2.1 Preamble

This chapter introduces a novel series of fluoro substituted pyrazolylpyrazolines. The targeted compounds were synthesized in good to excellent yield from pyrazole chalcones and substituted phenyl hydrazine hydrochlorides under microwave irradiation. 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 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.*

2.2 Pyrazoline

Pyrazoline is five membered heterocyclic compounds having ring structure. It is composed of three carbon atoms and two nitrogen atoms in adjacent positions. This nitrogen heterocycle and its derivatives are versatile synthetic blocks, frequent structural skeleton of natural products, or potential drug molecules. It consists of only one endocyclic double bond in the structure. It is a reduction product of pyrazole, which is further reduced to pyrazolidine. Pyrazoline and pyrazolidine are stronger bases than pyrazole. Depending on the position of the double bond three partially reduced forms of pyrazoline structure are possible namely 1-pyrazoline, 2-pyrazoline and 3-pyrazoline which exist in equilibrium with each other. 2-Pyrazoline exhibits the mono amino character and hence is more stable than the rest of the reduced forms. This abundance in existence can be accounted for the fact that 2-pyrazolines have a considerably easy route of synthesis and rich biological activity.

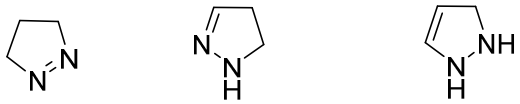
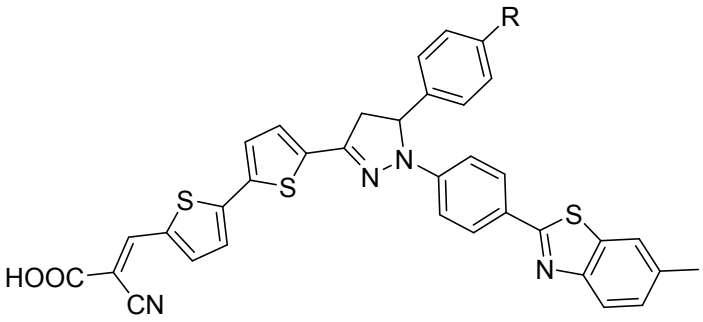


Figure 2.1 Structures of 1-pyrazoline, 2- pyrazoline and 3-pyrazoline.

2.3 Synthesis of pyrazoline

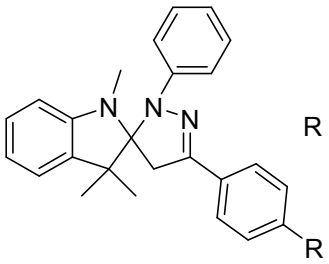
Takeshi Mori and co-workers[1] described synthesis of Influence of 1,3,5-triaryl-2-pyrazoline-based photosensitizers and studied on the photophysical properties. They tested the performance of these compounds in dye-sensitized solar cell (Scheme 2.1).



R = -H, -COOEt, $-OC_6H_{13}$, $-NMe_2$

Scheme 2.1 Photosensitizers based on a 1,3,5-triaryl-2-pyrazoline core for DSSCs.

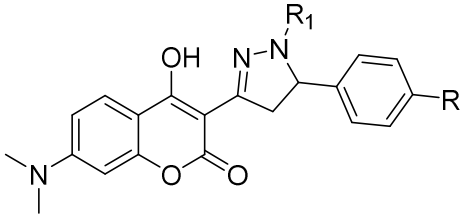
Ashton T. Hamme and co-workers[2] reported the spiro-pyrazolines achieved through a [3+2]-dipolar cycloaddition of an alkene with nitrile imines generated in situ and was isolated in high yield (Scheme 2.2).



R = -H, -Br, -Cl, $-OCH_3$

Scheme 2.2 Spiro-pyrazoline derivatives isolated from 1,3-dipolar cycloaddition.

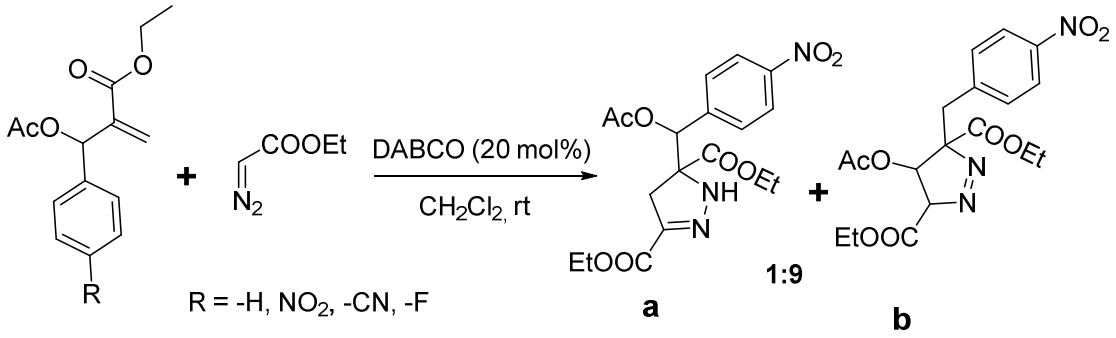
Jiun-Han Lin *et. al*.[3] reported the synthesis of 4-hydroxy-7-dimethylamino-3-pyrazolinylcoumarins and evaluated their polarity-sensitive properties (Scheme 2.3).



R = $N(CH_3)_2$, NO_2,
R_1 = Ac, Ph

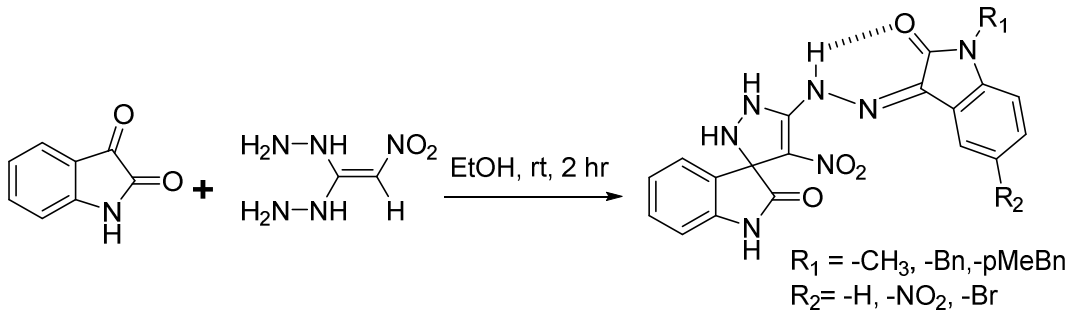
Scheme 2.3 4-hydroxy-7-dimethylamino-3-pyrazolinylcoumarins derivative.

Palakodety Radha Krishna and co-workers[4] reported 1,4 Diazabicyclo[2.2.2] octane (DABCO) catalyzed facile synthesis of highly functionalized pyrazolines from Baylis–Hillman acetates and ethyl diazoacetate in good to excellent yields (Scheme 2.4).



Scheme 2.4 The synthesis of highly functionalized pyrazolines from Baylis–Hillman acetates.

Abdolali Alizadeh and co-workers[5] reported one-pot synthesis of highly substituted pyrazoline-spirooxindoles *via* Domino SN/condensation/Aza-ene addition cyclization reaction sequence (Scheme 2.5).

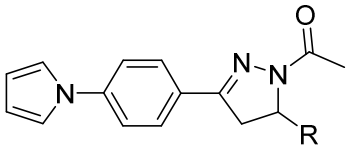


Scheme 2.5 The synthesis of Pyrazoline-Spirooxindoles derivatives.

2.4 Biological screening

2.4.1 Biological screening of pyrazoline motifs

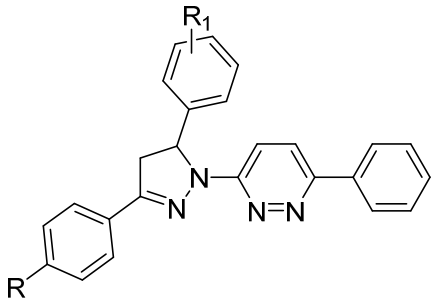
Shrinivas D. Joshi and co-workers[6] synthesized novel series of pyrrolyl derivatives bearing pyrazoline moiety and screened for their *in vitro* antimycobacterial screening and ligand-based molecular docking studies (Figure 2.2).



R = -H, 2,4-Cl, 2,3-Cl, 2,6,-Cl, 4-$CH(CH_3)_2$, 3-Phenoxy, 2-Furfuraldehyde, 3-OCH_3, 2,4-OCH_3, 4-Cl, 2-Cl, 4-CH_3, 4-OCH_3, 3-Br, 4-F, 2-Br, 3,4-OCH_3

Figure 2.2 Antimycobacterial agents of pyrrolyl derivatives bearing pyrazoline moiety.

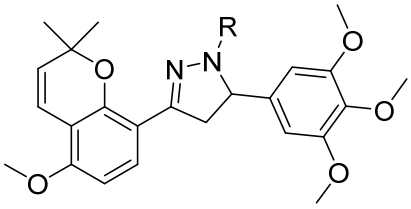
Riham F. George and co-workers[7] synthesized pyrazoline derivatives by cyclization of chalcones with 3-hydrazinyl-6-phenylpyridazine under basic condition screened for their antiproliferative activities against A549 (lung), HepG-2 (liver), CaCo-2 (intestinal) and MCF-7 (breast) cancer cell lines (Figure 2.3).



R= -H, -Cl, -OCH_3
R_1= -H, 4-F, 4-CF_3, 4-Cl, 4-OCH_3, 2,4-$(OCH_3)_2$, 3,4,5-$(OCH_3)_3$,

Figure 2.3 Antiproliferative agents from pyrazoline derivatives.

Jiqiang He *et. al.*[8] reported twenty-eight pyrazoline derivatives originated from pyranochalcones and evaluated for their inhibitory potency on the production of inflammatory mediator nitric oxide (NO) in LPS-stimulated RAW 264.7 cells lines (Figure 2.4).



R= phenyl, sulfonyl, acyl

Figure 2.4 Biologically active pyrazoline derivatives.

S. Shahavar Sulthana *et. al.*[9] reported synthesis of novel thiophene and benzodioxole appended thiazolyl-pyrazoline derivatives. The compounds were

investigated for their antimicrobial activity and molecular docking studies (Figure. 2.5).

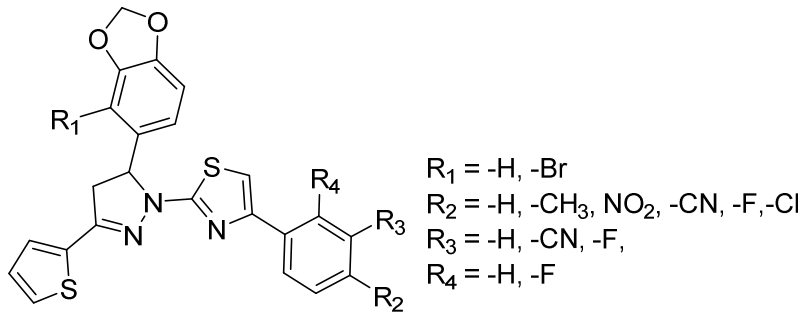


Figure 2.5 Antimicrobial agents from pyrazoline derivatives.

Braulio Insuasty and co-workers[10] reported a new series of chalcones synthesized from caffeine-based aldehyde and substituted acetophenones. Treatment of compounds with hydrazine hydrate led to pyrazolines, and their subsequent reaction with acetic anhydride or formic acid afforded the corresponding N-substituted pyrazolines respectively. They evaluated their *in vitro* antimalarial, antileishmanial and antitrypanozomal activities (Figure 2.6).

