RSC Advances

ROYAL SOCIETY OF CHEMISTRY

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Cite this: DOI: 10.1039/c0xx00000x

www.rsc.org/xxxxxx

ARTICLE TYPE

Design, Synthesis and characterization of fluoro substituted novel pyrazole nucleus clubbed with 1,3,4-oxadiazole scaffolds and their biological applications

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Received (in XXX, XXX) Xth XXXXXXXXX 20XX, Accepted Xth XXXXXXXXX 20XX
DOI: 10.1039/b000000x

In the current work, novel series of fluoro substituted pyrazole nucleus clubbed with 1,3,4-oxadiazole scaffolds (**7a-p**), were synthesized in good yield (79-89%). The structures of all the compounds were confirmed on the basis of elemental analysis, IR, ^{1}H NMR, and mass spectral data. The newly synthesized compounds were screened for their preliminary *in vitro* antibacterial activity against a panel of pathogenic strains of bacteria and fungi; antituberculosis activity against *Mycobacterium tuberculosis* $H_{37}Rv$ and antimalarial activity against *Plasmodium falciparum*. Compounds **7e, 7o** and **7h** were found to possess promising antibacterial potency. while Compounds **7c**, **7h** and **7j** demonstrated better potency against *M. tuberculosis* $H_{37}Rv$ as compared to that of refampicin. While compound **7b**, **7h**, **7i**, **7l** and **7o** were found to possess excellent activity against *P. falciparum* strain as compared to quinine IC_{50}=0.826µM.

1. Introduction

Malaria is the most lethal human parasitic infection caused by *Plasmodium falciparum* and *Plasmodium vivax*[1]. A number of vaccine candidates are being clinically tested, which may become significant tool for the treatment of malaria in future. However, the first signs of resistance to artemisinin, the first-line antimalarial treatment, have appeared in Southeast Asia.[2-4]. It is therefore important to develop new antimalarial medications with novel modes of action[5]. On the other hand, tuberculosis (TB), a lung infectious disease mostly caused by *Mycobacterium tuberculosis* (MTB), has become worldwide public health problem and is responsible for death of 2–3 million people annually [6, 7]. Moreover, TB repeatedly occurs in HIV/AIDS patients who have a more reduced response to TB treatment. The emergence and distribution of multi-drug resistant (MDR-TB) and extensively drug-resistant (XDR-TB) strains of *Mycobacterium tuberculosis* have become the major challenges in treatment with modern anti-TB drugs. Modern anti-TB drugs also suffer from low tolerability or adverse effects.

Over the past two decades the world population is suffering cruelly with the life threatening infectious disease caused by multidrug-resistant pathogenic bacteria (Gram-positive and Gram-negative bacteria)[8, 9]. Microbial infections are the second most leading death causing diseases after heart attack in the world, due to their impulsive extend, toxicity and resistance towards the available antibiotic drugs.

Owing to such an emergency there is an urgent need for the development of novel drugs with fewer side effects and improved efficacy to cure malaria, tuberculosis (TB) and microbial infections. We have designed and synthesized fluoro substituted pyrazole based 1,3,4-oxadiazole scaffolds. The improvement of hybrid molecules through the combination of diverse pharmacophores in one frame may lead to path way for finding out a better solution.

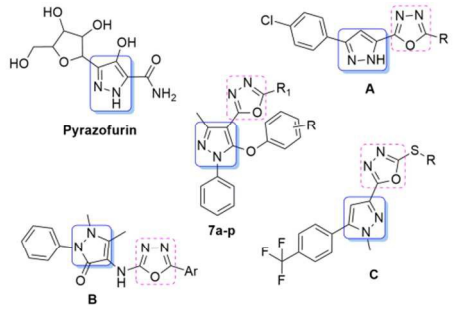


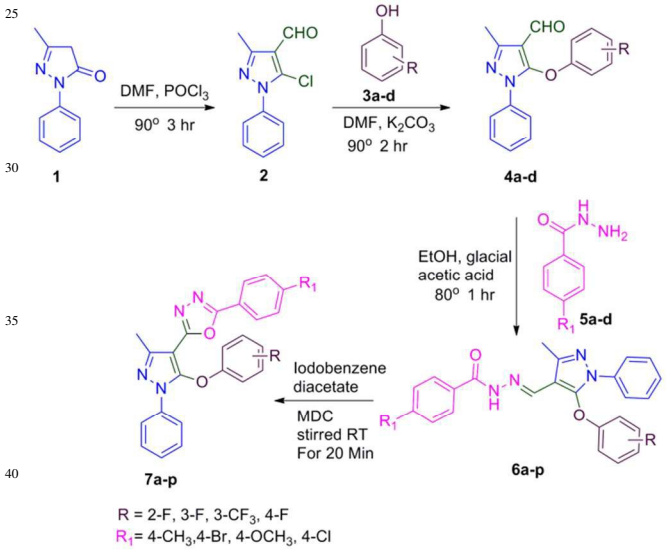
Figure 1. Structures of pyrazofurin and some reported biologically active pyrazole based 1,3,4-oxadiazoles scaffold **A**, **B**, **C** and synthesized compounds **7a–p.**

Pyrazole ring is a ubiquitous core in heterocyclic chemistry and represents a key motif in medicinal chemistry due to their potential to exhibit an array of bioactivities such as antimicrobial[10], anti-inflammatory [11], antipyretic [12], anticancer [13], anti-viral, antitumor [14, 15] , analgesic [16], fungistatic [17], and anti-hyperglycemic activity[18, 19]. 1,3,4-Oxadiazole forms important class of heterocyclic bioactive compounds which have extensive attracted attention, owing to their remarkable biological and pharmacological properties such as antibacterial[20], and anti-tubercular activities [21, 22], anti-inflammatory[23], antifungal [24], antidepressant [25], anti-proliferative[26], anti-anxiety[27]. Moreover,

1,3,4-oxadiazole heterocycles are very good bioisosteres of amides and esters, which contribute substantially to growing pharmacological potency by participating in hydrogen bonding interactions with the receptors. Also several biologically active pyrazofurin and some reported pyrazole based 1,3,4-oxadiazole scaffolds (**Fig. 1A–C**)[21, 28, 29].

2. Chemistry

The synthetic protocol for novel series of fluoro substituted pyrazole bearing 1,3,4-oxadiazole scaffolds was performed as outlined in **Scheme 1.** The starting material 5-chloro-3-methyl-1-phenyl-1H-pyrazole-4-carbaldehyde **2** was prepared according to Vilsmeier–Haack reaction of 3-methyl-1-phenyl-1H-pyrazol-5(4H)-one[30]**.** 3-methyl-5-substituted aryloxy-1-phenyl-1H-pyrazole-4-carbaldehydes **4a-d** were prepared by refluxing compound **2** and fluoro substituted phenols **3a-d** in presence of anhydrous K_2CO_3 as basic catalyst in DMF as solvent. Then these derivatives **4a-d** were treated with 4-substitutedbenzohydrazide **5a-d** in the presence of few drops of glacial acetic acid in ethanol. The mixture was refluxed for 1 h to obtain corresponding hydrazones **6a-p.** The obtained hydrazones **6a-p** were then subjected to oxidative cyclization using phenyliodiodiacetate ($PhI(OAc)_2$) in dichloromethane (MDC) by stirring at room temperature for 20 min to afford corresponding 1,3,4-oxadiazoles **7a-p**.



2.2 Analytical results

The structure of the targeted fluoro substituted pyrazole motifs clubbed with 1,3,4-oxadiazole scaffolds **7a-p** were confirmed by mass spectrometry, ^{1}H NMR, FT-IR and elemental analysis. The mass spectrum of all the compounds showed molecular ion peak (M+) corresponding to their respective molecular weights, which additionally confirmed the molecular frame work. The aromatic region resonates in the range of 6.83-7.92 ppm (Ar-H) as multiplet in ^{1}H NMR spectra of the compounds. In IR spectra, the absorption bands in the range of 1621-1638 cm^{-1} was observed for all the compounds which may be due to –C=N stretching. -C=C- stretching appeared at 1589-1598 cm^{-1}. The absorption around 3051- 3067cm^{-1} is due to aromatic C-H stretching. IR spectra of the synthesized scaffolds exhibited characteristic absorption bands in the range 1213 -1237cm^{-1} due to the presence of ether linkage.

3. Pharmacology

3.1. *In vitro* antimicrobial activity

The antimicrobial activity of the newly synthesized fluoro substituted pyrazole bearing 1,3,4-oxadiazole derivative was carried out by broth micro dilution method according to National Committee for Clinical Laboratory Standards (NCCLS) [31]. Antibacterial activity was screened against three Gram positive (*Bacillus subtilis* MTCC 441, *Clostridium tetani* MTCC 449, and *Streptococcus pneumoniae* MTCC 1936) and three Gram negative (*Salmonella typhi* MTCC 98, *Escherichia coli* MTCC 443, and *Vibrio cholerae* MTCC 3906) bacteria using ampicillin, norfloxacin, chloramphenicol and ciprofloxacin as the standard antibacterial drugs. Antifungal activity was screened against two fungal species (*Aspergillus fumigatus* MTCC 3008 and *Candida albicans* MTCC 227) where nystatin and griseofulvin were used as the standard antifungal drugs. The strains employed for the activity were procured from the Institute of Microbial Technology, Chandigarh (MTCC-Micro Type Culture Collection). Mueller Hinton broth was used as nutrient medium to grow and dilute the drug suspension for the test. DMSO was used as the diluent to get the desired concentration of compounds to test upon the standard bacterial strains. The result of antimicrobial screening data is shown in **Table 1**.

3.2. *In vitro* antituberculosis activity

A primary *in vitro* antituberculosis activity of the newly synthesized fluoro substituted pyrazole bearing 1,3,4-oxadiazole derivatives was conducted at 250 µg/mL against *Mycobacterium tuberculosis* H37Rv strain by using Lowenstein-Jensen medium as described by Rattan[32]. The obtained results are presented in **Table 2** in form of % inhibition. Rifampicin and Isoniazid were used as the standard drugs.

