European Journal of Medicinal Chemistry 45 (2010) 3374–3383

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Original article

New quinoline derivatives: Synthesis and investigation of antibacterial and antituberculosis properties

Sumesh Eswaran [a], Airody Vasudeva Adhikari [b,*], Imran H. Chowdhury [c], Nishith K. Pal [c], K.D. Thomas [a]

[a] *Anthem Biosciences Pvt. Ltd, 49 Bommasandra Industrial Area, Bommasandra, Bangalore 560099, Karnataka, India*
[b] *Department of Chemistry, National Institute of Technology Karnataka, Surathkal, Srinivasnagar, Mangalore 575025, Karnataka, India*
[c] *Department of Bacteriology and Serology, Calcutta School of Tropical Medicine, Kolkata 700073, West Bengal, India*

ARTICLE INFO

Article history:
Received 15 January 2010
Received in revised form
17 April 2010
Accepted 21 April 2010
Available online 28 April 2010

Keywords:
Quinoline
Mefloquine
Antibacterial
Antituberculosis activity
X-ray crystallography

ABSTRACT

Four new series of quinoline derivatives were synthesized starting from 2-trifluoromethyl aniline through multi-step reactions. In the reaction sequence, substituted aniline was cyclized to 4-hydroxy quinoline **1**, which was then transformed to 4-chloro-2,8-bis(trifluoromethyl)quinoline **2**. The key scaffold 4-hydrazinyl-2,8-bis(trifluoromethyl)quinoline **3**, obtained from the compound **2**, was successfully converted to target quinoline derivatives, viz. hydrazones **4a**–**t**, ureas **5a**–**e**, thioureas **6a**–**c** and pyrazoles **7a**–**d**, in good yields. The newly synthesized title compounds were evaluated for their *in vitro* antibacterial activity against *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* (recultured) and antituberculosis activity against *Mycobacterium tuberculosis* $H_{37}Rv$ and MDR-TB. Preliminary results indicated that most of the hydrazone derivatives demonstrated very good antibacterial and antituberculosis activities while other derivatives showed moderate activity.

1. Introduction

Tuberculosis (TB) is an infectious disease caused by different species of *mycobacteria*. Though it is a treatable epidemic disease, the latest statistics reveals that around 2 million people throughout the world die annually from tuberculosis and there are around 9 million new cases each year. In the statistics, developing countries show major share [1]. As a result, it has become a major public health and socioeconomic problem in most of the developing countries. Amongst HIV-infected people with weakened immune system, TB is a leading killer pandemic. Every year about 0.2 million people living with HIV/AIDS die from TB [2]. Furthermore, in recent times the appearance of multidrug-resistant TB (MDR-TB), a form of TB that does not respond to the first-line TB drugs has become a serious threat to TB control and its treatment. It is a shocking revelation that MDR-TB is present in almost all countries as per the current survey, made by the World Health Organization (WHO) and its partners. A recent estimation by WHO has revealed that within next 20 years approximately 30 million people will be infected with the *bacillus* [3]. Keeping in view of the above statistics, WHO declared TB as a global health emergency and aimed at saving 14 million lives between 2006 and 2015 [4]. All the above facts reveal that there is an urgent need for development of new drugs with divergent and unique structure and with a mechanism of action possibly different from that of existing drugs.

In the recent time, quinoline nucleus has gathered an immense attention among chemists as well as biologists as it is one of the key building elements for many naturally occurring compounds. Among the important heterocyclic moieties of biological and pharmacological interest, the quinoline ring is endowed with various activities, such as antituberculosis [5], antimalarial [6], anti-inflammatory [7], anticancer [8], antibiotic [9], antihypertensive [10], tyrokinase PDGF-RTK inhibiting agents [11], and antiHIV [12,13]. In spite of its wide range of pharmacological activities, very few activity studies have been reported against tuberculosis in comparison with other classes. Keeping this in view, we have designed four new series of quinoline derivatives with possibly a new mode of action. The design concepts have been drawn in Fig. 1, which explains the structural similarity of our new target compounds with renowned drug mefloquine.

Mefloquine, a well-known antimalarial drug is still being used today in spite of its numerous side-effects [14]. Further, a number of its analogues have been reported to possess very good antibacterial as well as antituberculosis activities [15–18]. Moreover, it is important to note that quinoline is a core pharmacophore in the

---

* Corresponding author. Tel.: +91 (0)824 2474000x3203; fax: +91 (0)824 2474033.
E-mail addresses: avadhikari123@yahoo.co.in, avchem@nitk.ac.in (A.V. Adhikari).

0223-5234/$ – see front matter © 2010 Elsevier Masson SAS. All rights reserved.
doi:10.1016/j.ejmech.2010.04.022

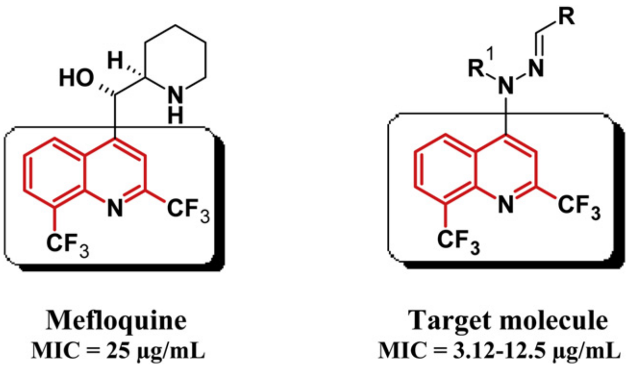


Fig. 1. Design concept for new quinoline derivatives.

recently developed anti tuberculosis drug, viz. TMC207, a diarylquinoline (DARQ), whose activity is mainly due to its interaction with the proton pump of the ATP synthase of *Mycobacterium tuberculosis* [19].

On the basis of these observations and as a part of our general program in the continued research for new antibacterials and antitubercular agents [20–22], we have designed some new quinoline derivatives, wherein active pharmacophores, viz. hydrazones, ureas, thioureas and pyrazoles have been attached at the 4th position of the quinoline ring containing active trifluoromethyl groups at 2nd and 8th positions, hoping that the newly designed molecules would exhibit improved biological activity. Structures of target molecules have been designed on the basis of combinatorial synthesis, which is the current trend being practiced in most of the drug discoveries. In this communication, we report the synthesis of hitherto unknown title compounds **4a**–**t**, **5a**–**e**, **6a**–**c** and **7a**–**d** starting from 2-trifluorometհy aniline (**1**) and evaluation of their *in vitro* antibacterial property against four pathogenic strains, viz. *Escherichia coli* (ATTC-25922), *Staphylococcus aureus* (ATTC-25923), *Pseudomonas aeruginosa* (ATCC 27853) and *Klebsiella pneumoniae* (recultured) and antituberculosis activity against *M. tuberculosis H37Rv* (ATCC 27294).

