4.1 Preamble

This chapter deals with synthesis of a novel series of fluoro substituted pyrazole nucleus clubbed with 1,3,4-oxadiazole scaffolds. The targeted compound were synthesized in good yield. The structures of all the compounds were confirmed on the basis of elemental analysis, IR, ^{1}H NMR, ^{13}C 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* H37Rv and antimalarial activity against *Plasmodium falciparum.*

4.2 1,3,4-Oxadiazole

Oxadiazole is a five membered heterocycle having two carbons, two nitrogens, one oxygen and two double bonds having general formula $C_2H_2ON_2$. Oxadiazole is considered to be derived from furan by replacement of two methine (-CH=) groups by two pyridine type nitrogen (-N=). There are four possible isomers of oxadiazole depending on the position of nitrogen atom in the ring and are numbered as shown in **Figure 4.1**

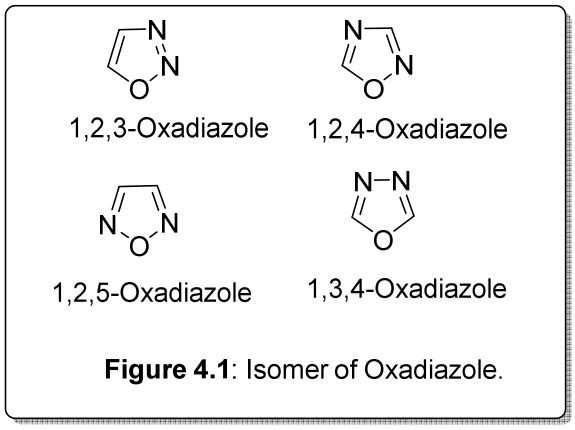


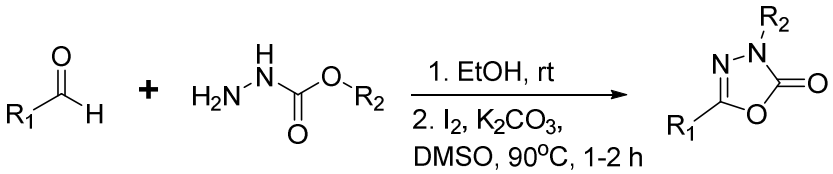
Figure 4.1: Isomer of Oxadiazole.

Out of its four possible isomers, 1,3,4-oxadiazole is widely exploited for various applications. A variety of substituted 1,3,4-oxadiazoles have attracted considerable attention in the field of drug discovery because of their wide range of pharmacological activities. The capacity of 1,3,4-oxadiazole nucleus to undergo variety of chemical reactions including electrophillic substitution, nucleophilic

substitution, thermal and photochemical reactions makes it medicinal backbone on which a number of potential molecules can be constructed.

4.3 Reported method for synthesis of 1,3,4-oxadiazole

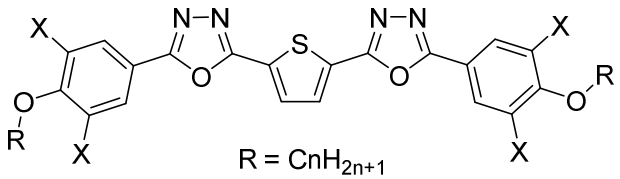
Nidhi Jain and co-workers[1] reported a simple and efficient iodine-assisted protocol for synthesis of 5-substituted-3-methyl/benzyl-1,3,4-oxadiazol-2(3H)-ones *via* sequential condensation/ oxidative cyclization and rearrangement. (Scheme 4.1).



R_1 = aryl/ heteroaryl
R_2 = methyl/ benzyl

Scheme 4.1 Iodine assisted synthesis of 5-substituted-3-methyl/ benzyl-1,3,4-oxadiazol-2(3H)-ones .

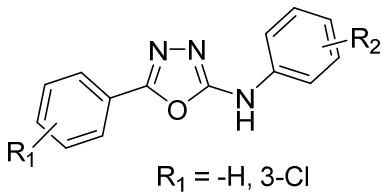
Chung K. Lai and co-workers[2] synthesized three new series of bis-pyrazoles and bis-1,3,4-oxadiazoles scaffolds (Figure 4.2).



R = C_nH_{2n+1}
X = -H
X = OR

Figure 4.2 Bis-1,3,4-oxadiazoles scaffolds.

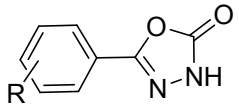
Shokoofeh Maghari *et. al* [3] prepared an efficient method for the synthesis of 2,5-disubstituted 1,3,4-oxadiazoles from isothiocyanates and hydrazides through cyclodesulfurization in the presence of (O-(benzotriazol-1-yl)-N,N,N0,N0-tetramethyluroniumtetrafluoroborate) TBTU as an uranium coupling reagent. (Figure 4.3).



R_1 = -H, 3-Cl
R_2 = -H, 4-CH_3, 3-CH_3, 4-OCH_3, 4-Cl

Figure 4.3 2,5-disubstituted 1,3,4-oxadiazoles.

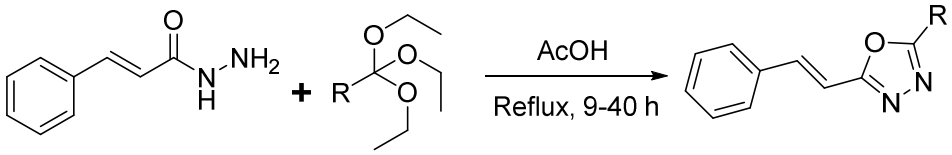
Animesh Pramanik and co-workers[4] prepared a new and efficient route for the synthesis of biologically important 5-aryl-3H-[1,3,4]oxadiazol-2-ones from N0-(chloro-aryl-methylene)-tert-butylcarbazates using basic alumina as a solid support under solvent-free condition (Figure 4.4).



R = -H, 4-CH_3, 3-NO_2, 4-NO_2, 4-F, 2-Cl, 3-Cl,
2-Br, 3-Br, 4-Br, 4-CN, 4-OCH_3, 4-Cl

Figure 4.4 Synthesis of 5-aryl-3H-[1,3,4]oxadiazol-2-ones.

Agnieszka Kudelko and his co-worker[5] develop a novel and efficient synthesis of 2-styryl-1,3,4-oxadiazoles by cyclocondensation of cinnamic acid hydrazide and triethyl orthoesters under microwave irradiation (Scheme 4.2).



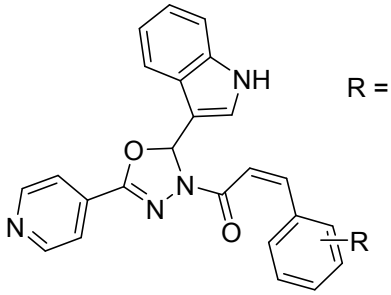
R = -H, Me, Et, Ph

Scheme 4.2 Synthesis of 2-styryl-1,3,4-oxadiazole scaffolds.

4.4 Biological screening

4.4.1 Biological screening of 1,3,4-oxadiazole scaffolds

N. C. Desai and his co-workers[6] reported a series of indole and pyridine based 1,3,4-oxadiazole derivatives and evaluated their *in vitro* antitubercular activity against *Mycobacterium tuberculosis* H37Ra (MTB) and Mycobacterium bovis BCG both in active and dormant state (Figure 4.5).



R = -H, 2-OH, 3-OH, 4-OH, 2-NO_2,
3-NO_2, 4-NO_2, 4-CH_3, 4-OCH_3, 2-F, 3-F,
4-F, 2-Cl, 3-Cl, 4-Cl, 2,3-$(Cl)_2$, 2,4-$(Cl)_2$,
2,6-$(Cl)_2$, 3-Br, 4-Br

Figure 4.5 Antitubercular activity of pyridine based 1,3,4-oxadiazole derivatives.

Simona Distinto *et. al* [7] reported the synthesis of 3-acetyl-2-dichlorophenyl-5-aryl-2,3-dihydro-1,3,4-oxadiazole scaffold and evaluated them for their *in vitro* and *in silico* selective monoaminoxidase B inhibitors activity (Figure 4.6).

