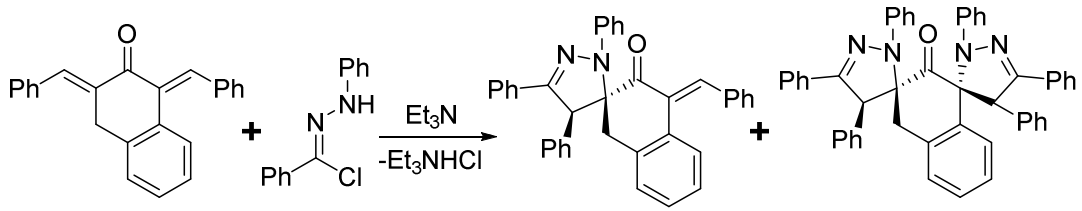
5.1 Preamble

This chapter describes microwave assisted synthesis of series of novel morpholinoquinoline based sixteen conjugates with pyrazoline moiety. The structures of all the sixteen compounds were confirmed on the basis of elemental analysis, IR, ^{1}H NMR, ^{13}C NMR and mass spectral analysis. 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*. The cytotoxicity of the synthesized compounds was tested using bioassay of *Schizosaccharomyces pombe* cells at cellular level.

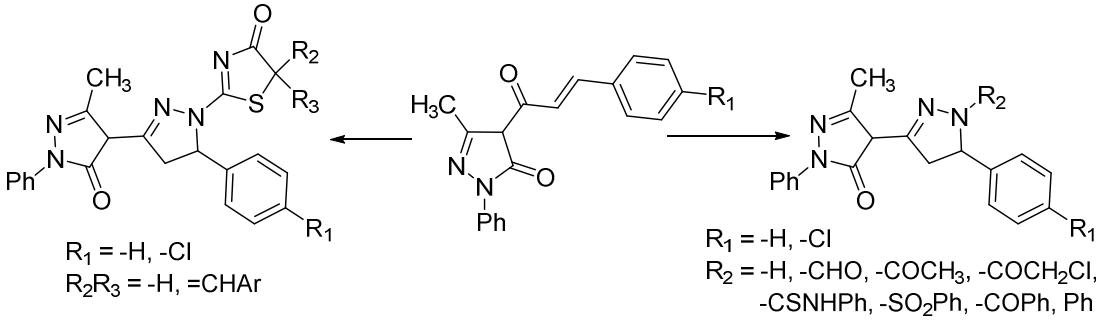
5.2 Synthesis of quinoline based pyrazoline scaffolds

Houda Gazzeh *et al*.[1] reported microwave-assisted 1,3-dipolar cycloaddition of *(E,E)*-1,3-bisarylidenetetral-2-ones with nitrilimines, generated *in situ* by dehydrohalogenation of the corresponding hydrazonoyl chlorides which, afforded a series of spiropyrazolines in good to excellent yields (Scheme 5.1).



Scheme 5.1 Synthesis ofspiro pyrazoline scaffolds.

Ahmed Abdou O. Abeed [2] prepared a novel series of pyrazoline and thiazole derivatives incorporating 2-pyrazolin-5-one moiety starting from α,β-unsaturated ketones under the effect of hydrazine derivatives and thiosemicarbazide (Scheme 5.2).



Scheme 5.2 Synthesis of pyrazoline and thiazole derivatives incorporating 2-pyrazolin-5-one moiety.

Albert Lévai and József Jeko[3] synthesized new 1-substituted 3-styryl-2-pyrazolines and 5-styryl-2-pyrazolines by the reaction of dibenzylideneacetones or *E,E*-cinnamylidene-acetophenones with hydrazines in either hot acetic acid or propionic acid solutions (Figure 5.1).

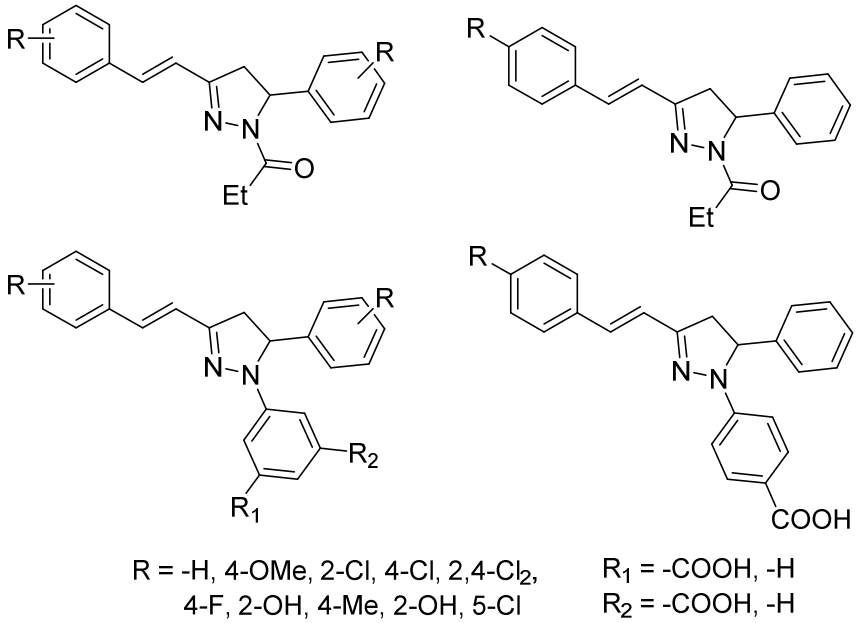
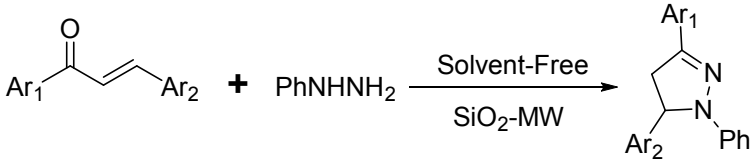


Figure 5.1 Synthesis of 1-substituted 3-styryl-2-pyrazolines and 5-styryl-2-pyrazolines derivatives.

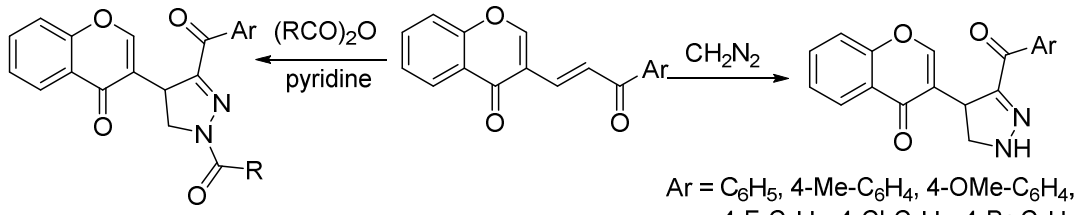
Davood Azarifar and Behrooz Maleki[4] reported silica-supported synthesis of some 1,3,5-trisubstituted 2-pyrazolines under solvent-free and microwave irradiation conditions (Scheme 5.3).



Ar_1 = 2-Naphthyl, Ph, 4-MeC_6H_4, 4-$MeOC_6H_4$, 3-MeC_6H_4
Ar_2 = Ph, 2-MeC_6H_4, 3-MeC_6H_4, 2-ClC_6H_4, 4-ClC_6H_4

Scheme 5.3 Synthesis of Silica-supported Synthesis of 1,3,5-Substituted 2-Pyrazolines derivatives.

József Jeko[5] reported new 3-aroyl-4-(3-chromonyl)-2-pyrazolines h by the reaction of 3-(3-aryl-3-oxopropenyl) chromen-4-ones and diazomethane. Some of these 2-pyrazolines were N-acylated with a mixture of anhydrous pyridine and acetic anhydride or propionic anhydride (Scheme 5.4).



R = Et, Me
Ar = 4-Me-C_6H_4, 4-F-C_6H_4, 4-Br-C_6H_4, 4-Cl-C_6H_4, 1-naphthyl, 2-naphthyl

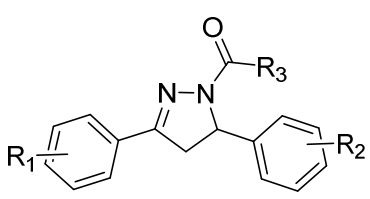
Ar = C_6H_5, 4-Me-C_6H_4, 4-OMe-C_6H_4, 4-F-C_6H_4, 4-Cl-C_6H_4, 4-Br-C_6H_4

Scheme 5.4 Synthesis pyrazoline scaffolds

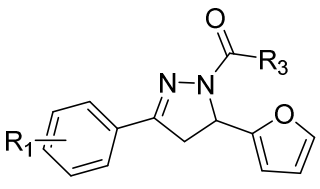
5.3 Biological screening

5.3.1 Biological screening of quinoline based pyrazoline scaffolds

A. F. M. Motiur Rahman and his co-workers[6] designed and synthesized a series of novel pyrazoline derivatives in two steps *via* chalcones and 2-carbohydrazide. These compounds were evaluated for their antiproliferative activity and topo I and II inhibitory activities (Figure 5.2).



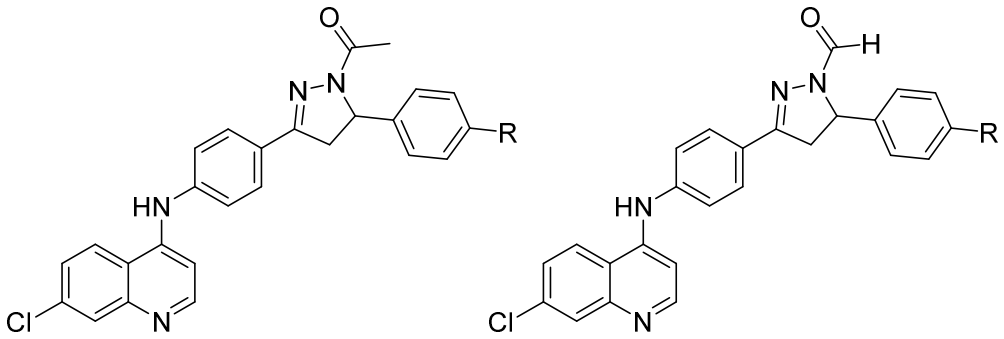
R_1 = H, 2'-OMe, 4'-Cl
R_2 = 4-OMe, 4-NO_2
R_3 = 2-fury, 5-NO_2-2-furyl, 2-benzo[d]thiazole, 4-pyridyl-



R_1 = 4-Cl, 4-OMe
R_3 = 2-furyl, 4-pyridyl-

Figure 5.2 Antiproliferative agents from pyrazoline derivatives.

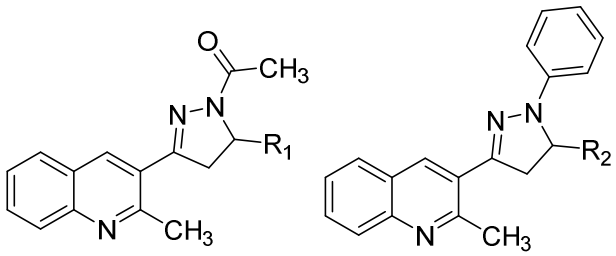
Justo Cobo *et al*.[7] prepared a new series of N-acetyl and N-formyl-pyrazoline derivatives by cyclocondensation reaction of [(7-chloroquinolin-4-yl)amino]chalcones with hydrazine hydrate in acetic acid and hydrazine hydrate in formic acid respectively. These compounds were evaluated in vitro as antitumor and as antimalarial agents (Figure 5.3).



R = -Br, -Cl, -H, -OCH_3, -3,4,5,-$(OCH_3)_3$, -CH_3

Figure 5.3 Antitumor and Antimalarial agents from N-acetyl and N-formyl-pyrazoline.

Venkatesham Rachakonda *et al*.[8] reported design and diversity oriented synthesis of novel bis heterocycles with a common 2-methyl, C-4 unsubstituted quinoline moiety as the central key heterocycles and evaluated for their antimycobacterial activity (Figure 5.4).



R_1 = 4-CN-phenyl, 4-N,Ndimethyl phenyl, -OCH_3 phenyl,
2,5-OCH_3 phenyl, 3,4,5-OCH_3 phenyl, 2,4,6-OCH_3 phenyl,
2,3,4-OCH_3 phenyl, 3,4,-OCH_3 phenyl,
R_2 = 4-Br phenyl, 3,4,5-OCH_3 phenyl, 2,3,4-OCH_3 phenyl

Figure 5.4 Antimycobacterial active quinoline based pyrazoline scaffolds.

Charansingh H. Gill and his co-workers[9] designed and synthesized a series of 3,2-(4,5-dihydro-5-(4-morphilinophenyl)-1H-pyarazol-3-yl)phenols and its N-phenylpyrazol-1-carbothioamide by Claisan–Schmidt condensation followed by the

reaction of hydrazine hydrate. All the synthesized compounds were assayed for their in vivo analgesic and anti-inflammatory activities (Figure 5.5).