3.3. *In vitro* antimalarial activity

In vitro antimalarial activity of the newly synthesized fluoro substituted pyrazole bearing 1,3,4-oxadiazole derivatives against *P. falciparum strain* was performed using chloroquine and quinine as the reference compounds. The consequences of the antimalarial screening are expressed as the drug concentration resulting in 50% inhibition (IC_{50}) of parasite growth and are listed in **Table 3**.

4.1. Biological section

4.1.1. *In vitro* antibacterial activity

Evaluation of antibacterial data (**Table 1**) revealed that, most of the tested compounds exhibited moderate to excellent antibacterial activity and good to moderate antifungal activity against all the tested microbial strains.

Among them, the compound **7e** (139 µM) and **7o** (102 µM) has exhibited excellent potency against *S. pneumoniae* as compared to ciprofloxacin (150 µM), chloramphenicol (154 µM) and ampicillin (286 µM), while compounds **7f** (230 µM), **7i** (0.282 mM), **7k** (254 µM), **7l** (223 µM) and **7n** (226 µM) displayed comparable activities to that of ampicillin (286 µM).

Table 1. *In vitro* antimicrobial activity (MIC, µM) of compounds **7a-p**

	Gram positive bacteria			*Gram negative bacteria*			*Fungi*	
Compound	S.P. MTCC 1936	C.T. MTCC 449	B.S. MTCC 441	S.T. MTCC 98	V.C. MTCC 3906	E.C. MTCC 443	C.A. MTCC 227	A.F. MTCC 3008
7a	469	**586**	**134**	586	469	469	1173	**104**
7b	1017	**508**	**203**	**203**	508	**203**	**1017**	508
7c	468	**468**	**234**	586	**234**	**146**	2344	1172
7d	524	**524**	**209**	**209**	**131**	**209**	2098	>2098
7e	**139**	**559**	**279**	**139**	447	447	>2237	>2237
7f	**230**	**184**	**115**	**184**	369	461	>1847	**159**
7g	452	**282**	**452**	452	565	452	**1130**	>2260
7h	293	**152**	**508**	508	**223**	293	>2035	>2035
7i	**282**	**565**	**452**	**252**	452	**226**	**1130**	1130
7j	468	**586**	**586**	586	**283**	**234**	>2344	**172**
7k	**254**	**407**	**407**	407	508	508	>2035	**135**
7l	**223**	**279**	**559**	**123**	447	559	>2237	2237
7m	447	**447**	**447**	**223**	**279**	1118	2237	>2237
7n	**226**	**282**	**226**	**282**	565	**265**	**1130**	**130**
7o	**102**	**503**	**201**	503	1006	402	2012	2012
7p	507	**507**	1015	406	1015	**126**	**1015**	>2203
A	286	715	715	286	286	286	n. t.[a]	n. t.
B	154	154	154	154	154	154	n. t.	n. t.
C	150	301	150	75	75	75	n. t.	n. t.
D	31	313	310	31	31	31	n. t.	n. t.
E	n. t.	n. t.	n. t.	n. t.	n. t.	n. t.	107	107
F	n. t.	n. t.	n. t.	n. t.	n. t.	n. t.	1147	283

S.P.: *Streptococcus pneumoniae*, B.S.: *Bacillus subtilis*, C.T.: *Clostridium tetani*, E.C.: *Escherichia coli* S.T.: *Salmonella typhi*, V.C.: *Vibrio cholerae*, C.A.: *Candida albicans*, A.F.: *Aspergillus fumigatus*, MTCC: Microbial Type Culture Collection. A: Ampicillin, B: Chloramphenicol, C: Ciprofloxacin, D: Norfloxacin, E: Nystatin, F: Griseofulvin, [a] n.t.: not tested.

Compound **7h** (152 µM) illustrated superior potency against *C. tetani* as compared to all the standard drugs. Compound **7a** (134 µM) and **7f** (115 µM) exhibited greater activity against *C. tetani* as compared to all the standard drugs. Majority of the compounds displayed excellent activity towards gram positive bacteria i.e *B. subtilis* and *C. tetani* as compared to ampicillin as well as norfloxacin.

In case of gram negative bacteria against *S. typhi*, Compounds **7e** (139 µM) and **7l** (123 µM) demonstrated excellent potency as contrast to that of chloramphenicol (154 µM) as well as ampicillin (286 µM), while compounds **7b** (203 µM), **7d** (209 µM), **7f** (184 µM), **7i** (252 mM), **7m** (223 µM) and **7n** (282 µM) exhibited comparable potency to that of ampicillin (286 µM).

Against *V. cholerae,* compound **7d** (131 µM) showed brilliant activity as compared to chloramphenicol (154 µM) as well as ampicillin (286 µM), while compound **7c** (234 µM), **7h** (223 µM), **7j** (283 mM) and **7m** (279 mM) demonstrate less potency to that of chloramphenicol (154 µM) but they showed comparable potency to hat of ampicillin (286 µM).

The compounds **7c** (146 µM) and **7p** (126 µM) illustrated highest activity in inhibiting gram negative bacteria *E. coli* as compared to chloramphenicol (154 µM) as well as ampicillin (286 µM), while compounds **7b** (203 µM), **7d** (209 µM), **7i** (226 µM), **7j** (234 µM) and **7c** (265 µM) illustrated good potency to that of ampicillin (286 µM).

4.1.2. *In vitro* antifungal activity

Evaluation of antifungal activity revealed that (**Table 1**), all the compounds shown moderate activity against *C. albicans* as compared to standard drugs nystatin as well as griseofulvin. Against *C. albicans*, Compounds **7b** (1017 µM), **7g** (1130 µM), **7i** (1130 µM), **7n** (1130 µM) and **7p** (1015 µM) illustrated good potency as compared to that of griseofulvin (1147 µM) but they were found to be less active as compared to nystatin (107 µM). Compound **7a** (104 µM) showed comparable potency against *A. fumigatus* as that of nystatin (107 µM) but superior than griseofulvin (283 µM). Compounds **7f** (159 µM), **7j** (172 µM), **7k** (135 µM) and **7n** (130 µM) also exhibited better activity against *A. fumigatus* as contrast to that of griseofulvin (283 µM).

4.1.1.2. *In vitro* Antituberculosis activity

Antituberculosis screening of all the synthesized fluoro substituted pyrazole nucleus clubbed with 1,3,4-oxadiazole scaffolds were conducted at 250 µg/mL concentrations against *M. tuberculosis* $H_{37}Rv$ strain.

Compounds **7c**, **7f**, **7h**, **7j**, **7n** and **7o** demonstrated excellent activity i.e. 95%, 91%, 94%, 93%, 88% and 87% at 250 µg/mL respectively against *M. tuberculosis* $H_{37}Rv$ (**Table 2**) as compared to that of refampicin 98%. The remaining compounds disclosed poor inhibition against *M. tuberculosis* growth. From the above results, it can be concluded that compounds **7c**, **7h** and **7j** may become new member of antituberculosis agents in this series.

Table 2. *In vitro* antituberculosis activity (% inhibition) of pyrazole based 1,3,4-oxadiazole derivative against *M. tuberculosis* $H_{37}Rv$ (at concentration 250 µg/mL).

Comp.	% Inhibition	Comp.	% Inhibition
7a	54	**7j**	**93**
7b	23	7k	85
7c	**95**	7l	65
7d	62	7m	35
7e	88	**7n**	**88**
7f	**91**	**7o**	**87**
7g	20	7p	65
7h	**94**	Rifampicin	98
7i	32	Isoniazid	99

4.1.3. *In vitro* Antimalarial activity

All the synthesized fluoro substituted pyrazole nucleus clubbed with 1,3,4-oxadiazole scaffolds were evaluated for their antimalarial activity against chloroquine and quinine sensitive strain of *P. falciparum.* All experiments were performed in duplicate and a mean value of IC_{50} is mentioned in **Table 3**.

Table 3. *In vitro* antimalarial activity of pyrazole based 1,3,4-oxadiazole scaffolds**.**

Compound	IC_{50} (µM)	Compound	IC_{50} (µM)
7a	2.956	7j	2.884
7b	**0.709**	7k	2.361
7c	4.385	**7l**	**0.797**
7d	2.329	7m	2.081
7e	2.304	7n	2.712
7f	1.570	**7o**	**0.610**
7g	2.825	7p	2.396
7h	**0.506**	Chloroquine	0.062
7i	**0.536**	Quinine	0.826

As shown in **Table 3**, compounds **7b**, **7h**, **7i**, **7l** and **7o** were found to have IC_{50} in the range of 0.506 μM to 0.797 μM against *P. falciparum* strain. These compounds displayed promising activity against *P. falciparum* strain as compared to quinine IC_{50} = 0.826 μM. Remaining other compounds were found to be less active against chloroquine sensitive strain of *P. falciparum*.

5. Conclusion

Novel fluoro substituted pyrazole bearing 1,3,4-oxadiazole derivatives (**7a-p**) were synthesized in good yields *via* four step protocol from accessible 3-methyl-1-phenyl-1-H-pyrazol-5-(4H)-one using on the final step phenyliododiacetate ($PhI(OAc)_2$) in dichloromethane at room temperature. They were evaluated for their *in vitro* antimicrobial, antituberculosis and antimalarial studies. Among the series, compounds **7e, 7o** and **7h** were found to be promising against two gram positive bacteria i.e *S. pneumoniae* and *C. tetani.* Compounds **7e** and **7l** displayed superior potency against gram negative bacteria i.e *S. typhi.* The antifungal activity revealed that, all the compounds showed moderate activity against *C. albicans.* Compounds **7c**, **7h** and **7j** demonstrated better potency against *M. tuberculosis* $H_{37}Rv$ strain. Compounds **7b**, **7h**, **7i**, **7l** and **7o** were found to possess excellent activity against *P. falciparum* strain as compared to quinine IC_{50}=0.826μM. Compound **7h** was identified as the most biologically active member which exhibited admirable antimicrobial, antituberculosis, and antimalarial activity as compared to standard drugs.