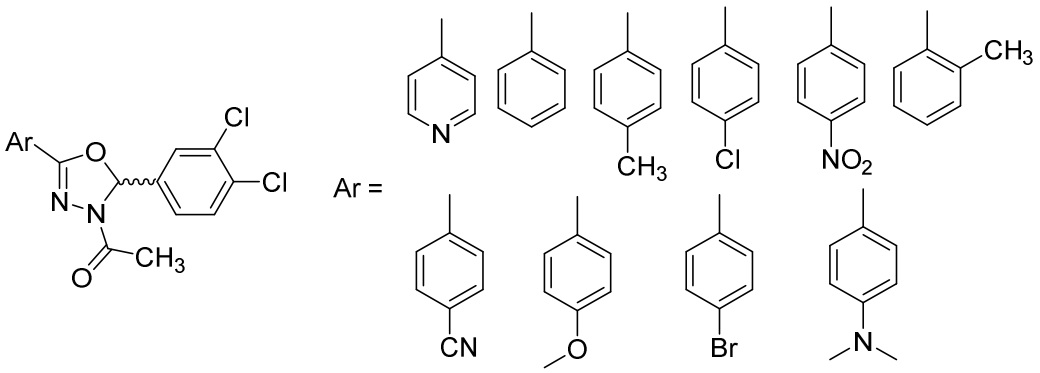
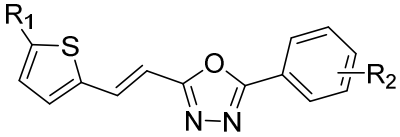


Figure 4.6 Biologically active1,3,4-oxadiazole derivatives.

Fatiha Benmansour *et. al* [8] prepared a series of new 2-phenyl-5-[(E)-2-(thiophen-2-yl)ethenyl]-1,3,4-oxadiazole derivatives and evaluated for anti-DENV-2 RdRp activity (Figure 4.7).

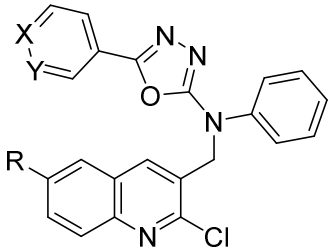


R_1= -Br, Ph

R_2 = 4-F, 4-OCH_3, 3-Cl, 3,4-Cl, 4-CF_3, 4-Br

Figure 4.7 2-phenyl-5-[(E)-2-(thiophen-2-yl)ethenyl]-1,3,4-oxadiazole derivatives for anti-DENV-2 RdRp activity.

Manish P. Patel and his co-worker[9] reported novel 1,3,4-oxadiazole motifs bearing quinoline nucleus and evaluated for their *in vitro* antimicrobial, antitubercular, antimalarial and cytotoxic activity (Figure 4.8).



R = -H, -CH_3, -Cl, -OCH_3
X = -CH, N
Y = -CH, N

Figure 4.8 Biologically active 1,3,4-oxadiazole motifs bearing quinoline nucleus.

Chang-Hyun Oh and co-workers[10] prepared a series of diarylamides and diarylureas possessing 1,3,4-oxadiazole scaffold and evaluated *in vitro*

antiproliferative activities against a panel of 58 cell lines of nine different cancer types at the NCI taking compared with Sorafenib as a reference compound (Figure 4.9).

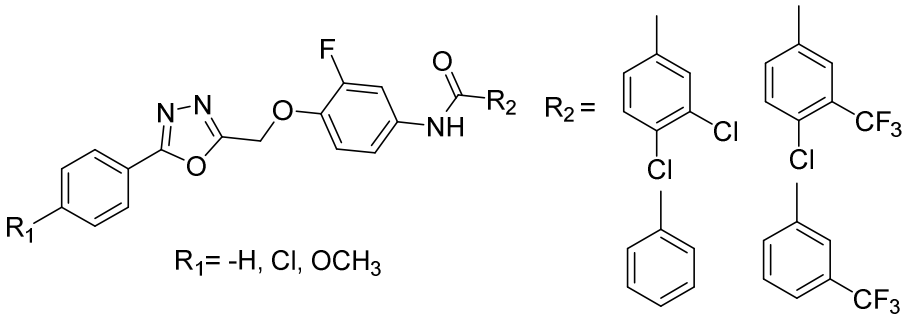
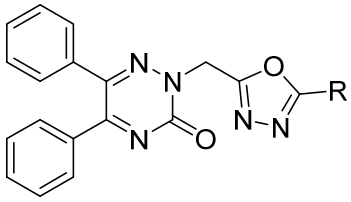


Figure 4.9 Antiproliferative active 1,3,4-oxadiazole scaffolds.

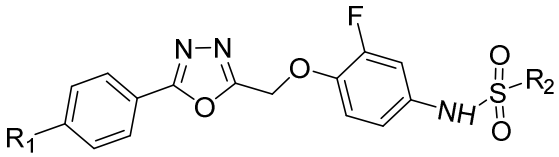
Anupam G. Banerjee *et. al.*[11] prepared and chatrecterized a series of triazine-3(2H)-one derivatives bearing 1,3,4-oxadiazole. The compounds were evaluated for anti-inflammatory and analgesic activities. Preliminary in vitro anti-inflammatory activity was assessed using an albumin denaturation assay (Figure 4.10).



R = Aryl, Sustituted aryl / Heteroaryl

Figure 4.10 Anti-inflammatory and analgesic 1,3,4-oxadiazole scaffolds.

Mahmoud M. Gamal El-Din *et. al.*[12] reported a new series of 1,3,4-oxadiazole derivatives possessing sulfonamide moiety. The *in vitro* antiproliferative activities against NCI-58 human cancer cell lines of nine different cancer types were tested for the compounds (Figure 4.11).



R_1 = -H, Cl, OCH_3

R_2 = -CH_3, n-Pr, C_6H_5, 4-Cl(C_6H_4), 4-Tolyl, 4-MeO(C_6H_4)

Figure 4.11 Antiproliferative agents from 1,3,4-oxadiazole scaffolds.

Lingaiah Nagarapu and co-worker[13] reported a series of novel analogs of benzosuberone embedded with 1,3,4-oxadiazole, 1,3,4-thiadiazole and 1,2,4-triazole moieties were synthesized in excellent yields and evaluation of their in vitro anti proliferative activity (Figure 4.12).

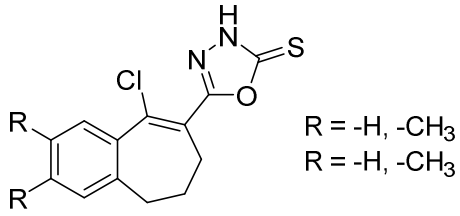
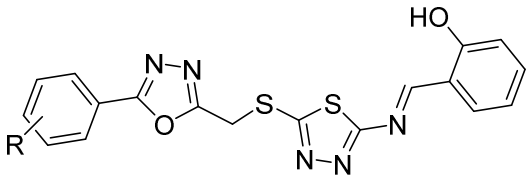


Figure 4.12 Antiproliferative agents from 1,3,4-oxadiazole scaffolds.

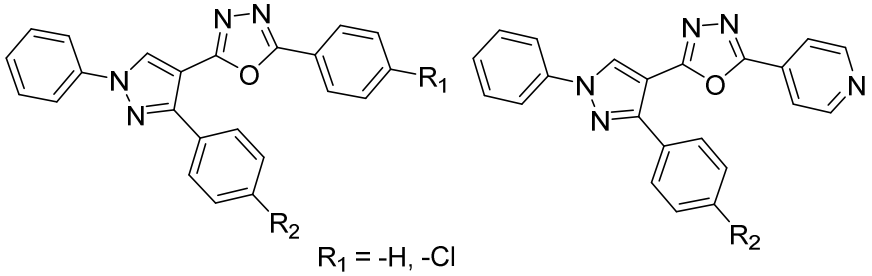
Bao-Quan Chen and co-workers[14] reported series of novel hybrid molecules containing 1,3,4-oxadiazole and 1,3,4-thiadiazole bearing Schiff base moiety and evaluated for their in vitro antitumor activities against SMMC-7721, MCF-7 and A549 human tumor cell lines by CCK-8 assay (Figure 4.13).



R = -H, 4-F, 2-Cl, 4-Cl, 2-Br,
2-CH_3, 3-CH_3, 3-OCH_3,
3,4,5-Tri-OCH_3, 3-NO_2, 4-NO_2

Figure 4.13 Antitumor active 1,3,4-oxadiazole scaffolds.