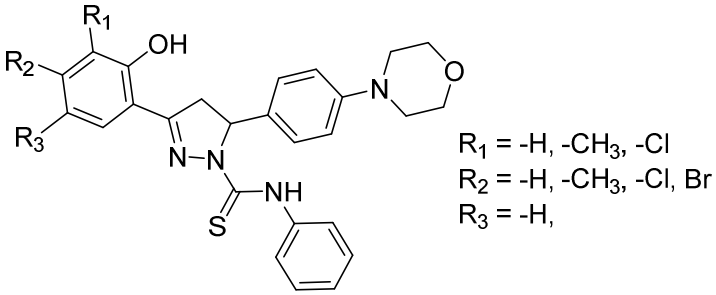


Figure 5.5 Analgesic and anti-inflammatory agents from pyrazoline scaffolds.

Faisal Hayat *et al*.[10] investigated novel series of pyrazoline derivatives bearing quinoline tail and evaluated for their *in vitro* antiamoebic activity was performed against HM1: IMSS strain of *Entamoeba histolytica* and cytotoxicity of the synthesized compounds (Figure 5.6).

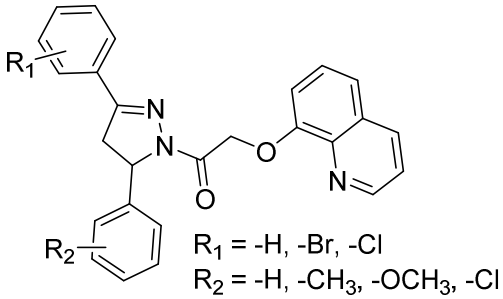


Figure 5.6 Antiamoebic agents from quinoline containing pyrazoline derivatives.

5.4 Present work

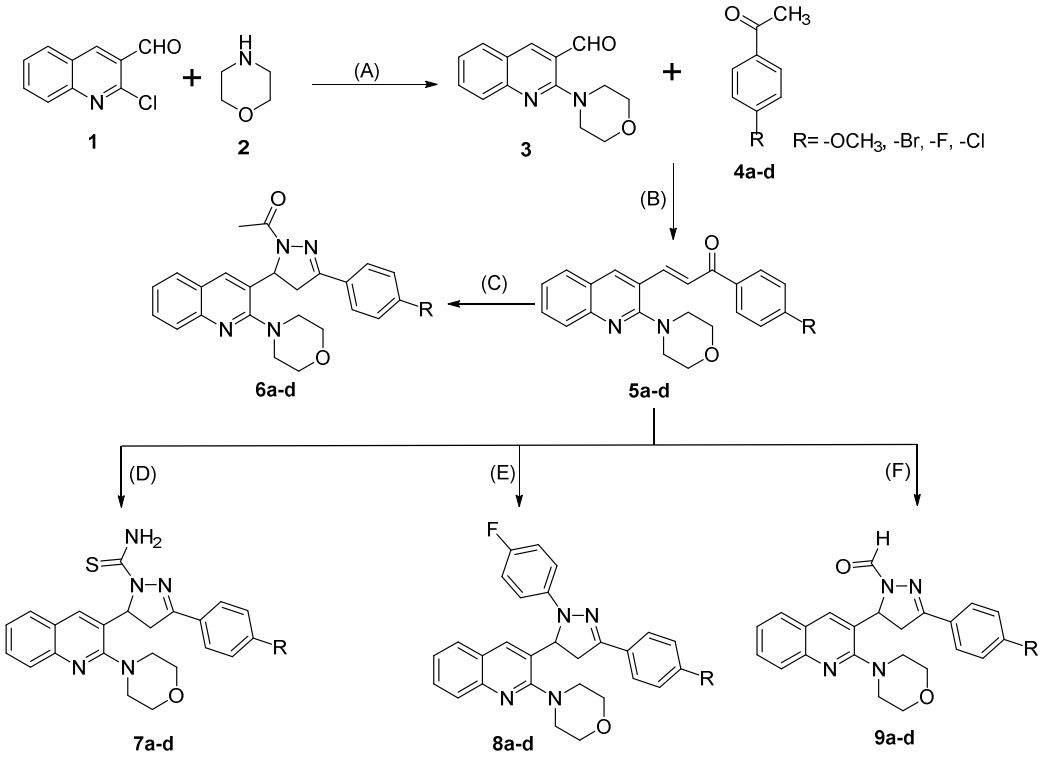
Microwave (MW) promoted reactions provided reputable method and environmentally benign protocol for the high-speed synthesis of novel chemical entities. Many chemical reactions are accelerated by the selective absorption of MW energy. The application of MW irradiation is advantageous in terms of shorter reaction times, cleaner reaction products, higher yields and better selectivity.

Quinoline nucleus is the key building element in many naturally occurring (cinchona alkaloids) compounds and pharmacologically active substances. It displays a broad range of biological activity such as antituberculosis [11], antimalarial [12], antifungal, antibacterial, antiprotozoic, antibiotic[13], and anti-HIV activities [14]. N-Functionalized morpholine motifs possess diversified biological activities such as

antidiabetic [15], antiemetic [16, 17], platelet aggregation inhibitors [15], inflammatory migraine and asthma[18, 19]. Pyrazoline derivatives are the electron rich nitrogen heterocycles which play a significant role in the diverse biological activities with strong efficacy. Scaffolds containing the 2-pyrazoline (4,5-dihydropyrazole) moiety have demonstrated a broad range of pharmacological activities including antimalarial [20], antimicrobial [21], antituberculosis, antioxidant [22], anti-inflammatory, anti-cancer, anti-microbial [23], anti-parasitary [24], anti-depressive and anticonvulsant property [25].

In context of the above consequences and our current interest directed towards the synthesis of biologically important heterocyclic compounds[26-32], herein microwave assisted synthesis of some novel morpholino quinoline based pyrazoline scaffolds by using [(2-morpholinoquinolin-3-yl)]chalcones and substituted hydrazines thiosemicarbazide has been attempted. The antimalarial, antitubercular and antimicrobial activities of synthesized compounds have been determined.

5.5 Reaction scheme



Scheme 5.5 Synthesis of quinoline based pyrazoline scaffolds (A) DMF, K_2CO_3, Reflux 2 h. (B) 20 % ethanolic NaOH, room temperature. (C) Glacial acetic acid, hydrazine hydrate (99%), MW, 12-15 Min, 350 W. (D) EtOH, NaOH, thiosemicarbazide, MW, 5-7 Min, 350 W. (E) Ethanol, 4-fluoro phenyl hydrazine hydrochloride catalytic glacial acetic acid, MW, 15-18 Min, 350 W. (F) DMF, hydrazine hydrate (99 %), Formic acid, MW, 4-8 Min, 350 W.

The synthesis of novel series of pyrazoline scaffolds was performed as outlined in **Scheme 5.5.** The starting material 2-chloroquinoline-3-carbaldehydes **1** was prepared according to Vilsmeier-Haack reaction according to a literature procedure [33]. 2-Morpholinoquinoline-3-carbaldehyde **3** was prepared by refluxing 2-chloroquinoline-3-carbaldehydes **1** and morpholine **2** in presence of anhydrous K_2CO_3 as basic catalyst in DMF as solvent. 2-Morpholinoquinoline-3-carbaldehyde **3** was subjected to base catalyzed Claisen-Schmidt condensation reaction with 4-substituted acetophenones **4a-d** to produce the required (*E*)-1-(4-substituted phenyl)-3-(2-morpholinoquinolin-3-yl)prop-2-en-1-ones **5a-d**. These [(2-morpholinoquinolin-3-yl)]chalcones **5a–d** were treated with (C) hydrazine hydrate/acetic acid, (D) thiosemicarbazide/EtOH in glacial acetic acid, (E) 4-Fluorophenyl hydrazine hydrochlorides/EtOH in glacial acetic acid and (F) hydrazine hydrate/DMF in formic acid under microwave irradiation to afford pyrazoline derivatives **6a–d**, **7a-d**, **8a-d** and **9a–d** respectively.

5.6 Experimental

- All reactions were performed with commercially available reagents without further purification. All the reactions were carried out at atmospheric pressure using a multimode microwave reactor (Microwave Synthesis System, Model: Cata-R, Catalyst Systems, Pune, - India).
- Microwave were generated by magnetron at a frequency of 2450 MHz having an adjustable output power level (i.e. 1 to 10 levels from 140 to 700 Watts) and with an individual sensor for temperature control through attachment of the reflux condenser with constant stirring.
- The temperature was monitored with an external flexible probe. All reactions were monitored by thin-layer chromatography on aluminium plates coated with silica gel 60 F_{254}, 0.25 mm thickness (Merck). Detection of the components was made by exposure to iodine vapors or UV light.
- Melting points were determined in an open capillary using µThermoCal10 melting point apparatus (Analab Scientific Pvt. Ltd, India) and are uncorrected.

- ^{1}H NMR spectra and ^{13}C NMR were recorded on Bruker Avance 400F (MHz) spectrometer (Bruker Scientific Corporation Ltd., Switzerland) using $CDCl_3$ as solvent and tetramethylsilane (TMS) as the internal standard.
- 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}.
- The elemental analysis was performed on a 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.

5.7 Synthesis of novel morpholinoquinoline nucleus clubbed with pyrazoline scaffolds 6a–d, 7a-d, 8a-d and 9a–d.

The title compounds (**6a–d, 7a-d, 8a-d AND 9a–d**) were synthesized in following steps:

5.7.1. General procedure for the synthesis 2-morpholinoquinoline-3-carbaldehyde (3)

2-chloroquinoline-3-carbaldehyde **1** (1 mmol), morpholine **2** (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 the reaction as confirmed by TLC, the reaction mixture was poured into 100 mL ice-water, filtered, washed thoroughly with water, dried and recrystallized from ethanol to obtain a yellow solid.

5.7.2 General procedure for the synthesis of (E)-1-(4-substituted phenyl)-3-(2-morpholinoquinolin-3-yl)prop-2-en-1-one (5a-d)

To a solution of 2-morpholinoquinoline-3-carbaldehyde **3** (1.0 mmol) and 1-(4-substituted phenyl)ethanone **4a-d** (1.0 mmol), 20 % ethanolic NaOH (5 mL) solution was added dropwise over a peried of 15 min. The reaction mixture was stirred at ambient temperature until the formation of precipitate. The solid obtained was isolated by filtration, washed with cold ethanol and recrystallized from hot $CHCl_3$.

Comp.	IUPAC Name	M. F. (MW)	Yield (%)	m.p. (°C)
5a	*(E)*-1-(4-fluorophenyl)-3-(2-morpholinoquinolin-3-yl)prop-2-en-1-one	$C_{22}H_{19}FN_2O_2$ (363.2)	82	148-150
5b	*(E)*-1-(4-bromophenyl)-3-(2-morpholinoquinolin-3-yl)prop-2-en-1-one	$C_{22}H_{19}BrN_2O_2$ (424.1)	72	150-152
5c	*(E)*-1-(4-methoxyphenyl)-3-(2-morpholinoquinolin-3-yl)prop-2-en-1-one	$C_{23}H_{22}N_2O_3$ (375.2)	75	157-159
5d	*(E)*-1-(4-chlorophenyl)-3-(2-morpholinoquinolin-3-yl)prop-2-en-1-one	$C_{22}H_{19}ClN_2O_2$ (379.1)	76	130-132

5.7.3 General procedure for the synthesis 1-(3-(4-substituted phenyl)-5-(2-morpholino quinolin-3-yl)-4,5-dihydro-1H-pyrazol-1-yl)ethanone (6a-d).

[(2-morpholinoquinolin-3-yl)]chalcones **5a-d,** hydrazine hydrate (99 %) (0.01 mol) and glacial acetic acid (4.0 mL) were charged in a 50 mL round bottom flask. The reaction mixture was subjected to microwave irradiation at 350 W for 12-15 min. The progress of the reaction was monitored by TLC. After completion of the reaction, resulting solution was cooled to room temperature, neutralized with 10% NaOH solution and poured into crushed ice with continuous stirring. The solid obtained was isolated by filtration, washed with cold ethanol and recrystallized from hot ethanol, affording compounds (**6a-d**).

5.7.4 General procedure for the synthesis 3-(4-substituted phenyl)-5-(2-morpholino quinolin-3-yl)-4,5-dihydro-1H-pyrazole-1-carbothioamide (7a-d).

[(2-morpholinoquinolin-3-yl)]chalcones **5a-d** (0.01 mol) (**5a–d**), sodium hydroxide (0.025 mol) and thiosemicarbazide (0.01 mol) were thoroughly mixed in ethanol (5 mL) in a 50 mL round bottom flask. The reaction mixture was subjected to microwave irradiation at 350 W for 5-7 min. The progress of the reaction was monitored by TLC. After completion of the reaction, the reaction mixture was cooled to room temperature. The solid was filtered and recrystallized from hot ethanol, affording compounds (**7a-d**).

7.5.5 General procedure for the synthesis 4-(3-(3-(4-substituted phenyl)-1-(4-fluorophenyl)-4,5-dihydro-1H-pyrazol-5-yl)quinolin-2-yl)morpholine (8a-d)

[(2-morpholinoquinolin-3-yl)]chalcones **5a-d** (1.0 mmol) and 4-fluoro phenyl hydrazine hydrochloride (1.1 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 for 15-18 min. After completion of the reaction as monitored by TLC (ethyl acetate: hexane: 2:8), reaction mixture was cooled to room temperature. The solid thus separated was filtered, washed with cold ethanol (10 mL), dried and recrystallized from hot ethanol, affording compounds (**8a-d**).