6. Experimental section

All the reagents and solvents used were of commercial grade and employed without any further purification. The progress of the reactions as well as the purity of the compounds were checked by thin-layer chromatography on aluminium plates coated with silica gel 60 F_{254}, 0.25 mm thickness (Merck), and the developed chromatograms were visualized under UV light and iodine vapors. Melting points were determined in open capillaries using using μThermoCal10 melting point apparatus (Analab Scientific Pvt. Ltd, India) and are uncorrected. IR spectra were recorded on Shimadzu FTIR 8401 spectrophotometer using potassium bromide pellets in the range 4000-400 cm^{-1} and frequencies of only characteristic peaks are expressed in cm^{-1}. Mass Spectra were recorded on Shimadzu LCMS 2010 spectrometer.^{1}H NMR spectra were recorded on Bruker Avance 400F (MHz) spectrometer (Bruker Scientific Corporation Ltd., Switzerland) using $CDCl_3$ as solvent and tetramethylsilane (TMS) as internal standard. Elemental analyses were performed on Perkin-Elmer 2400 series-II elemental analyzer (Perkin- Elmer, USA). All compounds were found within ±0.4% of their theoretical values.

6.1 General procedure for the synthesis of 3-methyl-5-substituted aryloxy-1-phenyl-1H-pyrazole-4-carbaldehyde (4a-d)

5-chloro-3-methyl-1-phenyl-1H-pyrazole-4-carbaldehyde **2** (1 mmol), substituted phenols **3a-d** (1 mmol) and anhydrous potassium carbonate (2 mmol) in dimethylformamide (10 mL) were charged in a 100 mL round bottom flask equipped with a mechanical stirrer and a condenser. The reaction mixture was heated at 90°C for 2 h. The progress of the reaction was monitored by TLC. After the completion of reaction as confirmed by TLC, the reaction mixture was poured in to 100 mL ice-water and filtered, washed thoroughly with water, dried and recrystallized from hot ethanol to obtain a white solid.

6.1.1 5-(2-fluorophenoxy)-3-methyl-1-phenyl-1H-pyrazole-4-carbaldehyde (4a).

Yield 85 %, m.p. 225-227 °C; IR (KBr, ν_{max}, cm^{-1}): 1215 (C–O–C); 1720 (-C=O str.), 3053 (Ar, -CH str.); ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.56 (s, 3H, pyrazole-CH_3), 6.99 – 7.11 (m, 2H, Ar–H), 7.12-720 (m, 2H, Ar–H), 7.34-7.37 (m, 1H, Ar–H), 7.36-7.45 (m, 2H, Ar–H), 7.47-7.69 (m, 2H, Ar–H), 9.61(s, 1H, -CHO); ^{13}C APT (400 MHz, $CDCl_3$) δ 14.5, 108.5, 117.6, 118.3, 123.4, 125.8, 128.1, 128.9, 136.8, 144.2, 150.8, 151.0, 152.2, 153.4, 182.6; ESI-MS (m/z): 297.2 (M^+); Anal. % Calculated for $C_{17}H_{13}FN_2O_2$: C, 68.91; H, 4.42; N, 9.45; Found: C, 68.69; H, 4.20; N, 9.22.

6.1.2 5-(3-fluorophenoxy)-3-methyl-1-phenyl-1H-pyrazole-4-carbaldehyde (4b).

Yield 78 %, m.p. 210-212 °C; IR (KBr, ν_{max}, cm^{-1}): 1218 (C–O–C); 1715 (-C=O str.); 3051 (Ar, -CH str.); ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.58(s, 3H, pyrazole-CH_3), 675-6.87 (m, 3H, Ar–H), 7.25-7.33 (m, 2H, Ar–H), 7.37-7.45 (m, 2H, Ar–H), 7.61-7.63 (m, 2H, Ar–H), 9.70 (s, 1H, -CHO); ^{13}C APT (400 MHz, $CDCl_3$) δ 14.3,104.4, 109.1, 117.7, 122.8, 128.2, 129.3, 131.1, 136.7, 150.9, 151.3, 157.7, 162.2, 164.6, 182.7; ESI-MS (m/z): 297.3 (M^+); Anal. % Calculated for $C_{17}H_{13}FN_2O_2$: C, 68.91; H, 4.42; N, 9.45; Found: C, 68.69; H, 4.21; N, 9.22.

6.1.3 3-methyl-1-phenyl-5-(3-(trifluoromethyl)phenoxy)-1H-pyrazole-4-carbaldehyde (4c).

Yield 81 %, m.p. 226-228 °C; IR (KBr, ν_{max}, cm^{-1}): 1219 (C–O–C); 1719 (-C=O str.); 3056 (Ar, -CH str.); ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.59 (s, 3H, pyrazole-CH_3), 7.03-7.06 (m, 4H, Ar–H), 7.29-7.39(m, 1H, Ar–H), 7.42-7.46 (m, 2H, Ar–H), 7.63-7.65 (m, 2H, Ar–H), 9.66 (s, 1H, -CHO); ^{13}C APT (400 MHz, $CDCl_3$) δ 14.4, 101.9, 116.9, 116.9, 118.5, 124.8, 128.7, 129.2, 137.8, 151.9, 152.3, 152.9, 158.2, 160.5, 182.9; ESI-MS (m/z): 347.2 (M^+); Anal. % Calculated for $C_{18}H_{13}F_3N_2O_2$: C, 62.42; H, 3.78; N, 8.09; Found: C, 62.19; H, 3.54; N, 7.87.

6.1.4 5-(4-fluorophenoxy)-3-methyl-1-phenyl-1H-pyrazole-4-carbaldehyde (4d).

Yield 82 %, m.p. 245-247 °C; IR (KBr, ν_{max}, cm^{-1}): 1218 (C–O–C); 1717 (-C=O str.); 3055 (Ar, -CH str.); ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.57 (s, 3H, pyrazole-CH_3), 7.00-7.05 (m, 4H, Ar–H), 7.28-7.37 (m, 1H, Ar–H), 7.42-7.46 (m, 2H, Ar–H), 7.63-7.65 (m, 2H, Ar–H), 9.65 (s, 1H, -CHO); ^{13}C APT (400 MHz, $CDCl_3$) δ 14.4, 101.8, 116.7, 116.9, 117.5, 122.8, 128.1, 129.2, 136.8, 150.9, 152.3, 152.8, 158.0, 160.4, 182.8; ESI-MS (m/z): 297.1 (M^+); Anal. % Calculated for $C_{17}H_{13}FN_2O_2$: C, 68.91; H, 4.42; N, 9.45; Found: C, 68.66; H, 4.21; N, 9.74.

6.2 Synthesis of (*E*)-N'-((5-(substituted-fluorophenoxy)-3-methyl-1-phenyl-1H-pyrazol-4-yl)methylene)-4-substitutedbenzohydrazide (6a-p).

A mixture of 3-methyl-5-substituted aryloxy-1-phenyl-1H-pyrazole-4-carbaldehydes **4a–d** (10 mmol), 4-substitutedbenzohydrazide **5a-d** (10 mmol) and catalytic amount

of glacial acetic acid in ethanol (50 mL) was refluxed for 1 h. After the completion of reaction, the reaction mixture was stirred magnetically for further 10 min. After cooling the separated solid mass was collected by filtration, washed well with ethanol (10 mL) dried, and crystallized from hot ethanol (10 mL) to affording compounds (**6a-p**).

6.2.1 (E)-N'-((5-(2-fluorophenoxy)-3-methyl-1-phenyl-1H-pyrazol-4-yl)methylene)-4-methylbenzohydrazide (6a).

Yield 77 %, m.p. 166–168 °C; IR (KBr, ν_{max}, cm^{-1}): 3441 (-NH str.); 1722 (C=O); 1630 (C=N); 1230 (C-O-C); 3028 (Ar, -CH str.);^{1}H NMR (400 MHz, $CDCl_3$) δ 2.35 (s, 3H, Ar-CH_3), 2.61 (s, 3H, pyrazole-CH_3), 7.04(m, 1H, Ar–H), 7.19-7.22 (m, 3H, Ar–H), 7.27-7.31 (m, 2H, Ar–H), 7.34 (m, 3H, Ar–H), 7.36-7.41 (m, 2H, Ar–H), 7.56-7.70 (m, 2H, Ar–H); 8.15 (s, 1H, =CH-), 9.40 (s, 1H, -NH); ^{13}C APT (400 MHz, $CDCl_3$) δ 14.8, 21.4, 104.5, 112.5, 118.7, 120.5, 121.9, 122.4, 124.8, 126.8, 126.9, 127.7, 129.6, 130.6, 132.5, 137.4, 139.6, 143.6, 149.9, 154.5, 156.6, 164.6; ESI-MS (m/z): 429.2 (M^+); Anal. % Calculated for $C_{25}H_{21}FN_4O_2$: C, 70.08; H, 4.94; N, 13.08; Found: C, 69.87; H, 4.69; N, 12.87.

6.2.2 (E)-4-bromo-N'-((5-(3-fluorophenoxy)-3-methyl-1-phenyl-1H-pyrazol-4-yl)methylene)benzohydrazide (6b).

Yield 80 %, m.p. 208–210 °C; IR (KBr, ν_{max}, cm^{-1}): 3446 (-NH str.); 1720 (C=O); 1629 (C=N); 685 (C-Br); 1232 (C-O-C); 3026 (Ar, -CH str.); ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.62 (s, 3H, pyrazole-CH_3), 6.65 (m, 2H, Ar–H), 6.72-6.77 (m, 1H, Ar–H), 7.21-7.25 (m, 1H, Ar–H), 7.29-7.32 (m, 1H, Ar–H), 7.38-7.42 (m, 4H, Ar–H), 7.57 (m, 2H, Ar–H), 7.74-7.80 (m, 2H, Ar–H), 8.10 (s, 1H, =CH-), 9.35 (s, 1H, -NH); ^{13}C APT (400 MHz, $CDCl_3$) δ 14.8, 103.6, 103.8, 110.8, 110.9, 111.5, 118.9, 122.6, 127.5, 128.7, 129.4, 130.8, 130.9, 137.4, 143.8, 149.6, 154.5, 156.4, 162.3, 164.7; ESI-MS (m/z): 494.3 (M^+); Anal. % Calculated for $C_{24}H_{18}BrFN_4O_2$: C, 58.43; H, 3.68; N, 11.36; Found: C, 58.19; H, 3.43; N, 11.13

6.2.3 (E)-N'-((5-(3-fluorophenoxy)-3-methyl-1-phenyl-1H-pyrazol-4-yl)methylene)-4-methylbenzohydrazide (6c).