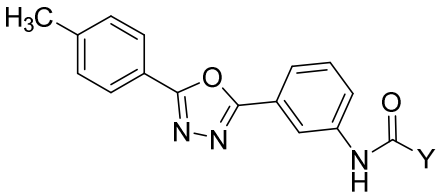
Sumit Bansal *et. al*. [15] prepared a novel series of 2-phenyl-5-(1,3-diphenyl-1H-pyrazol-4-yl)-1,3,4-oxadiazoles for selective COX-2 inhibition with potent anti-inflammatory activity (Figure 4.14).



R_1 = -H, -Cl
R_2 = -H, -F, -Cl, -Br, -CH_3, -OCH_3,-NO_2,

Figure 4.14 Anti-inflammatory agents from 1,3,4-oxadiazole scaffolds.

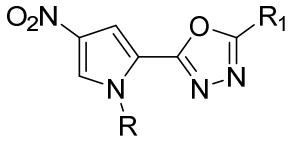
Fan Yang and co-workers[16] prepared synthesisized of 2,5-Diphenyl-1,3,4-oxadiazoles and evaluated structure-activity relationship for non-phosphorus-based fructose-1,6-bisphosphatase inhibitors activity (Figure 4.15).



Y = Et, i-Pr, n-Bu, t-Bu

Figure 4.15 Biologically active1,3,4-oxadiazole scaffolds.

Rajesh A. Rane and co-workers[17] prepared novel 4-nitropyrrole-based 1,3,4-oxadiazoles and evaluated for anti-bacterial, anti-fungal and anti-tubercular activities (Figure 4.16).



R = -H, -CH_3
R_1 = aryl/ heteroaryl

Figure 4.16 Biologically active1,3,4-oxadiazole scaffolds.

4.5 Present work

Pyrazole ring is a ubiquitous core in heterocyclic chemistry and represents a key motif in medicinal chemistry due to its potential to exhibit an array of bioactivities such as antimicrobial[18], anti-inflammatory [19], antipyretic [20], anticancer [21], anti-viral, antitumor [22, 23] , analgesic [24], fungistatic [25], and anti-hyperglycemic activity[26, 27]. 1,3,4-Oxadiazole forms important class of heterocyclic bioactive compounds which has extensively attracted attention, owing to its remarkable biological and pharmacological properties such as antibacterial[28], and anti-tubercular activities[29, 30], anti-inflammatory[31], antifungal[32], antidepressant[33], anti-proliferative[34], anti-anxiety[35]. Moreover, 1,3,4-oxadiazole heterocycles are very good bioisosteres of amides and esters, which contribute substantially to pharmacological potency by participating in hydrogen bonding interactions with the receptors. Several biologically active pyrazofurin and pyrazole based 1,3,4-oxadiazole scaffolds have been reported (**Fig. 4.17 A–C**)[29, 36, 37].

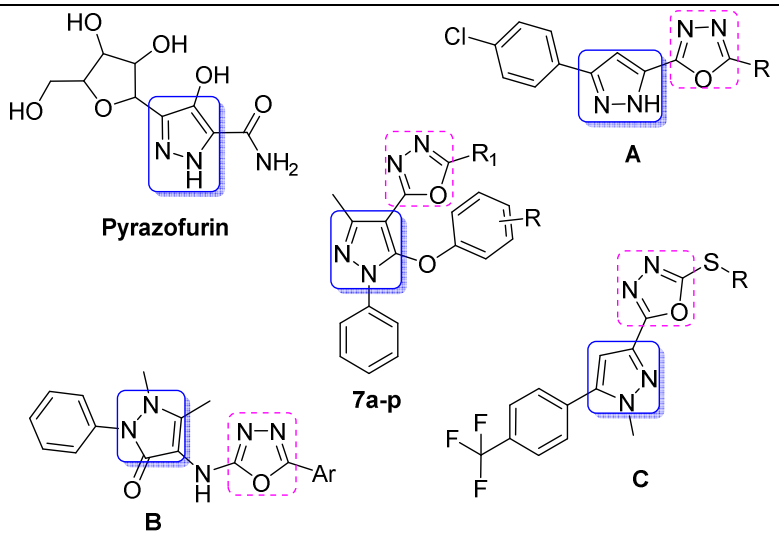
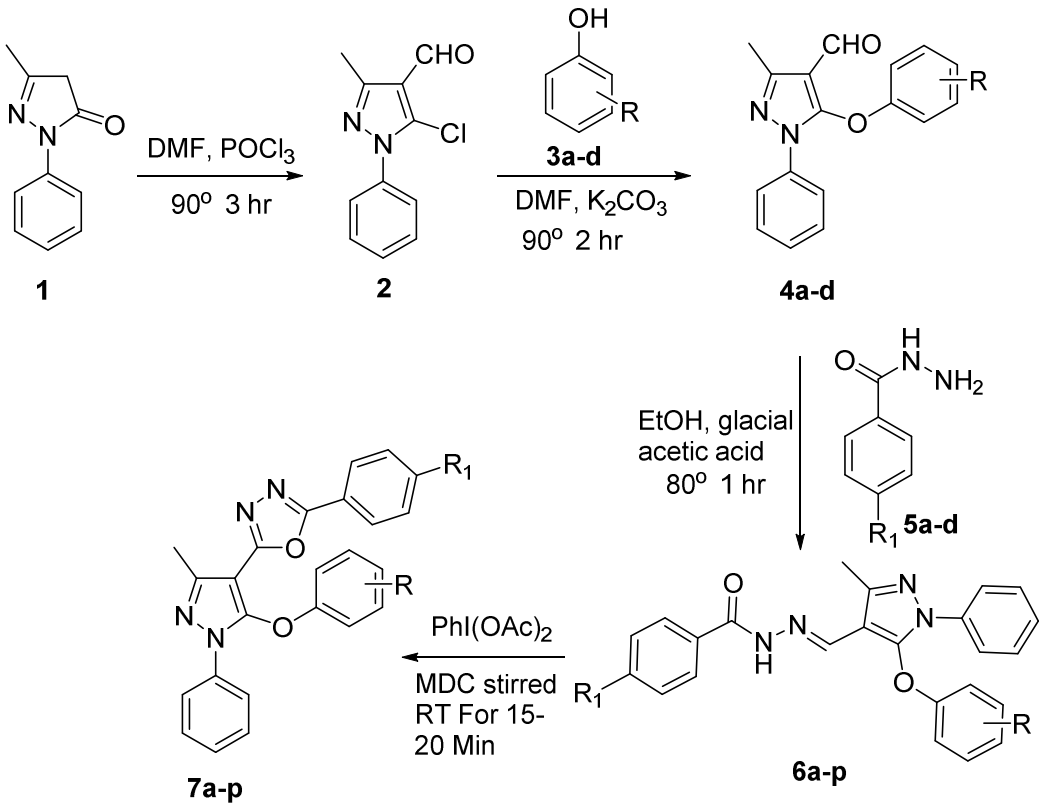


Figure 4.17. Structures of pyrazofurin and some reported biologically active pyrazole based 1,3,4-oxadiazoles scaffold **A**, **B**, **C** and synthesized compounds **7a–p.**

In context of above, We have designed and synthesized fluoro substituted pyrazole based 1,3,4-oxadiazole scaffolds **7a-p**. 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.

4.6 Reaction scheme

The synthetic protocol for novel series of fluoro substituted pyrazole bearing 1,3,4-oxadiazole scaffolds was performed as outlined in **Scheme 4.3.** 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[38]**.** 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-substituted benzohydrazide **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 phenyliododiacetate ($PhI(OAc)_2$) in dichloromethane (MDC) by stirring at room temperature for 20 min to afford corresponding 1,3,4-oxadiazoles **7a-p**.



Scheme 4.3 Sythesis of fluoro substituted pyrazole bearing 1,3,4-oxadiazole scaffolds

4.7 Experimental

- 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.

4.8 Synthesis fluoro substituted novel pyrazole nucleus clubbed with 1,3,4-oxadiazole derivatives

The title compounds (**7a-7p**) were synthesized in following steps:

4.8.1 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.

Table 4.1 Physical data of 3-methyl-5-substituted aryloxy-1-phenyl-1H-pyrazole-4-carbaldehyde (4a-d).