5.7.6 General procedure for the synthesis 3-(4-substituted phenyl)-5-(2-morpholino quinolin-3-yl)-4,5-dihydro-1H-pyrazole-1-carbaldehyde (9a-d)

[(2-morpholinoquinolin-3-yl)]chalcones **5a-d** (1.0 mmol) and hydrazine hydrate (1.0 mmol) were thoroughly mixed in DMF (5 mL) in a 50 mL round bottom flask. The reaction mixture was subjected to microwave irradiation at 350 W for 6-8 min until complete consumption of the chalcone **5a-d** (TLC control). Then, formic acid (4.0 mL) was dropwise added to the reaction mixture and it was further subjected to microwave irradiation at 350 W for 4-6 min. After completion of the reaction as monitored by TLC (ethyl acetate: hexane: 4:6), 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 (**9a-d**).

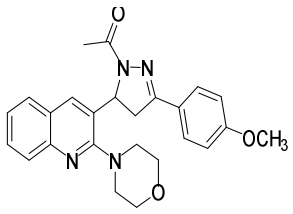
5.8 Preliminary and spectral characterization

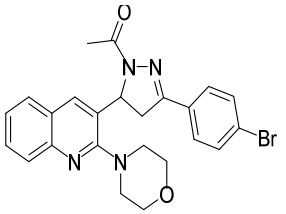
The formation of newly synthesized compounds was confirmed by ^{1}H NMR, FT-IR, mass spectrometry and elemental analysis. The IR spectrum of synthesized scaffolds exhibited characteristic absorption band in the range 1234-1211 cm^{-1}. This can be attributed to the presence of ether linkage. The absorption band around 3062-3051 cm^{-1} is due to aromatic C-H stretching. The carbonyl group stretching frequency was observed at 1649-1640 cm^{-1}. The absorption band in the range of 1640-1595 cm^{-1}

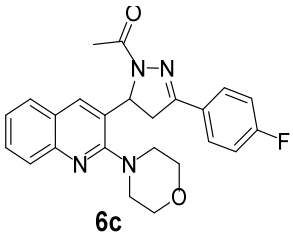
observed for all the compounds may be due to –C=N stretching. Stretching frequency of -C=S- bond appeared at 1287-1280 cm^{-1}. The characteristic absorption band in the range 3480-3336 cm^{-1} may be attributed to asymmetric & symmetric stretching of $-NH_2$. The ^1H NMR spectra of the target pyrazoline scaffolds displayed a typical ABX type pattern of doublet of doublet due to three pyrazoline protons. Methine proton of pyrazoline scaffolds **6a-d**, **7a-d**, **8a-d** and **9a-d** were respectively found at around 5.95-5.92 δ (17.6-4.8 Hz), 6.46-6.33 δ (12.4-3.6 Hz), 5.58-5.48 δ (12.0-6.8 Hz) and 5.92-5.80 δ (11.6-4.8 Hz) as a doublet of doublet. Two methylene protons of pyrazoline scaffolds **6a-d**, **7a-d**, **8a-d** and **9a-d** displayed a signal having doublet of doublet pattern at around 3.07-3.00 δ (17.6-4.4 Hz), 3.02-3.01 δ (18.0-3.6 Hz), 3.26-3.24 δ (17.2-16.8 Hz) and 3.18-3.07 δ (17.6-4.8 Hz) respectively. Another doublet of doublet due to the same methylene hydrogen resonating at around 4.07-3.82 δ was found to be merged with the signal of $-CH_2-O-CH_2-$ of morpholine for each series. The mass spectrum of all the compounds showed molecular ion peak at (M^+) corresponding to their respective molecular weights, which confirmed the molecular frame work.

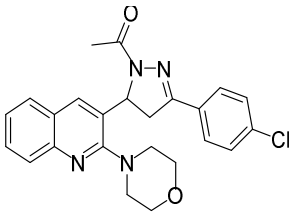
- ❖ The spectral Characterization of all synthesized compounds **6a-d, 7a-d, 8a-d and 9a-d** are depicted in following tables.

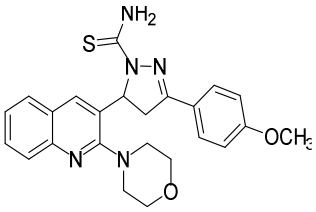
- ✓ The ^1H NMR spectra of Compounds **3, 5a, 6a, 7a, 8a** and **9a** are represented in Figures. 5.7, 5.8, 5.9, 5.11, 5.13 and 5.15 respectively.
- ✓ The ^{13}C spectra of Compounds **6a, 7a, 8a** and **9a** are represented in Figures. 5.10, 5.12, 5.14 and 5.16 respectively.

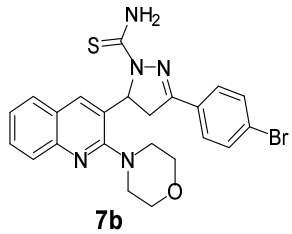
6a	***1-(3-(4-methoxyphenyl)-5-(2-morpholinoquinolin-3-yl)-4,5-dihydro-1H-pyrazol-1-yl)ethanone***		
Mol. for. M. P. (°C) Mol. Wt.	$C_{25}H_{26}N_4O_3$ 248-250 431.2		 **6a**
Ele. Ana. Calcd.(Obs)	C 69.75 (69.68)	H 6.09 (6.03)	N 13.01 (12.95)
FT-IR ν_{max} cm^{-1} (KBr)	3058 (Ar, -CH str.); 1614 and 1578 (C=N and C=C); 1640 (C=O); 1227 (C–O–C).		
^{1}H NMR δ ppm DMSO-d_6	2.52 (s, 3H, $-CH_3$), 3.07 (dd, *J* = 4.4 and 17.6 Hz, 1H, C_4-H pyrazoline); 3.17-3.76 (m, 4H, $-CH_2-N-CH_2-$ of morpholine); 3.81 (s, 3H, OCH_3), 3.84-4.04 (m, 5H, C_4-H pyrazoline + $-CH_2-O-CH_2-$ of morpholine); 5.92 (dd, *J* = 4.4 and 11.6 Hz, 1H, C_5-H pyrazoline); 6.93-7.91 (m, 9H, Ar-H).		
^{13}C NMR δ ppm DMSO-d_6	21.9, 42.7, 51.4, 55.4, 56.4, 67.2, 114.2, 123.8, 125.1, 126.2, 127.3, 127.8, 129.2, 130.6, 133.2, 146.6, 154.1, 159.1, 159.4, 161.5, 168.7		

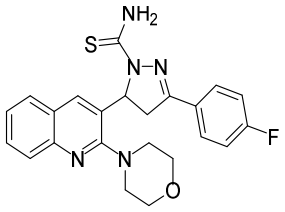
6b	***1-(3-(4-bromophenyl)-5-(2-morpholinoquinolin-3-yl)-4,5-dihydro-1H-pyrazol-1-yl)ethanone***		
Mol. for. M. P. (°C) Mol. Wt.	$C_{24}H_{23}BrN_4O_2$ 212-214 480.1		 **6b**
Ele. Ana. Calcd.(Obs)	C 60.13 (60.06)	H 4.84 (4.77)	N 11.69 (11.63)
FT-IR ν_{max} cm^{-1} (KBr)	1622 and 1572 (C=N and C=C); 1646 (C=O); 1221 (C–O–C)		
^{1}H NMR δ ppm DMSO-d_6	2.52 (s, 3H, $-CH_3$), 2.99 (dd, *J* = 4.4 and 17.6 Hz, 1H, C_4-H pyrazoline); 3.16-3.68 (m, 4H, $-CH_2-N-CH_2-$ of morpholine); 3.82 (s, 3H, OCH_3), 3.85-3.97 (m, 5H, C_4-H pyrazoline + $-CH_2-O-CH_2-$ of morpholine); 5.95 (dd, *J* = 4.4 and 11.6 Hz, 1H, C_5-H pyrazoline); 7.37-7.92 (m, 9H, Ar-H)		
^{13}C NMR δ ppm DMSO-d_6	21.9, 42.4, 51.4, 56.7, 67.2, 124.8, 125.2, 126.1, 127.3, 127.9, 128.0, 129.3, 130.1, 130.3, 132.0, 133.2, 146.7, 153.2, 159.3, 169.0		

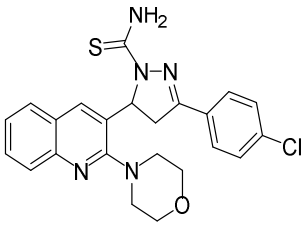
6c	***1-(3-(4-fluorophenyl)-5-(2-morpholinoquinolin-3-yl)-4,5-dihydro-1H-pyrazol-1-yl)ethanone***		
Mol. for. M. P. (°C) Mol. Wt.	$C_{24}H_{23}FN_4O_2$ 182-184 419.3		 **6c**
Ele. Ana. Calcd.(Obs)	C 68.88 (68.22)	H 5.54 (5.47)	N 13.39 (13.32)
FT-IR ν_{max} cm^{-1} (KBr)	3058 (Ar, -CH str.); 1624 and 1592 (C=N and C=C); 1649 (C=O); 1229 (C–O–C).		
^{1}H NMR δ ppm DMSO-d_6	2.53 (s, 3H, $-CH_3$), 3.00 (dd, *J* = 4.8 and 17.6 Hz, 1H, C_4-H pyrazoline); 3.16-3.70 (m, 4H, $-CH_2$-N-CH_2- of morpholine); 3.83-3.96 (m, 5H, C_4-H pyrazoline + $-CH_2$-O-CH_2- of morpholine); 5.95 (dd, *J* = 4.8 and 11.6 Hz, 1H, C_5-H pyrazoline); 7.10-7.92 (m, 9H, Ar-H)		
^{13}C NMR δ ppm DMSO-d_6	21.9, 42.6, 51.4, 56.6, 67.2, 115.8, 125.2, 126.2, 127.3, 127.5, 127.8, 128.5, 128.6, 129.3, 130.4, 133.2, 146.7, 153.2, 159.3, 168.9.		

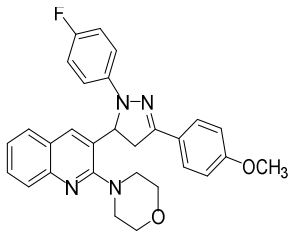
6d	***1-(3-(4-chlorophenyl)-5-(2-morpholinoquinolin-3-yl)-4,5-dihydro-1H-pyrazol-1-yl)ethanone***		
Mol. for. M. P. (°C) Mol. Wt.	$C_{24}H_{23}ClN_4O_2$ 190-192 435.1		 **6d**
Ele. Ana. Calcd.(Obs)	C 66.28 (66.21)	H 5.33 (5.27)	N 12.88 (12.81)
FT-IR ν_{max} cm^{-1} (KBr)	3055 (Ar, -CH str.); 1630 and 1585 (C=N and C=C); 1641 (C=O); 1232 (C–O–C)		
^{1}H NMR δ ppm DMSO-d_6	2.58 (s, 3H, $-CH_3$), 3.00 (dd, *J* = 4.8 and 17.6 Hz, 1H, C_4-H pyrazoline); 3.16-3.68 (m, 4H, $-CH_2$-N-CH_2- of morpholine); 3.82-3.98 (m, 5H, C_4-H pyrazoline + $-CH_2$-O-CH_2- of morpholine); 5.95 (dd, *J* = 4.8 and 11.6 Hz, 1H, C_5-H pyrazoline); 7.37-7.92 (m, 9H, Ar-H)		
^{13}C NMR δ ppm DMSO-d_6	21.9, 42.5, 51.4, 56.7, 67.2, 125.2, 126.2, 127.3, 127.8, 127.9, 129.0, 129.3, 129.7, 130.3, 133.2, 136.5, 146.7, 153.1, 159.3, 168.9		

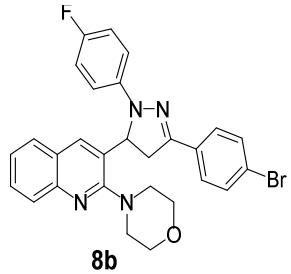
7a	***3-(4-methoxyphenyl)-5-(2-morpholinoquinolin-3-yl)-4,5-dihydro-1H-pyrazole-1-carbothioamide***			
Mol. for. M. P. (°C) Mol. Wt.	$C_{24}H_{25}N_5O_2S$ 251-253 448.3			 **7a**
Ele. Ana. Calcd.(Obs)	C 64.41 (64.34)	H 5.63 (5.57)	N 15.65 (15.59)	
FT-IR ν_{max} cm^{-1} (KBr)	3478 & 3336 (asym. & sym. str. of –NH2), 3051 (Ar, -CH str.); 1211 (C–O–C); 1624 and 1592 (C=N and C=C); 1286, (-C=S).			
^{1}H NMR δ ppm DMSO-d_6	3.02 (dd, *J* = 3.6 and 17.6 Hz, 1H, C_4-H pyrazoline); 3.10-3.67, (m, 4H, -CH_2-N-CH_2- of morpholine); 3.75 (s, 3H, OCH_3), 3.90-4.03 (m, 5H, C_4-H pyrazoline + -CH_2-O-CH_2- of morpholine); 6.33 (dd, *J* = 3.6 and 11.2 Hz, 1H, C_5-H pyrazoline); 6.37-8.11(m, 11H, Ar-H +-NH_2)			
^{13}C NMR δ ppm DMSO-d_6	42.8, 51.2, 55.4, 60.6, 67.3, 114.3, 122.9, 125.1, 126.1, 127.5, 127.8, 128.6, 129.2, 129.9, 133.0, 146.7, 156.6, 159.1, 162.0, 176.4.			