2. Chemistry

The reaction sequence employed for synthesis of the key scaffold, 4-hydrazinyl-2,8-bis(trifluoromethyl)quinoline (**3**) is shown in Scheme 1. The starting material 2-(trifluoromethyl) aniline was conveniently cyclized to 2,8-bis(trifluoromethyl)quinolin-4-ol (**1**), by heating it with ethyl 4,4,4-trifluoroacetoactate in presence of polyphosphoric acid (PPA) at 150 °C. The compound **1** on refluxing with freshly distilled phosphorus oxychloride yielded the corresponding 4-chloro derivative **2**, which on condensation with hydrazine hydrate in alcoholic medium smoothly underwent nucleophilic substitution reaction to give 4-hydrazinyl-2,8-bis(trifluoromethyl)quinoline (**3**) in good yield.

The key intermediate 4-hydrazinyl-2,8-bis(trifluoromethyl) quinoline (**3**) was readily converted to different hydrazones **4a**–**t** by reacting it with aliphatic and (hetero)aromatic aldehydes in presence of catalytic amount of acetic acid in alcoholic medium. Further, the compound **3** on heating with substituted isocyanate in toluene at 110 °C gave the corresponding urea derivatives **5a**–**e**, while **3** on condensing with substituted isothiocyanate at 110 °C in the presence of toluene yielded the corresponding thiourea derivatives **6a**–**c**. The target compounds **7a**–**d** were conveniently prepared from the compound **3** by the sequential addition of substituted acetoacetate to it, followed by cyclization of the resulting intermediate by treating with sodium ethoxide in ethanol at 80 °C. The crude compounds thus obtained were purified by column chromatography. The reaction sequences employed for the synthesis of title compounds, viz. **4a**–**t**, **5a**–**e**, **6a**–**c** and **7a**–**d** have been described in Scheme 2.

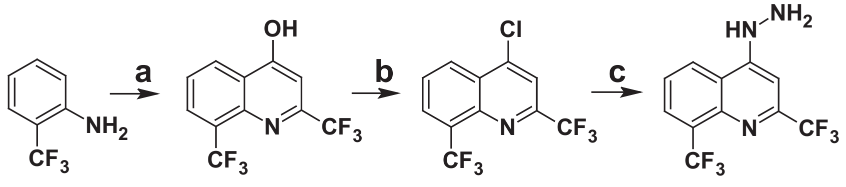
The structures of all the newly synthesized compounds were confirmed by ^{1}H, ^{13}C NMR and LC–MS studies. The cyclization of 2,8-bis(trifluoromethyl)quinolin-4-ol, (**1**) from 2-trifluoromethyl aniline was evidenced by its ^{1}H NMR spectrum and LC–MS. Its ^{1}H NMR spectrum showed a sharp singlet at δ 7.27 due to C_3 proton of quinoline ring and the appearance of a broad singlet at δ 12.78, which disappeared on D_2O exchange, attributed to –OH proton, clearly indicating the smooth cyclization. In fact, the total proton count for compound **1** perfectly matched with its structure. The LC–MS spectrum of **1** showed a molecular ion peak at m/z 282 (M + 1), which matches with its molecular formula $C_{11}H_5F_6NO$.

The formation of 4-chloro-2,8-bis(trifluoromethyl)quinoline (**2**) from the compound **1** was confirmed by its ^{1}H NMR spectral and LC–MS data. In the ^{1}H NMR spectrum, shifting of the singlet from δ 7.27 to 7.95 due to C_3 proton and the disappearance of a broad singlet at δ 12.78 confirmed the chlorination at the C_4 position of the quinoline ring. Further, the LC–MS spectrum of **2** showed a molecular ion peak at m/z 300 (M + 1), which matches with its molecular formula $C_{11}H_4ClF_6N$.

The structure of compound **3** was elucidated by their NMR spectral and LC–MS analyses. The ^{1}H NMR spectrum of **3** showed broad singlets at δ 4.72 and 9.34 corresponding to $-NH_2$ and –NH protons, respectively. Further, the total proton count for compound **3** perfectly matched with its structure. The LC–MS of it showed a molecular ion peak at m/z 296 (M + 1), which corresponds to its molecular formula $C_{11}H_7F_6N_3$.

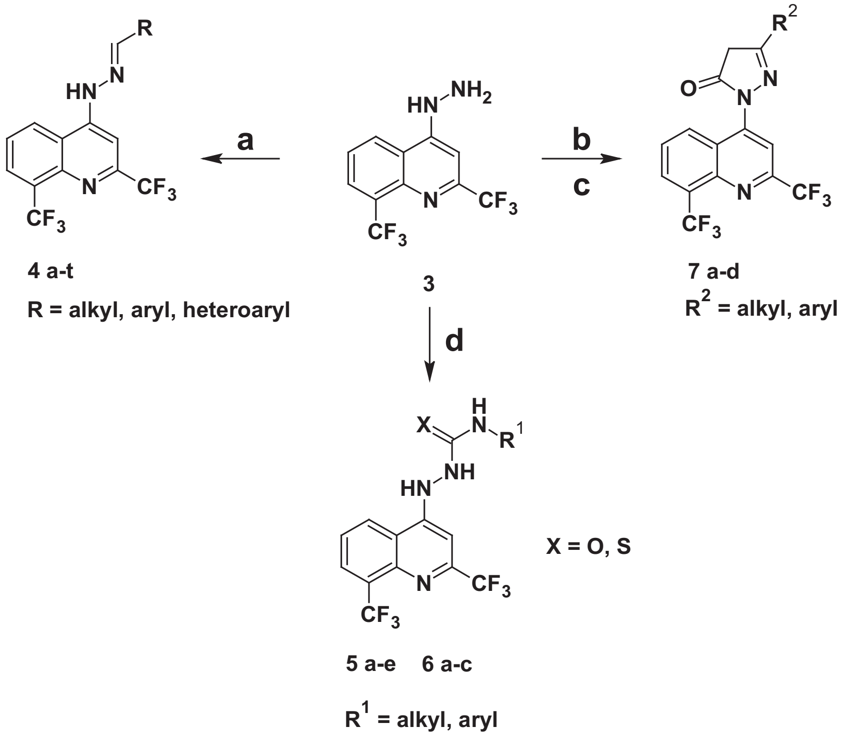
The formation of the title compounds, **4a**–**t**, **5a**–**e**, **6a**–**c** and **7a**–**d** from the hydrazine derivative, **3** were evidenced by ^{1}H, ^{13}C NMR spectral, LC–MS and elemental analysis data, explained in experimental part. In addition, the molecular structure of the compound **4l** was established by single crystal X-ray diffraction studies. The ORTEP view of the molecular structure shows the spatial atomic positions of compound **4l**, as shown in Fig. 2.