Comp.	IUPAC Name	M. F. (MW)	Yield (%)	m.p. (°C)
4a	*5-(2-fluorophenoxy)-3-methyl-1-phenyl-1H-pyrazole-4-carbaldehyde*	$C_{17}H_{13}FN_2O_2$ (297.2)	85	225-227
4b	*5-(3-fluorophenoxy)-3-methyl-1-phenyl-1H-pyrazole-4-carbaldehyde*	$C_{17}H_{13}FN_2O_2$ (297.3)	78	210-212
4c	3-methyl-1-phenyl-5-(3-(trifluoromethyl)phenoxy)-1H-pyrazole-4-carbaldehyde	$C_{18}H_{13}F_3N_2O_2$ (347.2)	81	226-228
4d	*5-(4-fluorophenoxy)-3-methyl-1-phenyl-1H-pyrazole-4-carbaldehyde*	$C_{17}H_{13}FN_2O_2$ (297.1)	82	245-247

4.8.2 General procedure for the 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 afford compounds (**6a-p**).

Table 4.2 Physical data of substituted pyrazole based hydrazones (6a-p).

Comp.	R	R_1	Yield[a](%)	Comp.	R	R_1	Yield[a](%)
6a	2-F	$-CH_3$	77	**6i**	4-F	$-OCH_3$	76
6b	3-F	-Br	80	**6j**	4-F	$-CH_3$	78
6c	3-F	$-CH_3$	75	**6k**	2-F	-Br	74
6d	$3-CF_3$	$-CH_3$	79	**6l**	2-F	-Cl	75
6e	4-F	-Cl	81	**6m**	3-F	-Cl	73
6f	$3-CF_3$	-Br	72	**6n**	3-F	$-OCH_3$	73
6g	2-F	$-OCH_3$	79	**6o**	$3-CF_3$	-Cl	70
6h	4-F	-Br	77	**6p**	$3-CF_3$	$-OCH_3$	68

4.8.3 General procedure for the 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 affording target compounds (**7a-p**).

4.9 Preliminary and spectral characterization

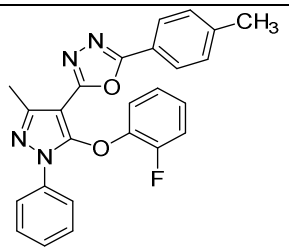
The structure of the targeted fluoro substituted pyrazole motifs clubbed with 1,3,4-oxadiazole scaffolds **7a-p** were confirmed by mass spectrometry, ^{1}H NMR, FT-IR and elemental analysis. The mass spectrum of all the compounds showed molecular ion peak (M+) corresponding to their respective molecular 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.

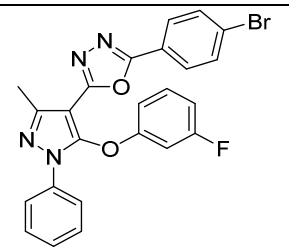
Table 4.3 Preliminary Characterization of all synthesized compounds 7a-p

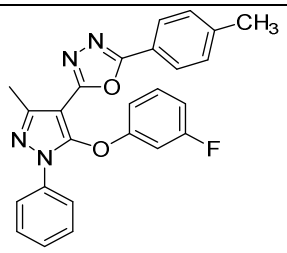
Comp.	R	R_1	Yield[a](%)	Comp.	R	R_1	Yield[a](%)
7a	2-F	$-CH_3$	79	**7i**	4-F	$-OCH_3$	85
7b	3-F	-Br	81	**7j**	4-F	$-CH_3$	86
7c	3-F	$-CH_3$	80	**7k**	2-F	-Br	86
7d	3-CF_3	$-CH_3$	82	**7l**	2-F	-Cl	79
7e	4-F	-Cl	81	**7m**	3-F	-Cl	87
7f	3-CF_3	-Br	86	**7n**	3-F	$-OCH_3$	82
7g	2-F	$-OCH_3$	80	**7o**	3-CF_3	-Cl	88
7h	4-F	-Br	80	**7p**	3-CF_3	$-OCH_3$	84

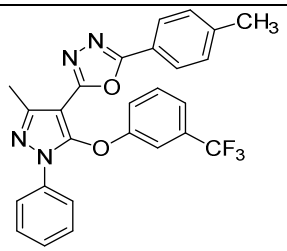
[a] Isolated yield

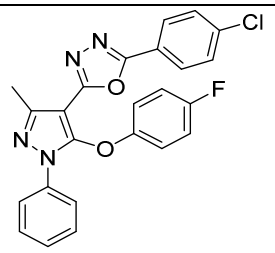
- ❖ The spectral Characterization of all synthesized compounds **7a-p** are depicted in following tables.
- ✓ The ^{1}H NMR spectra of Compounds **6a, 6b, 7a** and **7b** are represented in Figures. 4.18, 4.20, 4.22 and 4.24 respectively.
- ✓ The ^{13}C APT spectra of Compounds **6a, 6b, 7a** and **7b** are represented in Figures. 4.19, 4.21, 4.23 and 4.25 respectively.

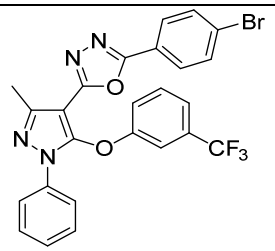
7a	***2-(5-(2-fluorophenoxy)-3-methyl-1-phenyl-1H-pyrazol-4-yl)-5-(p-tolyl)-1,3,4-oxadiazole***		
Mol. for. M. P. (°C) Mol. Wt.	$C_{25}H_{19}FN_4O_2$ 176-178 427.1		 **7a**
Ele. Ana. Calcd.(Obs)	C 70.41 (70.17)	H 4.49 (4.26)	N 13.14 (12.93)
FT-IR ν_{max} cm^{-1} (KBr)	1215 (C–O–C); 1622 and 1594 (C=N and C=C); 3054 (Ar, -CH str.)		
^{1}H NMR δ ppm DMSO-d_6	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 NMR δ ppm DMSO-d_6	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		

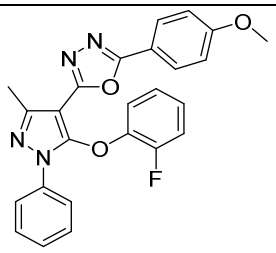
7b	***2-(4-bromophenyl)-5-(5-(3-fluorophenoxy)-3-methyl-1-phenyl-1H-pyrazol-4-yl)-1,3,4-oxadiazole***		
Mol. for. M. P. (°C) Mol. Wt.	$C_{24}H_{16}BrFN_4O_2$ 154-156 492.2		 **7b**
Ele. Ana. Calcd.(Obs)	C 58.67 (58.44)	H 3.28 (3.03)	N 11.40 (11.14)
FT-IR ν_{max} cm^{-1} (KBr)	1215 (C–O–C); 1621 and 1592 (C=N and C=C); 3051 (Ar, -CH str.)		
^{s1}H NMR δ ppm DMSO-d_6	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 NMR δ ppm DMSO-d_6	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		

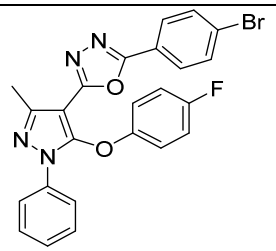
7c	***2-(5-(3-fluorophenoxy)-3-methyl-1-phenyl-1H-pyrazol-4-yl)-5-(p-tolyl)-1,3,4-oxadiazole***		
Mol. for. M. P. (°C) Mol. Wt.	$C_{26}H_{19}F_3N_4O_2$ 181-183 427.4		 **7c**
Ele. Ana. Calcd.(Obs)	C 70.41 (70.18)	H 4.49 (4.25)	N 13.14 (12.87)
FT-IR ν_{max} cm^{-1} (KBr)	1213 (C–O–C); 1622 and 1589 (C=N and C=C); 3051 (Ar, -CH str.)		
^{s1}H NMR δ ppm DMSO-d_6	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 NMR δ ppm DMSO-d_6	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		

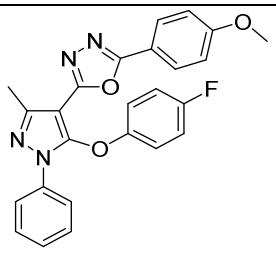
7d	***2-(3-methyl-1-phenyl-5-(3-(trifluoromethyl)phenoxy)-1H-pyrazol-4-yl)-5-(p-tolyl)-1,3,4-oxadiazole***		
Mol. for. M. P. (°C) Mol. Wt.	$C_{25}H_{19}FN_4O_2$ 172-174 477.4		 **7d**
Ele. Ana. Calcd.(Obs)	C 65.54 (65.30)	H 4.02 (3.81)	N 11.76 (11.54)
FT-IR ν_{max} cm^{-1} (KBr)	1216 (C–O–C); 1617 and 1598 (C=N and C=C); 3052 (Ar, -CH str.)		
^{s1}H NMR δ ppm DMSO-d_6	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 NMR δ ppm DMSO-d_6	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		