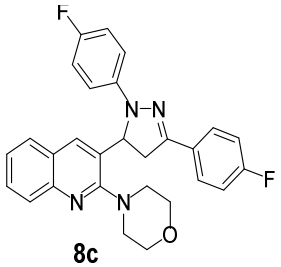
7b	***3-(4-bromophenyl)-5-(2-morpholinoquinolin-3-yl)-4,5-dihydro-1H-pyrazole-1-carbothioamide***			
Mol. for. M. P. (°C) Mol. Wt.	$C_{23}H_{22}BrN_5OS$ 272-274 497.2			 **7b**
Ele. Ana. Calcd.(Obs)	C 55.65 (55.58)	H 4.47 (4.41)	N 14.11 (14.05)	
FT-IR ν_{max} cm^{-1} (KBr)	3480 & 3340 (asym. & sym. str. of –NH2), 3053 (Ar, -CH str.); 1218 (C–O–C); 1622 and 1594 (C=N and C=C); 1282, (-C=S)			
^{1}H NMR δ ppm DMSO-d_6	3.01 (dd, *J* = 4.0 and 18.0 Hz, 1H, C_4-H pyrazoline); 3.09-3.75, (m, 4H, -CH_2-N-CH_2- of morpholine); 3.90-4.01 (m, 5H, C_4-H pyrazoline + -CH_2-O-CH_2- of morpholine); 6.46 (dd, *J* = 4.0 and 12.4 Hz, 1H, C_5-H pyrazoline); 6.51-7.97 (m, 11H, Ar-H+-NH_2).			
^{13}C NMR δ ppm DMSO-d_6	42.8, 51.2, 60.2, 67.3, 117.5, 118.7, 123.2, 126.5, 128.9, 127.5, 127.7, 128.4, 129.2, 131.2, 134.2, 136.2, 138.2, 154.5, 176.4.			

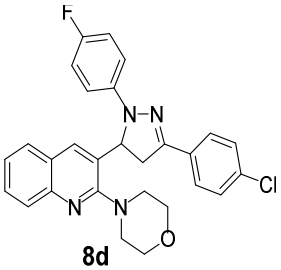
7c	***3-(4-fluorophenyl)-5-(2-morpholinoquinolin-3-yl)-4,5-dihydro-1H-pyrazole-1-carbothioamide***			
Mol. for. M. P. (°C) Mol. Wt.	$C_{23}H_{22}FN_5OS$ 240-242 436.4			 **7c**
Ele. Ana. Calcd.(Obs)	C 63.43 (63.36)	H 5.09 (5.03)	N 16.08 (16.01)	
FT-IR ν_{max} cm^{-1} (KBr)	3479 & 3343 (asym. & sym. str. of $-NH_2$), 3051 (Ar, -CH str.); 1219 (C–O–C); 1629 and 1595 (C=N and C=C); 1287, (-C=S)			
^{1}H NMR δ ppm DMSO-d_6	3.02 (dd, *J* = 4.0 and 18.0 Hz, 1H, C_4-H pyrazoline); 3.09-3.77 (m, 4H, -CH_2-N-CH_2- of morpholine); 3.90-4.01 (m, 5H, C_4-H pyrazoline + -CH_2-O-CH_2- of morpholine); 6.36 (dd, *J* = 4.0 and 11.2 Hz, 1H, C_5-H pyrazoline); 7.09-7.92 (m, 11H, Ar-H + -NH_2).			
^{13}C NMR δ ppm DMSO-d_6	42.8, 51.2, 60.8, 67.3, 116.2, 125.2, 126.1, 126.7, 126.7, 127.5, 127.8, 129.0, 129.0, 129.3, 129.7, 146.7, 155.7, 159.1, 176.8			

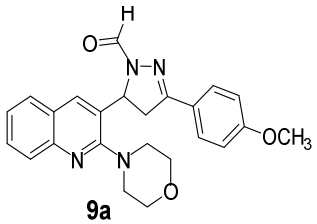
7d	***3-(4-chlorophenyl)-5-(2-morpholinoquinolin-3-yl)-4,5-dihydro-1H-pyrazole-1-carbothioamide***			
Mol. for. M. P. (°C) Mol. Wt.	$C_{23}H_{22}ClN_5OS$ 271-273 453.1			 **7d**
Ele. Ana. Calcd.(Obs)	C 61.12 (61.05)	H 4.91 (4.84)	N 15.50 (15.44)	
FT-IR ν_{max} cm^{-1} (KBr)	3472 & 3348 (asym. & sym. str. of $-NH_2$), 3051 (Ar, -CH str.); 1222 (C–O–C); 1627 and 1591 (C=N and C=C); 1280, (-C=S).			
^{1}H NMR δ ppm DMSO-d_6	3.01 (dd, *J* = 3.6 and 17.6 Hz, 1H, C_4-H pyrazoline); 3.09-3.74 (m, 4H, -CH_2-N-CH_2- of morpholine); 3.90-4.00 (m, 5H, C_4-H pyrazoline + -CH_2-O-CH_2- of morpholine); 6.36 (dd, *J* = 3.6 and 11.2 Hz, 1H, C_5-H pyrazoline); 7.18-7.92 (m, 11H, Ar-H + -NH_2)			
^{13}C NMR δ ppm DMSO-d_6	41.6, 51.2, 59.1, 66.2, 118.5, 118.8, 123.4, 126.7, 126.8, 127.6, 127.8, 128.3, 129.5, 131.0, 134.3, 136.3, 138.2, 154.6, 176.3			

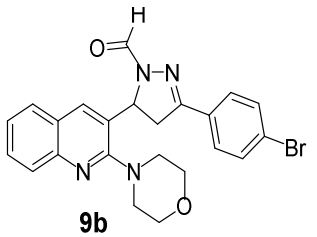
8a	***4-(3-(1-(4-fluorophenyl)-3-(4-methoxyphenyl)-4,5-dihydro-1H-pyrazol-5-yl)quinolin-2-yl)morpholine***			
Mol. for. M. P. (°C) Mol. Wt.	$C_{29}H_{27}FN_4O_2$ 224-226 483.4			 **8a**
Ele. Ana. Calcd.(Obs)	C 72.18 (72.12)	H 5.64 (5.57)	N 11.61 (11.57)	
FT-IR ν_{max} cm^{-1} (KBr)	3051 (Ar, -CH str.); 1612 and 1573 (C=N and C=C); 1222 (C–O–C).			
^{1}H NMR δ ppm DMSO-d_6	3.25 (dd, *J* = 7.2 and 16.8 Hz, 1H, C_4-H pyrazoline); 3.51-3.75 (m, 4H, -CH_2-N-CH_2- of morpholine); 3.82 (s, 3H, OCH_3), 3.86-4.07 (m, 5H, C_4-H pyrazoline + -CH_2-O-CH_2- of morpholine); 5.48 (dd, *J* = 7.2 and 11.6 Hz, 1H, C_5-H pyrazoline); 6.86-8.12 (m, 13H, Ar-H).			
^{13}C NMR δ ppm DMSO-d_6	43.5, 51.8, 55.3, 60.0, 67.1, 113.9, 114.1, 114.5, 114.9, 115.5, 115.7, 124.9, 125.5, 127.3, 127.5, 130.5, 132.4, 133.8, 134.8, 140.2, 141.3, 145.2, 146.6, 155.8, 158.4, 160.4			

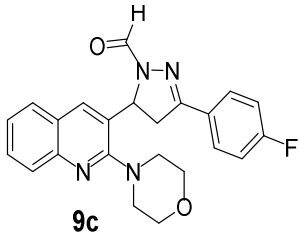
8b	***4-(3-(3-(4-bromophenyl)-1-(4-fluorophenyl)-4,5-dihydro-1H-pyrazol-5-yl)quinolin-2-yl)morpholine***			
Mol. for. M. P. (°C) Mol. Wt.	$C_{28}H_{24}BrFN_4O$ 234-236 531.2			 **8b**
Ele. Ana. Calcd.(Obs)	C 63.28 (63.21)	H 4.55 (4.48)	N 10.54 (10.48)	
FT-IR ν_{max} cm^{-1} (KBr)	3054 (Ar, -CH str.); 1618 and 1574 (C=N and C=C); 1225 (C–O–C).			
^{1}H NMR δ ppm DMSO-d_6	3.24 (dd, *J* = 7.2 and 16.8 Hz, 1H, C_4-H pyrazoline); 3.41 (m, 4H, -CH_2-N-CH_2- of morpholine); 3.95-4.06 (m, 5H, C_4-H pyrazoline + -CH_2-O-CH_2- of morpholine); 5.58 (dd, *J* = 7.2 and 12.0 Hz, 1H, C_5-H pyrazoline); 6.86-7.97 (m, 13H, Ar-H).			
^{13}C NMR δ ppm DMSO-d_6	43.1, 51.7, 60.1, 67.1, 114.0, 114.1, 115.5, 115.8, 122.8, 125.3, 126.1, 127.1, 127.5, 127.8, 129.7, 130.2, 131.4, 131.8, 135.9, 140.7, 145.4, 146.6, 155.9, 158.2, 159.3			

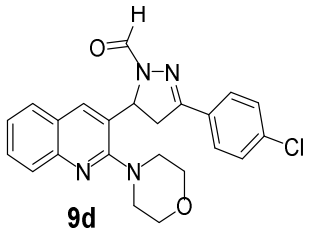
8c	***4-(3-(1,3-bis(4-fluorophenyl)-4,5-dihydro-1H-pyrazol-5-yl)quinolin-2-yl) morpholine***		
Mol. for. M. P. (°C) Mol. Wt.	$C_{28}H_{24}F_2N_4O$ 249-251 471.3		 **8c**
Ele. Ana. Calcd.(Obs)	C 71.48 (71.42)	H 5.14 (5.07)	N 11.91 (11.85)
FT-IR ν_{max} cm^{-1} (KBr)	3056 (Ar, -CH str.); 1614 and 1571 (C=N and C=C); 1227 (C–O–C).		
^{1}H NMR δ ppm DMSO-d_6	3.25 (dd, *J* = 7.2 and 17.2 Hz, 1H, C_4-H pyrazoline); 3.41 (m, 4H, -CH_2-N-CH_2- of morpholine); 3.95-4.07 (m, 5H, C_4-H pyrazoline + -CH_2-O-CH_2- of morpholine); 5.56 (dd, *J* = 7.2 and 11.6 Hz, 1H, C_5-H pyrazoline); 6.86-8.19 (m, 13H, Ar-H).		
^{13}C NMR δ ppm DMSO-d_6	43.3, 51.6, 60.2, 67.1, 114.0, 115.5, 115.6, 115.8, 125.3, 126.2, 127.3, 127.5, 127.6, 127.9, 128.7, 129.6, 130.3, 135.9, 141.1, 145.6, 155.8, 158.2, 159.4, 161.9, 164.4		

8d	***4-(3-(3-(4-chlorophenyl)-1-(4-fluorophenyl)-4,5-dihydro-1H-pyrazol-5-yl) quinolin-2-yl)morpholine***		
Mol. for. M. P. (°C) Mol. Wt.	$C_{28}H_{24}ClFN_4O$ 267-269 488.4		 **8d**
Ele. Ana. Calcd.(Obs)	C 69.06 (68.99)	H 4.97 (4.91)	N 11.51 (11.44)
FT-IR ν_{max} cm^{-1} (KBr)	3051 (Ar, -CH str.); 1614 and 1573 (C=N and C=C); 1222 (C–O–C).		
^{1}H NMR δ ppm DMSO-d_6	3.26 (dd, *J* = 6.8 and 16.8 Hz, 1H, C_4-H pyrazoline); 3.53-3.77 (m, 4H, -CH_2-N-CH_2- of morpholine); 3.99-4.07 (m, 5H, C_4-H pyrazoline + -CH_2-O-CH_2- of morpholine); 5.54 (dd, *J* = 6.8 and 12.0 Hz, 1H, C_5-H pyrazoline); 6.87-8.15 (m, 13H, Ar-H).		
^{13}C NMR δ ppm DMSO-d_6	43.2, 51.8, 60.1, 67.1, 114.0, 114.1, 115.5, 115.6, 115.8, 125.2, 125.6, 125.8, 126.3, 126.9, 127.5, 128.4, 128.7, 129.9, 130.2, 134.7, 140.7, 141.2, 145.3, 155.9, 158.3		