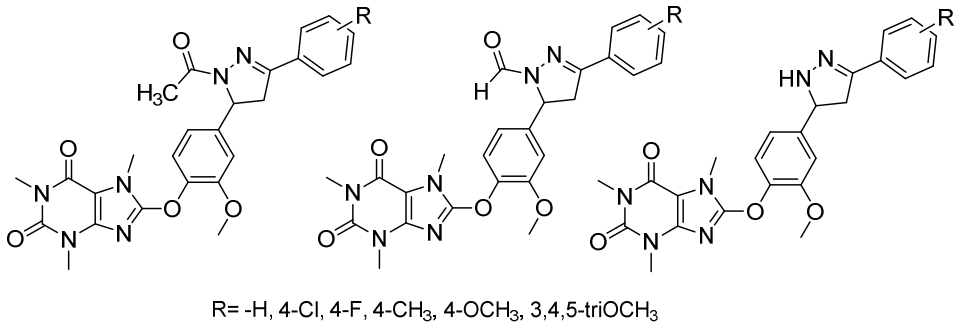
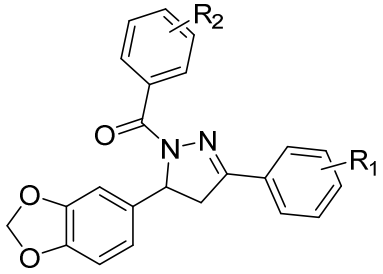


Figure 2.6 Antimalarial, Antitrypanosomal and Antileishmanial active new caffeine-based pyrazolines derivatives

Hai-Liang Zhu and co-workers[11] reported a series of novel pyrazoline-containing derivatives and evaluated their antiproliferative activity against A549, MCF-7 and HepG-2 cells line and *in vitro* tubulin polymerization inhibitory activity (Figure 2.7).

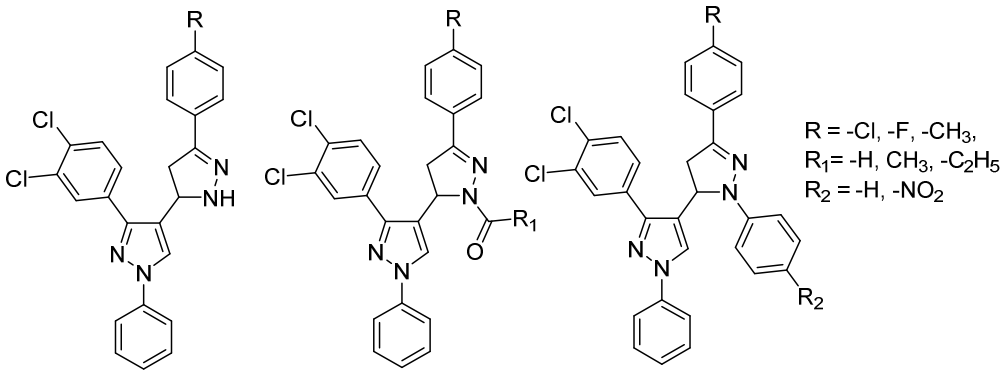


R_1= 4-OC_2H_5, 2-OCH_3, 3-OCH_3, 4-OCH_3, 3,4$(OCH_3)_2$, 4-Br, 4-Cl, 4-CH_3, -H, 4-F, 3-CH_3, 3,4,5,$(OCH_3)_3$

R_2= 3,4,5,$(OCH_3)_3$, 4-F, 4-Cl, 3-OCH_3, 4-OCH_3, 3,4$(OCH_3)_2$, 4-Br

Figure 2.7 Biologically active pyrazoline derivatives.

An efficient synthesis of some new pyrazoline derivatives linked to a substituted pyrazole scaffold was performed by a multistep reaction sequences and compounds were screened for their anti-inflammatory, analgesic and antibacterial activities by Shivapura Viveka *et. al* [12] (Figure 2.8).



R = -Cl, -F, -CH_3,
R_1= -H, CH_3, -C_2H_5
R_2 = -H, -NO_2

Figure 2.8 Anti-inflammatory, Analgesic and Antibacterial agents from pyrazoline derivatives.

Jia-Jia Liu and co-workers[13] reported a series of novel compounds containing the 4,5- dihydropyrazole core with a dinitrobenzotrifluoride moiety and evaluated for their antibacterial activity against *E. coli* ATCC 25922, *P. aeruginosa* ATCC 27853, *B. subtilis* ATCC 530 and *S. aureus* ATCC 25923 in order to achieve new and better potential antibacterial DNA gyrase inhibitors (Figure 2.9)

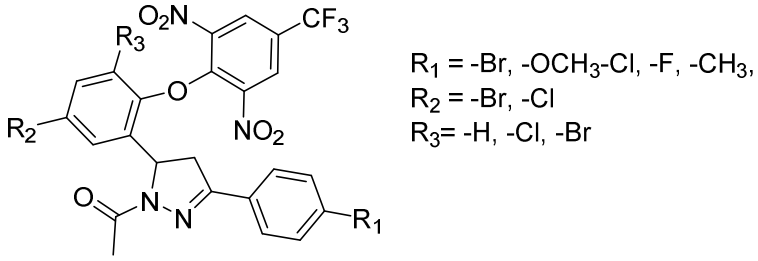


Figure 2.9 Potential antibacterial DNA gyrase inhibitors from pyrazoline derivatives.

Alexander Ciupa et al[14] reported the synthesis and antiproliferative activity of eleven 3-(pyrid-2-yl)-pyrazolines against two cancer cell lines.(Figure 2.10).

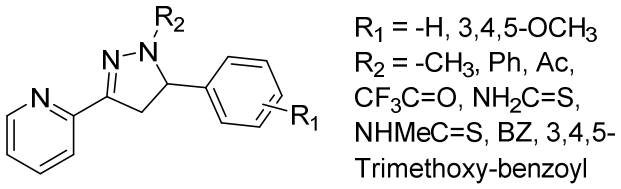


Figure 2.10 Antiproliferative agents from 3-(pyrid-2-yl)-pyrazoline derivatives.

Xin-Hua Liu and co-workers[15] synthesized thirty seven novel 2-pyrazoline-1-ethanone derivatives and evaluated as selective hMAO inhibitors (Figure 2.11).

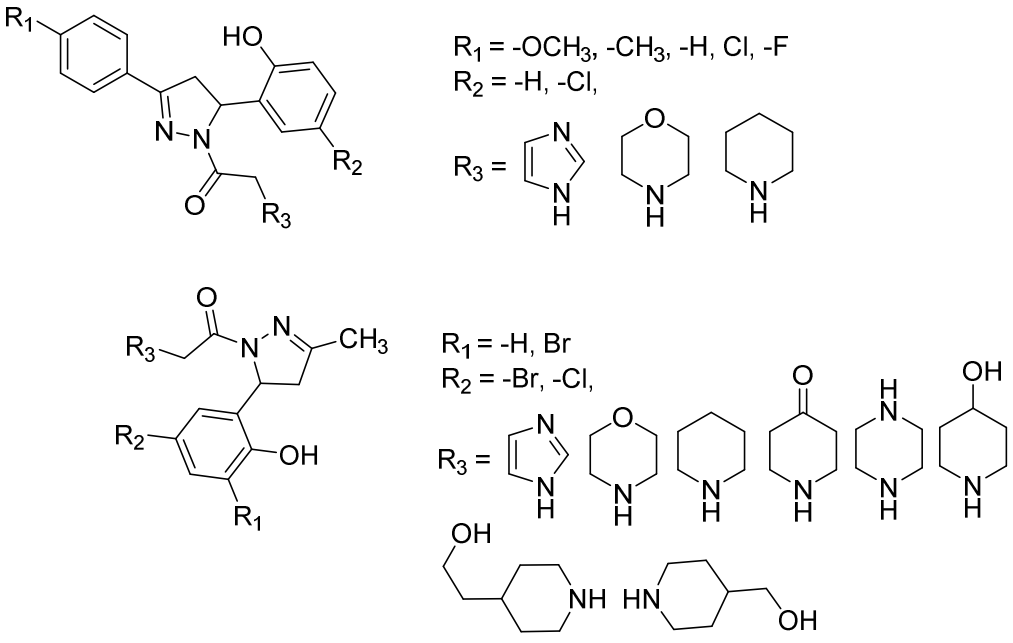


Figure 2.11 Selective hMAO inhibitos from pyrazoline scaffolds.

2.5 Present work

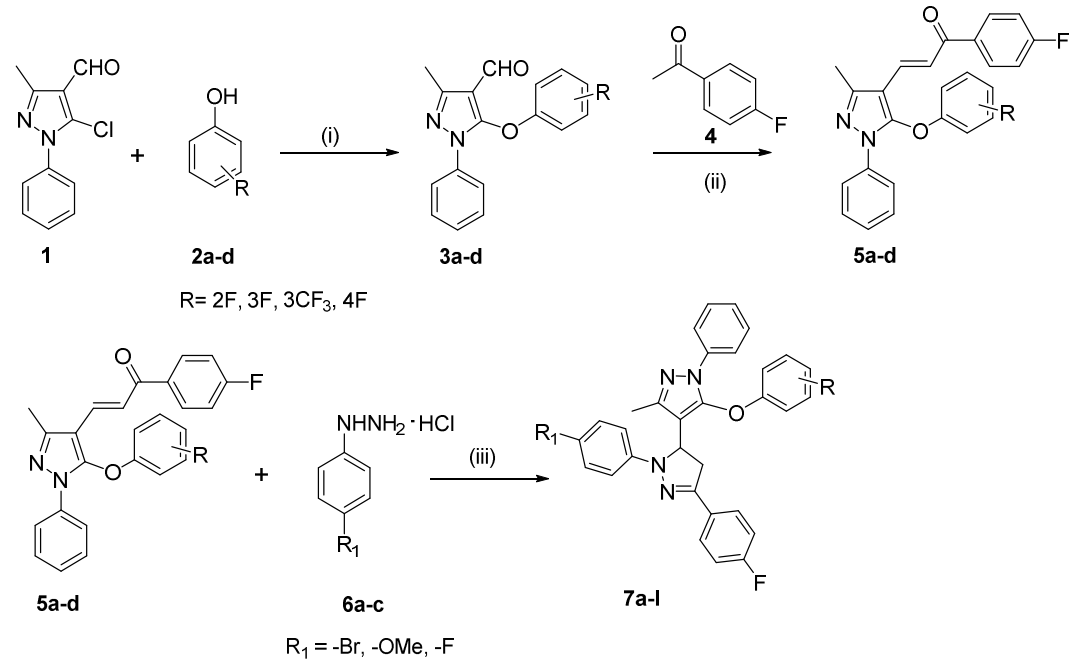
Pyrazoline has become of much interest in the field of medicinal chemistry. This is due to the fact that the scaffold has a rich and diverse range of biological activities such as antitumor[16], anti-inflammatory[17], anticancer[18], antimicrobial[19], and antidepressant properties [20]. The recent success of pyrazole COX-2 inhibitor [21] has further highlighted the importance of these heterocycles in medicinal chemistry. A systematic investigation of this class of compounds showed that pyrazole containing pharmacoactive agents play significant role in medicinal chemistry.

The substitution of fluorine in to a potential drug molecule can improve efficacy of drugs by extending pharmacokinetic and pharmacodynamics properties [22]. Trifluoromethyl group is a well-known substituent of unique qualities. Its high lipophilicity enables to improve pharmacological activities of the molecule [23, 24]. Pyrazoles and their derivatives possess numerous medicinal applications because of their versatile biological activities [25-31]. They have occupied a distinct place due to a range of bioactivities such as antiproliferative [32], antimicrobial [33-35], antidepressant [36], antipyretic [37], anti-inflammatory [17] and anticonvulsant [38]. Pyrazoline is also an important nitrogenous heterocyclic moiety in many drugs. Literature survey revealed that various pyrazoline derivatives have displayed significant biological roles [39-45].

Microwave irradiation as a source of energy leads to environmentally benign protocols in terms of reduction in reaction time, energy saving with high efficiency, improved yields and selectivity [46]. In context of the above consequences and in continuation to our previous studies directed toward the synthesis of biologically active novel heterocyclic scaffolds [46-51], herein we attempted microwave assisted synthesis of some fluorinated novel pyrazolylpyrazoline derivatives. The synthesized compounds exhibited an interesting profile as antimalarial, antitubercular and antimicrobial agents.

2.6 Reaction scheme

The synthesis of novel series of pyrazolylpyrazolines **7a-l** was performed as outlined in **Scheme 2.6** The starting material 5-chloro-3-methyl-1-phenyl-1H-pyrazole-4-carbaldehyde **1** was prepared according to Vilsmeier-Haack reaction of 3-methyl-1-phenyl-1H-pyrazol-5(4H)-one [52]. 3-methyl-5-substituted aryloxy-1-phenyl-1H-pyrazole-4-carbaldehydes **3a-d** were prepared by refluxing compound **1** and substituted phenols **2a-d** in presence of anhydrous K_2CO_3 as basic catalyst in DMF as solvent. 3-methyl-5-substituted aryloxy-1-phenyl-1H-pyrazole-4-carbaldehydes **3a-d** were subjected to base catalyzed Claisen-Schmidt condensation reaction with 4-Fluoro acetophenone **4** generating the required (*E*)-1-(4-fluorophenyl)-3-(3-methyl-5-substituted aryloxy-1-phenyl-1H-pyrazol-4-yl)prop-2-en-1-ones **5a-d**. Finally pyrazolyl-pyrazolines **7a-l** were obtained by the condensation of **5a-d** and substituted phenyl hydrazine hydrochlorides **6a-c** in ethanol containing catalytic amount of glacial acetic acid under microwave irradiation at 350 W power level for 8-10 min.