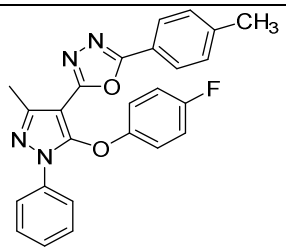
7e	***2-(4-chlorophenyl)-5-(5-(4-fluorophenoxy)-3-methyl-1-phenyl-1H-pyrazol-4-yl)-1,3,4-oxadiazole***		
Mol. for. M. P. (°C) Mol. Wt.	$C_{24}H_{16}ClFN_4O_2$ 168-170 447.7		 **7e**
Ele. Ana. Calcd.(Obs)	C 64.51 (64.29)	H 3.61 (3.38)	N 12.54 (12.28)
FT-IR ν_{max} cm^{-1} (KBr)	1218 (C–O–C); 1625 and 1595 (C=N and C=C); 3053 (Ar, -CH str.)		
^{s1}H NMR δ ppm DMSO-d_6	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 NMR δ ppm DMSO-d_6	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		

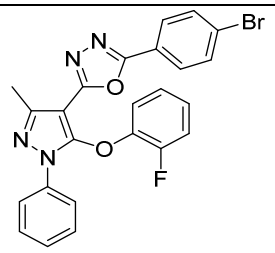
7f	***2-(4-bromophenyl)-5-(3-methyl-1-phenyl-5-(3-(trifluoromethyl)phenoxy)-1H-pyrazol-4-yl)-1,3,4-oxadiazole***		
Mol. for. M. P. (°C) Mol. Wt.	$C_{25}H_{16}BrF_3N_4O_2$ 158-160 542.2		 **7f**
Ele. Ana. Calcd.(Obs)	C 55.47 (55.24)	H 2.98 (2.77)	N 10.35 (10.09)
FT-IR ν_{max} cm^{-1} (KBr)	1225 (C–O–C); 1625 and 1590 (C=N and C=C); 3057 (Ar, -CH str.)		
^{s1}H NMR δ ppm DMSO-d_6	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 NMR δ ppm DMSO-d_6	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		

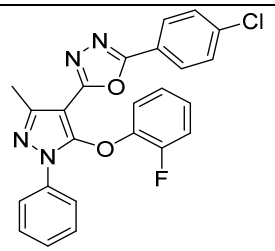
7g	***2-(5-(2-fluorophenoxy)-3-methyl-1-phenyl-1H-pyrazol-4-yl)-5-(4-methoxyphenyl)-1,3,4-oxadiazole***		
Mol. for. M. P. (°C) Mol. Wt.	$C_{25}H_{19}FN_4O_3$ 172-174 443.4		 **7g**
Ele. Ana. Calcd.(Obs)	C 67.87 (67.64)	H 4.33 (4.11)	N 12.66 (12.39)
FT-IR ν_{max} cm^{-1} (KBr)	1229 (C–O–C); 1621 and 1598 (C=N and C=C); 3056 (Ar, -CH str.)		
^{s1}H NMR δ ppm DMSO-d_6	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 NMR δ ppm DMSO-d_6	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		

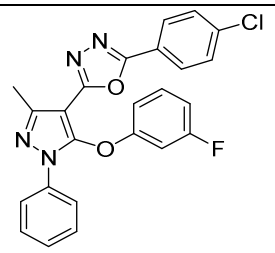
7h	***2-(4-bromophenyl)-5-(5-(4-fluorophenoxy)-3-methyl-1-phenyl-1H-pyrazol-4-yl)-1,3,4-oxadiazole***		
Mol. for. M. P. (°C) Mol. Wt.	$C_{24}H_{16}BrFN_4O_2$ 177-179 492.3		 **7h**
Ele. Ana. Calcd.(Obs)	C 58.67 (58.39)	H 3.28 (3.07)	N 11.40 (11.15)
FT-IR ν_{max} cm^{-1} (KBr)	1237 (C–O–C); 1636 and 1594 (C=N and C=C); 3053 (Ar, -CH str.)		
^{s1}H NMR δ ppm DMSO-d_6	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 NMR δ ppm DMSO-d_6	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		

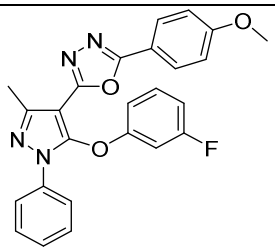
7i	***2-(5-(4-fluorophenoxy)-3-methyl-1-phenyl-1H-pyrazol-4-yl)-5-(4-methoxyphenyl)-1,3,4-oxadiazole***		
Mol. for. M. P. (°C) Mol. Wt.	$C_{25}H_{19}FN_4O_3$ 157-159 443.4		 **7i**
Ele. Ana. Calcd.(Obs)	C 67.87 (67.63)	H 4.33 (4.07)	N 12.66 (12.44)
FT-IR ν_{max} cm^{-1} (KBr)	1227 (C–O–C); 1623 and 1593 (C=N and C=C); 3058 (Ar, -CH str.)		
^{s1}H NMR δ ppm DMSO-d_6	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 NMR δ ppm DMSO-d_6	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		

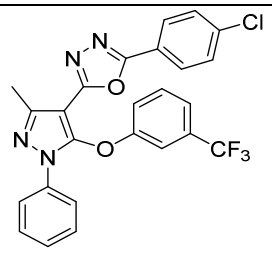
7j	***2-(5-(4-fluorophenoxy)-3-methyl-1-phenyl-1H-pyrazol-4-yl)-5-(p-tolyl)-1,3,4-oxadiazole***		
Mol. for. M. P. (°C) Mol. Wt.	$C_{25}H_{19}FN_4O_2$ 178-180 425.4		 **7j**
Ele. Ana. Calcd.(Obs)	C 70.41 (70.18)	H 4.49 (4.26)	N 13.14 (12.88)
FT-IR ν_{max} cm^{-1} (KBr)	1229 (C–O–C); 1625 and 1595 (C=N and C=C); 3054 (Ar, -CH str.)		
^{s1}H NMR δ ppm DMSO-d_6	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 NMR δ ppm DMSO-d_6	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		

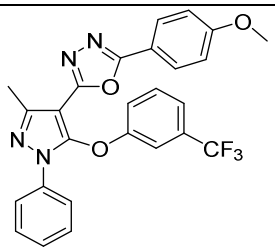
7k	***2-(4-bromophenyl)-5-(5-(2-fluorophenoxy)-3-methyl-1-phenyl-1H-pyrazol-4-yl)-1,3,4-oxadiazole***		
Mol. for. M. P. (°C) Mol. Wt.	$C_{24}H_{16}BrFN_4O_2$ 184-186 492.3		 **7k**
Ele. Ana. Calcd.(Obs)	C 58.67 (58.41)	H 3.28 (3.07)	N 11.40 (11.13)
FT-IR ν_{max} cm^{-1} (KBr)	1227 (C–O–C); 1638 and 1595 (C=N and C=C); 3060 (Ar, -CH str.)		
^{s1}H NMR δ ppm DMSO-d_6	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 NMR δ ppm DMSO-d_6	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		

7l	***2-(4-chlorophenyl)-5-(5-(2-fluorophenoxy)-3-methyl-1-phenyl-1H-pyrazol-4-yl)-1,3,4-oxadiazole***		
Mol. for. M. P. (°C) Mol. Wt.	$C_{24}H_{16}ClFN_4O_2$ 190-192 447.7		 **7l**
Ele. Ana. Calcd.(Obs)	C 64.51 (64.28)	H 3.61 (3.36)	N 12.54 (12.27)
FT-IR ν_{max} cm^{-1} (KBr)	1226 (C–O–C); 1638 and 1598 (C=N and C=C); 3061 (Ar, -CH str.)		
^{s1}H NMR δ ppm DMSO-d_6	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 NMR δ ppm DMSO-d_6	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		

7m	***2-(4-chlorophenyl)-5-(5-(3-fluorophenoxy)-3-methyl-1-phenyl-1H-pyrazol-4-yl)-1,3,4-oxadiazole***		
Mol. for. M. P. (°C) Mol. Wt.	$C_{24}H_{16}ClFN_4O_2$ 177-179 447.8		 **7m**
Ele. Ana. Calcd.(Obs)	C 64.51 (64.26)	H 3.61 (3.37)	N 12.54 (12.28)
FT-IR ν_{max} cm^{-1} (KBr)	1227 (C–O–C); 1622 and 1597 (C=N and C=C); 3056 (Ar, -CH str.)		
^{s1}H NMR δ ppm DMSO-d_6	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 NMR δ ppm DMSO-d_6	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		