9a	***3-(4-methoxyphenyl)-5-(2-morpholinoquinolin-3-yl)-4,5-dihydro-1H-pyrazole-1-carbaldehyde***			
Mol. for. M. P. (°C) Mol. Wt.	$C_{24}H_{24}N_4O_3$ 180-182 417.6			 **9a**
Ele. Ana. Calcd.(Obs)	C 69.21 (69.15)	H 5.81 (5.74)	N 13.45 (13.38)	
FT-IR ν_{max} cm^{-1} (KBr)	3057 (Ar, -CH str.); 1632 and 1587 (C=N and C=C); 1641 (C=O); 1233 (C–O–C).			
^{1}H NMR δ ppm DMSO-d_6	3.07 (dd, *J* = 4.8 and 17.6 Hz, 1H, C_4-H pyrazoline); 3.19-3.65 (m, 4H, -CH_2-N-CH_2- of morpholine); 3.86 (s, 3H, OCH_3), 3.92-3.95 (m, 5H, C_4-H pyrazoline + -CH_2-O-CH_2- of morpholine); 5.89 (dd, *J* = 4.8 and 11.6 Hz, 1H, C_5-H pyrazoline); 6.94-7.92 (m, 9H, Ar-H); 9.06 (s, 1H, CHO).			
^{13}C NMR δ ppm DMSO-d_6	43.0, 51.4, 55.3, 55.4, 67.2, 114.2, 123.3, 125.3, 126.1, 127.3, 127.9, 128.3, 129.3, 129.5, 133.8, 146.7, 155.8, 159.4, 159.9, 161.0			

9b	***3-(4-bromophenyl)-5-(2-morpholinoquinolin-3-yl)-4,5-dihydro-1H-pyrazole-1-carbaldehyde***			
Mol. for. M. P. (°C) Mol. Wt.	$C_{23}H_{21}BrN_4O_2$ 202-204 466.1			 **9b**
Ele. Ana. Calcd.(Obs)	C 59.36 (59.29)	H 4.55 (4.49)	N 12.04 (11.97)	
FT-IR ν_{max} cm^{-1} (KBr)	1627 and 1592 (C=N and C=C); 1644 (C=O); 1229 (C–O–C)			
^{1}H NMR δ ppm DMSO-d_6	3.07 (dd, *J* = 5.2 and 17.6 Hz, 1H, C_4-H pyrazoline); 3.20-3.64 (m, 4H, -CH_2-N-CH_2- of morpholine); 3.89-3.98 (m, 5H, C_4-H pyrazoline + -CH_2-O-CH_2- of morpholine); 5.92 (dd, *J* = 5.2 and 11.6 Hz, 1H, C_5-H pyrazoline); 7.23-8.08 (m, 9H, Ar-H); 9.08 (s, 1H, CHO).			
^{13}C NMR δ ppm DMSO-d_6	42.7, 51.5, 55.7, 67.1, 125.2, 125.4, 126.1, 127.3, 127.9, 128.1, 129.1, 129.6, 129.7, 132.1, 133.7, 146.8, 155.0, 159.3, 160.0			

9c	***3-(4-fluorophenyl)-5-(2-morpholinoquinolin-3-yl)-4,5-dihydro-1H-pyrazole-1-carbaldehyde***			
Mol. for. M. P. (°C) Mol. Wt.	$C_{23}H_{21}FN_4O_2$ 197-199 405.5			 **9c**
Ele. Ana. Calcd.(Obs)	C 68.30 (68.23)	H 5.23 (5.17)	N 13.85 (13.78)	
FT-IR ν_{max} cm^{-1} (KBr)	3062 (Ar, -CH str.); 1630 and 1596 (C=N and C=C); 1647 (C=O); 1232 (C–O–C).			
^{1}H NMR δ ppm DMSO-d_6	3.08 (dd, *J* = 4.8 and 17.6 Hz, 1H, C_4-H pyrazoline); 3.20-3.65 (m, 4H, -CH_2-N-CH_2- of morpholine); 3.90-3.99 (m, 5H, C_4-H pyrazoline + -CH_2-O-CH_2- of morpholine); 5.92 (dd, *J* = 4.8 and 11.6 Hz, 1H, C_5-H pyrazoline); 7.11-8.03 (m, 9H, Ar-H); 9.08 (s, 1H, CHO).			
^{13}C NMR δ ppm DMSO-d_6	42.9, 51.5, 55.6, 67.1, 115.9, 116.1, 125.4, 126.1, 127.0, 127.3, 127.9, 128.8, 129.1, 129.5, 133.7, 146.8, 155.0, 159.3, 160.0			

9d	***3-(4-chlorophenyl)-5-(2-morpholinoquinolin-3-yl)-4,5-dihydro-1H-pyrazole-1-carbaldehyde***			
Mol. for. M. P. (°C) Mol. Wt.	$C_{23}H_{21}ClN_4O_2$ 203-205 421.9			 **9d**
Ele. Ana. Calcd.(Obs)	C 65.63 (65.57)	H 5.03 (4.96)	N 13.31 (13.24)	
FT-IR ν_{max} cm^{-1} (KBr)	3054 (Ar, -CH str.); 1635 and 1591 (C=N and C=C); 1640 (C=O); 1230 (C–O–C).			
^{1}H NMR δ ppm DMSO-d_6	3.07 (dd, *J* = 4.8 and 17.6 Hz, 1H, C_4-H pyrazoline); 3.21-3.65 (m, 4H, -CH_2-N-CH_2- of morpholine); 3.89-3.98 (m, 5H, C_4-H pyrazoline + -CH_2-O-CH_2- of morpholine); 5.92 (dd, *J* = 4.8 and 11.6 Hz, 1H, C_5-H pyrazoline); 7.39-7.93 (m, 9H, Ar-H); 9.08 (s, 1H, CHO)			
^{13}C NMR δ ppm DMSO-d_6	42.8, 51.5, 55.6, 67.1, 125.4, 126.1, 127.3, 127.9, 128.2, 129.1, 129.4, 129.5, 129.6, 133.7, 136.9, 146.8, 155.0, 159.3, 160.0			