The structure of 1-(2,8-bis(trifluoromethyl)quinolin-4-yl)-4-(4-fluorophenyl)semicarbazide (**5a**) was determined by ^{1}H, ^{13}C NMR



1 2 3

Scheme 1. Synthesis of 4-hydrazinyl-2,8-bis(trifluoromethyl)quinoline Reagents and conditions: (a) PPA, ethyl 4,4,4-trifluoroacetoactate, 150 °C, 2 h; (b) $POCl_3$, 80 °C, 4 h; (c) Hydrazine hydrate, EtOH, 90 °C, 4 h.



Scheme 2. Reagents and conditions: (a) substituted aldehyde, EtOH, RT, 30 min; (b) substituted isocyanate, toluene, 110 °C, 30 min; (c) substituted isothiocyanate, toluene, 110 °C, 30 min; (d) substituted acetoacetate, EtOH, RT, 30 min, NaOEt, 80 °C, 30 min.

and LC–MS data. In the ^{1}H NMR spectrum, appearance of sharp signals at δ 8.80, 9.13 and 10.00 corresponding to –NH (attached to aromatic ring), –NH (attached to –NH) and –CONH, respectively indicated the smooth condensation between 4-fluorobenzaldehyde and hydrazine derivative **3**. In addition, its mass spectrum showed molecular ion peak at m/z 433 (M + 1), which corresponds to its molecular formula $C_{18}H_{11}F_7N_4O$.

The structures of compounds **6a**–**c** were elucidated by their ^{1}H NMR and LC–MS analyses. The ^{1}H NMR spectrum of **6a**, the appearance of broad signals at δ 9.96, 10.38, and 10.41 revealed the presence of –CSNH, and two –NH protons, respectively. Finally the structure was confirmed by its LC–MS, which showed its molecular ion peak at m/z 449 (M + 1). This is in accordance with its molecular formula $C_{18}H_{11}F_7N_4S$.

The cyclization of quinoline hydrazine **3** to the corresponding 1-(2,8-bis(trifluoromethyl)quinolin-4-yl)-3-methyl-1H-pyrazol-5 (4H)-one (**7a**) was evidenced by its ^{1}H NMR spectrum. In its spectrum, the disappearance of broad singlet from δ 4.72 and 9.34 corresponding to –NH$_2$ and –NH protons, respectively, and the appearance of two singlets at δ 2.32 and 3.59 corresponding to –CH$_3$, and –CH$_2$ of pyrazole ring, respectively, confirmed the cyclization. Further, the total proton count for compound **7a** perfectly matched with its structure, which further established the cyclization. The LC–MS of it showed a molecular ion peak at m/z 362 (M + 1), which matches with its molecular formula $C_{15}H_9F_6N_3O$. The characterization data of newly synthesized compounds are summarized in experimental section.

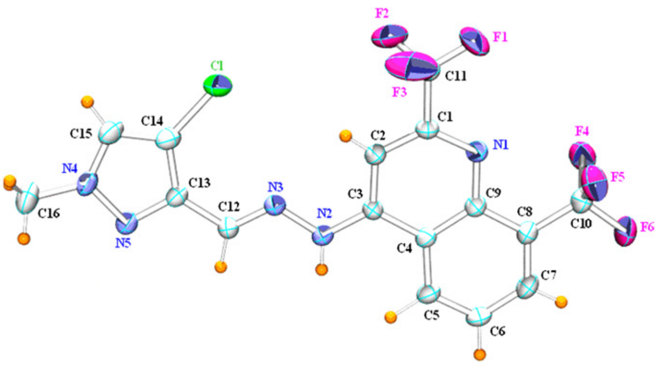


Fig. 2. ORTEP diagram showing the X-ray crystal structure of **4l**.

3. Pharmacology

3.1. Antibacterial studies

The newly synthesized compounds were screened for their *in vitro* antibacterial activity against *E. coli* (ATTC 25922), *S. aureus* (ATTC 25923), *P. aeruginosa* (ATCC 27853) and *K. pneumoniae* (recultured) bacterial stains by serial plate dilution method [23,24] using ciprofloxacin as standard. The MICs (μM) and zone of inhibition (mm) were determined for **4a**–**t**, **5a**–**e**, **6a**–**c** and **7a**–**d** and their results are summarized along with that of ciprofloxacin in Table 1.

3.2. Antituberculosis studies

The encouraging results from the antibacterial studies impelled us to go for the preliminary screening of the title compounds for their *in vitro* antituberculosis activity. The compounds were evaluated against *M. tuberculosis* H$_{37}$Rv and MDR-TB using broth microdilution method with Resazurin as indicator [25,26] and the observed MICs are presented in Table 2. Isoniazid (INH) and Rifampicin (RIF) were used as standard drugs.

Table 1
Antibacterial activity of the title compounds **4a–t**, **5a–e**, **6a–c** and **7a–d**.

Compounds	MIC in µg/mL and zone of inhibition in mm			
	S. aureus (ATCC 25923)	*E. coli* (ATCC 25922)	*P. aeruginosa* (ATCC 27853)	*K. pneumoniae* (recultured)
4a	12.5 (13)	6.25 (22)	12.5 (12)	12.5 (13)
4b	6.25 (22)	6.25 (21)	6.25 (26)	6.25 (18)
4c	12.5 (14)	12.5 (13)	12.5 (15)	12.5 (12)
4d	6.25 (23)	6.25 (25)	6.25 (24)	6.25 (22)
4e	50 (<10)	50 (<10)	50 (<10)	50 (<10)
4f	6.25 (22)	6.25 (24)	6.25 (25)	6.25 (20)
4g	6.25 (24)	6.25 (25)	6.25 (23)	6.25 (19)
4h	6.25 (21)	6.25 (24)	6.25 (26)	6.25 (20)
4i	12.5 (12)	6.25 (24)	12.5 (14)	12.5 (12)
4j	50 (<10)	50 (<10)	50 (<10)	50 (<10)
4k	6.25 (24)	6.25 (25)	6.25 (23)	6.25 (18)
4l	6.25 (22)	6.25 (24)	6.25 (28)	6.25 (18)
4m	12.5 (14)	6.25 (21)	12.5 (15)	12.5 (12)
4n	6.25 (22)	6.25 (20)	6.25 (24)	6.25 (21)
4o	6.25 (18)	6.25 (22)	6.25 (24)	6.25 (20)
4p	6.25 (22)	6.25 (20)	6.25 (24)	6.25 (21)
4q	6.25 (24)	6.25 (27)	6.25 (24)	6.25 (19)
4r	12.5 (10)	6.25 (20)	12.5 (14)	12.5 (11)
4s	6.25 (24)	6.25 (26)	6.25 (25)	6.25 (22)
4t	6.25 (22)	6.25 (23)	6.25 (25)	6.25 (20)
5a	12.5 (10)	12.5 (10)	12.5 (14)	50 (<10)
5b	50 (<10)	50 (<10)	50 (<10)	50 (<10)
5c	6.25 (21)	6.25 (24)	6.25 (22)	6.25 (18)
5d	6.25 (23)	6.25 (20)	6.25 (24)	6.25 (22)
5e	12.5 (10)	12.5 (10)	12.5 (14)	50 (<10)
6a	50 (<10)	50 (<10)	50 (<10)	50 (<10)
6b	12.5 (11)	12.5 (10)	12.5 (12)	50 (<10)
6c	50 (<10)	50 (<10)	50 (<10)	50 (<10)
7a	6.25 (24)	6.25 (22)	6.25 (25)	6.25 (20)
7b	6.25 (22)	6.25 (21)	6.25 (24)	6.25 (22)
7c	6.25 (18)	12.5 (10)	12.5 (12)	12.5 (10)
7d	6.25 (20)	12.5 (10)	12.5 (12)	12.5 (10)
Ciprofloxacin (Standard)	6.25 (26)	6.25 (28)	6.25 (31)	3.12 (24)

Note: the MIC values were evaluated at concentration range, 3.125–50 µg/mL. The figures in the table show the MIC values in µg/mL and the corresponding zone of inhibition in mm.