7n	***2-(5-(3-fluorophenoxy)-3-methyl-1-phenyl-1H-pyrazol-4-yl)-5-(4-methoxyphenyl)-1,3,4-oxadiazole***		
Mol. for. M. P. (°C) Mol. Wt.	$C_{25}H_{19}FN_4O_3$ 169-171 443.4		 **7n**
Ele. Ana. Calcd.(Obs)	C 67.87 (67.66)	H 4.33 (4.07)	N 12.66 (12.38)
FT-IR ν_{max} cm^{-1} (KBr)	1231 (C–O–C); 1631and 1593 (C=N and C=C); 3061 (Ar, -CH str.)		
^{s1}H NMR δ ppm DMSO-d_6	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 NMR δ ppm DMSO-d_6	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		

7o	***2-(4-chlorophenyl)-5-(3-methyl-1-phenyl-5-(3-(trifluoromethyl)phenoxy)-1H-pyrazol-4-yl)-1,3,4-oxadiazole***		
Mol. for. M. P. (°C) Mol. Wt.	$C_{25}H_{16}ClF_3N_4O_2$ 194-196 497.8		 **7o**
Ele. Ana. Calcd.(Obs)	C 60.43 (60.15)	H 3.25 (3.04)	N 11.28 (11.05)
FT-IR ν_{max} cm^{-1} (KBr)	1234 (C–O–C); 1634 and 1594 (C=N and C=C); 3062 (Ar, -CH str.)		
^{s1}H NMR δ ppm DMSO-d_6	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 NMR δ ppm DMSO-d_6	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		

7p	***2-(4-methoxyphenyl)-5-(3-methyl-1-phenyl-5-(3-(trifluoromethyl)phenoxy)-1H-pyrazol-4-yl)-1,3,4-oxadiazole***		
Mol. for. M. P. (°C) Mol. Wt.	$C_{26}H_{19}F_3N_4O_3$ 188-190 493.3		 **7p**
Ele. Ana. Calcd.(Obs)	C 63.41 (63.18)	H 3.89 (3.61)	N 11.38 (11.14)
FT-IR ν_{max} cm^{-1} (KBr)	1237 (C–O–C); 1637 and 1595 (C=N and C=C); 3067 (Ar, -CH str.)		
^{s1}H NMR δ ppm DMSO-d_6	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 NMR δ ppm DMSO-d_6	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		