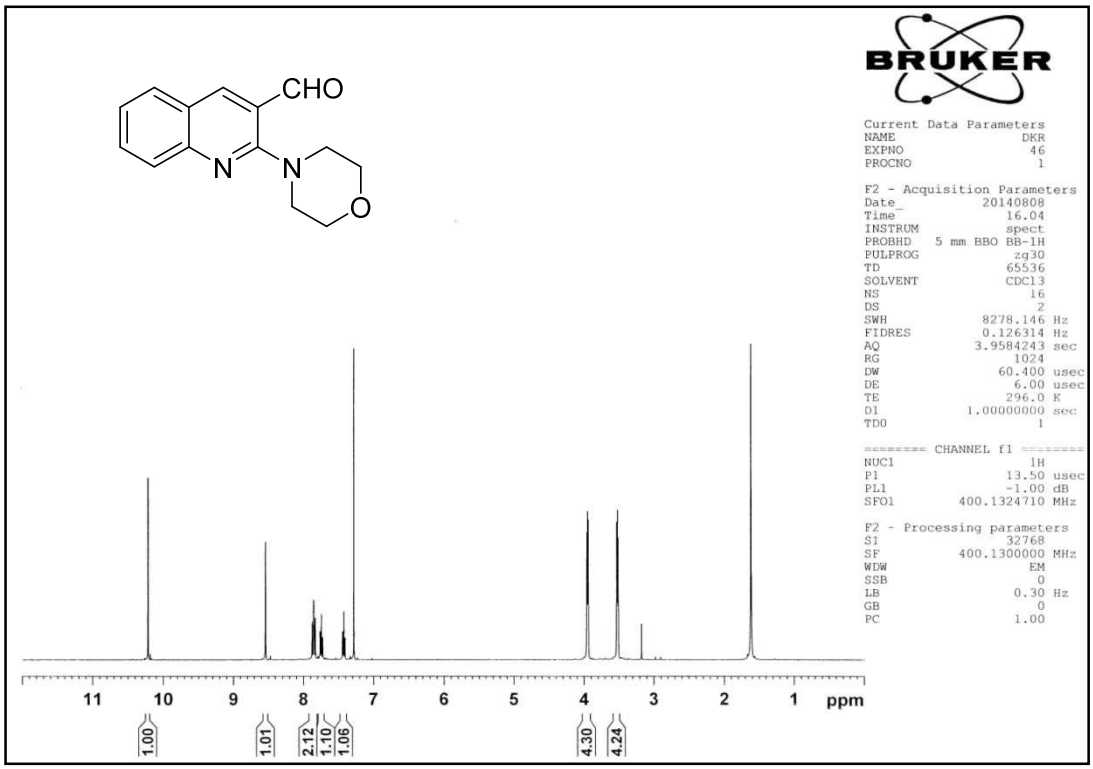


Figure 5.7 ^{1}H NMR spectra of compound **3**

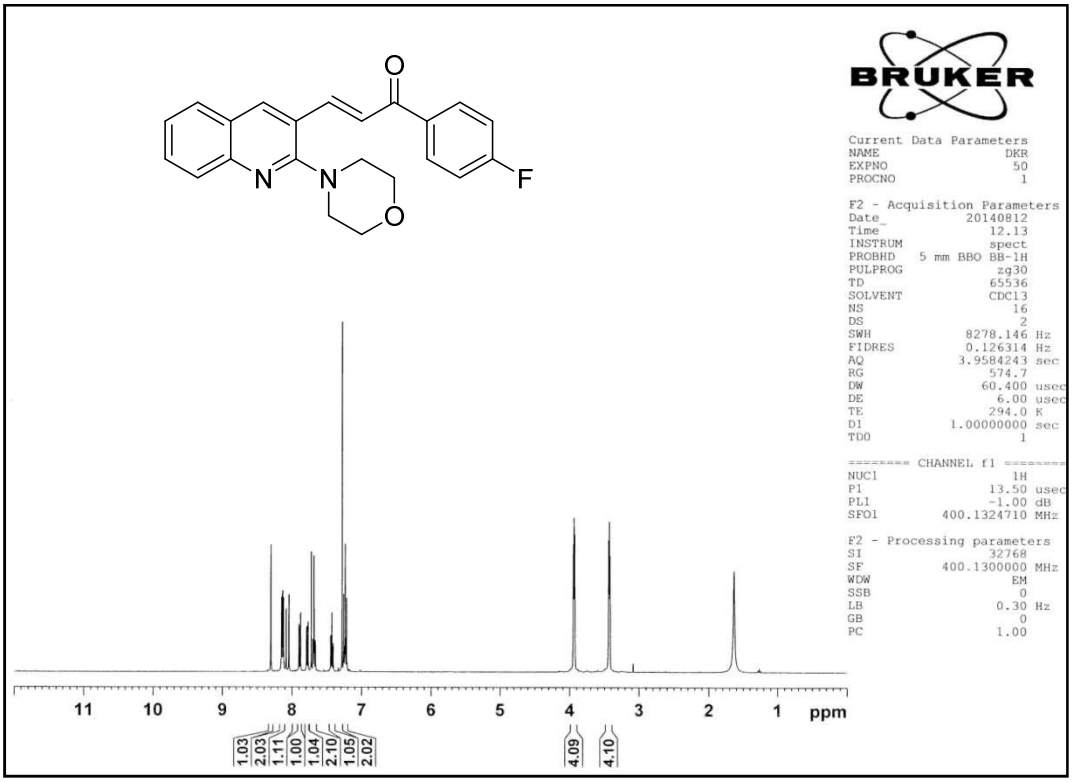


Figure 5.8 ^{1}H NMR spectra of compound **5a**

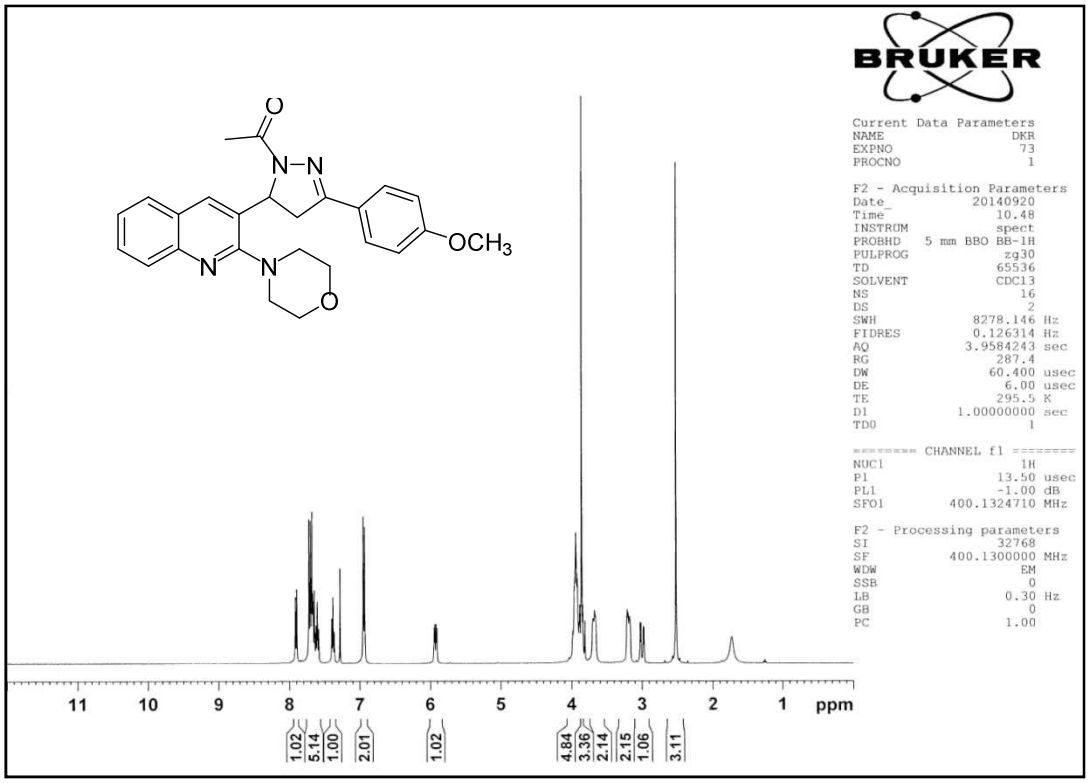


Figure 5.9 ^{1}H NMR spectra of compound **6a**

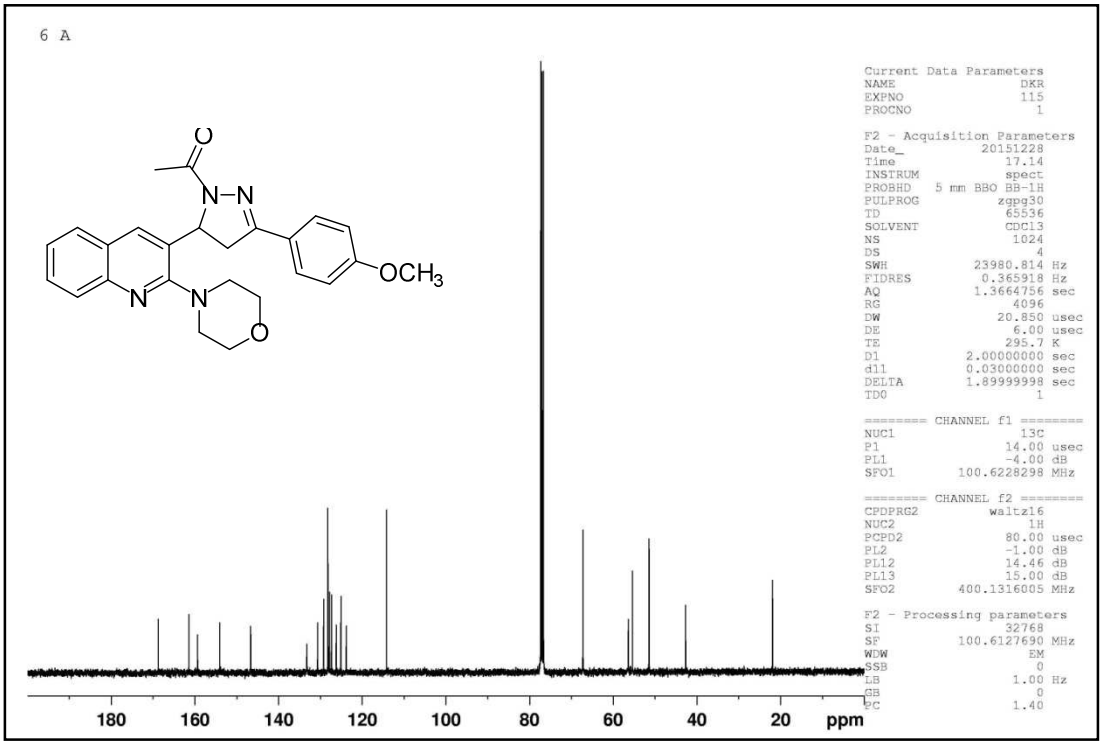


Figure 5.10 ^{13}C NMR spectra of compound **6a**

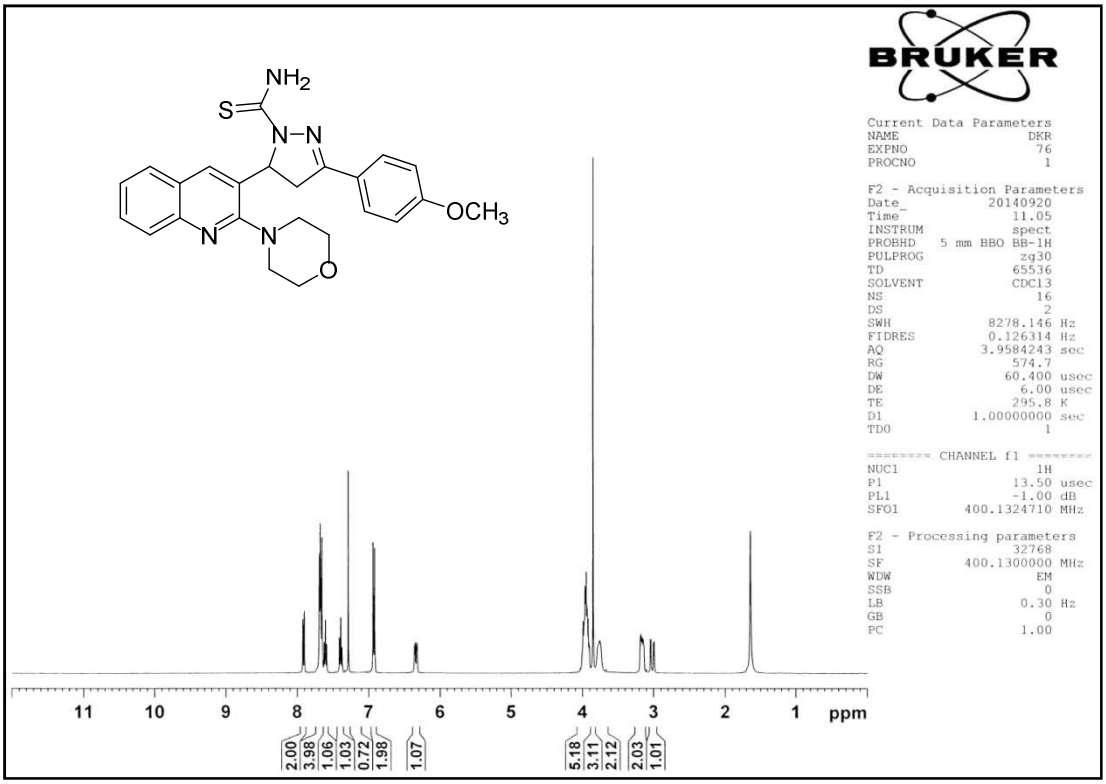


Figure 5.11 ^{1}H NMR spectra of compound **7a**

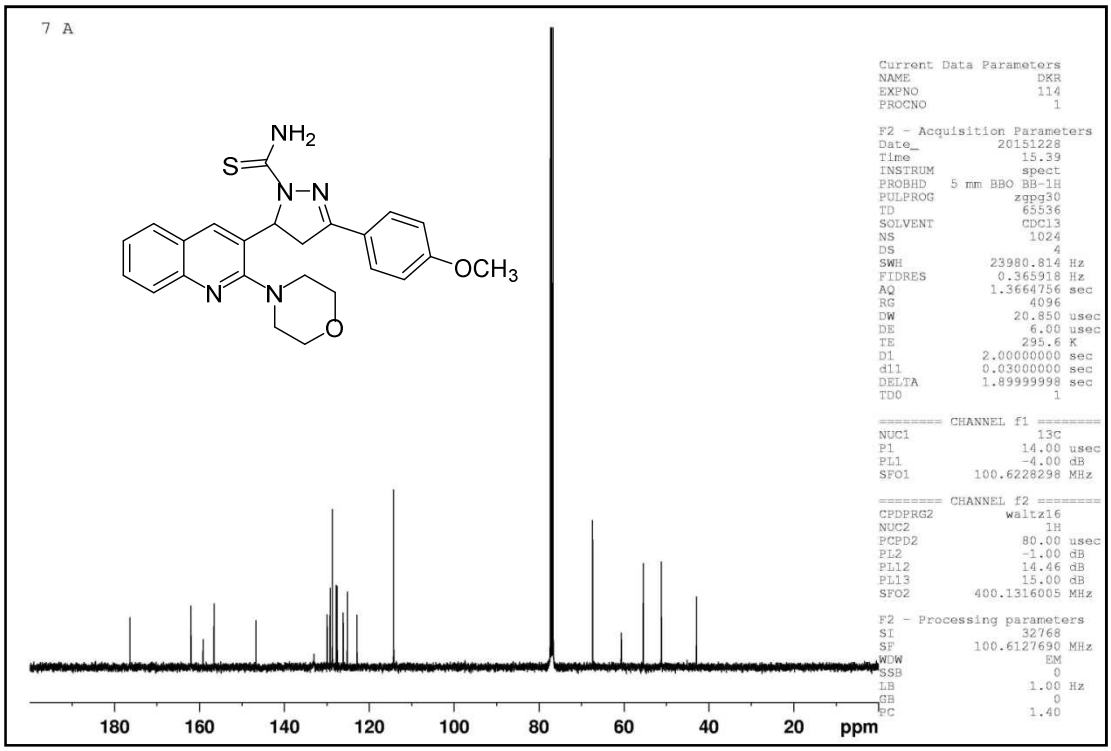


Figure 5.12 ^{13}C NMR spectra of compound **7a**

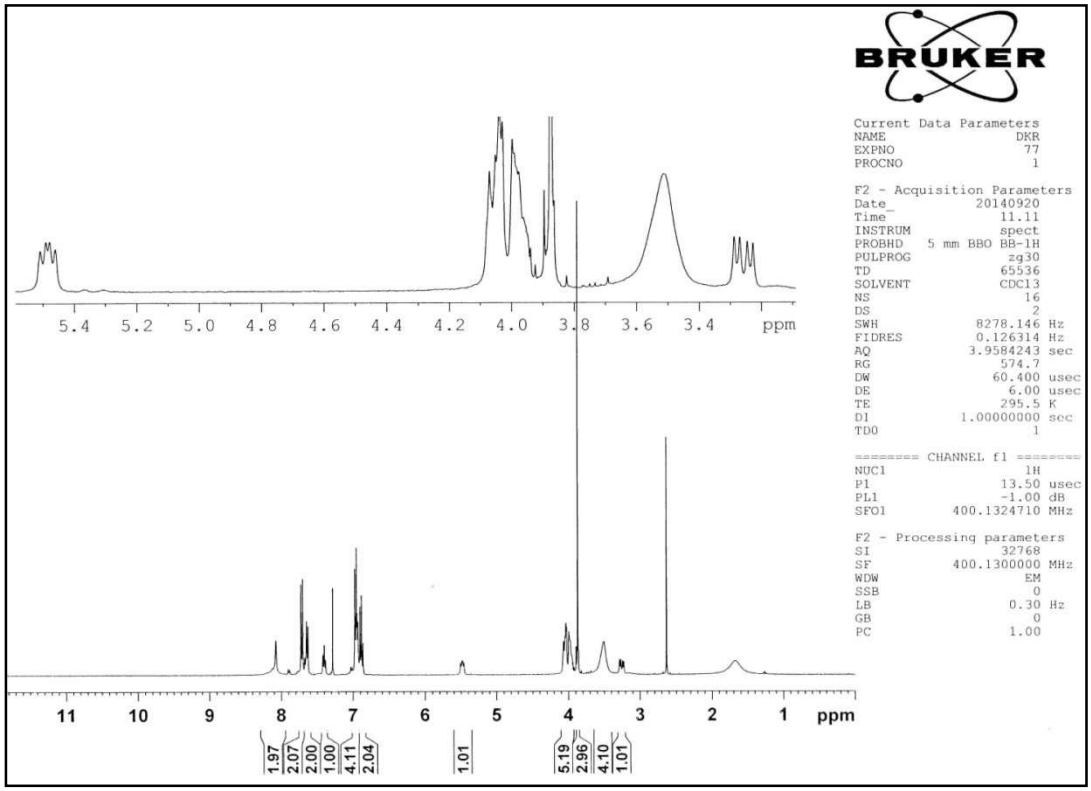


Figure 5.13 ^{1}H NMR spectra of compound **8a**

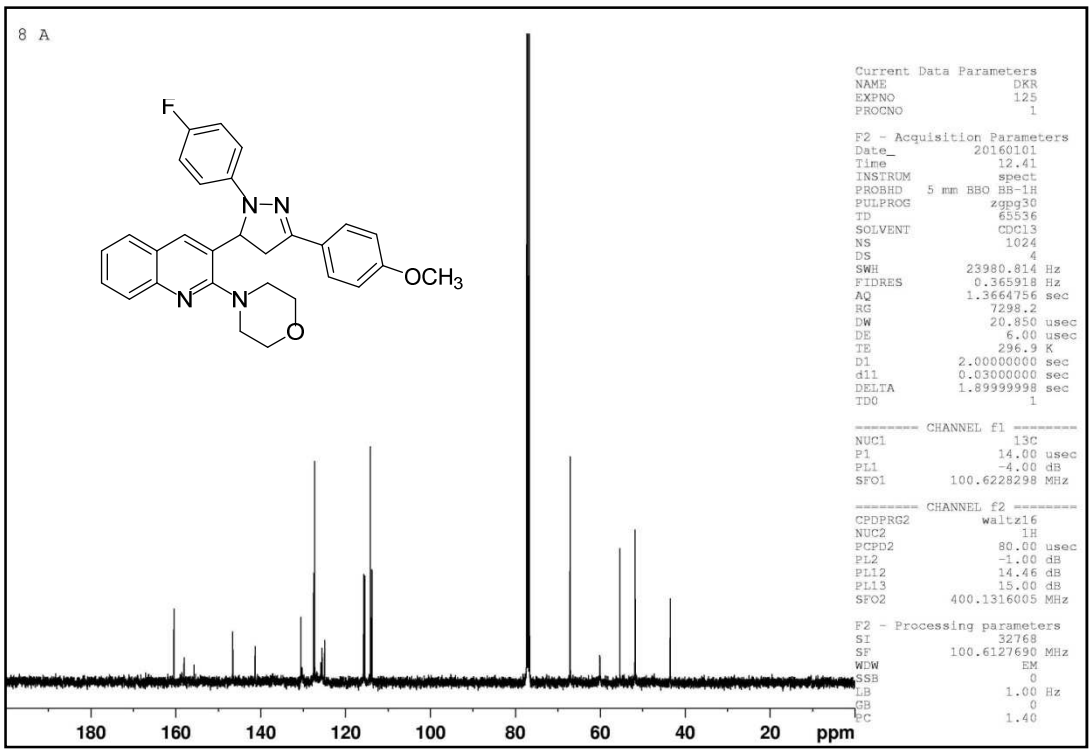


Figure 5.14 ^{13}C NMR spectra of compound **8a**

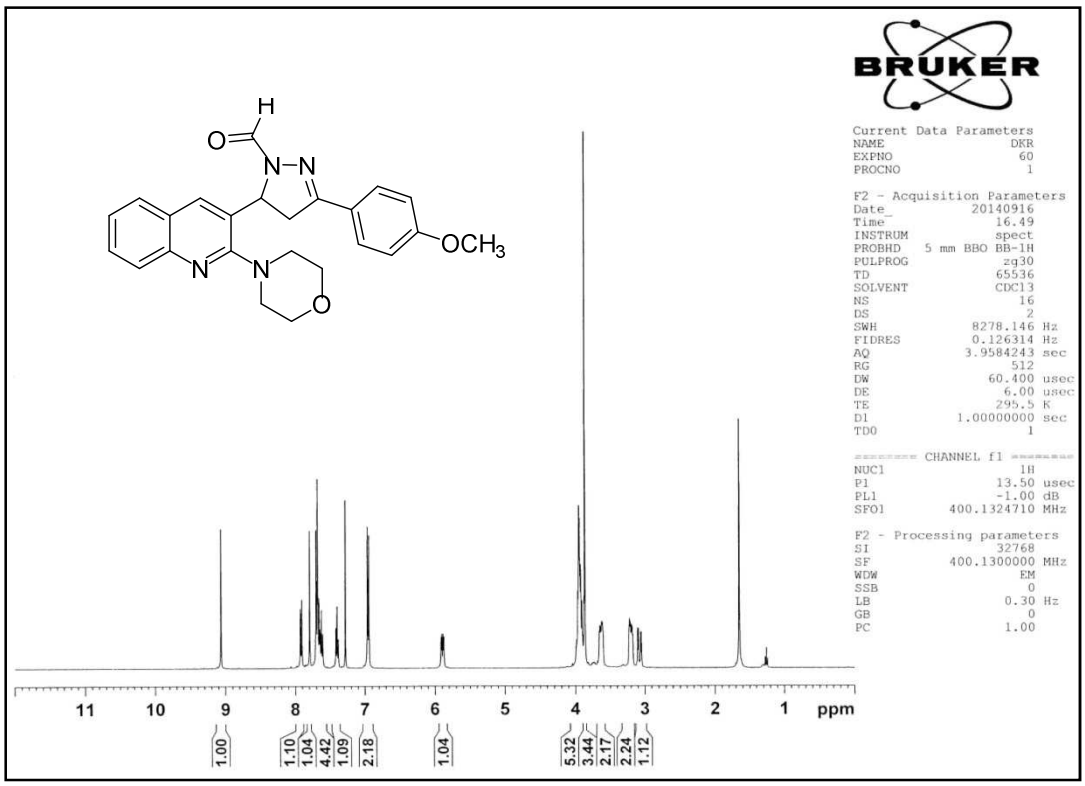


Figure 5.15 ^{1}H NMR spectra of compound 9**a**

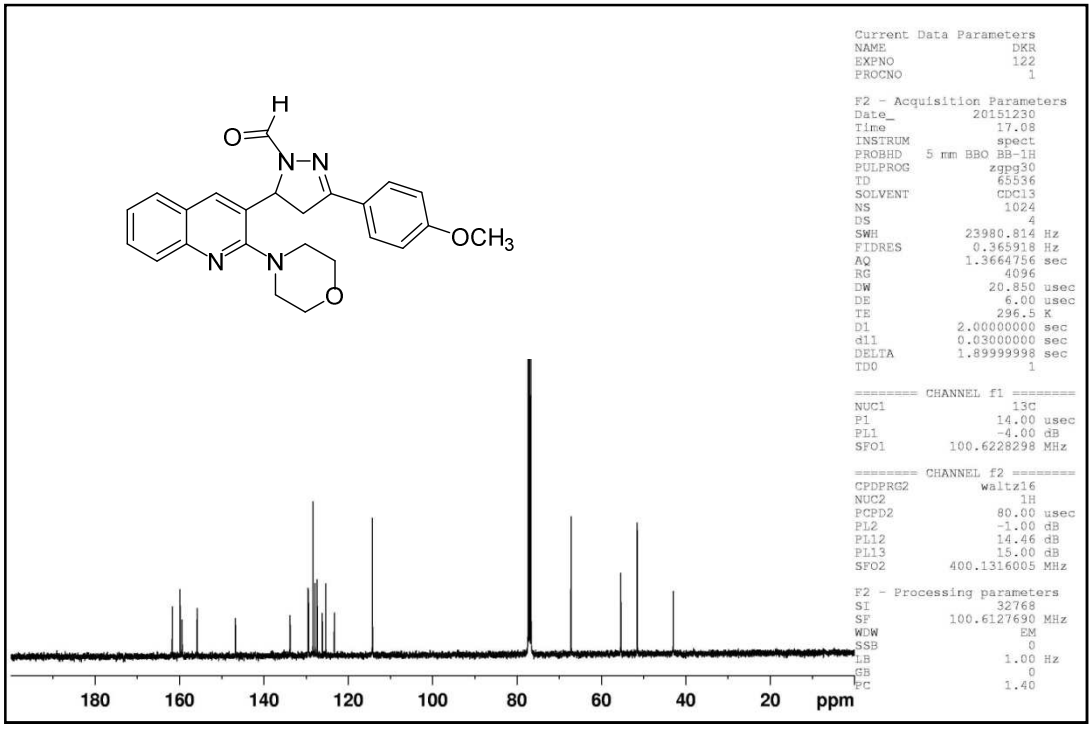


Figure 5.16 ^{13}C NMR spectra of compound **9a**

5.9 Biological results

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

5.9.1 Antibacterial activity

The antimicrobial activity of the newly synthesized quinoline based pyrazoline derivatives at minimal inhibitory concentration (MIC) in millimolar (μM) were carried out by broth micro dilution method according to the National Committee for Clinical Laboratory Standards (NCCLS)[34]. 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 study 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 5.1**.

Upon investigation of antimicrobial activity data (**Table 5.1**), it was revealed that all the synthesized quinoline based pyrazoline scaffolds were found to possess moderate to high inhibitory activity. Against *S. pneumoniae*, compounds **6a** and **9d** were found to possess excellent potency i.e. (145 μM) and (148 μM) respectively as compared to ampicillin (286 μM), chloramphenicol (154 μM) and ciprofloxacin (150 μM). While Compounds **6c** (238 μM), **7a** (223 μM), **7c** (229 μM), **8a** (259 μM), **8b** (188 μM), **9a** (240 μM) and **9c** (247 μM) exhibited comparable potency to that of ampicillin (286 μM).

Compound **8c** was found to be more potent (132 μM) against *C. tetani* as compared to all the standard drugs i.e ampicillin (715 μM), chloramphenicol (154 μM), Ciprofloxacin (i.e. 301 μM) and norfloxacin (313 μM). Compounds **6b** (208 μM), **6c**

(238 µM), **7b** (201 µM), **8b** (188 µM) and **7d** (221 µM) showed better activity as compared to ciprofloxacin (301 µM) as well as norfloxacin (i.e. 313 mM). Compound **7a** (446 µM), **9b** (429 µM) and **9d** (475 µM) were found to be more effective as compared to ampicillin (715 µM) But all were found to be less potent as compared to chloramphenicol (154 µM).

Against *B. subtillis*, compound **9c** was found to possess significant potency (154 µM) as compared to ampicillin (715 µM) as well as norfloxacin (310 µM). But it exhibited less potency as compared to ciprofloxacin (i.e. 150 µM) and equivalent potency to that of chloramphenicol (154 µM). Compounds **6a** (232 µM), **6d** (229 µM), **7d** (221 µM), **8c** (212 µM) and **9b** (214 µM) were found to possess higher potency to that of norfloxacin (310 µM) as well as ampicillin (715 µM). While compound **6c** (597 µM), **7a** (446 µM), **7b** (402 µM), **8d** (410 µM), **9a** (600 µM) were found to be more potent as compared to ampicillin (715 µM).