Table 2
In vitro antituberculosis evaluation of the synthesized compounds **4a–t**, **5a–e**, **6a–c** and **7a–d**.

Compounds	MIC (µM)		% Inhibition
	MTB	MDR-TB[a]	
4a	>50[b]	>50[b]	–
4b	12.5	25	<90
4c	12.5	12.5	<90
4d	6.25	6.25	95
4e	>50[b]	>50[b]	–
4f	12.5	>50	<90
4g	6.25	6.25	95
4h	12.5	12.5	<90
4i	6.25	6.25	95
4j	>50[b]	>50[b]	–
4k	3.12	6.25	99
4l	6.25	6.25	95
4m	25	12.5	–
4n	12.5	25	<90
4o	12.5	12.5	<90
4p	12.5	25	<90
4q	6.25	12.5	95
4r	>50[b]	>50[b]	–
4s	3.12	6.25	99
4t	25	>50[b]	–
5a	12.5	12.5	<90
5b	25	>50[b]	–
5c	12.5	12.5	<90
5d	6.25	6.25	95
5e	25	>50[b]	–
6a	>50[b]	>50[b]	–
6b	25	25	–
6c	>50[b]	>50[b]	–
7a	3.12	6.25	99
7b	6.25	6.25	95
7c	12.5	12.5	<90
7d	6.25	12.5	95
Isoniazid (INH)	1.5	12.5	95
Rifampicin (RIF)	0.5	25	99

[a] Mycobacterial tuberculosis resistant to three drugs viz., isoniazid (INH), Rifampicin (RFP) and ethambutol (EB).
[b] Compound inactive up to MIC 50 µM.

4. Results and discussion

The preliminary antibacterial screening revealed that most of the tested compounds in series **4a–t**, **5a–e** and **7a–d** showed moderate to very good inhibitory activity against all the strains, where as **6a-c** compounds were inactive. It was noteworthy to see that among the hydrazone series, the compounds **4b**, **4d**, **4f–h**, **4k**, **4l**, **4n–q**, **4s**, **4t** showed very good activity against all the pathogenic bacterial strains with MIC 6.25 µg/mL, comparable to standards used. In the urea series, compounds **5c** and **5d** displayed good activity, while all the three compounds in thiourea series did not exhibit any activity. The compounds **7a** and **7b** which belong to pyrazole series also exhibited very good activity same as it was with hydrazone series. The good antibacterial activity of **4a–t** is attributed to the presence of active hetero-aryl groups in their structures. In the urea derivatives **5c** and **5d**, the presence of electron donating group, viz. $-OCH_3$ and $-CH_3$ attached to the aryl ring enhanced the activity considerably. While the decreased activity in series **6a–c** may be due to the reduced H-bonding ability of $>C{=}S$ group. Among pyrazole derivatives **7a–d**, it was observed that the presence of electron donating alkyl group attached to the pyrazole ring brought about enhanced activity while presence of electron withdrawing phenyl and pyridine rings (**7c** and **7d**) resulted in reduced activity. It is interesting to note that compounds **4a**, **4i**, **4m** and **4r** showed very good activity against gram negative strains and poor activity against other strains, whereas compounds **7c** and **7d** showed very good activity against gram positive strains and showed poor activity against other strains.

On the other hand antituberculosis screening data revealed that all the tested compounds in series **4a–t**, **5a–e** and **7a–d** showed good to moderate inhibitory activity, whereas all the compounds in series **6a–c** were inactive against both MTB and MDR-TB. Compounds **4k**, **4s**, and **7a** were found to be very potent inhibitor, being able to inhibit 99% growth of *M. tuberculosis* at a concentration of 3.12 µg/mL, while compounds **4d**, **4g**, **4i**, **4l**, **4q**, **5b**, **7b** and **7d** showed moderate to good activity with 95% growth inhibition of mycobacterium at 6.25 µg/mL.

In addition, the compounds were screened against MDR-TB (*M. tuberculosis* resistant to three drugs, viz. isoniazid (INH), rifampicin (RFP) and ethambutol (EB)). Among the thirty two compounds screened, most of them showed good activity against MDR-TB strain with MIC ranging from 6.25 to 25 µg/mL and were found to be more active than isoniazid (INH), rifampicin (RFP). It is interesting to note that 9 compounds, viz. **4d**, **4g**, **4i**, **4k**, **4l**, **4s**, **5d**, **7a** and **7b** were found to be more potent than INH (MIC: 12.5µg/mL) with MIC 6.25 µg/mL, while 9 compounds, viz. **4c**, **4h**, **4m**, **4o**, **4q**, **5a**, **5c**, **7c** and **6d** were found to be twofold potent than RFP (MIC: 25 µg/mL) with MIC 12.5 µg/mL. Further, compounds **4b**, **4n**, **4p** and **6b** were shown to be as potent as RFP (MIC: 25 µg/mL) with MIC 25 µg/mL. The good anti-TB activity is attributed to the presence of pharmacologically active hetero-aryl groups, viz. pyrazole, imidazole, indole, etc, attached to the quinoline ring. It is surprising and encouraging to see that compound **7a** showed very good anti tuberculosis activity against both the TB strains. It may be attributed to the presence of electron donating $-CH_3$ group, which is

Table 3
Crystal data and measurement detail for compound **4l**.

Crystal data	
Empirical formula	$C_{16}H_{10}ClF_6N_5$
Formula weight	421.05
Crystal system	Triclinic
Crystal dimension	0.30 mm × 0.25 mm × 0.15 mm
Space group	p111
a (Å)	8.6445(6)
b (Å)	10.0487(8)
c (Å)	11.7731(9)
Volume (Å^3)	917.40(12)
Angle α, β, γ	84.131(6), 65.530(7), 80.495(6)
Z	4
Crystal density, g/cm^3	1.592
F_{000}	444
μ (mm^{-1})	0.285
Absorption coefficient	0.285
Cut-off used in R-factor calculations	$Fo^2 > 2\sigma\ (Fo^2)$
R (Fo)	0.0705
R_w (Fo^2)	0.1910
Temperature (T)	293(2)
Radiation wavelength	0.71073
Radiation type	MoKα
Radiation source	Fine-focus sealed tube
Radiation monochromator	Graphite
$h_{\min}$	−10
$h_{\max}$	10
$k_{\min}$	−11
$k_{\max}$	11
$l_{\min}$	−13
$l_{\max}$	13
Reflns (Fo)	4212
Structure refinement	SHELXL97

responsible for stabilizing the pyrazole ring, thereby making the quinoline ring more active species.

