61%), 527.2 ($C_{31}H^{37}_{24}Cl_2N_4$+H, 10%); Anal. Calcd. for $C_{31}H_{24}Cl_2N_4$: C, 71.13; H, 4.62; N, 10.70. Found: C, 71.11; H, 4.63; N, 10.66.

*4.1.5.4. 4-(3-(4-Chlorophenyl)-1-(4-nitrophenyl)-4,5-dihydro-1H-pyrazol-5-yl)-3-(3,4dichlorophenyl) -1-phenyl-1H-pyrazole (**6d**).* Dark brown amorphous solid, Yield (3.94 g, 67%); FT-IR (KBr) ν_{max} (cm^{-1}): 3064 (Ar C–H), 1598 (C=N), 1477 (C=C), 765 (C–Cl); ^{1}H NMR (DMSO-d_6, 400 MHz, δ ppm): 3.32 (dd, 1H, H_a, J_{ab} = 17.0 Hz, J_{ax} = 7.6 Hz), 3.84 (dd, 1H, H_b, J_{ba} = 17.0 Hz, J_{bx} = 11.8 Hz), 5.61 (dd, 1H, H_x, J_{xa} = 7.6 Hz, J_{xb} = 11.8 Hz), 7.27 (t, 1H, Ring A–H, J = 7.4 Hz), 7.42–7.46 (m, 4H, Ring C–H, Ring A–H), 7.65–7.73 (m, 4H, Ring C–H, Ring B–H), 7.85 (d, 2H, Ring A–H, J = 8.2 Hz), 7.90 (d, 1H, Ring B–H, J = 2.0 Hz), 7.97 (d, 2H, Ring D–H, J = 8.8 Hz), 8.30 (d, 2H, Ring D–H, J = 8.9 Hz), 8.46 (s, 1H, pyrazole-H); ^{13}C NMR ($CDCl_3$, 100 MHz, δ ppm): 43.0, 55.9 (C-4, C-5 pyrazoline), 115.8, 119.1, 121.4, 123.0, 127.2, 127.4, 129.0, 129.3, 129.7, 130.0, 130.5, 130.6, 130.9, 132.5, 133.6, 133.9, 136.0, 134.2, 139.2, 144.6, 146.3, 147.8; MS (m/z, %): 588.2 ($C_{30}H^{35}_{20}Cl_3N_5O_2$+H, 100%), 590.2 ($C_{30}H^{35}_{20}Cl^{37}_2ClN_5O_2$+H, 98%), 592.2 ($C_{30}H^{35}_{20}Cl^{37}Cl_2N_5O_2$+H, 35%), 594.2 ($C_{30}H^{37}_{20}Cl_3N_5O_2$+H, 6%); Anal. Calcd. for $C_{30}H_{20}Cl_3N_5O_2$: C, 61.19; H, 3.42; N, 11.89. Found: C, 61.12; H, 3.41; N, 11.83.

*4.1.5.5. 3-(3,4-Dichlorophenyl)-4-(3-(4-fluorophenyl)-1-(4-nitrophenyl)-4,5-dihydro-1H-pyrazol-5-yl) -1-phenyl-1H-pyrazole (**6e**).* Dark brown amorphous solid, Yield (3.90 g, 74%); FT-IR (KBr) ν_{max} (cm^{-1}): 3027 (Ar C–H), 1594 (C=N), 1498 (C=C), 1123 (C–F), 798 (C–Cl); ^{1}H NMR (DMSO-d_6, 400 MHz, δ ppm): 3.18 (dd, 1H, H_a, J_{ab} = 16.4 Hz, J_{ax} = 7.8 Hz), 3.96 (dd, 1H, H_b, J_{ba} = 16.4 Hz, J_{bx} = 11.6 Hz), 5.61 (dd, 1H, H_x, J_{xa} = 7.8 Hz, J_{xb} = 11.6 Hz), 7.12 (t, 2H, Ring C–H, J = 8.6 Hz), 7.25 (t, 1H, Ring A–H, J = 7.2 Hz), 7.48 (t, 2H, Ring A–H, J = 8.4 Hz), 7.60–7.75 (m, 2H, Ring B–H), 7.82 (d, 2H, Ring A–H, J = 8.4 Hz), 7.88 (d, 1H, Ring B–H, J = 2.0 Hz), 8.04–8.26 (m, 4H, Ring C–H, Ring D–H), 8.29 (d, 2H, Ring D–H, J = 8.6 Hz), 8.42 (s, 1H, pyrazole-H); ^{13}C NMR ($CDCl_3$, 100 MHz, δ ppm): 40.3, 55.8 (C-4, C-5 pyrazoline), 115.1, 115.8, 118.9, 122.2, 122.9, 126.0, 126.4, 127.1, 128.8, 129.4, 129.7, 130.3, 131.2, 131.7, 133.1, 135.2, 137.6, 139.9, 144.0, 150.3, 152.5, 166.4 (C–F); MS (m/z, %): 572.2 ($C_{30}H^{35}_{20}Cl_2FN_5O_2$+H, 98%), 574.2 ($C_{30}H^{35}_{20}Cl^{37}ClFN_5O_2$+H, 64%), 575.2 ($C_{30}H^{37}_{20}Cl_2FN_5O_2$+H, 12%); Anal. Calcd. for $C_{30}H_{20}Cl_2FN_5O_2$: C, 62.95; H, 3.52; N, 12.23. Found: C, 62.94; H, 3.53; N, 12.20.