Yield 75 %, m.p. 172–174 °C; IR (KBr, ν_{max}, cm^{-1}): 3447 (-NH str.); 1715 (C=O); 16276 (C=N); 1235 (C-O-C); 3025 (Ar, -CH str.);^{1}H NMR (400 MHz, $CDCl_3$) δ 2.41 (s, 3H, Ar-CH_3), 2.64 (s, 3H, pyrazole-CH_3), 6.69 (m, 2H, Ar–H), 6.75-6.79 (m, 1H, Ar–H), 7.18-7.23 (m, 3H, Ar–H), 7.37-7.41 (m, 2H, Ar–H), 7.58-7.60 (m, 2H, Ar–H), 7.70 (m, 2H, Ar–H), 8.11(s, 1H, =CH-), 9.17 (s, 1H, -NH); ^{13}C APT (400 MHz, $CDCl_3$) δ 15.1, 21.5, 103.7, 103.9, 110.8, 111.1, 122.2, 126.8, 127.4, 129.2, 129.8, 130.5, 130.9, 137.2, 142.8, 143.5, 149.4, 154.5, 156.9, 162.2, 164.6; ESI-MS (m/z): 429.3 (M^+); Anal. % Calculated for $C_{25}H_{21}FN_4O_2$: C, 70.08; H, 4.94; N, 13.08; Found: C, 69.86; H, 4.71; N, 12.86.

6.2.4 (E)-4-methyl-N'-((3-methyl-1-phenyl-5-(3-(trifluoromethyl)phenoxy)-1H-pyrazol-4-yl)methylene)benzohydrazide (6d).

Yield 79 %, m.p. 177–179 °C; IR (KBr, ν_{max}, cm^{-1}): 3413 (-NH str.); 1721 (C=O); 1627 (C=N); 1232 (C-O-C); 3029 (Ar, -CH str.);^{1}H NMR (400 MHz, $CDCl_3$) δ 2.39 (s, 3H, Ar-CH_3), 2.62 (s, 3H, pyrazole-CH_3), 7.06 (m, 1H, Ar–H), 7.18-7.20 (m, 3H, Ar–H), 7.26-7.30 (m, 2H, Ar–H), 7.33 (m, 3H, Ar–H), 7.36-7.40 (m, 2H, Ar–H), 7.56-7.69 (m, 2H, Ar–H); 8.16 (s, 1H, =CH-), 9.41 (s, 1H, -NH); ^{13}C APT (400 MHz, $CDCl_3$) δ 14.9, 21.4, 104.4, 112.9, 118.5, 120.5, 121.9, 122.3, 124.7, 126.8, 126.9, 127.6, 129.5, 130.6, 132.6, 137.1, 139.6, 143.5, 149.8, 154.5, 156.6, 164.6; ESI-MS (m/z): 479.2 (M^+); Anal. % Calculated for $C_{26}H_{21}F_3N_4O_2$: C, 65.27; H, 4.42; N, 11.71; Found: C, 65.05; H, 4.21; N, 11.48.

6.2.5 (E)-4-chloro-N'-((5-(4-fluorophenoxy)-3-methyl-1-phenyl-1H-pyrazol-4-yl)methylene)benzohydrazide (6e).

Yield 81 %, m.p. 212–214 °C; IR (KBr, ν_{max}, cm^{-1}) 3435 (-NH str.); 1713 (C=O); 1630 (C=N); 750 (C-Cl); 1231 (C-O-C); 3030 (Ar, -CH str.);^{1}H NMR (400 MHz, $CDCl_3$) δ 2.62 (s, 3H, pyrazole-CH_3), 6.84-6.97 (m, 4H, Ar–H), 7.28-7.31 (m, 1H, Ar–H), 7.37-7.41 (m, 4H, Ar–H), 7.59 (m, 2H, Ar–H), 7.75-7.82 (m, 2H, Ar–H), 8.10 (s, 1H, =CH-), 9.27 (s, 1H, -NH);^{13}C APT (400 MHz, $CDCl_3$) δ 14.8, 116.4, 116.7, 116.9, 122.2, 124.8, 124.9, 126.9, 129.2, 129.8, 130.5, 130.7, 130.9, 137.2, 143.5, 149.8, 150.9, 157.6, 158.2, 160.1; ESI-MS (m/z): 449.4 (M^+); Anal. % Calculated for $C_{24}H_{18}ClFN_4O_2$: C, 64.22; H, 4.04; N, 12.48; Found: 63.99; H, 3.82; N, 12.24.

6.2.6 (E)-4-bromo-N'-((3-methyl-1-phenyl-5-(3-(trifluoromethyl)phenoxy)-1H-pyrazol-4-yl)methylene)benzohydrazide (6f).

Yield 72 %, m.p. 190–192 °C; IR (KBr, ν_{max}, cm^{-1}): 3436 (-NH str.); 1720 (C=O); 1631 (C=N); 684 (C-Br); 1230 (C-O-C); 3024 (Ar, -CH str.);^{1}H NMR (400 MHz, $CDCl_3$) δ 2.63 (s, 3H, pyrazole-CH_3), 7.00-7.11 (m, 2H, Ar–H), 7.22 (m, 5H, Ar–H), 7.29-7.33 (m, 4H, Ar–H), 7.37-7.72 (m, 2H, Ar–H), 8.15 (s, 1H, =CH-), 9.29 (s, 1H, -NH);^{13}C APT (400 MHz, $CDCl_3$) δ 14.9, 118.5, 120.6, 122.4, 122.6, 126.8, 126.9, 129.4, 129.8, 130.2, 130.8, 131.5, 131.9, 132.4, 135.6, 137.2, 143.5, 149.8, 154.6, 155.7, 163.6; ESI-MS (m/z): 544.2 (M^+); Anal. % Calculated for $C_{25}H_{18}BrF_3N_4O_2$: C, 55.26; H, 3.34; N, 10.31; Found: C, 55.05; H, 3.09; N, 10.09.

6.2.7 (E)-N'-((5-(2-fluorophenoxy)-3-methyl-1-phenyl-1H-pyrazol-4-yl)methylene)-4-methoxybenzohydrazide (6g).

Yield 79 %, m.p. 210–212 °C; IR (KBr, ν_{max}, cm^{-1}): 3443 (-NH str.); 1718 (C=O); 1625 (C=N); 1235 (C-O-C); 3025 (Ar, -CH str.); ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.53 (s, 3H, pyrazole-CH_3), 3.83 (s, 3H, -OCH_3), 6.85 (m, 6H, Ar–H), 7.25-7.29 (m, 1H, Ar–H), 7.35-7.39 (m, 2H, Ar–H), 7.59-7.62 (m, 2H, Ar–H), 7.79 (m, 2H, Ar–H), 8.09 (s, 1H, =CH-), 9.71 (s, 1H, -NH); ^{13}C APT (400 MHz, $CDCl_3$) δ 15.2, 55.3, 104.2, 113.6, 116.3, 116.7, 116.9, 122.2, 124.5, 125.8, 128.9, 128.9, 129.2, 137.5, 138.8, 143.5, 147.8, 152.7, 157.2, 159.8, 162.3; ESI-MS (m/z): 445.3 (M^+); Anal. % Calculated for $C_{25}H_{21}FN_4O_3$: C, 67.56; H, 4.76; N, 12.61; Found: 67.32; H, 4.51; N, 12.38.

6.2.8 (E)-4-bromo-N'-((5-(4-fluorophenoxy)-3-methyl-1-phenyl-1H-pyrazol-4-yl)methylene)benzohydrazide (6h).

Yield 77 %, m.p. 195–197 °C; IR (KBr, ν_{max}, cm^{-1}): 3437 (-NH str.); 1723 (C=O); 1638 (C=N); 686 (C-Br); 1239 (C-O-C); 3027 (Ar, -CH str.); ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.61 (s, 3H, pyrazole-CH_3), 6.85-6.99 (m, 4H, Ar–H), 7.25-7.35 (m, 1H, Ar–H), 7.38-7.43 (m, 4H, Ar–H), 7.57 (m, 2H, Ar–H), 7.75-7.85 (m, 2H, Ar–H), 8.09 (s, 1H, =CH-), 9.29 (s, 1H, -NH);^{13}C APT (400

MHz, $CDCl_3$) δ 14.8, 116.3, 116.7, 116.8, 122.2, 124.7, 124.9, 126.9, 129.5, 129.8, 130.6, 130.7, 130.9, 137.4, 143.4, 149.7, 150.9, 157.6, 158.3, 160.2; ESI-MS (m/z): 494.2 (M^+); Anal. % Calculated for $C_{24}H_{18}BrFN_4O_2$: C, 58.43; H, 3.68; N, 11.36; Found: C, 58.21; H, 3.45; N, 11.12.

6.2.9 (E)-N'-((5-(4-fluorophenoxy)-3-methyl-1-phenyl-1H-pyrazol-4-yl)methylene)-4-methoxybenzohydrazide (6i).

Yield 76 %, m.p. 189–191 °C; IR (KBr, ν_{max}, cm^{-1}): 3438 (-NH str.); 1720 (C=O); 1634 (C=N); 1234 (C-O-C); 3022 (Ar, -CH str.); ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.55 (s, 3H, pyrazole-CH_3), 3.82 (s, 3H, -OCH_3), 6.83 (m, 6H, Ar–H), 7.24-7.28 (m, 1H, Ar–H), 7.35-7.38 (m, 2H, Ar–H), 7.57-7.59 (m, 2H, Ar–H), 7.80 (m, 2H, Ar–H), 8.10(s, 1H, =CH-), 9.73 (s, 1H, -NH); ^{13}C APT (400 MHz, $CDCl_3$) δ 15.1, 55.3, 104.2, 113.7, 116.3, 116.6, 116.8, 122.2, 124.3, 125.4, 128.8, 128.9, 129.2, 137.3, 138.3, 143.5, 147.8, 152.8, 157.5, 159.9, 162.3; ESI-MS (m/z): 445.3 (M^+); Anal. % Calculated for $C_{25}H_{21}FN_4O_3$: C, 67.56; H, 4.76; N, 12.61; Found: C, 67.35; H, 4.52; N, 12.36.