The study reveals that presence of C–N–N linkage with quinoline at its position-4 is the desired structural feature for enhanced antituberculosis activity and hence the compound **7a** has a good scope for further derivatization with active pharmacophores in order to establish the SAR.

4.1. *X-Ray Crystallographic Analysis of* ***4l***

The X-ray crystallographic analysis of **4l** was determined on a colorless plate crystal, with approximate dimensions of 0.30 mm × 0.25 mm × 0.15 mm, grown from the slow evaporation of a dilute ethanol solution at room temperature. The crystal structure solution was solved by full matrix least-squares method using SHELXL97. All the atoms were located in different Fourier maps and refined isotropically, using a riding model and all the projections were generated using ORTEP. The details of the crystal data and refinement are shown in Table 3. Also the single crystal images for compound **4l** are given in Fig. 3.

5. Conclusion

The present research study reports the successful synthesis, antibacterial and antituberculosis studies of a four new series of quinoline derivatives carrying biologically active entities viz., hydrazones, ureas, thioureas and pyrazoles. Their screening results revealed that all the compounds showed moderate to very good activities against pathogenic strains. On the basis of structure–biological activity relationship it can be concluded that a combination of hetero-aryl group at position-4 of quinoline core showed an increased antibacterial and antituberculosis activity and hence they are ideally suited for further modifications to obtain more efficacious antibacterial and antituberculosis compounds.

6. Experimental section

6.1. *General*

All reagents were purchased from Aldrich. Solvents used were extra dried. Final purifications were carried out using Quad biotage

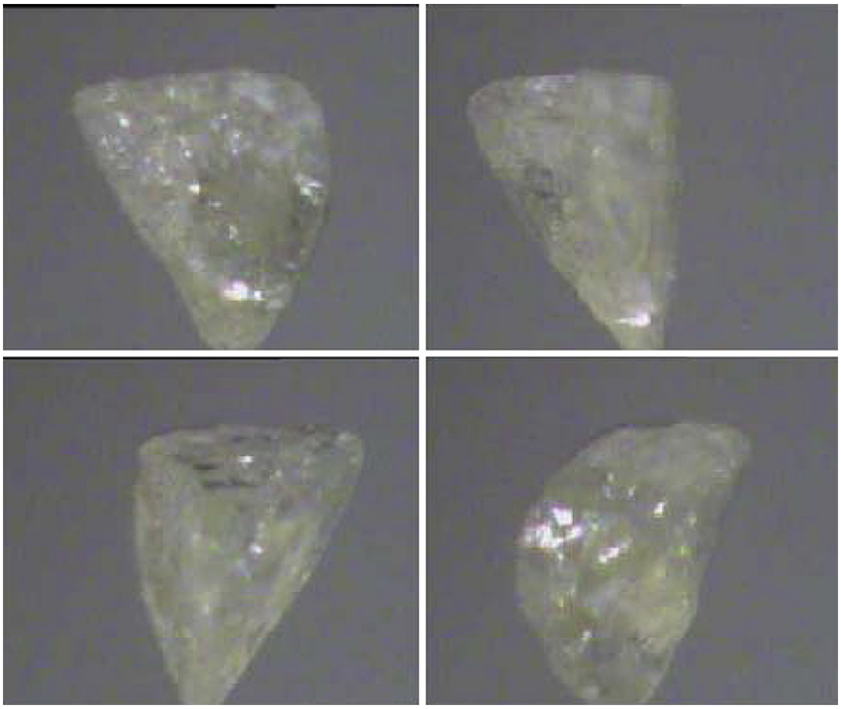


Fig. 3. Single crystal images of compound **4l**.

Flash purifier (A Dyax Corp. Company). TLC experiments were performed on alumina-backed silica gel 40 F254 plates (Merck, Darmstadt, Germany). The plates were illuminated under UV (254 nm) and molybidinic acid. Melting points were determined using Buchi B-540 and are uncorrected. Elemental analyses were carried out on an automatic Flash EA 1112 Series, CHNSO Analyzer (Thermo). X-Ray diffraction studies where carried on an Xcalibur E Oxford Diffraction system (Varian, California, USA). All ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AM-300 (300.12 MHz), Bruker BioSpin Corp., Germany. Molecular weights of unknown compounds were characterized by LC–MS 6200 series Agilent Technology. Chemical shifts are reported in ppm (δ) with reference to internal standard TMS. The signals are designated as follows: s, singlet; d, doublet; dd, doublet of doublets; t, triplet; m, multiplet; brs, broad singlet, brt, broad triplet.

The newly synthesized compounds **4 a–h**, **4 j–t**, **5 a–e**, **6a–c** were recrystallized from absolute ethanol where as compounds **4i**, **7a–d** were purified by column chromatography.

6.2. *Preparation of 2,8-bis(trifluoromethyl)quinolin-4-ol (**1**)*

To an equimolar solution of 2-trifluoromethyl aniline (25 g, 155.3 mmol) and ethyl 4,4,4-trifluoroacetoacetate (28.6 g, 155.3 mmol) was added polyphosphoric acid (125 g, 5 w/w). The reaction mixture was stirred at 150 °C for 2 h. Reaction completion was monitored by TLC. The reaction mixture was poured into ice water (500 mL) slowly with vigorous stirring. The precipitated solid was filtered and dried in vacuum oven for 4 h to get the crude product as white solid. The crude product was taken as such for the next step without further purification.

Compound **1** was obtained as white solid. Yield 77%. ^{1}H NMR (300 MHz, DMSO-d_6) δ; 7.27 (s, –CH, 1H), 7.78 (t, –CH, 1H, J = 7.8 Hz), 8.28 (d, –CH, 1H, J = 7.2 Hz), 8.52 (d, –CH, 1H, J = 8.4 Hz), 12.78 (brs, –OH, 1H, disappeared on D_2O exchange).

6.3. *Preparation of 4-chloro-2,8-bis(trifluoromethyl)quinoline (**2**)*

A mixture of **1** (25 g, 88.9 mmol) and freshly distilled $POCl_3$ (125 mL) was heated at 80 °C for 4 h. The reaction was monitored by TLC. After completion of the reaction, excess of $POCl_3$ was distilled off. The residue thus obtained was stirred with ice water for 15 min. After this, the solid phase was filtered and dried.