In case of gram negative bacteria, Compounds **7c** and **9b** exhibited better potency (143 µM) and (134 µM) respectively against *S. typhi* compared to chloramphenicol (154 µM). Whereas compounds **6c** (238 µM), **7a** (223 µM), **8a** (207 µM), **8c** (212 µM) and **9a** (240 µM) displayed superior potency to that of ampicillin (286 µM).

Against *V. cholera* compound **8c** (132 µM) showed significant activity as compared to the standard drug chloramphenicol (154 µM) as well as ampicillin (286 µM). While compounds **6d** (229 µM), **7b** (201 µM), **7d** (221 µM), **9b** (214 µM) and **9d** (237 µM) showed comparable potency to that of ampicillin (286 µM).

Compound **8b** revealed highest inhibition (117 µM) as compared to chloramphenicol (154 µM) as well as ampicillin (i.e. 286 µM) against *E. coli*. While compounds **6a** (232 µM), **6d** (229 µM), **7b** (201 µM), **8c** (265 µM), **8d** (205 µM) **9b** (214 µM) and **9d** (237 µM) displayed higher potency to that of ampicillin (286 µM).

Table 5.1 *In vitro* antimicrobial activity (MIC, µM) of the synthesized quinoline based pyrazoline derivatives.

Comp.	*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
6a	**145**	1161	**232**	1161	1161	**232**	2322	1161
6b	417	**208**	1043	1043	1043	521	2086	**208**
6c	**238**	**238**	**597**	**238**	597	597	**597**	1194
6d	1149	149	**229**	574	**229**	229	1149	1149
7a	**223**	**446**	**446**	**223**	558	558	**1117**	**223**
7b	402	**201**	**402**	402	**201**	**201**	>2014	1007
7c	**229**	1148	1148	**143**	574	574	**229**	1148
7d	553	**221**	**221**	553	**221**	442	2212	**221**
8a	**259**	1036	**518**	**207**	518	414	**518**	**207**
8b	**188**	**188**	940	376	470	**117**	**470**	940
8c	**265**	**132**	**212**	**212**	**132**	265	**212**	2125
8d	1026	1026	**410**	410	513	**205**	2053	**205**
9a	**240**	1200	**600**	**240**	480	480	1200	2401
9b	537	**429**	**214**	**134**	**214**	**214**	**537**	1074
9c	**247**	1236	**154**	1236	618	494	2472	>2472
9d	**148**	**475**	1187	1187	**237**	237	2375	>2375
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.

5.9.2 Antifungal activity

The antifungal screening data (**Table 5.1**) revealed that, against *C. albicans*, compounds **6c** (597 µM), **7c** (229 µM), **8a** (518 µM), **8b** (470 µM), **8c** (212 µM) and **9b** (537 µM) showed significant activity as compared to griseofulvin (1147 µM). Whereas compound **7a** (1117 µM) showed almost the same potency as that of griseofulvin but it was found be less active as compared to nystatin (i.e. 107 µM). While compounds **6b** (208 µM), **7a** (223 µM), **7d** (221 µM) **8a** (207 µM) and **8d** (205

μM) were found to possess high potency against *A. fumigates* as compared to griseofulvin (283 μM) and less potency to that of nystatin (107 μM).

5.9.3. Antituberculosis activity

A primary *in vitro* antituberculosis activity of the newly synthesized pyrazoline derivatives was conducted at 250 μg/mL against *Mycobacterium tuberculosis* $H_{37}Rv$ strain by using Lowenstein-Jensen medium as described by Rattan [35]. The obtained results are presented in **Table 5.2** in form of % inhibition.

Table 5.2 *In vitro* antituberculosis activity (% inhibition) of quinoline pyrazoline derivative against *M. tuberculosis* $H_{37}Rv$ (at concentration 250 μg/mL).