Scheme 2.6. Synthesis of 5-(4-fluorophenyl)-3'-methyl-5'- substituted aryloxy-1',2-diphenyl-3,4-dihydro-1'H,2H-3,4'-bipyrazole (**7a-l**) (i) DMF, K_2CO_3, Reflux 2 h. (ii) 20 % ethanolic NaOH, room temperature. (iii) Ethanol, catalytic glacial acetic acid, MW, 8-10 Min, 350 W

2.7 Experimental

- Melting points in °C were determined in open capillaries using µThermoCal$_{10}$ melting point apparatus (Analab Scientific Pvt. Ltd, India) and are uncorrected. Precoated silica gel plates (silica gel 0.25 mm, 60 G F 254; Merck, Germany) were used for thin layer chromatography.
- The 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}.
- Electron impact Mass Spectra were recorded on Shimadzu LCMS 2010 spectrometer (Shimadzu, Tokyo, Japan) purchased under PURSE program of DST at Sardar Patel University, Vallabh Vidyanagar, India. NMR spectra (in DMSO-d$_6$) were recorded on Bruker Avance 400F NMR Spectrometer at 400 MHz using TMS as the internal standard.
- The elemental analysis was performed on Perkin-Elmer 2400 series-II elemental analyzer (Perkin-Elmer, USA) at Sophisticated Instrumentation Centre for Applied Research & Training (SICART), Vallabh Vidyanagar, India.
- All compounds were found within ±0.4% of their theoretical values.
- All the reactions were carried out at atmospheric pressure using a multimode microwave reactor (Microwave Synthesis System, Model: Cata-R, Catalyst Systems, Pune-India) with an individual sensor for temperature control through attachment of reflux condenser with constant stirring.
- Microwaves are generated by magnetron at a frequency of 2450 MHz having an adjustable output power levels (i.e. 1 to 10 levels from 140 to 700 Watts).

2.8 Synthesis of fluoro substituted novel pyrazolylpyrazolines scaffold

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

5-chloro-3-methyl-1-phenyl-1H-pyrazole-4-carbaldehyde **1** (1 mmol), substituted phenols **2a-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, filtered, washed thoroughly with water, dried and recrystallized from ethanol to obtain a white solid.

Table 2.1 Physical data of 3-methyl-5-substituted aryloxy-1-phenyl-1H-pyrazole-4-carbaldehyde (3a-d)

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

2.8.2 General procedure for synthesis of (E)-1-(4-fluorophenyl)-3-(3-methyl-5-substituted aryloxy-1-phenyl-1H-pyrazol-4-yl)prop-2-en-1-ones (5a-d)

To a mixture of 3-methyl-5-substituted aryloxy-1-phenyl-1H-pyrazole-4-carbaldehydes **3a-d** (5.0 mmol) and 4-fluoro acetophenone **4** (5.0 mmol), 20 % ethanolic NaOH (5 mL) was added. The reaction mixture was stirred at room temperature until formation of precipitate. The solid obtained was isolated by filtration, washed with cold ethanol and recrystallized from $CHCl_3$.

Table 2.2 Physical data of substituted pyrazolic chalcones.

Comp.	R_1	M. F. (MW)	Yield (%)	m.p. (°C)
5a	2-F	$C_{25}H_{18}F_2N_2O_2$ (417.3)	78	229-231
5b	3-F	$C_{25}H_{18}F_2N_2O_2$ (471.3)	83	201-203
5c	3-CF_3	$C_{26}H_{18}F_4N_2O_2$ (467.3)	80	258-260
5d	4-F	$C_{25}H_{18}F_2N_2O_2$ (417.3)	75	238-240

2.8.3 General procedure for synthesis of 5-(4-fluorophenyl)-3'-methyl-5'-substituted aryloxy-1',2-diphenyl-3,4-dihydro-1'H,2H-3,4'-bipyrazoles (7a-l).

Pyrazole chalcones **5a-d** (1.0 mmol) and substituted phenyl hydrazine hydrochlorides **6a-c** (1.0 mmol) were thoroughly mixed in ethanol (5 mL) with catalytic amount of glacial acetic acid (2-3 drops) in a 50 mL round bottom flask. The reaction mixture was subjected to microwave irradiation at 350 W (50% of output power) for 8-10 min. After the completion of the reaction as monitored by TLC (ethyl acetate:hexane::1:1), the reaction mixture was cooled to room temperature. The solid separated was filtered, washed with cold ethanol (10 mL), dried and recrystallized from ethanol, affording compounds **7a-l**.

2.9 Preliminary and spectral characterization

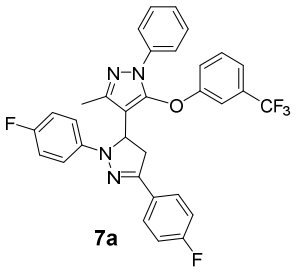
The structures of the synthesized compounds were confirmed by ^{1}H and ^{13}C NMR, FT-IR, mass spectrometry and elemental analysis. The IR spectra of compounds **7a-l** exhibited characteristic absorption band in the range of 1260-1253 cm^{-1} due to the presence of ether linkage. The absorption band around 3062-3051 cm^{-1} is due to aromatic C-H stretching. The absorption band observed for all the compounds in between of 1626-1598 cm^{-1} corresponds to -C=N stretching. The strong absorption band is also observed in the range of 1368-1357 cm^{-1} due to $-CH_3$ rocking. The ^{1}H NMR spectra of the target pyrazolylpyrazolines displayed a typical ABX type pattern of doublet of doublet due to three pyrazoline protons. Methine proton of pyrazoline was found at around 5.39-5.17 ppm as a doublet of doublet with coupling constants of nearly 12.8 Hz and 5.6 Hz. Two methylene protons displayed two signals; a doublet of doublet at around 3.74-3.62 ppm with coupling constants of nearly 17 Hz and 12 Hz and a doublet of doublet at around 3.32-3.22 ppm merging with the water signal from DMSO-d_6. At around 3.67 ppm C_4-H pyrazoline proton got merged with the methoxy signal in most of the cases. The data from ^{13}C NMR spectral studies is also in accordance with the suggested structures.

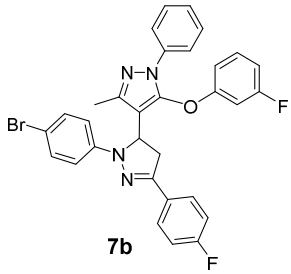
Table 2.3 Preliminary Characterization of all synthesized compounds **7a-l**.

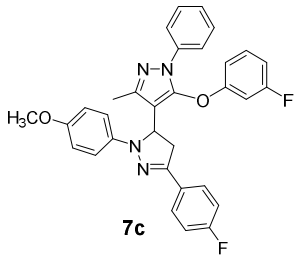
Comp.	R	R_1	Yield[a](%)	Comp.	R	R_1	Yield[a](%)
7a	3-CF_3	4-F	82	**7g**	4-F	4-F	83
7b	3-F	4-Br	79	**7h**	4-F	4-Br	79
7c	3-F	4-OCH_3	88	**7i**	4-F	4-OCH_3	84
7d	3-F	4-F	80	**7j**	2-F	4-F	85
7e	3-CF_3	4-Br	81	**7k**	2-F	4-Br	77
7f	3-CF_3	4-OCH_3	85	**7l**	2-F	4-OCH_3	87

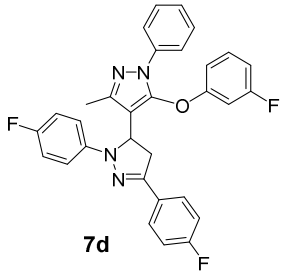
[a] Isolated yield

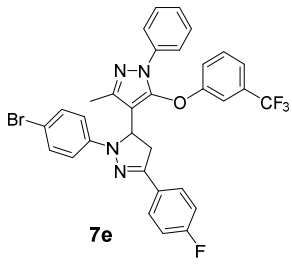
- ❖ The spectral Characterization of all synthesized compounds **7a-l** are depicted in following tables.
- ✓ The ^{1}H NMR spectra of compounds **3a, 3b, 3c, 7a, 7i and 7k** are represented in Figures 2.12, 2.14, 2.16, 2.18, 2.20 and 2.22 respectively.
- ✓ The ^{13}C APT spectra of compounds **3a, 3b, 3c, 7a, 7i and 7k** are represented in Figures 2.13, 2.15, 2.17, 2.19, 2.21 and 2.23 respectively.

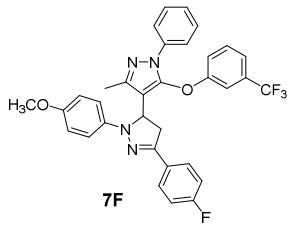
7a	***2,5-bis(4-fluorophenyl)-3'-methyl-1'-phenyl-5'-(3-(trifluoromethyl)phenoxy)-3,4-dihydro-1'H,2H-3,4'-bipyrazole***		
Mol. for. M. P. (°C) Mol. Wt.	$C_{32}H_{23}F_5N_4O$ 146-148 575.5		 7a
Ele. Ana. Calcd.(Obs)	C 66.90 (66.69)	H 4.03 (3.80)	N 9.75 (9.50)
FT-IR ν_{max} cm^{-1} (KBr)	1368 (-CH_3 rocking.), 1260 (C-O-C ether str.), 1608 (-C=N), 3059 (-CH aromatic).		
^{1}H NMR δ ppm DMSO-d_6	7.63-6.84 (17H, m, ArH), 5.34 (1H, dd, *J* = 6.4, 12.8 Hz, C_5-H pyrazoline), 3.71 (1H, dd, *J* = 12.8, 17.6 Hz, C_4-H pyrazoline), 3.27 (1H, dd, *J* = 6.4, 17.6 Hz, C_4-H pyrazoline), 2.27 (3H, s, CH_3).		
^{13}C NMR δ ppm DMSO-d_6	13.7 (-CH_3 of pyrazole), δ, 54.4 (-CH_2 of pyrazoline), δ, 109.0, 112.3, 114.3, 114.4, 115.6, 115.8, 115.9, 116.0, 119.3, 120.4, 122.0, 127.5, 127.9, 128.0, 129.1,129.7, 137.5, 137.7, 141.1, 144.6, 147.0, 147.3, 156.5, 164.3(24 signals, aromatic carbons, pyrazole carbons, C_3, C_4 of pyrazoline and -CF_3 of aryloxy ring of pyrazole).		

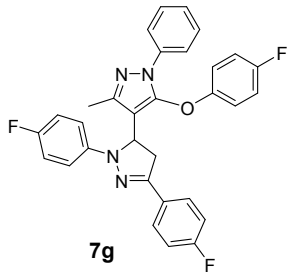
7b	***2-(4-bromophenyl)-5'-(3-fluorophenoxy)-5-(4-fluorophenyl)-3'-methyl-1'-phenyl-3,4-dihydro-1'H,2H-3,4'-bipyrazole***	
Mol. for. M. P. (°C) Mol. Wt.	$C_{31}H_{23}BrF_2N_4O$ 176-178 585.4	 **7b**
Ele. Ana. Calcd.(Obs)	C H N 63.60 (63.36) 3.96 (3.73) 9.57 (9.31)	
FT-IR ν_{max} cm^{-1} (KBr)	1366 (-CH_3 rocking.), 1259 (C-O-C ether str.), 1618 (-C=N), 3061 (-CH aromatic).	
^{1}H NMR δ ppm DMSO-d_6	7.68-6.56 (17H, m, ArH), 5.35 (1H, dd, *J* = 5.6, 12.4 Hz, C_5-H pyrazoline), 3.72 (1H, dd, *J* = 12.8, 17.6 Hz, C_4-H pyrazoline), 3.32 (1H, dd C_4-H pyrazoline merged with peak of H_2O), 2.19 (3H, s, CH_3)	
^{13}C NMR δ ppm DMSO-d_6	13.5 (-CH_3 of pyrazole), δ, 53.4 (-CH_2 of pyrazoline), δ, 108.3, 110.3, 115.2, 116.4, 117.3, 122.2, 124.8, 125.4, 127.3, 128.3, 128.7, 129.7, 132.0, 137.5, 143.5, 143.6, 144.8, 147.0, 147.6, 150.1, 152.4, 161.2, 164.8 (23 signals, aromatic carbons, pyrazole carbons, C_3 and C_4 of pyrazoline).	