6.2.10 (E)-N'-((5-(4-fluorophenoxy)-3-methyl-1-phenyl-1H-pyrazol-4-yl)methylene)-4-methylbenzohydrazide (6j).

Yield 78 %, m.p. 210–212 °C; IR (KBr, ν_{max}, cm^{-1}): 3435 (-NH str.); 1722 (C=O); 1608 (C=N); 1230 (C-O-C); 3021 (Ar, -CH str.);^{1}H NMR (400 MHz, $CDCl_3$) δ 2.40 (s, 3H, Ar-CH_3), 2.61 (s, 3H, pyrazole-CH_3), 9.60-6.94 (m, 4H, Ar–H), 7.22-7.29 (m, 2H, Ar–H), 7.36-7.40 (m, 1H, Ar–H), 7.59-7.60 (m, 2H, Ar–H), 7.70 (m, 2H, Ar–H), 7.79 (m, 2H, Ar–H), 8.09 (s, 1H, =CH-), 9.36 (s, 1H, -NH);^{13}C APT (400 MHz, $CDCl_3$) δ 15.1, 21.5, 116.4, 116.6, 116.8, 122.2, 122.8, 125.4, 126.8, 127.3, 129.1, 129.9, 131.8, 137.3, 142.5, 143.9, 145.2, 147.9, 157.5, 158.5, 160.1; ESI-MS (m/z): 429.3 (M^+); Anal. % Calculated for $C_{25}H_{21}FN_4O_2$: C, 70.08; H, 4.94; N, 13.08; Found: C, 69.85; H, 4.71; N, 12.85.

6.2.11 (E)-4-bromo-N'-((5-(2-fluorophenoxy)-3-methyl-1-phenyl-1H-pyrazol-4-yl)methylene)benzohydrazide (6k).

Yield 74 %, m.p. 180–182 °C; IR (KBr, ν_{max}, cm^{-1}): 3413 (-NH str.); 1723 (C=O); 1609 (C=N); 685 (C-Br); 1232 (C-O-C); 3024 (Ar, -CH str.); ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.63 (s, 3H, pyrazole-CH_3), 6.90-6.99 (m, 1H, Ar–H), 7.04 (m, 2H, Ar–H), 7.08-7.14 (m, 1H, Ar–H), 7.29-7.33 (m, 1H, Ar–H), 7.39-7.44 (m, 4H, Ar–H), 7.64-7.67 (m, 2H, Ar–H), 7.75-7.85 (m, 2H, Ar–H), 8.10 (s, 1H, =CH-), 9.35 (s, 1H, -NH); ^{13}C APT (400 MHz, $CDCl_3$) δ 14.8, 116.7, 117.5, 117.8, 122.4, 124.6, 127.6, 129.5, 129.7, 130.8, 130.9, 131.5, 137.5, 137.9, 141.7, 143.8, 149.8, 154.4, 159.6, 163.4; ESI-MS (m/z): 494.1 (M^+); Anal. % Calculated for $C_{24}H_{18}BrFN_4O_2$: C, 58.43; H, 3.68; N, 11.36; Found: C, 58.22; H, 3.45; N, 11.12.

6.2.12 (E)-4-chloro-N'-((5-(2-fluorophenoxy)-3-methyl-1-phenyl-1H-pyrazol-4-yl)methylene)benzohydrazide (6l).

Yield 75 %, m.p. 198–200 °C; IR (KBr, ν_{max}, cm^{-1}): 3435 (-NH str.); 1711 (C=O); 1599 (C=N); 751 (C-Cl); 1233 (C-O-C); 3025 (Ar, -CH str.); ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.62 (s, 3H, pyrazole-CH_3), 6.92-6.99 (m, 1H, Ar–H), 7.00 (m, 2H, Ar–H), 7.08-7.12 (m, 1H, Ar–H), 7.26-7.30 (m, 1H, Ar–H), 7.37-7.41 (m, 4H, Ar–H), 7.62-7.64 (m, 2H, Ar–H), 7.75-7.83 (m, 2H, Ar–H), 8.11 (s, 1H, =CH-), 9.37 (s, 1H, -NH); ^{13}C APT (400 MHz, $CDCl_3$) δ 14.8, 116.6, 117.2, 117.8, 122.2, 124.6, 127.5, 129.2, 129.3, 130.5, 130.8, 131.5, 137.2, 137.9, 141.5, 143.8, 149.5, 154.2, 159.2, 163.8; ESI-MS (m/z): 449.5 (M^+); Anal. % Calculated for $C_{24}H_{18}ClFN_4O_2$: C, 64.22; H, 4.04; N, 12.48; Found: C, 63.98; H, 3.81; N, 12.25.

6.2.13 (E)-4-chloro-N'-((5-(3-fluorophenoxy)-3-methyl-1-phenyl-1H-pyrazol-4-yl)methylene)benzohydrazide (6m).

Yield 73 %, m.p. 192–194 °C; IR (KBr, ν_{max}, cm^{-1}): 3412 (-NH str.); 1722 (C=O); 1605 (C=N); 752 (C-Cl); 1236 (C-O-C); 3029 (Ar, -CH str.); ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.63 (s, 3H, pyrazole-CH_3), 6.68 (m, 2H, Ar–H), 6.75-6.79 (m, 1H, Ar–H), 7.20-7.24 (m, 1H, Ar–H), 7.28-7.31 (m, 1H, Ar–H), 7.37-7.41 (m, 4H, Ar–H), 7.58 (m, 2H, Ar–H), 7.75-7.81 (m, 2H, Ar–H), 8.11 (s, 1H, =CH-), 9.39 (s, 1H, -NH); ^{13}C APT (400 MHz, $CDCl_3$) δ 14.9, 103.6, 103.9, 110.8, 110.9, 111.0, 118.9, 122.5, 127.5, 128.7, 129.2, 130.8, 130.9, 137.2, 143.8, 149.5, 154.5, 156.2, 162.4, 164.8; ESI-MS (m/z): 449.3 (M^+); Anal. % Calculated for $C_{24}H_{18}ClFN_4O_2$: C, 64.22; H, 4.04; N, 12.48; Found: C, 63.99; H, 3.81; N, 12.24.

6.2.14 (E)-N'-((5-(3-fluorophenoxy)-3-methyl-1-phenyl-1H-pyrazol-4-yl)methylene)-4-methoxybenzohydrazide (6n).

Yield 73 %, m.p. 192–194 °C; IR (KBr, ν_{max}, cm^{-1}): 3431 (-NH str.); 1720 (C=O); 1633 (C=N); 1239 (C-O-C); 3028 (Ar, -CH str.); ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.61 (s, 3H, pyrazole-CH_3), 3.86 (s, 3H, -OCH_3), 6.65-6.73 (m, 2H, Ar–H), 6.75-6.77 (m, 1H, Ar–H), 6.89-6.91 (m, 2H, Ar–H), 7.17-7.26 (m, 1H, Ar–H), 7.30-7.36 (m, 1H, Ar–H), 7.40 (m, 2H, Ar–H), 7.57-7.59 (m, 2H, Ar–H), 7.79- (m, 2H, Ar–H), 8.09 (s, 1H, =CH-), 9.34 (s, 1H, -NH); ^{13}C APT (400 MHz, $CDCl_3$) δ 15.8, 55.3, 103.7, 103.9, 110.7, 110.9, 122.2, 127.4, 129.2, 130.8, 130.9, 132.2, 135.7, 137.2, 143.4, 149.8, 150.2, 154.9, 158.2, 162.1, 164.6; ESI-MS (m/z): 445.3 (M^+); Anal. % Calculated for $C_{25}H_{21}FN_4O_3$: C, 67.56; H, 4.76; N, 12.61; Found: C, 67.33; H, 4.53; N, 12.38.

6.2.15 (E)-4-chloro-N'-((3-methyl-1-phenyl-5-(3-(trifluoromethyl)phenoxy)-1H-pyrazol-4-yl)methylene)benzohydrazide (6o).

Yield 70 %, m.p. 158–160 °C; IR (KBr, ν_{max}, cm^{-1}): 3445 (-NH str.); 1728 (C=O); 1621 (C=N); 750 (C-Cl); 1235 (C-O-C); 3022 (Ar, -CH str.); ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.59 (s, 3H, pyrazole-CH_3), 6.91-6.98 (m, 1H, Ar–H), 7.10-7.18 (m, 1H, Ar–H), 7.20-7.25 (m, 3H, Ar–H), 7.30-7.34 (m, 2H, Ar–H), 7.36-7.38 (m, 2H, Ar–H), 7.55 (m, 2H, Ar–H), 7.76 (m, 3H, Ar–H +=CH-), 9.88 (s, 1H, -NH); ^{13}C APT (400 MHz, $CDCl_3$) δ 14.8, 104.1, 112.8, 116.4, 118.5, 120.6, 121.8, 122.3, 124.6, 125.4, 126.5, 126.8, 128.9, 132.6, 137.1, 144.2, 150.1, 154.4, 155.8, 163.4, 164.1; ESI-MS (m/z): 499.3 (M^+); Anal. % Calculated for $C_{25}H_{18}ClF_3N_4O_2$: C, 60.19; H, 3.64; N, 11.23; Found: C, 59.96; H, 3.40; N, 10.99.

6.2.16 (E)-4-methoxy-N'-((3-methyl-1-phenyl-5-(3-(trifluoromethyl)phenoxy)-1H-pyrazol-4-yl)methylene)benzohydrazide (6p).