*4.1.5.6. 3-(3,4-Dichlorophenyl)-4-(1-(4-nitrophenyl)-3-p-tolyl-4,5-dihydro-1H-pyrazol-5-yl)-1-phenyl -1H-pyrazole (**6f**).* Dark brown amorphous solid, Yield (5.00 g, 88%); FT-IR (KBr) ν_{max} (cm^{-1}): 3034 (Ar C–H), 1602 (C=N), 1487 (C=C), 786 (C–Cl); ^{1}H NMR (DMSO-d_6, 400 MHz, δ ppm): 2.32 (s, 3H, Ring C–CH_3), 3.36 (dd, 1H, H_a, J_{ab} = 16.8 Hz, J_{ax} = 6.6 Hz), 3.86 (dd, 1H, H_b, J_{ba} = 16.8 Hz, J_{bx} = 12.2 Hz), 5.40 (dd, 1H, H_x, J_{xa} = 6.6 Hz, J_{xb} = 12.2 Hz), 7.21 (d, 2H, Ring C–H, J = 8.5 Hz), 7.40 (t, 1H, Ring A–H, J = 7.8 Hz), 7.53 (d, 2H, Ring A–H, J = 8.6 Hz), 7.62–7.68 (m, 4H, Ring C–H, Ring B–H), 7.93 (d, 2H, Ring A–H, J = 8.6 Hz), 7.97 (d, 1H, Ring B–H, J = 1.8 Hz), 8.05 (d, 2H, Ring D–H, J = 8.7 Hz), 8.29 (d, 2H, Ring D–H, J = 8.8 Hz), 8.52 (s, 1H, pyrazole-H); ^{13}C NMR ($CDCl_3$, 100 MHz, δ ppm): 22.5 (CH_3), 43.5, 56.2 (C-4, C-5 pyrazoline), 114.4, 120.1, 121.9, 123.8, 126.0, 126.6, 126.9, 128.7, 129.2, 129.7, 130.0, 130.5, 131.2, 132.3, 133.4, 133.7, 136.8, 139.6, 140.7, 146.0, 147.7, 151.9; MS (m/z, %): 568.2 ($C_{31}H^{35}_{23}Cl_2N_5O_2$+H, 96%), 570.2 ($C_{31}H^{35}_{23}Cl^{37}ClN_5O_2$+H, 60%), 572.2 ($C_{31}H^{37}_{23}Cl_2N_5O_2$+H, 9%); Anal. Calcd. for $C_{31}H_{23}Cl_2N_5O_2$: C, 65.50; H, 4.08; N, 12.32. Found: C, 65.49; H, 4.07; N, 12.36.

4.2. Anti-inflammatory activity

All the three series of synthesized compounds were evaluated for their anti-inflammatory activity against carrageenan-induced rat paw oedema assay model [29]. Male Wistar rats (200–250 g) were fasted with free access to water at least 12 h prior to the experiments and were divided randomly into different groups (control, standard and the test groups) of five rats each. The first group of rats was treated with 1 mL of 1% gum acacia suspension orally (control), the second group was administered in a dose of 20 mg/kg of the Diclofenac sodium (standard) and the third group was treated with 50 mg/kg of the suspension of the test compounds. After 30 min, the animals were injected with 0.1 mL of 1% carrageenan in normal saline, subcutaneously to the sub-planar region of right hind paw. The paw volume was measured after 30 min, 60 min and 120 min by using Plethysmometer.

4.3. Analgesic activity

The analgesic activity of the above mentioned derivatives were also evaluated by applying the tail flick method using Pentazocine as a standard reference [30]. Male Wistar rats (200–250 g) in the groups of six animals, each one were selected by random sampling technique. The test compounds at a dose level of 50 mg/kg were administered orally by intragastric tube. Pentazocin at a dose level of 20 mg/kg, was administered orally as a reference drug for comparison. The reading was recorded at regular intervals of 0 min, 30 min, 60 min and 90 min after administration of compounds. A cut off point of 10 s was observed to prevent the tail damage.

4.4. Acute toxicity studies

The acute toxicity for all test compounds was carried out in Swiss mice (25–35 g) maintained under standard conditions. The animals were fasted 3 h prior to the experiment and “up and down” (OECD guidelines NO: 425) method of CPCSEA was adopted for toxicity studies [31]. The dosage was varied 1000–100 mg/kg body weight. The toxic symptoms and mortality rates in each group were recorded 48 h after drug administration.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2015.07.002.

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