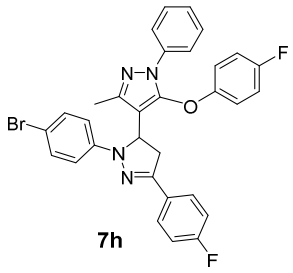
7c	***5'-(3-fluorophenoxy)-5-(4-fluorophenyl)-2-(4-methoxyphenyl)-3'-methyl-1'-phenyl-3,4-dihydro-1'H,2H-3,4'-bipyrazole***	
Mol. for. M. P. (°C) Mol. Wt.	$C_{32}H_{26}F_2N_4O_2$ 166-168 537.5	 **7c**
Ele. Ana. Calcd.(Obs)	C H N 71.63 (71.36) 4.88 (4.66) 10.44 (10.21)	
FT-IR ν_{max} cm^{-1} (KBr)	1367 (-CH_3 rocking.), 1253 (C-O-C ether str.), 1616 (-C=N), 3060 (-CH aromatic)	
^{1}H NMR δ ppm DMSO-d_6	7.66-6.61 (17H, m, ArH), 5.22 (1H, dd, *J* = 7.2, 12.8 Hz, C_5-H pyrazoline), 3.64 (4H, s, C_4-H pyrazoline merged with -OCH_3), 3.27 (1H, dd, *J* = 7.2, 17.6 Hz, C_4-H pyrazoline), 2.18 (3H, s, CH_3).	
^{13}C NMR δ ppm DMSO-d_6	13.9 (-CH_3 of pyrazole), δ, 54.9 (-CH_2 of pyrazoline), δ, 55.6 (-OCH_3), δ, 109.1, 110.7, 110.9, 111.5, 114.7, 115.0, 115.8, 116.0, 122.0, 127.4, 127.8, 129.8, 131.6, 137.9, 138.8,144.9, 146.1, 147.2, 153.4, 157.6, 164.3 (23 signals, aromatic carbons, pyrazole carbons, C_3 and C_4 of pyrazoline).	

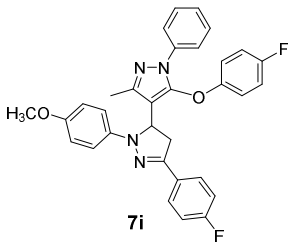
7d	***5'-(3-fluorophenoxy)-2,5-bis(4-fluorophenyl)-3'-methyl-1'-phenyl-3,4-dihydro-1'H,2H-3,4'-bipyrazole***		
Mol. for. M. P. (°C) Mol. Wt.	$C_{31}H_{23}F_3N_4O$ 144-146 525.4		 **7d**
Ele. Ana. Calcd.(Obs)	C 70.98 (70.73)	H 4.42 (4.20)	N 10.68 (10.41)
FT-IR ν_{max} cm^{-1} (KBr)	1361 (-CH_3 rocking.), 1256 (C-O-C ether str.), 1616 (-C=N), 3062 (-CH aromatic)		
^{1}H NMR δ ppm DMSO-d_6	7.68-6.57 (17H, m, ArH), 5.30 (1H, dd, *J* = 6.4, 12.4 Hz, C_5-H pyrazoline), 3.69 (1H, dd, *J* = 12.8, 17.6 Hz, C_4-H pyrazoline), 3.28 (1H, dd, C_4-H pyrazoline merged with peak of H_2O), 2.20 (3H, s, CH_3)		
^{13}C NMR δ ppm DMSO-d_6	13.5 (-CH_3 of pyrazole), δ, 54.7 (-CH_2 of pyrazoline), δ, 109.3, 112.8, 114.4, 114.6, 115.2, 115.4, 115.6, 116.0, 119.4, 120.5, 122.0, 127.7, 127.9, 128.3, 129.4,129.9, 137.6, 137.8, 141.4, 144.8, 147.5, 147.8, 156.7, 164.6 (23 signals, aromatic carbons, pyrazole carbons, C_3 and C_4 of pyrazoline)		

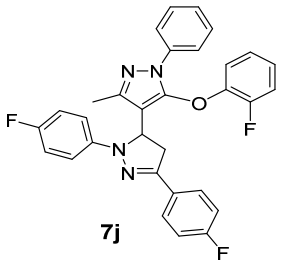
7e	***2-(4-bromophenyl)-5-(4-fluorophenyl)-3'-methyl-1'-phenyl-5'-(3-(trifluoromethyl) phenoxy)-3,4-dihydro-1'H,2H-3,4'-bipyrazole***		
Mol. for. M. P. (°C) Mol. Wt.	$C_{32}H_{23}BrF_4N_4O$ 138-140 635.5		 **7e**
Ele. Ana. Calcd.(Obs)	C 60.48 (60.25)	H 3.65 (3.44)	N 8.82 (8.57)
FT-IR ν_{max} cm^{-1} (KBr)	1361 (-CH_3 rocking.), 1259 (C-O-C ether str.), 1620 (-C=N), 3059 (-CH aromatic)		
^{1}H NMR δ ppm DMSO-d_6	7.63-6.80 (17H, m, ArH), 5.39 (1H, dd, *J* = 6.0, 12.8 Hz C_5-H pyrazoline), 3.74 (1H, dd, *J* = 12.4, 17.6 Hz, C_4-H pyrazoline) 3.27 (1H, dd, C_4-H pyrazoline merged with peak of H_2O), 2.27 (3H, s, CH_3)		
^{13}C NMR δ ppm DMSO-d_6	14.0 (-CH_3 of pyrazole), δ, 55.1 (-CH_2 of pyrazoline), δ, 108.9, 111.9, 114.1, 114.2, 115.1, 115.3, 115.8, 116.8, 119.5, 120.6, 122.2, 127.6, 127.9, 128.7, 129.4,129.9, 136.9, 137.2, 141.0, 144.3, 146.8, 147.3, 156.7, 164.7 (24 signals, aromatic carbons, pyrazole carbons, C_3, C_4 of pyrazoline and -CF_3 of aryloxy ring of pyrazole).		

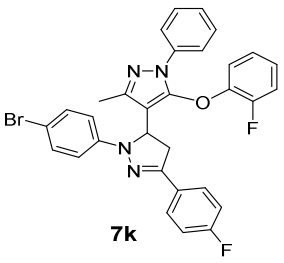
7f	***5-(4-fluorophenyl)-2-(4-methoxyphenyl)-3'-methyl-1'-phenyl-5'-(3-(trifluoromethyl) phenoxy)-3,4-dihydro-1'H,2H-3,4'-bipyrazole***	
Mol. for. M. P. (°C) Mol. Wt.	$C_{33}H_{26}F_4N_4O_2$ 134-136 587.4	 **7F**
Ele. Ana. Calcd.(Obs)	C H N 67.57 (67.34) 4.47 (4.24) 9.55 (9.27)	
FT-IR ν_{max} cm^{-1} (KBr)	1363 (-CH_3 rocking.), 1259 (C-O-C ether str.), 1598 (-C=N), 3062 (-CH aromatic)	
^{1}H NMR δ ppm DMSO-d_6	7.61-6.75 (17H, m, ArH), 5.27 (1H, dd, *J* = 7.2, 12.8 Hz, C_5-H pyrazoline), 3.66 (4H, s, C_4-H pyrazoline merged with -OCH_3), 3.24 (1H, dd, *J* = 6.8, 17.2 Hz, C_4-H pyrazoline), 2.24 (3H, s, CH_3).	
^{13}C NMR δ ppm DMSO-d_6	13.9 (-CH_3 of pyrazole), δ, 54.0 (-CH_2 of pyrazoline), δ, 55.5 (-OCH_3), δ, 109.8, 112.8, 114.1, 114.9, 115.2, 115.6, 115.9, 116.0, 119.7, 120.4, 122.5, 127.8, 127.9, 128.5, 129.5,129.9, 137.3, 137.9, 141.7, 144.5, 147.6, 147.9, 156.8, 164.4 (24 signals, aromatic carbons, pyrazole carbons, C_3, C_4 of pyrazoline and -CF_3 of aryloxy ring of pyrazole).	

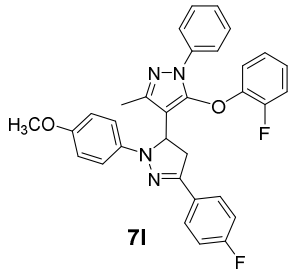
7g	***5'-(4-fluorophenoxy)-2,5-bis(4-fluorophenyl)-3'-methyl-1'-phenyl-3,4-dihydro-1'H,2H-3,4'-bipyrazole***	
Mol. for. M. P. (°C) Mol. Wt.	$C_{31}H_{23}F_3N_4O$ 148-150 525.4	 **7g**
Ele. Ana. Calcd.(Obs)	C H N 70.98 (70.73) 4.42 (4.21) 10.68 (10.43)	
FT-IR ν_{max} cm^{-1} (KBr)	1357 (-CH_3 rocking.), 1254 (C-O-C ether str.), 1610 (-C=N), 3059 (-CH aromatic)	
^{1}H NMR δ ppm DMSO-d_6	7.82-6.84 (17H, m, ArH), 5.34 (1H, dd, *J* = 6.0, 12.4 Hz, C_5-H pyrazoline), 3.72 (1H, dd, *J* = 12.4, 17.2 Hz, C_4-H pyrazoline), 3.27 (1H, dd, *J* = 6.0, 17.2 Hz, C_4-H pyrazoline), 2.27 (3H, s, CH_3)	
^{13}C NMR δ ppm DMSO-d_6	13.5 (-CH_3 of pyrazole), δ, 54.9 (-CH_2 of pyrazoline), δ, 109.7, 113.7, 114.4, 115.3, 116.9, 117.2, 122.2, 126.3, 127.8, 129.2, 136.9, 138.9, 145.4, 146.2, 147.5, 151.9, 152.3, 157.4, 159.9, 162.3, 164.8, (21signals, aromatic carbons, pyrazole carbons, C_3 and C_4 of pyrazoline).	

7h	***2-(4-bromophenyl)-5'-(4-fluorophenoxy)-5-(4-fluorophenyl)-3'-methyl-1'-phenyl-3,4-dihydro-1'H,2H-3,4'-bipyrazole***		
Mol. for. M. P. (°C) Mol. Wt.	$C_{31}H_{23}BrF_2N_4O$ 179-181 585.4		 7h
Ele. Ana. Calcd.(Obs)	C 63.60 (63.33)	H 3.96 (3.72)	N 9.57 (9.35)
FT-IR ν_{max} cm^{-1} (KBr)	1361 (-CH_3 rocking.), 1254 (C-O-C ether str.), 1615 (-C=N), 3051 (-CH aromatic)		
^{1}H NMR δ ppm DMSO-d_6	7.68-6.79 (17H, m, ArH), 5.30 (1H, dd, *J* = 6.0, 12.4 Hz, C_5-H pyrazoline), 3.69 (1H, dd, *J* = 12.8, 17.6 Hz, C_4-H pyrazoline), 3.27 (1H, dd, *J* = 6.0, 17.6 Hz, C_4-H pyrazoline), 2.16 (3H, s, CH_3)		
^{13}C NMR δ ppm DMSO-d_6	13.8 (-CH_3 of pyrazole), δ, 53.8 (-CH_2 of pyrazoline), δ, 108.3, 110.2, 115.9, 116.5, 117.1, 121.9, 127.4, 128.4, 128.9, 129.7, 131.9, 137.8, 143.4, 145.5, 147.1, 147.8, 152.8, 157.1, 159.5, 161.3, 164.0 (21 signals, aromatic carbons, pyrazole carbons, C_3 and C_4 of pyrazoline).		