Compound	% Inhibition	Compound	% Inhibition
6a	45	**8b**	**95**
6b	29	8c	48
6c	32	8d	24
6d	**96**	9a	36
7a	63	9b	32
7b	**90**	**9c**	**93**
7c	54	**9d**	**94**
7d	45	Rifampicin	98
8a	33	Isoniazid	99

Antituberculosis screening of all the synthesized pyrazoline scaffolds was conducted at 250 μg/mL concentrations against *M. tuberculosis* H37Rv strain. Compounds **6d**, **7b**, **8b**, **9c** and **9d** demonstrated excellent activity i.e. 96%, 90%, 95%, 93% and 94% at 250 μg/mL respectively against *M. tuberculosis* $H_{37}Rv$ (**Table 5.2**). All other remaining compounds disclosed poor inhibition against *M. tuberculosis* growth.

Table 5.3 *In vitro* antituberculosis activity of quinoline based pyrazoline derivative exhibiting higher % inhibition against *M. tuberculosis* H37Rv (MICs, μM).

Entry	% Inhibition	MIC, (μM)
6d	96	143
7b	90	201
8b	**95**	**47**
9c	93	247
9d	94	59
Rifampicin	98	48
Isoniazid	99	1

The compounds which showed higher inhibition against *M. tuberculosis* H37Rv, were they are further screened for their MICs. Among them compound **8b** (MIC = 47 µM) was found to be equipotent to rifampicin with 95% inhibition (**Table 5.3**). While compounds **6d, 7b**, **9c** and **9d** exhibited poor potency. From the above results, it can be accomplished that, compound **8b** may prove itself as an innovative member of antitubercular agents in future.

5.9.4 Antimalarial activity

All the synthesized morpholino quinoline based pyrazoline scaffolds were evaluated for their antimalarial screening against chloroquine and quinine sensitive strain of *P. falciparum*. All experiments were performed in triplicate and a mean value of IC_{50} is mentioned in **Table 5.4**.

Table 5.4 *In vitro* antimalarial activity of quinoline based pyrazoline derivatives.

Compound	IC_{50} (µM)	Compound	IC_{50} (µM)
6a	**0.034**	**8b**	**0.015**
6b	**0.018**	8c	3.421
6c	1.672	8d	1.786
6d	2.874	9a	1.872
7a	3.239	**9b**	**0.040**
7b	**0.044**	9c	1.038
7c	1.836	**9d**	**0.028**
7d	4.646	Chloroquine	0.062
8a	**0.051**	Quinine	0.826

As evident from **Table 5.4**, they were found to have IC_{50} between 0.015 µM and 4.646 µM against *P. falciparum* strain. It is important to note that compounds **8b** (0.015 µM), **6b** (0.018 µM), **9d** (0.028 µM), **6a** (0.034 µM), **9b** (0.040 µM), **7b** (0.044 µM) and **8a** (0.051 µM) displayed higher activity against *P. falciparum* strain as compared to chloroquine (0.062 µM) as well as quinine (0.826 µM). Remaining all other compounds were found to be less active against chloroquine sensitive strain of *P. falciparum*. From the above results, it can be concluded that compounds **6b** and **8b** having higher activity as compared to chloroquine (0.062 µM) may become a new potential member of antimalarial agents in future.

5.9.5 Cytotoxicity

Cytotoxicity of the synthesized compounds was tested using bioassay of *S. pombe* cells at the cellular level (**Figure 5.17**). Trypan blue dye exclusion method was used for the detection of dead *S. pombe* cells. Various 2-morpholinoquinoline based pyrazoline hybrids have changed the integrity of the cell membrane. Sensitive and selective cells have interacted with the compounds and allowed the dye to pass through the membrane. Live cells with intact membrane did not allow the dye to penetrate inside. As a result dead cells were found to be blue under microscope. The toxicity was found to vary with the type of substituent present and the concentrations of the synthesized compounds. Compounds **6a**, **6b** and **9d** were found to have maximum toxicity, while compounds **8a**, **7b**, **9b** and **8b** were found to be less cytotoxic. After 17 hrs of the treatment, many of the *S. pombe* cells died due to the toxic nature of the compounds.

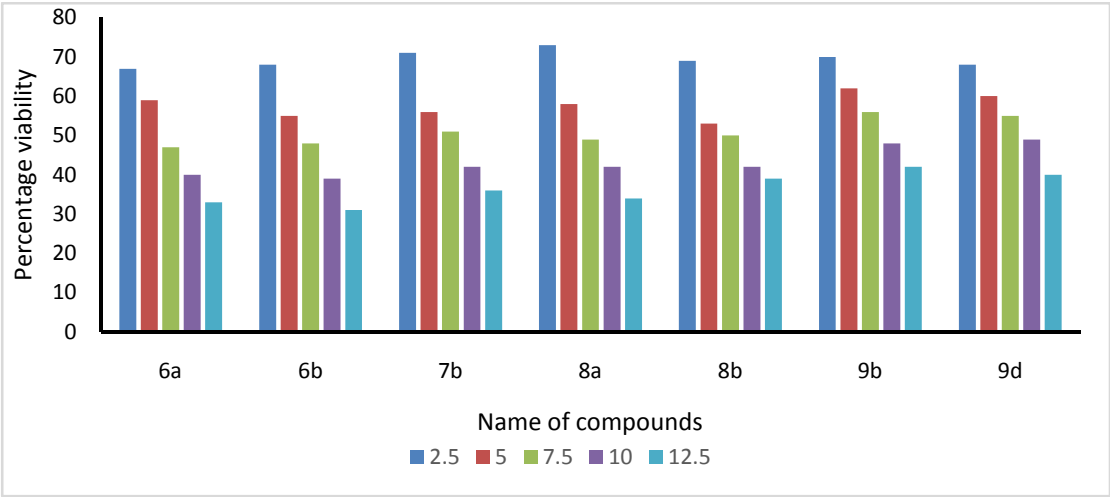


Figure 5.17 Effect of synthesized compounds on viability of *S. Pombe* at different concentrations.

5.10 Structure-activity relationship (SAR)

The consequences of the biological evaluation disclosed that the activity was significantly affected by introducing R_1 (-$CSNH_2$, -CHO, -$COCH_3$ and 4-F-Ar group) at N_1, substituent of postion-4 in phenyl ring at C-3 and 2-morpholinoquinoline nucleus at the C-5 position in pyrazoline scaffolds (**Figure 5.18**).

We perceived that the presence of -Br, -Cl group as R at position-4 in phenyl moiety and $–COCH_3$ group at N_1 position of pyrazoline nucleus exhibited excellent antimalarial activity against *P. falciparum strain* as compared to chloroquine (**6b**) and improved antituberculosis activity (**6d**) respectively. The existence of electron donating group ($-OCH_3$) as R at position-4 in phenyl moiety and $–COCH_3$ group at N_1 position of pyrazoline nucleus demonstrated superior antimalarial activity (**6a**, **8a**) as compared to quinine and improved antibacterial activity against *S. pneumoniae* (**6a**) and be less toxicity against *S. pombe* cells at the cellular level.

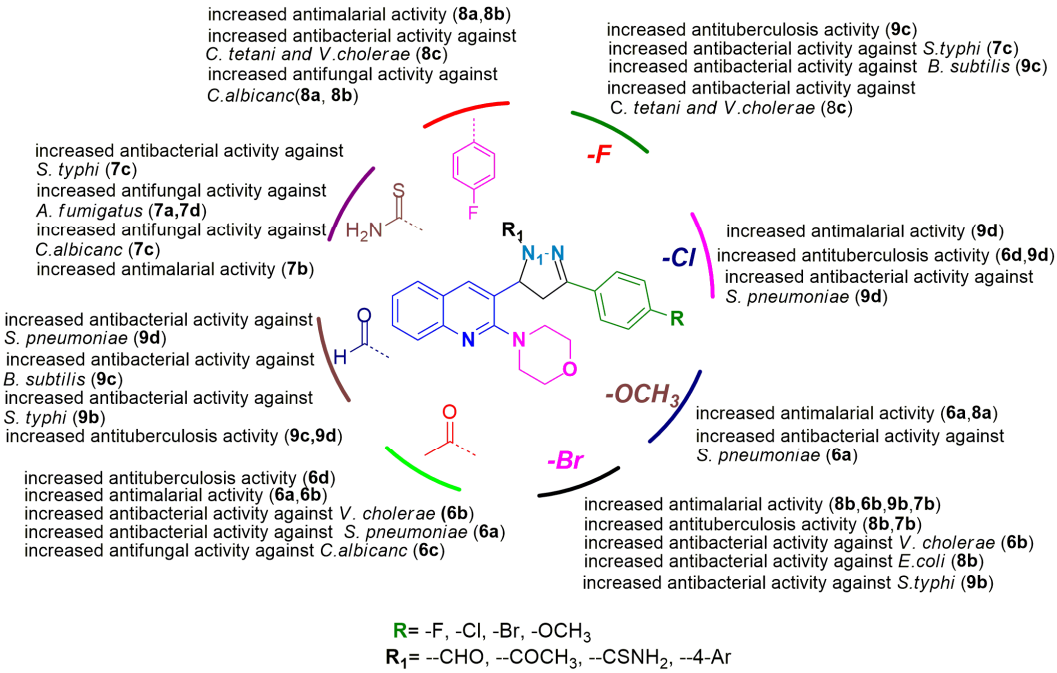


Figure 5.18 Structure–activity relationship for antimicrobial, antituberculosis and antimalarial activity of the synthesized morpholino quinoline based pyrazoline scaffolds.

The $–CSNH_2$ group at N_1 position of pyrazoline core and -Br and -Cl group existing as R at position-4 in phenyl moiety displayed notable antimalarial activity against *P. falciparum strain* as compared to quinine (**6a**, **6b**, **7b**, **8b**, **9b** and **9d** respectively). This has also improved antituberculosis activity (**7b**, **8b**, and **6d**, **9d** respectively) and enhanced antibacterial activity against *S. typhi* (**9b**). The $–CSNH_2$ group at N_1 position of pyrazoline core and -F group existing as R at position-4 in phenyl moiety displayed excellent antifungal potency against *C. albicans* as compared to griseofulvin (**6c**, **7c** and **8c**).

While –CHO group at N_1 position of pyrazoline core and -Br and -Cl group existing as R at position-4 in phenyl moiety demonstrated higher antimalarial (**6b**, **7b**, **8b**, and

9b), antituberculosis activity (**9d**) and good antibacterial potency against *V. cholera* **(6b** and **8b)** as compared to ampicillin. The presence of -F group existing as R at position-4 in phenyl moiety demonstrated superior antituberculosis activity (**9d**) and also displayed excellent antifungal potency against *C. albicans* as compared to griseofulvin (**6c**, **7c** and **8c**). But the presence of $–OCH_3$ group existing as R at position-4 in phenyl moiety exhibited greater antimalarial potency (**6a** and **8a**) and also increased antibacterial activity against *S. pneumonia* as compared to ampicillin (**6a**).

The presence of 4-F-Ar at N_1 position of pyrazoline core and –Br and -F group existing at R position in phenyl ring exihibited greater superior antimalarial activity (**6b**, **7b**, **8b**, and **9b**) and antituberculosis activity (**7b** and **8b)**. Increased antibacterial activities against *V. cholera* (**6b** and **8c**) as well as antifungal potency against *C. albicans* (**6c**, **7c** and **8c**) were also observed for the cited cases. Hence, it can be concluded that the presence of electron withdrawing group (-Br, -Cl, -F) at R position in phenyl moiety and $–COCH_3$, –CHO, $–CSNH_2$, 4-F-Ar group at N_1 position of pyrazoline nucleus were found responsible to increase antituberculosis and antimalarial activity. The existence of electron donating group ($-OCH_3$) at R position in phenyl moiety increased antibacterial activity and antimalarial potency. The presence of –CHO, $–CSNH_2$, 4-F-Ar group at N_1 position of pyrazoline nucleus and -Br, -Cl group existing at R position in phenyl ring led to the moieties possessing higher toxicity against *S. pombe* cells at the cellular level.

5.11 Conclusion

The objective of the present study was to design, synthesize and investigate the pharmacological efficacy of the novel pyrazoline scaffolds bearing 2-morpholinoquinoline nucleus. The synthesis were performed under MW irradiation with the hope of discovering new structural leads capable of serving as antimicrobial, antituberculosis and antimalarial agents. In antimalarial activity, Compounds **8b**, **6b**, **9d, 6a, 9b**, **7b** and **8a** displayed brilliant activity against *P. falciparum* strain as compared to chloroquine (IC_{50} 0.062 µM) as well as quinine (IC_{50} 0.826 µM). Most of the compounds were found to be active against *C. tetani* and *B. subtilis* and also illustrated good antituberculosis activity. Compounds **6c**, **7c**, **8a**, **8b**, **8c** and **9b**

showed significant antifungal activity as compared to griseofulvin (1147 µM). Finally, compounds **7b**, **8b** and **9d** could be identified as the biologically most active members with an interesting dual antimalarial and antituberculosis profile. Compounds **6a**, **6b** and **9d** were found to have maximum toxicity, while compounds **8a**, **7b**, **9b** and **8b** were found to be less cytotoxic. Consequently quinoline based pyrazoline scaffolds represent a class that needs further investigation with the hope of finding potent dual antimalarial – antituberculosis agents[36].

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