Yield 68 %, m.p. 172–174 °C; IR (KBr, ν_{max}, cm^{-1}): 3433 (-NH str.); 1726 (C=O); 1632 (C=N); 1231 (C-O-C); 3025 (Ar, -CH str.); ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.80 (s, 3H, pyrazole-CH_3), 3.86 (s, 3H, -OCH_3), 6.89-6.91 (m, 2H, Ar–H), 7.04-7.06 (m, 1H, Ar–H), 7.12-7.14 (m, 1H, Ar–H), 7.19 (m, 1H, Ar–H), 7.26-7.32

(m, 3H, Ar–H), 7.35-7.40 (m, , 3H, Ar–H), 7.57-7.88 (m, 2H, Ar–H), 8.12 (s, 1H, =CH-), 9.20 (s, 1H, -NH); ^{13}C APT (400 MHz, $CDCl_3$) δ 14.9, 55.3, 104.3, 112.9, 118.5, 119.1, 120.5, 122.3, 125.2, 126.3, 127.6, 128.5, 130.6, 132.6, 135.2, 137.1, 143.5, 149.4, 154.5, 156.6, 163.4, 164.2; ESI-MS (m/z): 495.2 (M^+); Anal. % Calculated for $C_{26}H_{21}F_3N_4O_3$: C, 63.16; H, 4.28; N, 11.33; Found: C, 62.93; H, 4.05; N, 11.10.

6.3 Synthesis of 2-(5-(substituted-fluorophenoxy)-3-methyl-1-phenyl-1H-pyrazol-4-yl)-5-(p-substituted)-1,3,4-oxadiazole (7a-p)

A mixture of compound **6a-p** (10 mmol) was dissolved in DCM (20 ml) and stirred. To this solution, $PhI(OAc)_2$ (10 mmol) was added and the mixture was stirred for 15-20 min at room temperature. After the completion of the reaction as monitored by TLC (ethyl acetate: hexane: 3:7), the solvent was evaporated and the residue was washed with diethyl ether, filtered (5 mL), dried and then crystallized from acetone to affording target compounds (**7a-p**).

6.3.1 2-(5-(2-fluorophenoxy)-3-methyl-1-phenyl-1H-pyrazol-4-yl)-5-(p-tolyl)-1,3,4-oxadiazole (7a)

Yield 79 %, m.p. 176–178 °C; IR (KBr, ν_{max}, cm^{-1}): 1215 (C–O–C); 1622 and 1594 (C=N and C=C); 3054 (Ar, -CH str.); ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.41 (s, 3H, Ar-CH_3), 2.74 (s, 3H, pyrazole-CH_3), 6.83 - 6.94 (m, 3H, Ar–H), 6.96-6.99 (m, 1H, Ar–H), 7.00-7.02 (m, 2H, Ar–H), 7.12 (m, 1H, Ar–H), 7.15-7.48 (m, 2H, Ar–H), 7.70 -7.92 (m, 4H, Ar–H); ^{13}C APT (400 MHz, $CDCl_3$) δ 14.9, 21.5, 95.0, 116.4, 117.0, 117.2, 120.9, 122.6, 124.5, 124.6, 126.6, 127.9, 129.3, 129.6, 137.1, 141.9, 144.1, 144.2, 146.9, 149.3, 150.6, 150.7, 153.1, 158.0, 163.7; ESI-MS (m/z): 427.1 (M^+); Anal. % Calculated for $C_{25}H_{19}FN_4O_2$: C, 70.41; H, 4.49; N, 13.14; Found: C, 70.17; H, 4.26; N, 12.93.

6.3.2 2-(4-bromophenyl)-5-(5-(3-fluorophenoxy)-3-methyl-1-phenyl-1H-pyrazol-4-yl)-1,3,4-oxadiazole (7b)

Yield 81 %, m.p. 154–156°C; IR (KBr, ν_{max}, cm^{-1}): 1215 (C–O–C); 1621 and 1592 (C=N and C=C); 3051 (Ar, -CH str.); ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.75 (s, 3H, pyrazole-CH_3), 6.80 - 6.82 (m, 3H, Ar–H), 7.28 (m, 1H, Ar–H), 7.36-7.38 (m, 1H, Ar–H), 7.43-7.7.47 (m, 2H, Ar–H), 7.57-7.65 (m, 4H, Ar–H), 7.67 - 7.68 (m, 2H, Ar–H); ^{13}C APT (400 MHz, $CDCl_3$) δ 14.9, 95.3, 103.6, 103.8, 110.8, 110.9, 111.1, 122.5, 122.6, 126.1, 127.9, 128.0, 129.3, 130.9, 131.0, 132.3, 137.1, 149.3, 150.1, 152.6, 158.5, 162.3, 162.7, 164.7; ESI-MS (m/z): 492.2 (M^+); Anal. % Calculated for $C_{24}H_{16}BrFN_4O_2$: C, 58.67; H, 3.28; N, 11.40; Found: C, 58.44; H, 3.03; N, 11.14.

6.3.3 2-(5-(3-fluorophenoxy)-3-methyl-1-phenyl-1H-pyrazol-4-yl)-5-(p-tolyl)-1,3,4-oxadiazole (7c)

Yield 80 %, m.p. 181-183 °C; IR (KBr, ν_{max}, cm^{-1}): 1213 (C–O–C); 1622 and 1589 (C=N and C=C); 3051 (Ar, -CH str.); ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.41 (s, 3H, Ar-CH_3), 2.76 (s, 3H, pyrazole-CH_3), 6.79-6.84 (m, 3H, Ar–H), 7.23-7.29 (m, 3H, Ar–H), 7.34-7.38 (m, 1H, Ar–H), 7.43-7.64 (m, 2H, Ar–H), 7.66 - 7.68 (m, 4H, Ar–H); ^{13}C APT (400 MHz, $CDCl_3$) δ 14.9, 21.5, 95.6, 103.7, 110.8, 11.09, 111.0, 120.1, 122.6, 126.6, 127.9, 129.3, 129.6, 130.8, 130.9, 137.0, 142.0, 146.4, 149.3, 157.6, 157.7, 158.0, 162.3, 163.2, 164.7; ESI-MS (m/z): 427.4 (M^+); Anal. % Calculated for $C_{25}H_{19}FN_4O_2$: C, 70.41; H, 4.49; N, 13.14; Found: C, 70.18; H, 4.25; N, 12.87.

6.3.4 2-(3-methyl-1-phenyl-5-(3-(trifluoromethyl)phenoxy)-1H-pyrazol-4-yl)-5-(p-tolyl)-1,3,4-oxadiazole (7d)

Yield 82 %, m.p. 172-174 °C; IR (KBr, ν_{max}, cm^{-1}): 1216 (C–O–C); 1617 and 1598 (C=N and C=C); 3052 (Ar, -CH str.); ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.41 (s, 3H, Ar-CH_3), 2.76 (s, 3H, pyrazole-CH_3), 7.10 -7.12 (m, 1H, Ar–H), 7.21 -7.23 (m, 2H, Ar–H), 7.35 -7.40 (m, 3H, Ar–H), 7.43 -7.48 (m, 3H, Ar–H), 7.57 - 7.59 (m, 2H, Ar–H), 7.65 -7.67 (m, 2H, Ar–H); ^{13}C APT (400 MHz, $CDCl_3$) δ 14.9, 21.5, 95.4, 113.2, 113.3, 117.9, 120.6, 120.6, 120.7, 122.7, 126.5, 127.1, 128.1, 129.4, 129.6, 130.7, 132.8, 135.2, 137.1, 142.1, 145.7, 149.4, 156.7, 157.9, 163.6; ESI-MS (m/z): 477.4 (M^+); Anal. % Calculated for $C_{26}H_{19}F_3N_4O_2$: C, 65.54; H, 4.02; N, 11.76; Found: C, 65.30; H, 3.81; N, 11.54.

6.3.5 2-(4-chlorophenyl)-5-(5-(4-fluorophenoxy)-3-methyl-1-phenyl-1H-pyrazol-4-yl)-1,3,4-oxadiazole (7e)

Yield 81%, m.p. 168-170 °C; IR (KBr, ν_{max}, cm^{-1}): 1218 (C–O–C); 1625 and 1595 (C=N and C=C); 3053 (Ar, -CH str.); ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.73 (s, 3H, pyrazole-CH_3), 6.99-7.00 (m, 4H, Ar–H), 7.34-7.37 (m, 1H, Ar–H), 7.41-7.46 (m, 4H, Ar–H), 7.66-7.71 (m, 4H, Ar–H); ^{13}C APT (400 MHz, $CDCl_3$) δ 14.9, 95.1, 116.4, 116.5, 116.6, 116.7, 121.7, 122.1, 122.6, 125.7, 127.6, 128.0, 129.3, 137.0, 137.7, 147.3, 149.3, 152.5, 152.6, 157.6, 158.5, 160.0, 161.4, 162.5; ESI-MS (m/z): 447.7 (M^+); Anal. % Calculated for $C_{24}H_{16}ClFN_4O_2$: C, 64.51; H, 3.61; N, 12.54; Found: C, 64.29; H, 3.38; N, 12.28.

6.3.6 2-(4-bromophenyl)-5-(3-methyl-1-phenyl-5-(3-(trifluoromethyl)phenoxy)-1H-pyrazol-4-yl)-1,3,4-oxadiazole (7f)

Yield 86 %, m.p. 158-160 °C; IR (KBr, ν_{max}, cm^{-1}): 1225 (C–O–C); 1625 and 1590 (C=N and C=C); 3057 (Ar, -CH str.); ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.76 (s, 3H, pyrazole-CH_3), 7.10-7.12 (d, 1H, Ar–H), 7.33 (m, 2H, Ar–H), 7.38-7.53 (m, 4H, Ar–H), 7.58-7.61 (m, 4H, Ar–H), 7.65-7.67 (d, 2H, Ar–H); ^{13}C APT (400 MHz, $CDCl_3$) δ 15.0, 113.2, 113.2, 117.9, 120.1, 120.7, 122.4, 122.7, 126.2, 127.2, 128.2, 129.4, 130.8, 135.4, 142.3, 149.5, 150.3, 152.6, 153.4, 154.8, 155.2, 156.7, 156.9, 163.7; ESI-MS (m/z): 542.2 (M^+); Anal. % Calculated for $C_{25}H_{16}BrF_3N_4O_2$: C, 55.47; H, 2.98; N, 10.35; Found: C, 55.24; H, 2.77; N, 10.09.