7i	***5'-(4-fluorophenoxy)-5-(4-fluorophenyl)-2-(4-methoxyphenyl)-3'-methyl-1'-phenyl-3,4-dihydro-1'H,2H-3,4'-bipyrazole***		
Mol. for. M. P. (°C) Mol. Wt.	$C_{32}H_{26}F_2N_4O_2$ 158-160 537.5		 7i
Ele. Ana. Calcd.(Obs)	C 71.63 (71.37)	H 4.88 (4.60)	N 10.44 (10.17)
FT-IR ν_{max} cm^{-1} (KBr)	1361 (-CH_3 rocking.), 1258 (C-O-C ether str.), 1619 (-C=N), 3053 (-CH aromatic)		
^{1}H NMR δ ppm DMSO-d_6	7.67-6.78 (17H, m, ArH), 5.17 (1H, dd, *J* = 7.2, 12.4Hz, C_5-H pyrazoline), 3.63 (4H, s, C_4-H pyrazoline merged with -OCH_3), 3.22 (1H, dd, *J* = 7.2, 17.6 Hz, C_4-H pyrazoline), 2.15 (3H, s, CH_3).		
^{13}C NMR δ ppm DMSO-d_6	13.9 (-CH_3 of pyrazole), δ, 55.0 (-CH_2 of pyrazoline), δ, 55.6 (-OCH_3), δ, 108.7, 114.7, 115.4, 116.3, 116.9, 117.2, 121, 127.3, 127.8, 129.5, 137.9, 138.9, 145.6, 146.1, 147.1, 152.9, 153.3, 157.2, 159.5, 161.3, 163.8, (21signals, aromatic carbons, pyrazole carbons, C_3 and C_4 of pyrazoline)		

7j	***5'-(2-fluorophenoxy)-2,5-bis(4-fluorophenyl)-3'-methyl-1'-phenyl-3,4-dihydro-1'H,2H-3,4'-bipyrazole***		
Mol. for. M. P. (°C) Mol. Wt.	$C_{31}H_{23}F_3N_4O$ 162-164 525.4		 **7j**
Ele. Ana. Calcd.(Obs)	C 70.98 (70.73)	H 4.42 (4.19)	N 10.68 (10.45)
FT-IR ν_{max} cm^{-1} (KBr)	1361 (-CH_3 rocking.), 1260 (C-O-C ether str.), 1622 (-C=N), 3052 (-CH aromatic)		
^{1}H NMR δ ppm DMSO-d_6	7.66-6.84 (17H, m, ArH), 5.32 (1H, dd, *J* = 6.4, 12.8 Hz, C_5-H pyrazoline), 3.71 (1H, dd, *J* = 12.4, 17.2 Hz, C_4-H pyrazoline), 3.27 (1H, dd, *J* = 6.4, 17.6 Hz, C_4-H pyrazoline), 2.27 (3H, s, CH_3)		
^{13}C NMR δ ppm DMSO-d_6	14.0 (-CH_3 of pyrazole), δ, 54.1 (-CH_2 of pyrazoline), δ, 109.5, 109.9, 115.7, 116.5, 117.9, 123.5, 124.9, 125.8, 127.7, 128.5, 128.9, 129.0, 132.9, 137.7, 143.2, 143.9, 144.8, 147.9, 148.9, 150.3, 152.6, 162.6, 164.7 (23 signals, aromatic carbons, pyrazole carbons, C_3 and C_4 of pyrazoline).		

7k	***2-(4-bromophenyl)-5'-(2-fluorophenoxy)-5-(4-fluorophenyl)-3'-methyl-1'-phenyl-3,4-dihydro-1'H,2H-3,4'-bipyrazole***		
Mol. for. M. P. (°C) Mol. Wt.	$C_{31}H_{23}BrF_2N_4O$ 165-167 585.4		 **7k**
Ele. Ana. Calcd.(Obs)	C 63.60 (63.32)	H 3.96 (3.73)	N 9.57 (9.36)
FT-IR ν_{max} cm^{-1} (KBr)	1357 (-CH_3 rocking.), 1258 (C-O-C ether str.), 1623 (-C=N), 3054 (-CH aromatic)		
^{1}H NMR δ ppm DMSO-d_6	7.67-6.62 (17H, m, ArH), 5.32 (1H, dd, *J* = 6.4, 12.8 Hz, C_5-H pyrazoline), 3.70 (1H, dd, *J* = 12.8, 17.6 Hz, C_4-H pyrazoline) 3.27 (1H, dd, C_4-H pyrazoline merged with peak of H_2O), 2.18 (3H, s, CH_3)		
^{13}C NMR δ ppm DMSO-d_6	13.7 (-CH_3 of pyrazole), δ, 53.7 (-CH_2 of pyrazoline), δ, 108.5, 110.3, 115.0, 116.0, 117.4, 122.0, 124.8, 125.3, 127.6, 128.1, 128.9, 129.7, 131.9, 137.7, 143.4, 143.9, 144.7, 147.0, 147.9, 150.0, 152.5, 161.6, 164.9 (23 signals, aromatic carbons, pyrazole carbons, C_3 and C_4 of pyrazoline).		

7l	***5'-(2-fluorophenoxy)-5-(4-fluorophenyl)-2-(4-methoxyphenyl)-3'-methyl-1'-phenyl-3,4-dihydro-1'H,2H-3,4'-bipyrazole***		
Mol. for. M. P. (°C) Mol. Wt.	$C_{32}H_{26}F_2N_4O_2$ 141.143 537.5		 **7l**
Ele. Ana. Calcd.(Obs)	C 71.63 (71.38)	H 4.88 (4.65)	N 10.11 (10.19)
FT-IR ν_{max} cm^{-1} (KBr)	1359 (-CH_3 rocking.), 1259 (C-O-C ether str.), 1626 (-C=N), 3055 (-CH aromatic)		
^{1}H NMR δ ppm DMSO-d_6	7.66-6.68 (17H, m, ArH), 5.18 (1H, dd, *J* = 7.2, 12.4Hz C_5-H pyrazoline), 3.67 (4H, s, C_4-H pyrazoline merged with -OCH_3), 3.62 (1H, dd, *J* = 12.8, 17.6 Hz, C_4-H pyrazoline), 3.23 (1H, dd, *J* = 7.2, 17.6 Hz C_4-H pyrazoline), 2.17 (3H, s, CH_3)		
^{13}C NMR δ ppm DMSO-d_6	14.0 (-CH_3 of pyrazole), δ, 55 (-CH_2 of pyrazoline), δ, 55.6 (-OCH_3), δ, 108.8, 114.7, 115.1, 115.8, 116.0, 116.8, 117.3, 117.5, 122, 124.9, 125, 125.4, 127.5, 127.8, 127.9, 129.7, 137.8, 138.9, 144.9, 146.2, 147.2, 153.4, 164.7 (23 signals, aromatic carbons, pyrazole carbons, C_3 and C_4 of pyrazoline).		