Compound **2** was obtained as pale yellow solid. Yield 82%. ^{1}H NMR (300 MHz, DMSO-d_6) δ; 7.86 (t, –CH, 1H, J = 7.8 Hz), 7.95 (s, –CH, 1H), 8.27 (d, –CH, 1H, J = 7.2 Hz), 8.56 (d, –CH, 1H, J = 8.4 Hz).

6.4. *Preparation of 4-hydrazinyl-2,8-bis(trifluoromethyl)-quinoline (**3**)*

Compound **2** (10 g, 33.4 mmol) and hydrazine hydrate 60% (50 mL) in 50 mL of ethanol was heated under reflux for 4 h. Completion of the reaction was monitored by TLC. The reaction mixture was concentrated and allowed to cool. The solid product obtained was filtered, washed with water and dried.

Compound **3** was obtained as off white solid. Yield 80%. ^{1}H NMR (300 MHz, DMSO-d_6) δ; 4.76 (brs, –NH_2, 2H), 7.35 (s, –CH, 1H), 7.62 (t, –CH, 1H, J = 7.8 Hz), 8.13 (d, –CH, 1H, J = 7.5 Hz), 8.54 (d, –CH, 1H, J = 8.4 Hz), 9.34 (s, –NH, 1H). LC–MS (ESI) m/z 296 (M + 1).

6.5. *General procedure for the synthesis of title compounds (**4a–t**)*

To a suspension of compound **3** (1 mmol) in dry ethanol, was added substituted aldehyde (1 mmol) and catalytic amount of acetic acid. The reaction mixture was stirred at room temperature for 30 min. Reaction completion was monitored by TLC. The reaction mixture was concentrated under reduced pressure. The solid separated was filtered, dried and recrystallized from diethyl ether. Some of the final compounds were purified by biotage column chromatography using pet ether/ethyl acetate as the eluent.

6.5.1. *2-[(E)-{2-[2,8-Bis(trifluoromethyl)quinolin-4-yl] hydrazinylidene}methyl]-5-fluorophenol (**4a**)*

Compound **4a** was obtained as yellow solid. M.P 246–249 °C. Yield 85%. ^{1}H NMR (300 MHz, DMSO-d_6) δ; 6.91–6.95 (m, –CH, 1H), 7.09–7.16 (m, –CH, 1H), 7.65–7.82 (m, –CH, 3H), 8.25 (d, –CH, 1H, J = 7.2 Hz), 8.72 (d, –CH, 1H, J = 8.4 Hz), 8.80 (s, –N=CH–, 1H), 10.22 (s, –OH, 1H), 11.75 (s, –NH, 1H). ^{13}C NMR (75 MHz, DMSO-d_6) δ; 97.1, 111.3, 111.6, 117.8,117.9, 118.1, 118.4, 120.1, 121.8, 121.9, 122.5, 123.8, 125.7, 126.1, 126.6, 127.0, 127.3, 129.7, 142.0, 144.4, 148.0, 148.4, 149.6, 153.2, 154.6, 157.7. LC–MS (ESI) m/z 418 (M + 1). Anal. Calcd for $C_{18}H_{10}F_7N_3O$; Calc: C, 51.81; H, 2.42; N, 10.07; found: C, 51.90; H, 2.47; N, 10.12.

6.5.2. *(E)-1-(2,8-Bis(trifluoromethyl)quinolin-4-yl)-2-(3-(1,1,2,2-tetrafluoroethoxy) benzylidene)hydrazine (**4b**)*

Compound **4b** was obtained as off white solid. M.P 194–196 °C. Yield 79%. ^{1}H NMR (300 MHz, DMSO-d_6) δ; 6.69–7.04 (m, –CH, 1H), 7.38 (d, –CH, 1H, J = 8.4 Hz), 7.60 (t, –CH, 1H, J = 7.8 Hz), 7.72–7.74 (m, –CH, 2H), 7.79–7.85 (m, –CH, 2H), 8.25 (d, –CH, 1H, J = 6.9 Hz), 8.50 (s, –N=CH–, 1H), 8.72 (d, –CH, 1H, J = 8.1 Hz), 11.89 (s, –NH, 1H). ^{13}C NMR (75 MHz, DMSO-d_6) δ; 97.3, 104.4, 104.9, 105.5, 107.7, 108.2, 108.8, 110.0, 111.0, 111.5, 116.5, 116.9, 117.3, 118.0, 119.7, 120.1, 120.5, 122.5, 123.1, 123.7, 125.7, 125.9, 126.3, 126.1, 126.7, 127.1, 127.3, 129.8, 131.2, 136.8, 144.4, 147.9, 148.4, 148.8, 149.0, 149.7. LC–MS (ESI) m/z 500 (M + 1). Anal. Calcd for $C_{20}H_{11}F_{10}N_3O$; Calc: C, 48.11; H, 2.22; N, 8.42; found: C, 48.14; H, 2.28; N, 8.50.

6.5.3. *(E)-1-(2,8-Bis(trifluoromethyl)quinolin-4-yl)-2-(3-hydroxy-4-methoxybenzylidene)hydrazine (**4c**)*

Compound **4c** was obtained as pale yellow solid. M.P. 203–206 °C. Yield 78%. ^{1}H NMR (300 MHz, DMSO-d_6) δ; 3.82 (s, –OCH_3, 3H), 6.99 (d, –CH, 1H, J = 8.4 Hz), 7.16 (d, –CH, 1H, J = 8.1 Hz), 7.37 (s, –CH, 1H), 7.67 (s, –CH, 1H), 7.79 (t, –CH, 1H, J = 7.8 Hz), 8.25 (d, –CH, 1H, J = 7.2 Hz), 8.36 (s, –N=CH–, 1H), 8.69 (d, –CH, 1H, J = 8.7 Hz), 9.37 (s, –OH, 1H), 11.60 (s, –NH, 1H). ^{13}C NMR (75 MHz, DMSO-d_6) δ; 56.2, 97.2, 115.8, 116.2, 119.1, 119.8, 122.8, 125.6, 126.1, 126.4, 129.2, 129.8, 144.1, 145.8, 151.8, 152.3, 153.6, 156.4. LC–MS (ESI) m/z 430 (M + 1). Anal. Calcd for $C_{19}H_{13}F_6N_3O_2$; Calc: C, 53.16; H, 3.05; N, 9.79; found: C, 53.12; H, 3.09; N, 9.84.