6.3.7 2-(5-(2-fluorophenoxy)-3-methyl-1-phenyl-1H-pyrazol-4-yl)-5-(4-methoxyphenyl)-1,3,4-oxadiazole (7g)

Yield 80%, m.p. 172-174 °C; IR (KBr, ν_{max}, cm^{-1}): 1229 (C–O–C); 1621 and 1598 (C=N and C=C); 3056 (Ar, -CH str.); ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.73 (s, 3H, pyrazole-CH_3), 3.87 (s, 3H, -OCH_3), 6.82-6.84 (m, 1H, Ar–H), 6.85-7.04 (m, 4H, Ar–H), 7.15-7.20 (m, 1H, Ar–H), 7.33-7.44 (m, 1H, Ar–H), 7.46-7.75 (m, 2H, Ar–H), 7.76 -7.78 (m, 4H, Ar–H); ^{13}C APT (400 MHz, $CDCl_3$) δ 14.9, 55.4, 95.1, 114.3, 116.2, 116.4, 117.2, 122.6, 124.5, 124.6, 124.6, 124.7, 127.9, 128.4, 129.3, 137.1, 144.1, 144.2, 146.8, 149.2, 150.6, 153.1, 157.8, 162.1, 163.5; ESI-MS (m/z): 443.4 (M^+); Anal. % Calculated for $C_{25}H_{19}FN_4O_3$: C, 67.87; H, 4.33; N, 12.66; Found: C, 67.64; H, 4.11; N, 12.39.

6.3.8 2-(4-bromophenyl)-5-(5-(4-fluorophenoxy)-3-methyl-1-phenyl-1H-pyrazol-4-yl)-1,3,4-oxadiazole (7h)

Yield 80%, m.p. 177-179 °C; IR (KBr, ν_{max}, cm^{-1}): 1237 (C–O–C); 1636 and 1594 (C=N and C=C); 3053 (Ar, -CH str.); ^1H NMR (400 MHz, $CDCl_3$) δ 2.75 (s, 3H, pyrazole-CH_3), 6.80 - 6.88 (m, 3H, Ar–H), 7.29 (m, 1H, Ar–H), 7.36-7.40 (m, 1H, Ar–H), 7.48-7.50 (m, 2H, Ar–H), 7.57-7.67 (m, 4H, Ar–H), 7.68-7.69 (m, 2H, Ar–H); ^{13}C APT (400 MHz, CDCl3) δ 14.9, 95.4, 103.6, 103.8, 110.7, 110.9, 111.5, 122.4, 122.6, 126.3, 127.9, 128.0, 129.5, 130.9, 131.3, 132.2, 137.4, 149.5, 150.4, 152.8, 158.7, 162.5, 162.7, 164.7; ESI-MS (m/z): 492.3 (M^+); Anal. % Calculated for $C_{24}H_{16}BrFN_4O_2$: C, 58.67; H, 3.28; N, 11.40; Found: C, 58.39; H, 3.07; N, 11.15.

6.3.9 2-(5-(4-fluorophenoxy)-3-methyl-1-phenyl-1H-pyrazol-4-yl)-5-(4-methoxyphenyl)-1,3,4-oxadiazole (7i)

Yield 85%, m.p. 157-159 °C; IR (KBr, ν_{max}, cm^{-1}): 1227 (C–O–C); 1623 and 1593 (C=N and C=C); 3058 (Ar, -CH str.); ^1H NMR (400 MHz, $CDCl_3$) δ δ 2.73 (s, 3H, pyrazole-CH_3), 3.87 (s, 3H, -OCH_3), 6.81-6.89 (m, 1H, Ar–H), 6.87-7.08 (m, 4H, Ar–H), 7.19-7.25 (m, 1H, Ar–H), 7.33-7.48 (m, 1H, Ar–H), 7.46-7.79 (m, 2H, Ar–H), 7.79 -7.81 (m, 4H, Ar–H); ^{13}C APT (400 MHz, CDCl3) δ 14.9, 55.3, 95.1, 114.2, 116.4, 116.8, 117.3, 122.5, 124.6, 124.7, 124.8, 124.9, 127.0, 128.4, 129.5, 137.4, 144.3, 144.2, 146.7, 149.3, 150.7, 153.3, 157.8, 162.3, 163.5; ESI-MS (m/z): 443.4 (M^+); Anal. % Calculated for $C_{25}H_{19}FN_4O_3$: C, 67.87; H, 4.33; N, 12.66; Found: C, 67.63; H, 4.07; N, 12.44.

6.3.10 2-(5-(4-fluorophenoxy)-3-methyl-1-phenyl-1H-pyrazol-4-yl)-5-(p-tolyl)-1,3,4-oxadiazole (7j)

Yield 86 %, m.p. 178-180 °C; IR (KBr, ν_{max}, cm^{-1}): 1229 (C–O–C); 1625 and 1595 (C=N and C=C); 3054 (Ar, -CH str.); ^1H NMR (400 MHz, $CDCl_3$) δ 2.42 (s, 3H, Ar-CH_3), 2.74 (s, 3H, pyrazole-CH_3), 6.99-7.04 (m, 4H, Ar–H), 7.24-7.26 (m, 2H, Ar–H), 7.33-7.37 (m, 1H, Ar–H), 7.43-7.47 (m, 2H, Ar–H), 7.65-7.68 (m, 4H, Ar–H); ^{13}C APT (400 MHz, $CDCl_3$) δ 14.9, 21.5, 95.3, 116.4, 116.4, 116.6, 116.7, 120.9, 122.6, 123.4, 126.5, 127.9, 128.2, 129.3, 129.6, 137.1, 142.0, 147.2, 149.2, 150.3, 152.6, 157.6, 158.1, 160.0, 163.6; ESI-MS (m/z): 425.4 (M^+); Anal. % Calculated for $C_{25}H_{19}FN_4O_2$: C, 70.41; H, 4.49; N, 13.14; Found: C, 70.18; H, 4.26; N, 12.88.

6.3.11 2-(4-bromophenyl)-5-(5-(2-fluorophenoxy)-3-methyl-1-phenyl-1H-pyrazol-4-yl)-1,3,4-oxadiazole (7k)

Yield 86 %, m.p. 184-186 °C; IR (KBr, ν_{max}, cm^{-1}): 1227 (C–O–C); 1638 and 1595 (C=N and C=C); 3060 (Ar, -CH str.); ^1H NMR (400 MHz, $CDCl_3$) δ 2.74 (s, 3H, pyrazole-CH_3), 6.82-6.84 (m, 1H, Ar–H), 6.82-6.84 (m, 1H, Ar–H), 6.86-6.99 (m, 2H, Ar–H), 7.00-7.04 (m, 1H, Ar–H), 7.16-7.21 (m, 1H, Ar–H), 7.34-7.56 (m, 2H, Ar–H), 7.59-7.73 (m, 4H, Ar–H); ^{13}C APT (400 MHz, $CDCl_3$) δ 14.9, 116.3, 117.0, 117.2, 122.6, 122.6, 124.6, 127.7, 124.8, 125.6, 126.1, 128.0, 129.3, 132.2, 135.7, 137.0, 144.1, 145.7, 149.3, 150.5, 153.0, 154.8, 158.5, 162.8; ESI-MS (m/z): 492.3 (M^+); Anal. % Calculated for $C_{24}H_{16}BrFN_4O_2$: C, 58.67; H, 3.28; N, 11.40; Found: C, 58.41; H, 3.07; N, 11.13.

6.3.12 2-(4-chlorophenyl)-5-(5-(2-fluorophenoxy)-3-methyl-1-phenyl-1H-pyrazol-4-yl)-1,3,4-oxadiazole (7l)

Yield 79 %, m.p. 190-192 °C; IR (KBr, ν_{max}, cm^{-1}): 1226 (C–O–C); 1638 and 1598 (C=N and C=C); 3061 (Ar, -CH str.); ^1H NMR (400 MHz, $CDCl_3$) δ 2.74 (s, 3H, pyrazole-CH_3), 6.82-6.84 (m, 1H, Ar–H), 6.86-6.93 (m, 1H, Ar–H), 6.95-7.01 (m, 1H, Ar–H), 7.02-7.04 (m, 2H, Ar–H), 7.16-7.34 (m, 4H, Ar–H), 7.36-7.74 (m, 4H, Ar–H); ^{13}C APT (400 MHz, $CDCl_3$) δ 14.9, 94.8, 116.4, 117.0, 117.2, 122.1, 122.6, 124.6, 124.7, 124.7, 127.8, 128.0, 129.3, 137.0, 137.7, 144.1, 144.2, 147.0, 149.3, 150.6, 153.0, 154.9, 158.4, 162.7; ESI-MS (m/z): 447.7 (M^+); Anal. % Calculated for $C_{24}H_{16}ClFN_4O_2$: C, 64.51; H, 3.61; N, 12.54; Found: C, 64.28; H, 3.36; N, 12.27.

6.3.13 2-(4-chlorophenyl)-5-(5-(3-fluorophenoxy)-3-methyl-1-phenyl-1H-pyrazol-4-yl)-1,3,4-oxadiazole (7m)

Yield 87 %, m.p. 177-179 °C; IR (KBr, ν_{max}, cm^{-1}): 1227 (C–O–C); 1622 and 1597 (C=N and C=C); 3056 (Ar, -CH str.); ^1H NMR (400 MHz, $CDCl_3$) δ 2.75 (s, 3H, pyrazole-CH_3), 6.80-6.83 (m, 3H, Ar–H), 7.24-7.30 (m, 1H, Ar–H), 7.34-7.36 (m, 1H, Ar–H), 7.38-7.65 (m, 4H, Ar–H), 7.65-7.68 (m, 4H, Ar–H); ^{13}C APT (400 MHz, $CDCl_3$) δ 14.9, 95.3, 103.6, 103.9, 110.8, 110.9, 111.1, 133.6, 127.8, 128.0, 129.3, 129.3, 130.9, 131.0, 137.0, 137.7, 14.02, 149.3, 157.7, 157.6, 158.4, 162.3, 162.6, 164.7; ESI-MS (m/z): 447.8 (M^+); Anal. % Calculated for $C_{24}H_{16}ClFN_4O_2$: C, 64.51; H, 3.61; N, 12.54; Found: C, 64.26; H, 3.37; N, 12.28.