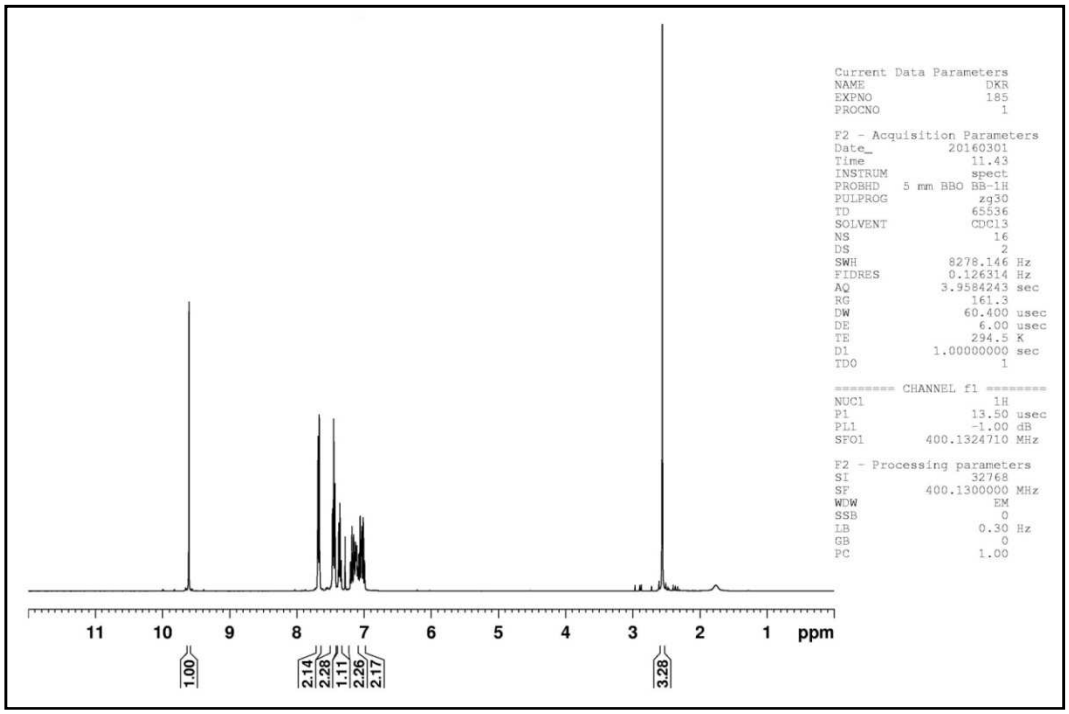


Figure 2.12 ^{1}H NMR spectrum of compound **3a**

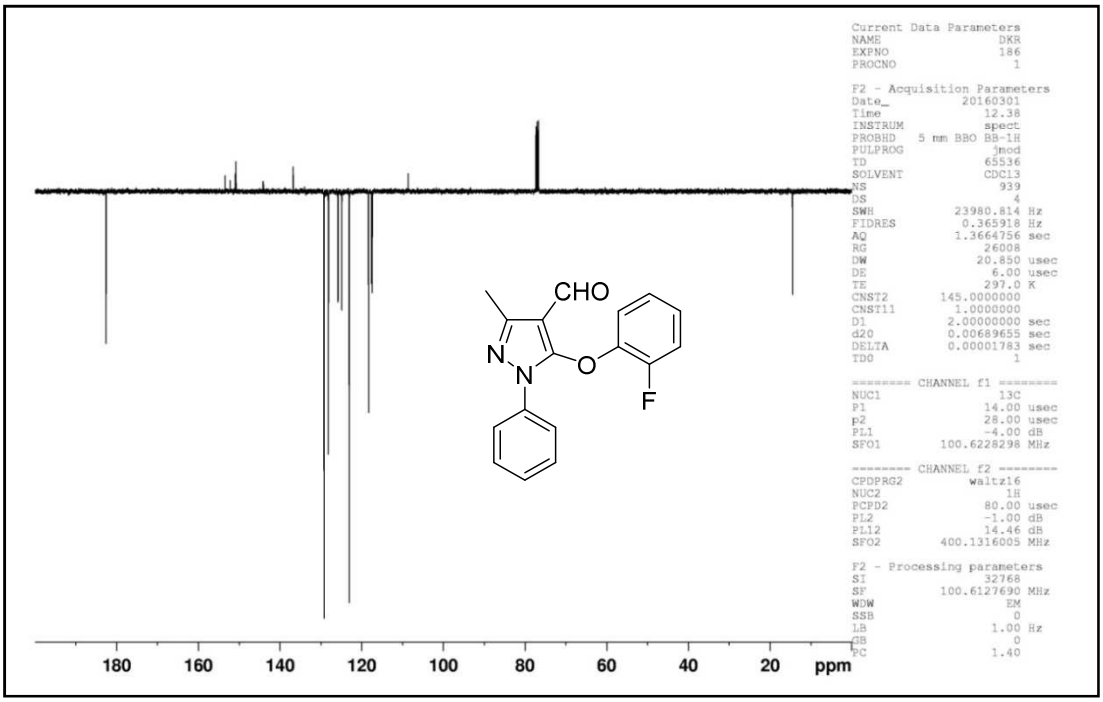


Figure 2.13 ^{13}C DEPT spectrum of compound **3a**

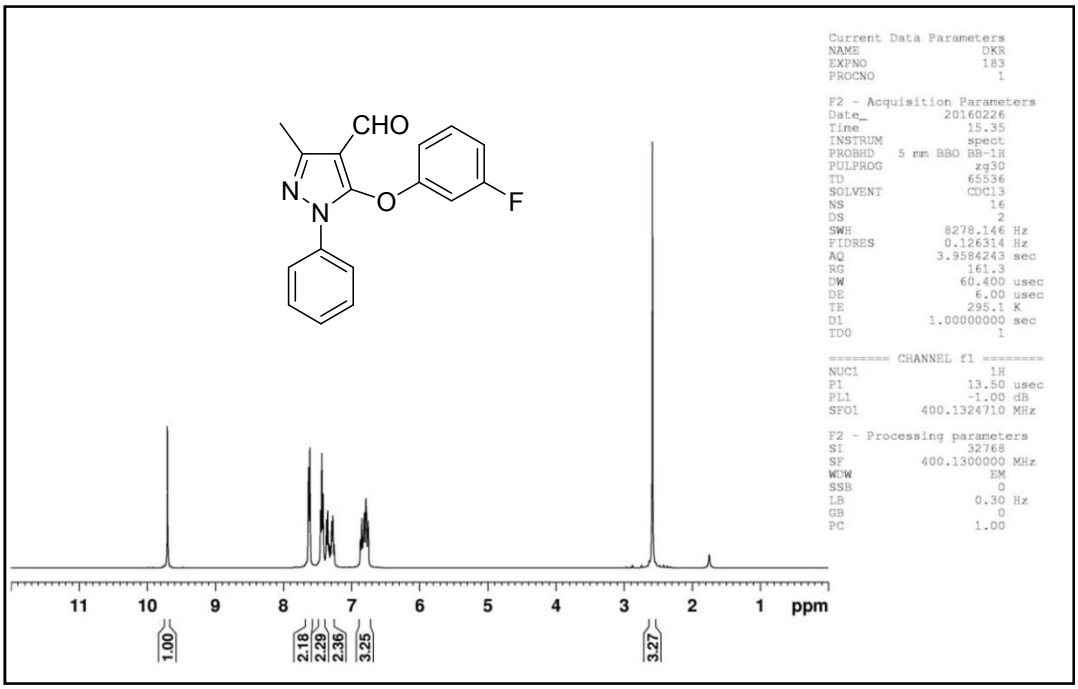


Figure 2.14 ^{1}H NMR spectrum of compound **3b**

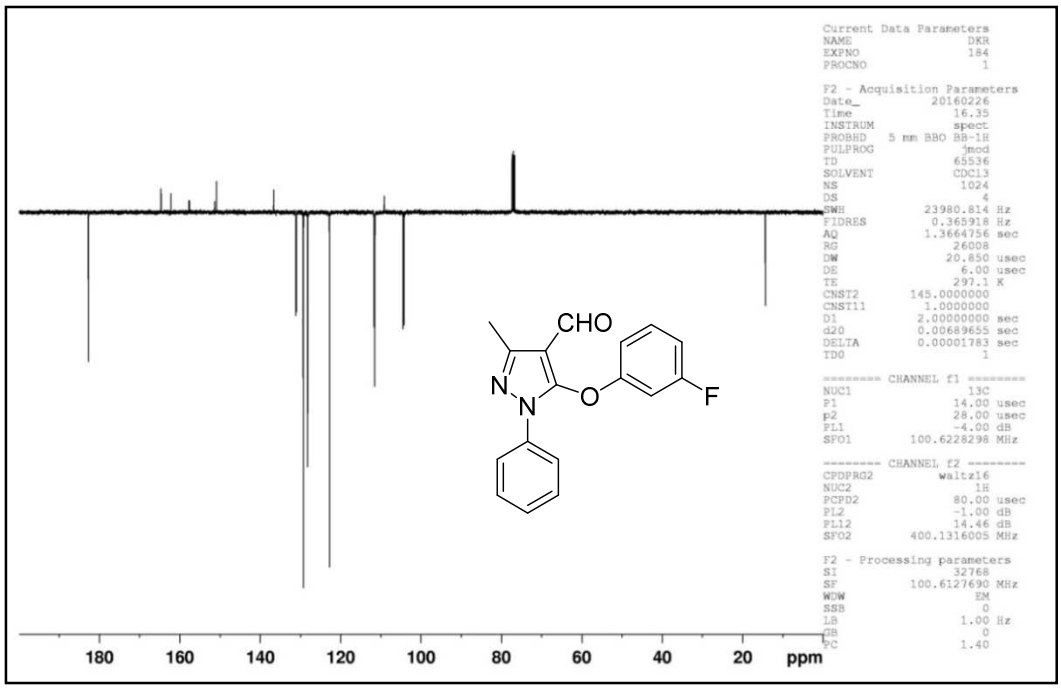


Figure 2.15 ^{13}C DEPT spectrum of compound **3b**

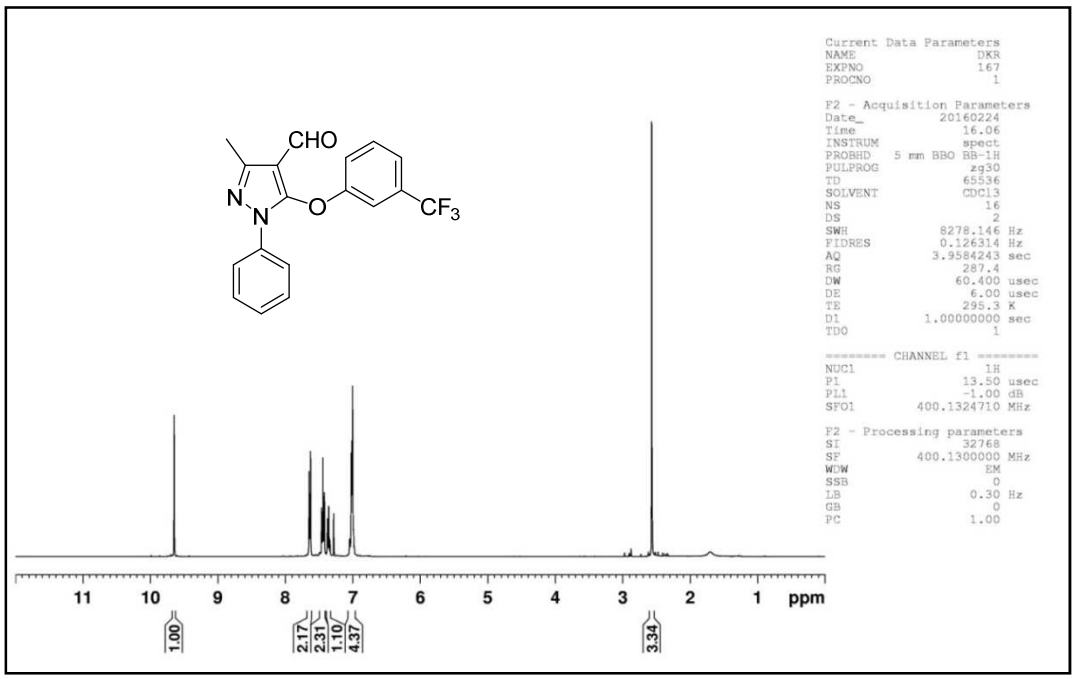


Figure 2.16 ^{1}H NMR spectrum of compound **3c**

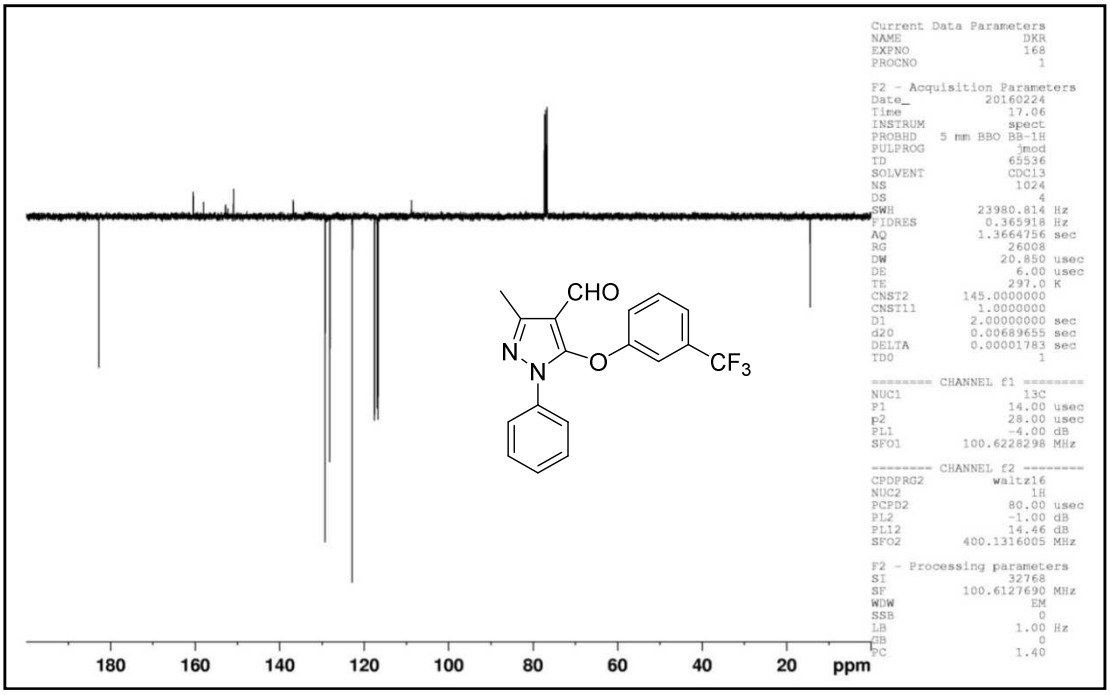


Figure 2.17 ^{13}C DEPT spectrum of compound **3c**

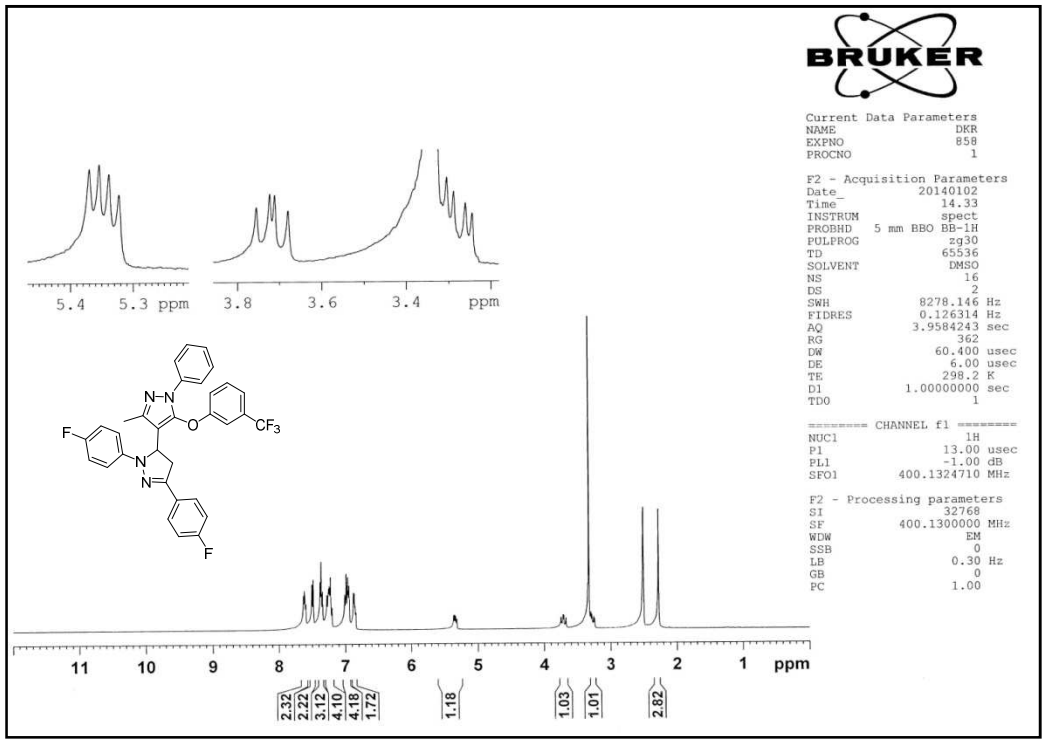


Figure 2.18 ^{1}H NMR spectrum of compound **7a**

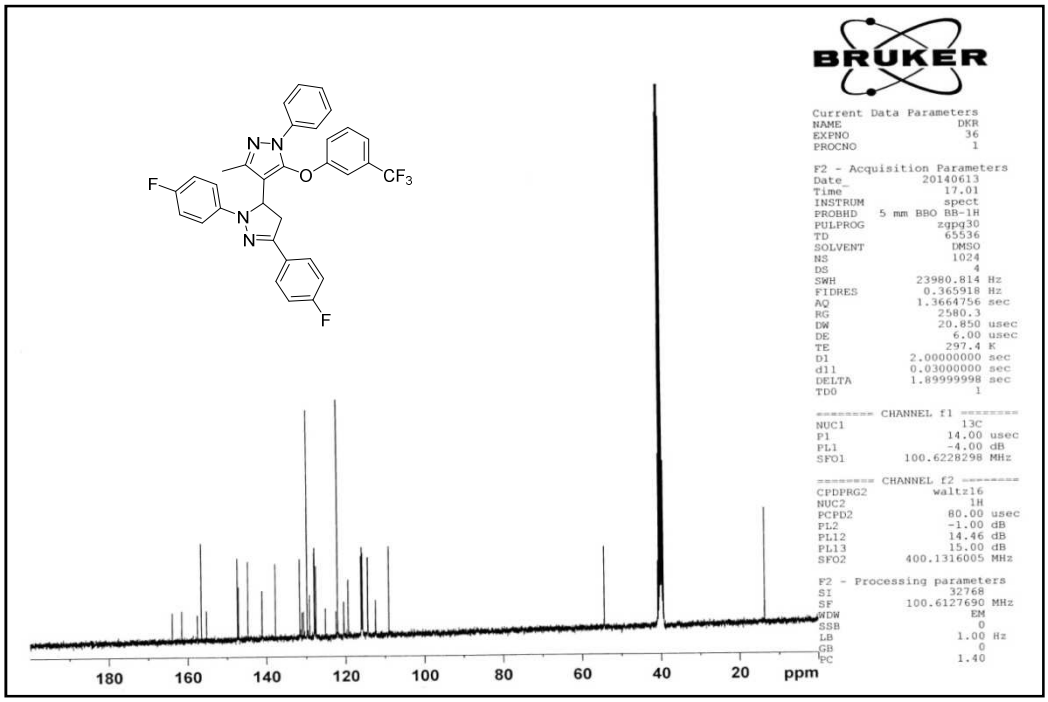


Figure 2.19 ^{13}C spectrum of compound **7a**

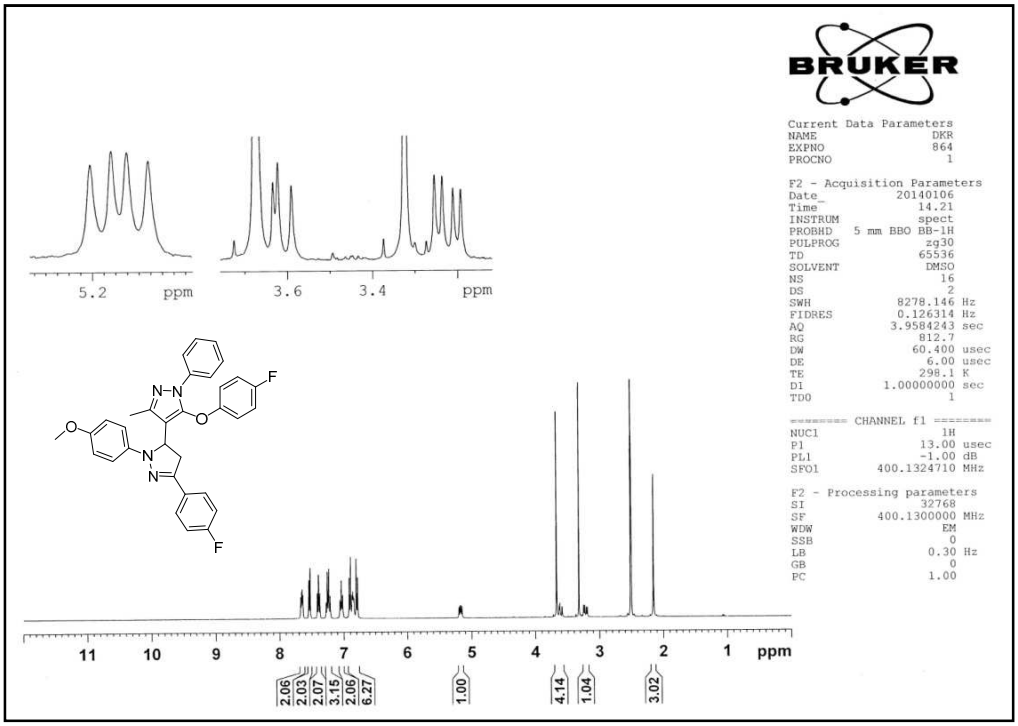


Figure 2.20 ^{1}H NMR spectrum of compound **7i**

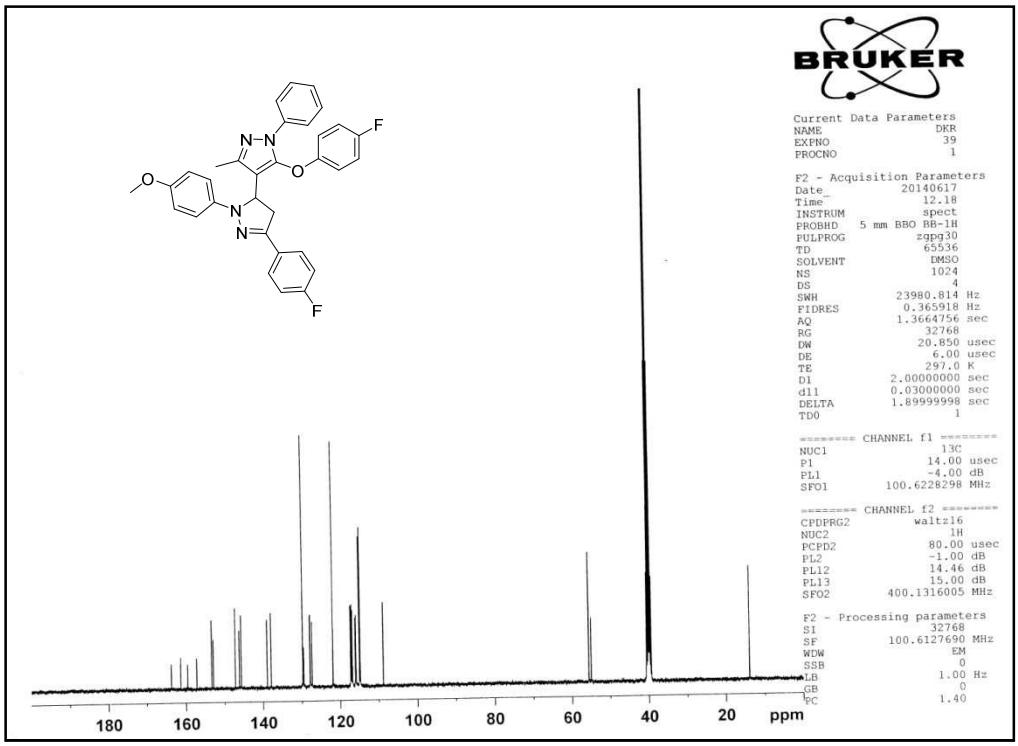


Figure 2.21 ^{13}C spectrum of compound **7i**

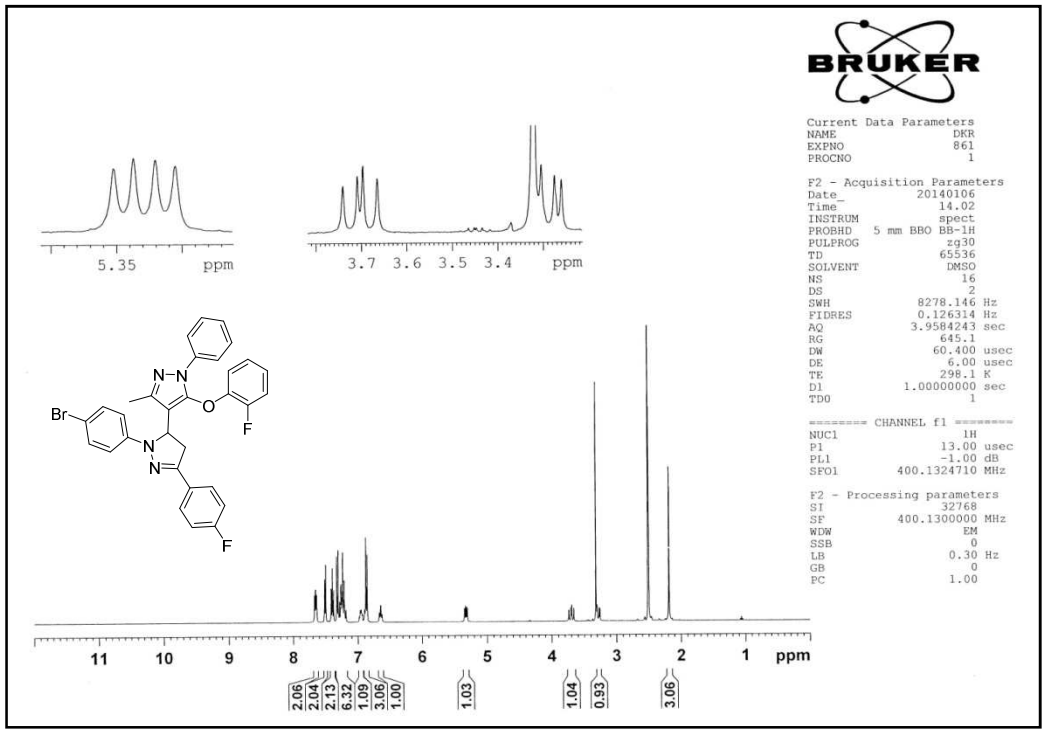


Figure 2.22 ^{1}H NMR spectrum of compound **7k**

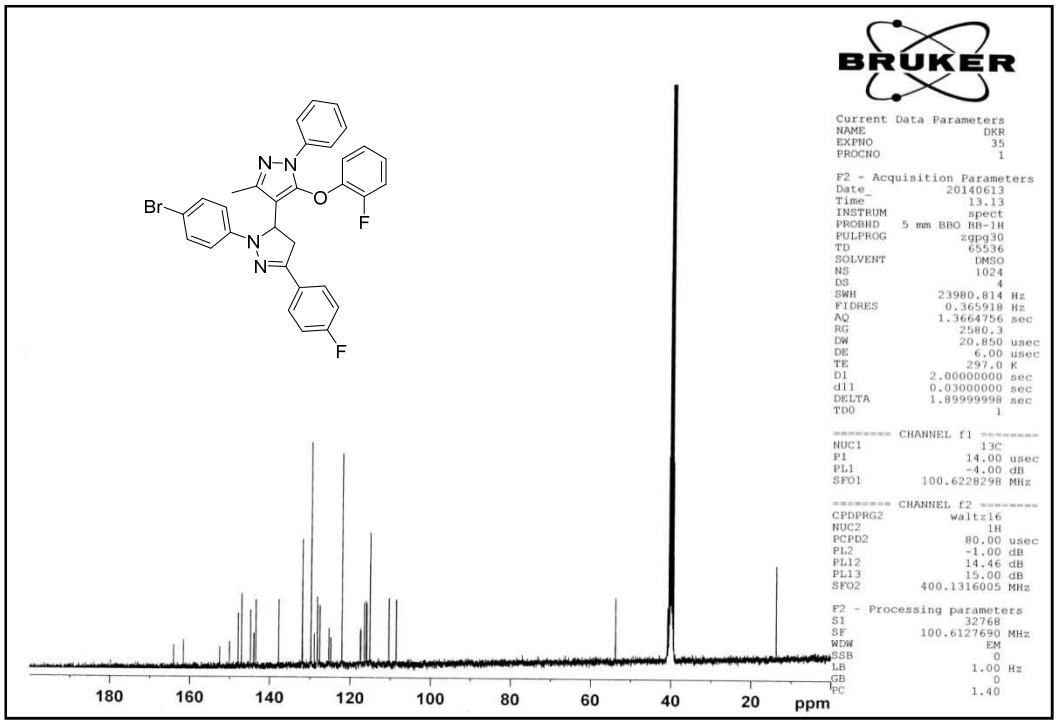


Figure 2.23 ^{13}C spectrum of compound **7k**

2.10. Biological results

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

2.10.1 Antibacterial activity

The synthesized pyrazolylpyrazoline derivatives **7a-l** were evaluated for their antimicrobial activity by broth micro dilution method according to National Committee for Clinical Laboratory Standards (NCCLS) [53]. The compounds were screened for antibacterial activity employing three Gram positive (*Clostridium tetani* MTCC 449, *Bacillus subtilis* MTCC 441, and *Streptococcus pneumoniae* MTCC 1936) and three Gram negative (*Escherichia coli* MTCC 443, *Salmonella typhi* MTCC 98 and *Vibrio cholerae* MTCC 3906) bacteria against ampicillin, norfloxacin, ciprofloxacin and chloramphenicol as the reference drugs. Antifungal activity was screened against two fungal species (*Candida albicans* MTCC 227 and *Aspergillus fumigats* MTCC 3008) where nystatin and griseofulvin were used as the standard drugs. The result of the antimicrobial screening data is shown in **Table 2.4**.