6.5.4. *(E)-1-(2,8-Bis(trifluoromethyl)quinolin-4-yl)-2-(2-fluoro-4-methoxybenzylidene)hydrazine (**4d**)*

Compound **4d** was obtained as pale yellow solid. M.P 198–199 °C. Yield 82%. ^{1}H NMR (300 MHz, DMSO-d_6) δ; 3.82 (s, –OCH_3, 3H), 6.88–6.95 (m, –CH, 2H), 7.69 (s, –CH, 1H), 7.78 (t, –CH, 1H, J = 7.8 Hz), 7.98 (t, –CH, 1H, J = 8.4 Hz), 8.23 (d, –CH, 1H, J = 7.2 Hz), 8.61 (s, –N=CH–, 1H), 8.68 (d, –CH, 1H, J = 8.7 Hz), 11.69 (s, –NH, 1H). ^{13}C NMR (75 MHz, DMSO-d_6) δ; 56.1, 95.7, 102.5, 110.3, 110.7, 119.0, 119.8, 125.6, 126.1, 126.4, 129.2, 129.4, 131.8, 143.0, 144.7, 151.8, 156.4, 160.5, 164.8. LC–MS (ESI) m/z 432 (M + 1). Anal. Calcd for $C_{19}H_{12}F_7N_3O$; Calc: C, 52.91; H, 2.80; N, 9.74; found: C, 52.96; H, 2.88; N, 9.79.

6.5.5. *(E)-1-(2,8-Bis(trifluoromethyl)quinolin-4-yl)-2-((thiophen-2-yl)methylene)hydrazine (**4e**)*

Compound **4e** was obtained as pale yellow solid. M.P. 184–186 °C. Yield 86%. ^{1}H NMR (300 MHz, DMSO-d_6) δ; 7.16–7.18 (m, –CH, 1H), 7.53–7.57 (m, –CH, 2H), 7.70 (d, –CH, 1H, J = 5.1 Hz), 7.79 (t, –CH, 1H, J = 8.1 Hz), 8.24 (d, –CH, 1H, J = 7.2 Hz), 8.66–8.69 (m, –N=CH–, –CH, 2H), 11.74 (s, –NH, 1H). ^{13}C NMR (75 MHz, DMSO-d_6) δ; 96.8, 117.9, 120.1, 122.5, 123.8, 125.5, 126.1, 126.7, 127.1, 127.2, 127.4, 128.4, 129.1, 129.7, 131.0, 139.3, 141.1, 144.4, 147.4, 147.9, 148.3, 149.4. LC−MS (ESI) *m*/*z* 390 (M + 1). Anal. Calcd for $C_{16}H_9F_6N_3S$; Calc: C, 49.36; H, 2.33; N, 10.79; S, 8.24; found: C, 49.39; H, 2.37; N, 10.72; S, 8.28.

*6.5.6. (E)-1-(2,8-Bis(trifluoromethyl)quinolin-4-yl)-2-((pyridin-3-yl)methylene)hydrazine (**4f**)*

Compound **4f** was obtained as white solid. M.P. 256–258 °C. Yield 72%. ^{1}H NMR (300 MHz, DMSO-d_6) δ; 7.50–7.54 (m, −CH, 1H), 7.80–7.85 (m, −CH, 2H), 8.25–8.31 (m, −CH, 2H), 8.53 (s, −N=CH−, 1H), 8.62 (d, −CH, 1H, *J* = 4.8 Hz), 8.76 (d, −CH, 1H, *J* = 8.7 Hz), 8.97 (s, −CH−, 1H), 11.90 (s, −NH, 1H). ^{13}C NMR (75 MHz, DMSO-d_6) δ; 97.4, 117.9, 120.1, 122.4, 123.8, 124.4, 125.7, 126.7, 127.1, 127.2, 129.8, 130.5, 133.7, 143.1, 144.3, 147.9, 148.4, 149.1, 149.6, 150.9. LC−MS (ESI) *m*/*z* 385 (M + 1). Anal. Calcd for $C_{17}H_{10}F_6N_4$; Calc: C, 53.13; H, 2.62; N, 14.58; found: C, 53.17; H, 2.52; N, 14.51.

*6.5.7. (E)-1-(2,8-Bis(trifluoromethyl)quinolin-4-yl)-2-(cyclohexylmethylene)hydrazine (**4g**)*

Compound **4g** was obtained as white solid. M.P. 204–206 °C. Yield 67%. ^{1}H NMR (300 MHz, DMSO-d_6) δ; 1.24–1.32 (m, cyclohexyl −CH_2, 5H), 1.63–1.88 (m, cyclohexyl −CH_2, 5H), 2.38 (m, cyclohexyl −CH, 1H), 7.51 (s, −CH, 1H), 7.72–7.77 (m, −CH, −N=CH−, 2H), 8.22 (d, −CH, 1H, *J* = 7.2 Hz), 8.62 (s, −CH, 1H, *J* = 8.4 Hz), 11.28 (s, −NH, 1H). ^{13}C NMR (75 MHz, DMSO-d_6) δ; 25.4, 25.9, 30.1, 96.4, 117.6, 120.2, 122.5, 123.8, 125.4, 126.1, 126.6, 127.0, 127.3, 129.7, 129.8, 144.4, 147.9, 148.3, 150.2, 154.6. LC−MS (ESI) *m*/*z* 390 (M + 1). Anal. Calcd for $C_{18}H_{17}F_6N_3$; Calc: C, 55.53; H, 4.40; N, 10.79; found: C, 55.61; H, 4.46; N, 10.88.

*6.5.8. (E)-1-(2,8-Bis(trifluoromethyl)quinolin-4-yl)-2-(3-(trifluoromethoxy)benzylidene)hydrazine (**4h**)*

Compound **4h** was obtained as white solid. M.P. 217–219 °C. Yield 77%. ^{1}H NMR (300 MHz, DMSO-d_6) δ; 7.43 (d, −CH, 1H, *J* = 8.4 Hz), 7.60 (t, −CH, 1H, *J* = 7.8 Hz), 7.74–7.86 (m, −CH, 4H), 8.24 (d, −CH, 1H, *J* = 7.2 Hz), 8.47 (s, −N=CH−, 1H), 8.71 (d, −CH, 1H, *J* = 8.4 Hz), 11.85 (s, −NH, 1H). ^{13}C NMR (75 MHz, DMSO-d_6) δ; 97.3, 117.9, 118.8, 119.1, 120.1, 122.3, 122.4, 123.7, 125.6, 126.1, 126.3, 126.7, 127.1, 127.2, 129.8, 131.2, 137.0, 144.1, 144.3, 147.9, 148.3, 149.2, 149.6. LC−MS (ESI) *m*/*z* 468 (M + 1). Anal. Calcd for $C_{19}H_{10}F_9N_3O$; Calc: C, 48.84; H, 2.16; N, 8.99; found: C, 48.89; H, 2.18; N, 9.08.