6.3.14 2-(5-(3-fluorophenoxy)-3-methyl-1-phenyl-1H-pyrazol-4-yl)-5-(4-methoxyphenyl)-1,3,4-oxadiazole (7n)

Yield 82 %, m.p. 169-171 °C; IR (KBr, ν_{max}, cm^{-1}): 1231 (C–O–C); 1631and 1593 (C=N and C=C); 3061 (Ar, -CH str.); ^1H NMR (400 MHz, $CDCl_3$) δ 2.57 (s, 3H, pyrazole-CH_3), 3.69 (s, 3H, -OCH_3), 6.62-6.65 (m, 3H, Ar–H), 6.75-6.77 (m, 2H, Ar–H), 7.05-7.11 (m, 1H, Ar–H), 7.16-7.19 (m, 1H, Ar–H), 7.25-7.29 (m, 2H, Ar–H), 7.48 -7.53 (m, 4H, Ar–H); ^{13}C APT (400 MHz, $CDCl_3$) δ 10.2, 50.6, , 99.2, 106.1, 106.2, 111.4, 117.8, 123.2, 126.6, 124.5, 126.1, 126.2, 132.3, 141.6, 144.5, 145.8, 152.8, 152.9, 153.0, 155.4, 157.4, 158.6, 160.2, 162.4, 163.7; ESI-MS (m/z): 443.4 (M^+); Anal. % Calculated for $C_{25}H_{19}FN_4O_3$: C, 67.87; H, 4.33; N, 12.66; Found: C, 67.66; H, 4.07; N, 12.38.

6.3.15 2-(4-chlorophenyl)-5-(3-methyl-1-phenyl-5-(3-(trifluoromethyl)phenoxy)-1H-pyrazol-4-yl)-1,3,4-oxadiazole (7o)

Yield 88 %, m.p. 194-196 °C; IR (KBr, ν_{max}, cm^{-1}): 1234 (C–O–C); 1634 and 1594 (C=N and C=C); 3062 (Ar, -CH str.); ^1H NMR (400 MHz, $CDCl_3$) δ 2.77 (s, 3H, pyrazole-CH_3), 7.10-7.12 (d, 1H, Ar–H), 7.33 (s, 3H, Ar–H), 7.36-7.38 (m, 3H, Ar–H), 7.42-7.54 (m, 2H, Ar–H), 7.61-7.67 (m, 4H, Ar–H); ^{13}C APT (400 MHz, $CDCl_3$) δ 15.0, 113.2, 113.2, 117.9, 120.1, 120.7, 122.4, 122.7, 126.2, 127.1, 128.2, 129.3, 130.8, 135.5, 142.4, 149.7, 150.2, 152.8, 153.6, 154.7, 155.3, 156.8, 156.9, 163.8; ESI-MS (m/z): 497.8 (M^+); Anal. % Calculated for $C_{25}H_{16}ClF_3N_4O_2$: C, 60.43; H, 3.25; N, 11.28; Found: C, 60.15; H, 3.04; N, 11.05.

6.3.16 2-(4-methoxyphenyl)-5-(3-methyl-1-phenyl-5-(3-(trifluoromethyl)phenoxy)-1H-pyrazol-4-yl)-1,3,4-oxadiazole (7p)

Yield 84 %, m.p. 188-190 °C; IR (KBr, ν_{max}, cm^{-1}): 1237 (C–O–C); 1637 and 1595 (C=N and C=C); 3067 (Ar, -CH str.); ^1H NMR (400 MHz, $CDCl_3$) δ 2.76 (s, 3H, pyrazole-CH_3), 3.86 (s, 3H, -OCH_3), 6.90-6.93 (d, 2H, Ar–H), 7.10-7.12(d, 1H, Ar–H),

7.36-7.40 (m, 2H, Ar–H), 7.44-7.47 (m, 4H, Ar–H), 7.62-7.67 (m, 4H, Ar–H); ^{13}C APT (400 MHz, $CDCl_3$) δ 14.9, 55.4, 95.4, 113.3, 113.3, 114.3, 116.0, 117.9, 130.6, 120.6, 122.7, 128.0, 128.2, 129.4, 130.7, 132.4, 132.8, 137.0, 146.1, 149.4, 156.7, 157.7, 160.4, 162.2, 163.4; ESI-MS (m/z): 493.3 (M^+); Anal. % Calculated for $C_{26}H_{19}F_3N_4O_3$: C, 63.41; H, 3.89; N, 11.38; Found: C, 63.18; H, 3.61; N, 11.14.

7. Biological evaluation

7.1. *In vitro* antimicrobial assay

The antimicrobial activity of fluoro substituted pyrazole containing 1,3,4-oxadiazole scaffolds was carried out by broth micro dilution method. DMSO was used as the diluent to get the desired concentration of compounds to test upon standard bacterial strains. Mueller-Hinton broth was used as nutrient medium to grow and dilute the compound suspension for the test bacteria. Sabouraud Dextrose broth was used for fungal nutrition. Inoculum size for test strain was adjusted to 10^8 CFU mL^{-1} by comparing the turbidity. Serial dilutions were prepared in primary and secondary screening. Each synthesized compound and the standard drugs were diluted obtaining 2000 µg/mL concentration as the stock solution. The compounds which were found to be active in their primary screening (i.e. 500, 250 and 200 µg/mL concentrations) were further screened in their second set of dilution at 100, 50, 25 and 12.5 µg/mL concentrations against all microorganisms. 10 µL suspensions were further inoculated on appropriate media and growth was noted after 24 and 48 h. The control tube containing no antibiotic was instantaneously subcultured (before inoculation) by evenly spreading a loopful over an area of plate of medium suitable for the growth of the test organism. The tubes were then put overnight for incubation at 37°C. The highest dilution preventing appearance of turbidity after spot subculture was considered as minimal inhibitory concentration (MIC, mM) and was listed in **Table 1**. All the tubes showing no visible growth (same as the control tube) were subcultured and incubated overnight at 37°C. The amount of growth from the control tube before incubation was compared. In this study ampicillin, norfloxacin, chloramphenicol and ciprofloxacin were used as the standard antibacterial drugs. Nystatin and Griseofulvin were used as the standard antifungal drugs. The results are summarized in **Table 1**.

7.2. *In vitro* antituberculosis assay

All fluoro substituted pyrazole containing 1,3,4-oxadiazole derivatives were screened for their antitubercular activity against *Mycobacterium tuberculosis* $H_{37}Rv$ performed by Lowensteine-Jensen method with minor modification where 250 µg/mL dilution of each compound was added to Lowensteine-Jensen medium and then media was uncontaminated by inspissation method. A culture of *Mycobacterium tuberculosis* $H_{37}Rv$ grown on Lowensteine-Jensen medium was harvested in 0.85% saline in bijou bottle. The stock solutions of title compounds (100 µg/mL) were prepared in DMSO. These tubes were then incubated at 37°C for 24 h followed by streaking of *Mycobacterium tuberculosis* H37Rv (5×10^4 bacilli per tube). The growth of bacilli was observed after 2 weeks, 3 weeks and finally after 4 weeks of incubation. The tubes having the compounds were compared with control tubes where medium alone was incubated with *Mycobacterium tuberculosis* $H_{37}Rv$. The concentration at which complete inhibition of colonies occurred was taken as active concentration of the tested compound. The standard strain *Mycobacterium tuberculosis* $H_{37}Rv$ was also tested with the known drugs rifampicin and isoniazid for comparison. The results are summarized in **Table 2**.

7.3. *In vitro* antimalarial assay

In vitro antimalarial activity of the fluoro substituted pyrazole based 1,3,4-oxadiazole derivatives was screened against *P. falciparum* strain. *P. falciparum* strain was acquired from Shree R. B Shah Mahavir Super-speciality hospital, Surat, Gujarat, India and was used in *in vitro* tests. *P. falciparum* strain was cultivated by a modified method described by Trager and Jensen [33]. Compounds were dissolved in DMSO. The final concentration of DMSO used was not toxic and did not interfere with the assay. The antiparasitic effect of the compounds was measured by growth inhibition percentage as described by Carvalho and Krettli [34]. For experimental purpose, the cultures were synchronized with 5% D-sorbitol when the parasites were in the ring stage [35]. The parasite suspension, consisting of predominately the ring stage, was adjusted to a 1-2 % parasitaemia and 2.5 % haematocrit in hypoxanthine-free RPMI-1640 culture medium with 10% human plasma and was exposed to 7 concentrations of each compound for a single cycle of parasitic growth for 48 h at 37°C. A positive control with reference to antimalarial drugs in standard concentrations was used for each experiment. The stock solutions were additionally diluted in whole medium (RPMI 1640 plus 10% human serum) to each of the used concentrations. The concentration that inhibited 50% of parasite growth (IC_{50} value) was determined by interpolation method using Microcal Origin software. The standard drugs chloroquine and quinine were used as the reference antimalarial drugs, blood smears were read blind and each duplicate experiment was repeated thrice. The results are summarized in **Table 3**.

Acknowledgements

The authors are thankful to Head, Department of Chemistry, Sardar Patel University for providing necessary research facilities and constant encouragement. SCK and VBP gratefully acknowledge the University Grants Commission, New Delhi, India for meritorious fellowships awards 2013-2015.

Notes

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The spectral data of synthesized compound are shown in supplementary data.

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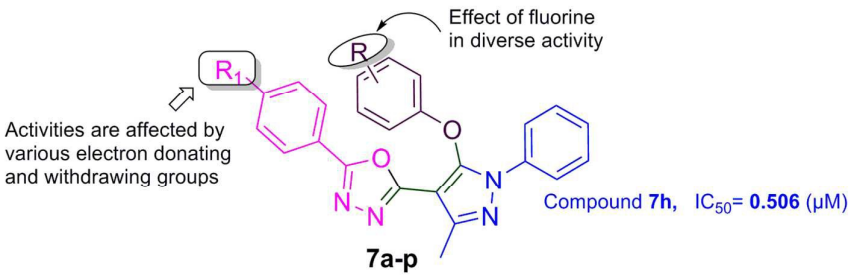

Graphical Abstract