Table 2.4 *In vitro* antimicrobial activity (MIC, µg/mL) of compounds **7a-l**

Comp.	*Gram positive bacteria*			*Gram negative bacteria*			*Fungi*	
	S.P. MTCC 1936	B.S. MTCC 441	C.T. MTCC 449	E.C. MTCC 443	S.T. MTCC 98	V.C. MTCC 3906	C.A. MTCC 227	A.F. MTCC 3008
7a	500	500	500	200	250	125	1000	250
7b	500	**250**	**250**	500	200	125	**250**	>1000
7c	**100**	**200**	**200**	500	500	200	1000	>1000
7d	200	**250**	500	200	200	250	**250**	1000
7e	500	**250**	**250**	250	200	250	1000	500
7f	200	**100**	**200**	**100**	**100**	250	**250**	500
7g	**100**	500	**250**	500	250	200	**200**	>1000
7h	500	500	**125**	200	200	500	**500**	>1000
7i	125	**62.5**	**200**	500	500	**100**	**500**	250
7j	250	500	**250**	200	250	250	1000	**100**
7k	250	**200**	500	**100**	200	500	**250**	1000
7l	500	**100**	500	500	500	500	**500**	250
A	100	250	250	100	100	100	n. t.[a]	n. t.
B	10	100	50	10	10	10	n. t.	n. t.
C	50	50	50	50	50	50	n. t.	n. t.
D	25	50	100	25	25	25	n. t.	n. t.
E	n. t.	n. t.	n. t.	n. t.	n. t.	n. t.	100	100
F	n. t.	n. t.	n. t.	n. t.	n. t.	n. t.	500	100

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: Norfloxacin, C: Chloramphenicol, D: Ciprofloxacin, E: Nystatin, F: Griseofulvin, [a] n.t.: not tested.

Upon investigation of antimicrobial activity data (**Table 2.4**), it has been observed that against *B. subtillis*, compound **7i** (R= 4-F, R_1= 4-OCH_3) was found to possess excellent potency i.e. 62.5 µg/mL as compared to ampicillin i.e. 250 µg/mL as well as norfloxacin i.e. 100 µg/mL. Compounds **7f** (R= 3-CF_3, R_1= 4-OCH_3) and **7l** (R= 2-F, R_1= 4-OCH_3) were found to be more potent against *B. subtillis* i.e. 100 µg/mL as compared to ampicillin. Compounds **7c** (R= 3-F, R_1= 4-OCH_3) and **7k** (R= 2-F, R_1= 4-Br) were found to be more effective (MIC = 200 µg/mL) against *B. subtillis* as compared to ampicillin. Compounds **7b** (R= 3-F, R_1= 4-Br), **7d** (R= 3-F, R_1= 4-F), **7e** (R= 3-CF_3, R_1= 4-Br) were equipotent as that of ampicillin against *B. subtillis*. Against *S. pneumonia* compounds **7c** (R= 3-F, R_1= 4-OCH_3) and **7g** (R= 4-F, R_1= 4-F) showed comparable activity to that of ampicillin. Against *C. tetani*, compounds **7b** (R= 3-F, R_1= 4-Br), **7e** (R= 3-CF_3, R_1= 4-Br), **7g** (R= 4-F, R_1= 4-F), and **7j** (R= 2-F, R_1= 4-F)

displayed same influence as that of ampicillin. Compounds **7h** (R= 4-F, R_1= 4-Br), **7c** (R= 3-F, R_1= 4-OCH_3), **7f** (R= 3-CF_3, R_1= 4-OCH_3), **7i** (R= 4-F, R_1= 4-OCH_3) were found to exhibit superior activity as compared to ampicillin against *C. tetani*. In case of inhibiting gram negative bacteria compounds **7f** (R= 3-CF_3, R_1= 4-OCH_3) and **7k** (R= 2-F, R_1= 4-Br) were found equipotent against *E. coli* as compared to ampicillin. Compounds **7f** (R= 3-CF_3, R_1= 4-OCH_3) and **7i** (R= 4-F, R_1= 4-OCH_3) also exhibited the same power as that of ampicillin i.e. 100 µg/mL against *S. typhi* and *V. cholera* respectively.

2.10.2 Antifungal activity

The antifungal screening data (**Table 2.4**) revealed that, against *C. albicans*, compounds **7g** (R= 4-F, R_1= 4-F) , **7b** (R= 3-F, R_1= 4-Br), **7d** (R= 3-F, R_1= 4-F), **7f** (R= 3-CF_3, R_1= 4-OCH_3) and **7k** (R= 2-F, R_1= 4-Br) were found to possess significant activity as compared to griseofulvin. Compounds **7h** (R= 4-F, R_1= 4-Br), **7i** (R= 4-F, R_1= 4-OCH_3) and **7l** (R= 2-F, R_1= 4-OCH_3) exhibited equivalent potency against *C. albicans* as compared to griseofulvin. Compound **7j** (R= 2-F, R_1= 4-F) showed equal potency against *A. fumigates* as compared to griseofulvin as well as nystatin.

2.10.3 Antituberculosis activity

A primary *in vitro* antituberculosis activity of novel pyrazolylpyrazolines **7a-l** was conducted at 250 µg/mL against *Mycobacterium tuberculosis* H37Rv strain by using Lowensteine-Jensen medium as described by Rattan [54]. The obtained result is presented in **Table 2.5** in the form of % inhibition. Rifampicin and Isoniazid were employed as the standard drugs.

Antituberculosis screening of all the synthesized compounds **7a-l** was conducted at 250 µg/mL concentrations against tuberculosis H37Rv strain. Compounds **7a** (R= 3-CF_3, R_1= 4-F), **7e** (R= 3-CF_3, R_1= 4-Br), **7h** (R= 4-F, R_1= 4-Br) and **7k** (R= 2-F, R_1= 4-Br) found to possess brilliant activity (i.e. 90%, 91%, 96 and 94% at 250 µg/mL) against *M. tuberculosis* H37Rv (**Table 2.5**). Remaining all other compounds showed poor inhibition against *M. tuberculosis* growth.

Table 2.5 *In vitro* antituberculosis activity (% inhibition) of compounds **7a-l** against *M. tuberculosis* H37Rv (at concentration 250 µg/mL).

Comp.	% Inhibition	Comp.	% Inhibition
7a	**90**	**7h**	**96**
7b	56	7i	74
7c	56	7j	10
7d	65	**7k**	**94**
7e	**91**	7l	22
7f	52	**Rifampicin**	98
7g	40	**Isoniazid**	99

2.10.4 Antimalarial activity

In vitro antimalarial activity of the novel pyrazolylpyrazolines derivatives **7a-l** against *P. falciparum* strain was performed using chloroquine and quinine as the reference drugs. The consequence of antimalarial screening is expressed as the drug concentration resulting in 50% inhibition of parasite growth (IC_{50}) and is listed in **Table 2.6.**

Table 2.6. *In vitro* antimalarial activity of compounds **7a-l**

Comp.	IC_{50} (µg/mL)	Comp.	IC_{50} (µg/mL)
7a	**0.034**	**7h**	**0.025**
7b	**0.060**	7i	0.25
7c	0.57	**7j**	**0.025**
7d	0.82	**7k**	**0.088**
7e	1.46	7l	0.65
7f	0.30	**Chloroquine**	0.020
7g	**0.022**	**Quinine**	0.268

Compounds **7a-l** were also evaluated for their antimalarial screening 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 2.6**. As shown in **Table 3**, they were found to have IC_{50} between 0.022 and 0.088 upon *P. falciparum* strain. It is important to note that compounds **7a** (R= 3-CF_3, R_1= 4-F), **7b** (R= 3-F, R_1= 4-Br), **7g** (R= 4-F, R_1= 4-F), **7h** (R= 4-F, R_1= 4-Br), **7j** (R= 2-F, R_1= 4-F) and **7k** (R= 2-F, R_1= 4-Br) displayed excellent activity against *P. falciparum* strain as

compared to quinine IC_{50} 0.268. From the above results, it can be concluded that compound **7g, 7h** and **7j** may prove themselves as new antimalarial agents in future.

2.11 Structure-activity relationship (SAR)

The results of the biological evaluation revealed that the activity was significantly affected by introducing fluorinated aryloxy nucleus at the C-5' position in pyrazoline scaffold (**Figure 2.24**).

It has been observed that electron donating group (4-OCH_3) existing at R_1 position and 3-CF_3 group on aryloxy ring on pyrazoline induced the greatest antibacterial activity against *B. subtilis* and *C. tetani.*

The presence of electron withdrawing group (4-F, 4-Br) at R_1 position and -CF_3 group at *meta* position of aryloxy ring illustrated superior antimalarial activity as well as antituberculosis activity. The presence of bromo group at R_1 position and fluoro group at *ortho*, *meta,* and *para* positions of aryloxy ring showed enhanced antituberculosis activity as well as remarkable antimalarial activity. The presence of fluoro group at R_1 position and the *ortho*, *meta,* and *para* positions of aryloxy ring displayed excellent antimalarial activity. It can be concluded that the presence of electron withdrawing groups (4-F, 4-Br) at R_1 position and fluoro group at different positions of aryloxy ring are responsible for increasing antimalarial activity.

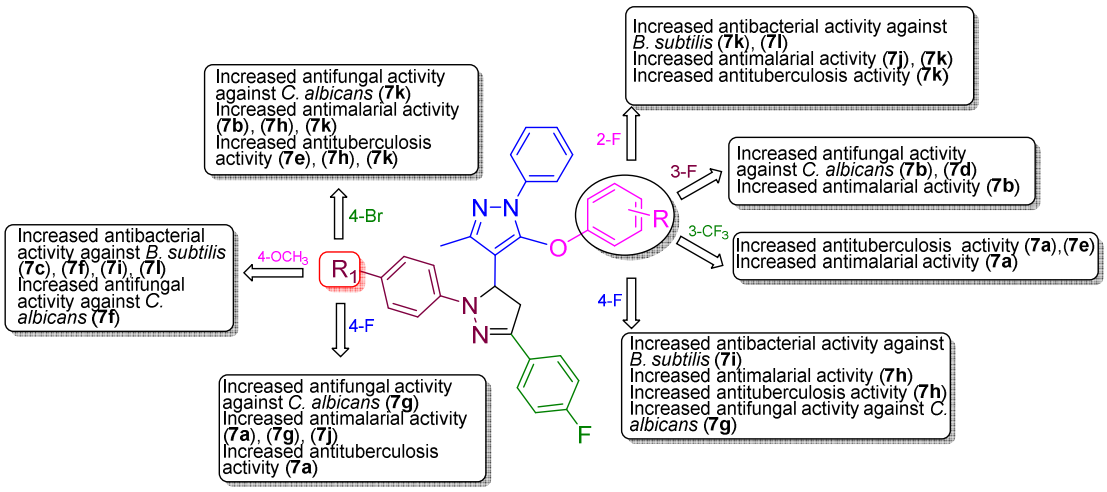


Figure 2.24 Structure-activity relationships for antimicrobial, antituberculosis and antimalarial activity of the synthesized compounds **7a-l.**

2.12 Conclusion

The novel series of fluoro substituted pyrazolylpyrazoline derivatives have been synthesized in excellent yield using microwave irradiation and examined for their pharmacological screening. The results demonstrated that some analogues of this series were found to have more potency against *C. tetani* and *B. subtilis*. The compounds **7b**, **7d**, **7f**, **7g** and **7k** illustrated remarkable antifungal activity as compared to griseofulvin. Amongst the tested compounds, **7a**, **7e**, **7h**, and **7k** showed pronounced antituberculosis activity against *M. tuberculosis* H37Rv. Whereas, compounds **7a**, **7b**, **7g, 7h, 7j** and **7k** displayed superior antimalarial activity against *P. falciparum* strain as compared to quinine. Compound **7h** with an excellent dual profile as antimalarial and antituberculosis agent was recognized as the most active member among the prepared series[55].

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