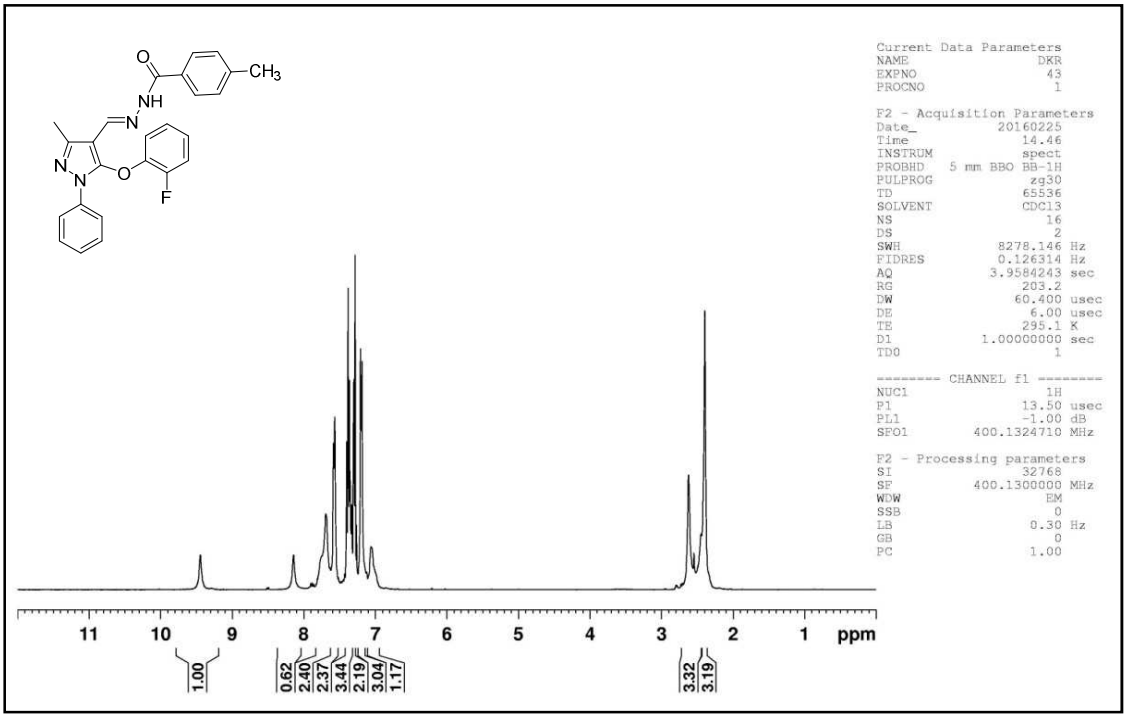


Figure 4.18 ^{1}H NMR spectra of compound **6a**

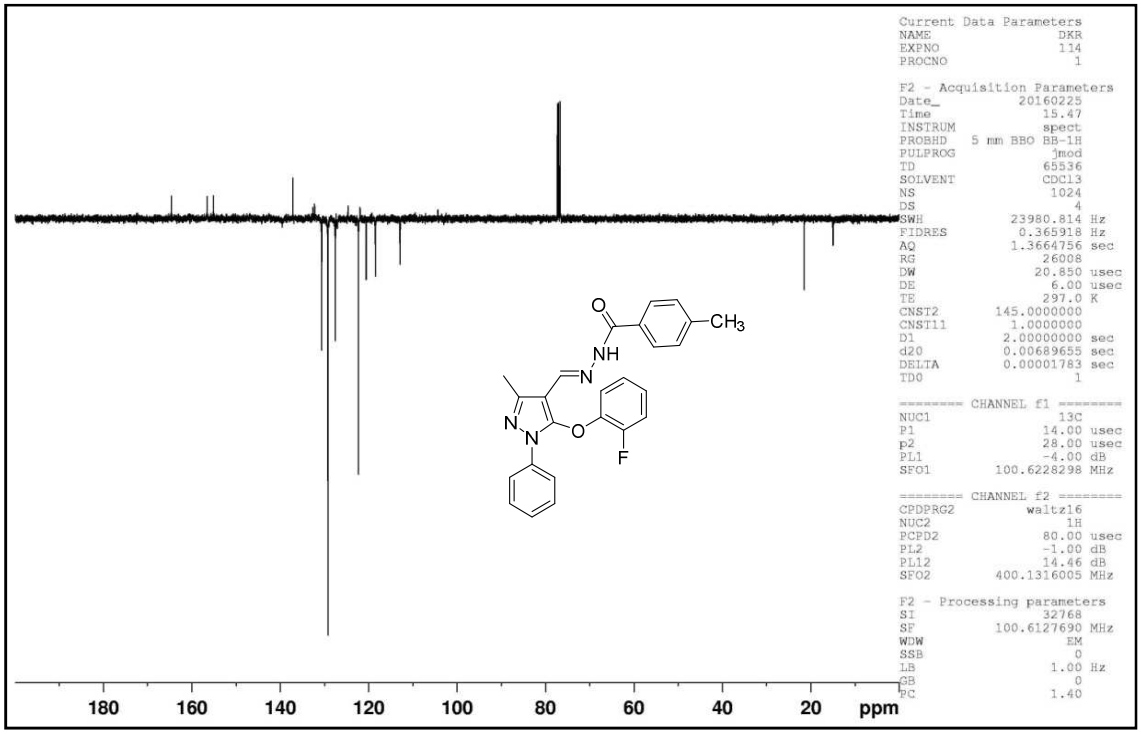


Figure 4.19 ^{13}C APT (Attached Proton Test) spectra of compound **6a**

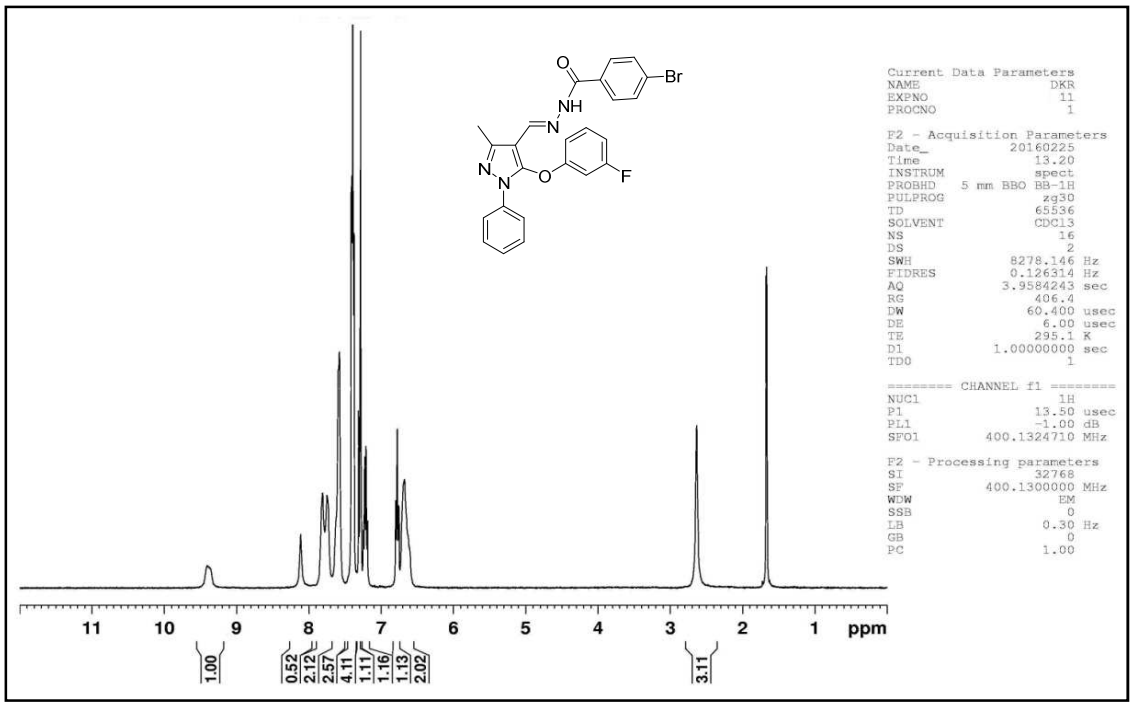


Figure 4.20 ^{1}H NMR spectra of compound **6b**

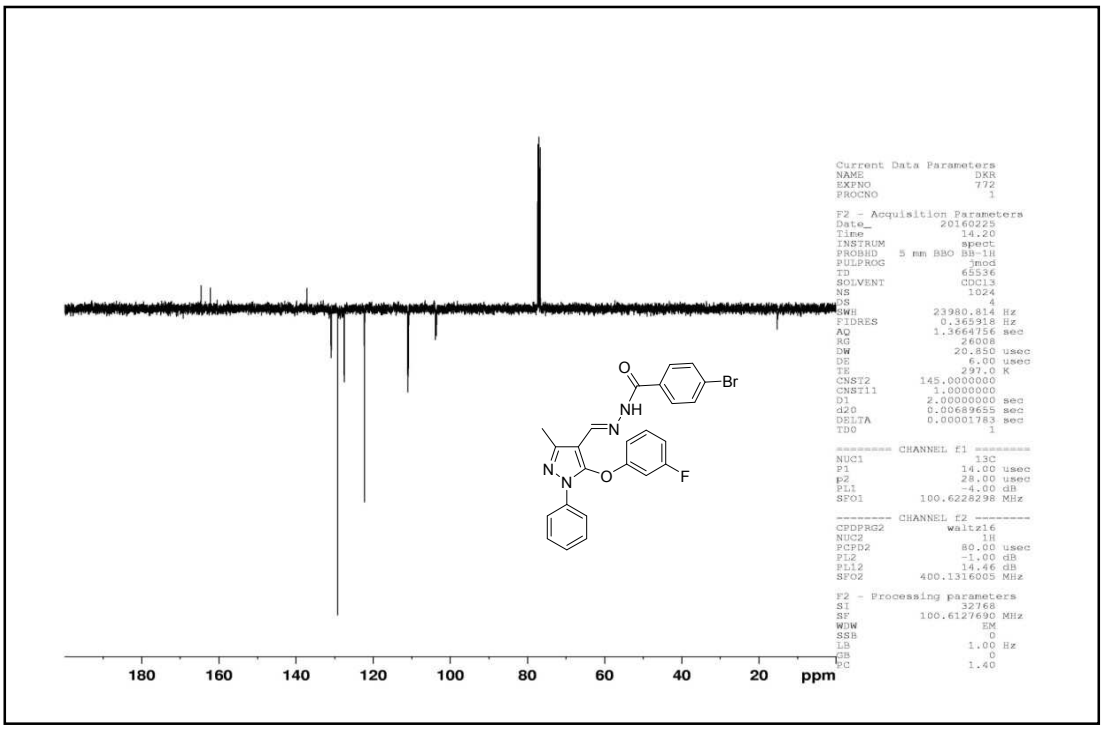


Figure 4.21 ^{13}C APT (Attached Proton Test) spectra of compound **6b**

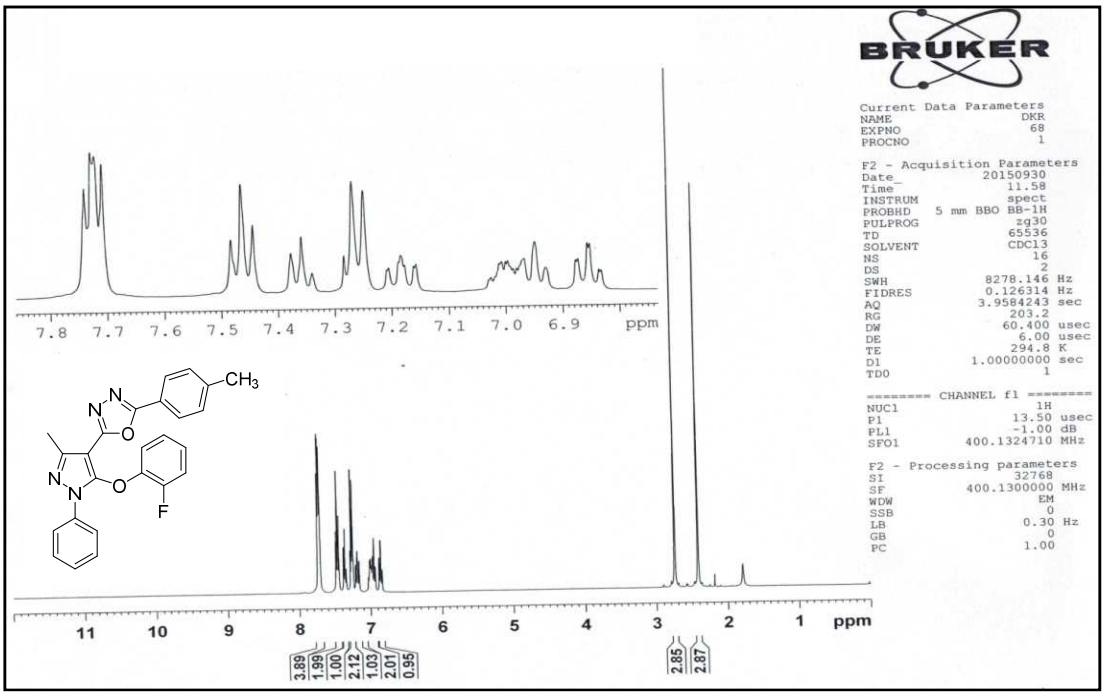


Figure 4.22 ^{1}H NMR spectra of compound **7a**

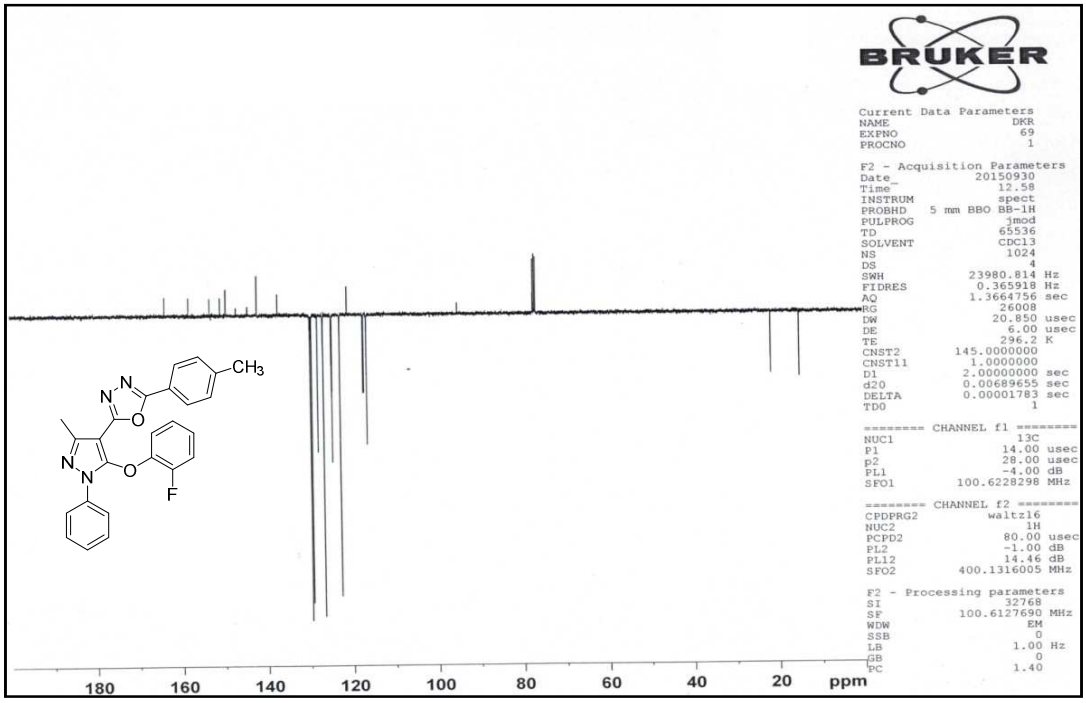


Figure 4.23 ^{13}C APT (Attached Proton Test) spectra of compound **7a**

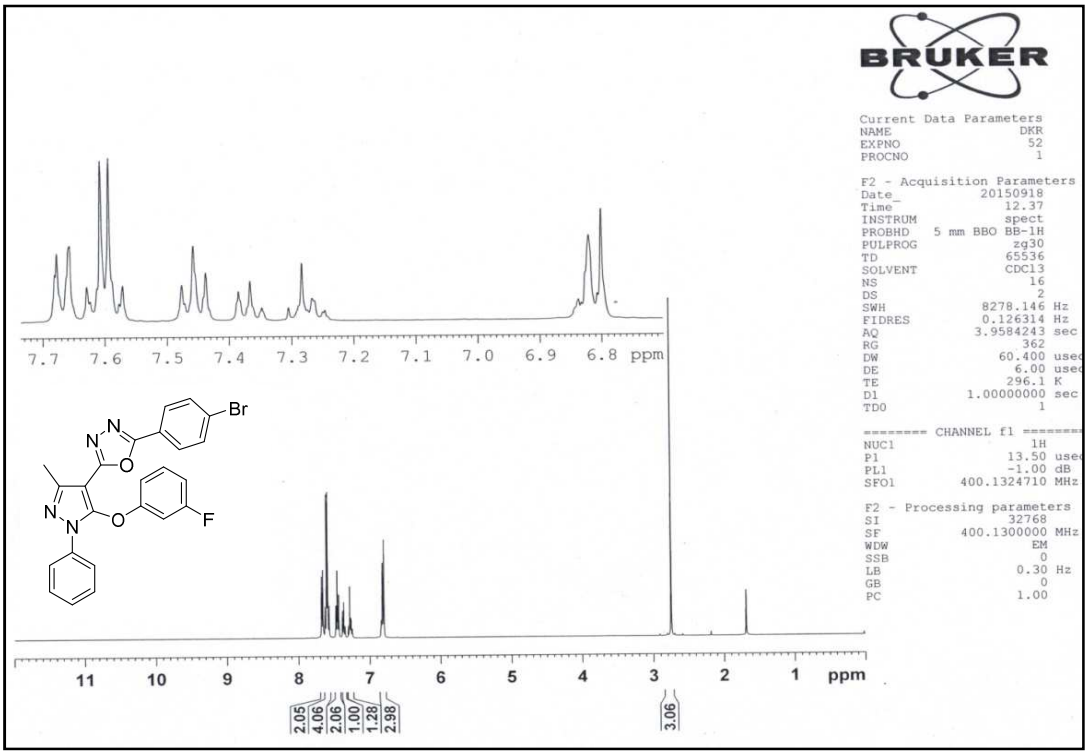


Figure 4.24 ^{1}H NMR spectra of compound **7b**

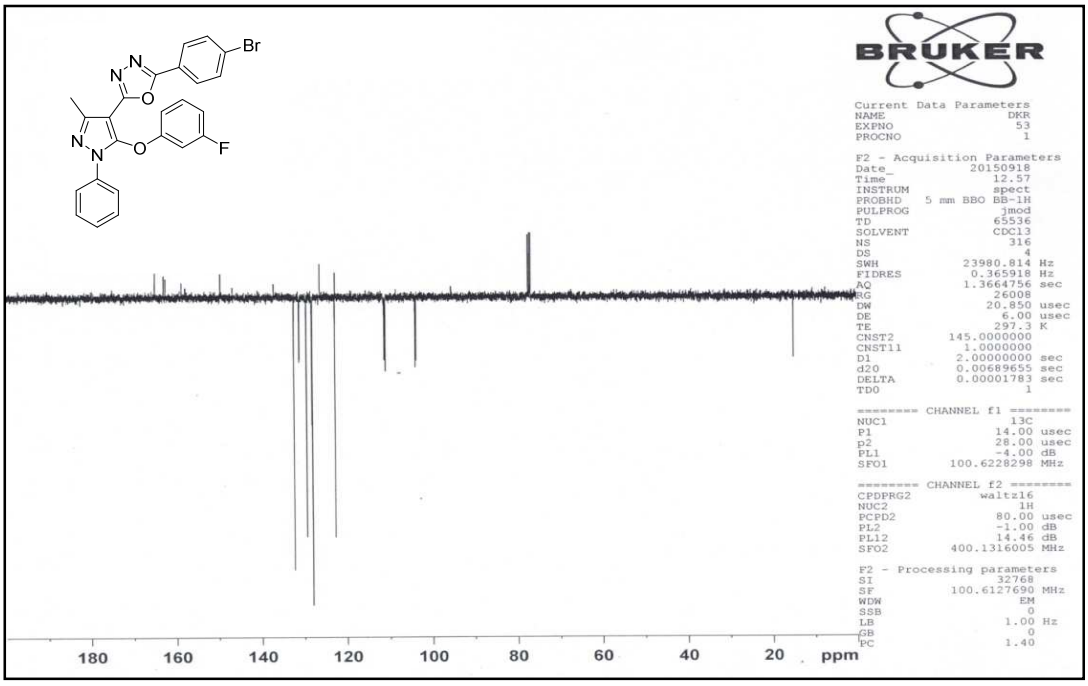


Figure 4.25 ^{13}C APT (Attached Proton Test) spectra of compound **7b**

4.10 Biological results

The synthesized compounds were further tested for their antimicrobial, antituberculosis and antimalarial activities. The detail results are discussed below.

4.10.1 Antibacterial 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) [35]. 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 4.4**.

Evaluation of antibacterial data (**Table 4.4**) 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** (282 µM), **7k** (254 µM), **7l** (223 µM) and **7n** (226 µM) displayed comparable activities to that of ampicillin (286 µM).

Table 4.4 *In vitro* antimicrobial activity (MIC, µM) of compounds **7a-p**

Compound	*Gram positive bacteria*			*Gram negative bacteria*			*Fungi*	
	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 μM), **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 μM) and **7m** (279 μM) 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).

4.10.2 Antifungal activity

Evaluation of antifungal activity revealed that (**Table 4.4**), all the compounds showd 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* in contrast to that of griseofulvin (283 μM).

4.10.3 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[39]. The obtained results are presented in **Table 4.5** in form of % inhibition. Rifampicin and Isoniazid were used as the standard drugs.

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 4.5**) 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 4.5 *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

4.10.4 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 4.6**.

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 4.6**.

As shown in **Table 4.6**, 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.*

Table 4.6 *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

4.11 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 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[40].

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