*6.5.9. (E)-1-(2,8-Bis(trifluoromethyl)quinolin-4-yl)-2-octylidenehydrazine (**4i**)*

Compound **4i** was obtained as brown liquid. Yield 56%. ^{1}H NMR (300 MHz, DMSO-d_6) δ; 0.91 (m, −CH_3, 3H), 1.23–1.52 (m, −CH_2, 6H), 1.59–1.61 (m, −CH_2, 4H), 1.69–1.73 (m, −CH_2, 2H), 2.40–2.49 (m, −CH_2, 2H), 7.46 (m, −N=CH−, 1H), 7.55–7.62 (m, −CH, 1H), 7.72 (s, −CH, 1H), 7.93–7.97 (m, −CH, 1H), 8.01 (d, −CH, 1H, *J* = 7.2 Hz), 8.28 (brs, −NH, 1H). ^{13}C NMR (75 MHz, DMSO-d_6) δ; 14.2, 22.8, 26.3, 26.4, 29.1, 29.4, 40.1, 95.9, 119.1, 119.8, 125.6, 126.1, 126.5, 129.2, 129.4, 144.6, 148.2, 150.5, 154.1. LC−MS (ESI) *m*/*z* 406 (M + 1). Anal. Calcd for $C_{19}H_{21}F_6N_3$; Calc: C, 56.29; H, 5.22; N, 10.37; found: C, 56.22; H, 5.26; N, 10.42.

*6.5.10. (E)-1-(2,8-Bis(trifluoromethyl)quinolin-4-yl)-2-((4-bromofuran-3-yl)methylene)hydrazine (**4j**)*

Compound **4j** was obtained as off white solid. M.P 186–188 °C. Yield 88%. ^{1}H NMR (300 MHz, DMSO-d_6) δ; 7.76–7.84 (m, −CH, 2H), 8.11 (s, −CH, 1H), 8.26 (d, −CH, 1H, *J* = 6.9 Hz), 8.39–8.41 (m, −N=CH−, −CH, 2H), 8.71 (d, −CH, 1H, *J* = 8.7 Hz), 11.77 (s, −NH, 1H). ^{13}C NMR (75 MHz, DMSO-d_6) δ; 97.3, 98.5, 117.8, 120.1, 121.1, 122.5, 123.5, 126.1, 126.7, 127.1, 127.3, 129.8, 129.9, 137.1, 144.1, 144.4, 145.8, 148.0, 148.4, 149.8. LC−MS (ESI) *m*/*z* 452 (M + 2). Anal. Calcd for $C_{16}H_8BrF_6N_3O$; Calc: C, 42.50; H, 1.78; N, 9.29; found: C, 42.55; H, 1.73; N, 9.36.

*6.5.11. (E)-2-((1H-imidazol-4-yl)methylene)-1-(2,8-bis(trifluoromethyl)quinolin-4-yl)hydrazine (**4k**)*

Compound **4k** was obtained as yellow solid. M.P. 252–254 °C. Yield 65%. ^{1}H NMR (300 MHz, DMSO-d_6) δ; 7.42–7.84 (m, −CH, 4H), 8.23 (d, −CH, 1H, *J* = 7.2 Hz), 8.42 (s, −N=CH−, 1H), 8.69 (d, −CH, 1H, *J* = 8.4 Hz), 11.51 (s, −NH, 1H), 12.92 (brs, −NH of imidazole, 1H). LC−MS (ESI) *m*/*z* 374 (M + 1). Anal. Calcd for $C_{15}H_9F_6N_5$; Calc: C, 48.27; H, 2.43; N, 18.76; found: C, 48.32; H, 2.49; N, 18.88.

*6.5.12. (E)-1-(2,8-Bis(trifluoromethyl)quinolin-4-yl)-2-((4-chloro-1-methyl-1H-pyrazol-3-yl)methylene)hydrazine (**4l**)*

Compound **4l** was obtained as white solid. M.P. 231–234 °C. Yield 70%. ^{1}H NMR (300 MHz, DMSO-d_6) δ; 3.89 (s, −NCH_3, 3H), 7.71 (s, −CH, 1H), 7.80 (t, −CH, 1H, *J* = 7.8 Hz), 8.10 (s, −CH, 1H), 8.26 (d, −CH, 1H, *J* = 7.2 Hz), 8.44 (s, −N=CH−, 1H), 8.69 (d, −CH, 1H, *J* = 8.4 Hz), 11.76 (s, −NH, 1H). ^{13}C NMR (75 MHz, DMSO-d_6) δ; 41.6, 97.3, 107.5, 117.9, 120.1, 122.5, 123.8, 125.8, 126.1, 126.7, 127.1, 127.3, 130.0, 131.8, 138.3, 142.2, 144.4, 147.9, 148.4, 149.8. LC−MS (ESI) *m*/*z* 422 (M + 1). Anal. Calcd for $C_{16}H_{10}ClF_6N_5$; Calc: C, 45.57; H, 2.39; N, 16.61; found: C, 45.62; H, 2.32; N, 16.68.

*6.5.13. (E)-1-(2,8-Bis(trifluoromethyl)quinolin-4-yl)-2-((2,3-dihydrobenzofuran-5-yl)methylene)hydrazine (**4m**)*

Compound **4m** was obtained as yellow solid. M.P. 220–221 °C. Yield 73%. ^{1}H NMR (300 MHz, DMSO-d_6) δ; 3.25 (t, −CH_2, 2H, *J* = 8.4 Hz), 4.60 (t, −OCH_2, 2H, *J* = 8.7 Hz), 6.87 (d, −CH, 1H, *J* = 8.4 Hz), 7.54 (d, −CH, 1H, *J* = 7.8 Hz), 7.68 (s, −CH, 1H), 7.75–7.79 (m, −CH, 2H), 8.23 (d, −CH, 1H, *J* = 7.2 Hz), 8.42 (s, −N=CH−, 1H), 8.71 (d, −CH, 1H, *J* = 8.4 Hz), 11.57 (s, −NH, 1H). ^{13}C NMR (75 MHz, DMSO-d_6) δ; 29.1, 72.0, 96.7, 109.7, 117.9, 120.2, 123.8, 123.8, 125.5, 126.1, 126.6, 127.7, 127.1, 127.3, 128.8, 129.0, 129.7, 129.8, 144.4, 146.7, 147.9, 148.4, 149.8, 162.1. LC−MS (ESI) *m*/*z* 426 (M + 1). Anal. Calcd for $C_{20}H_{13}F_6N_3O$; Calc: C, 56.58; H, 3.01; N, 9.88; found: C, 56.66; H, 3.08; N, 9.99.

*6.5.14. (E)-1-(2,8-Bis(trifluoromethyl)quinolin-4-yl)-2-((1-methyl-1H-pyrrol-2-yl)methylene)hydrazine (**4n**)*

Compound **4n** was obtained as green solid. M.P 169–171 °C. Yield 70%. ^{1}H NMR (300 MHz, DMSO-d_6) δ; 3.94 (s, −NCH_3, 3H), 6.14 (m, pyrrole −CH, 1H), 6.61 (m, pyrrole −CH, 1H), 7.04 (m, pyrrole −NCH, 1H), 7.51 (s, −CH, 1H), 7.71 (t, −CH, 1H, *J* = 7.8 Hz), 8.22 (d, −CH, 1H, *J* = 6.9 Hz), 8.43 (s, −N=CH−, 1H), 8.69 (d, −CH, 1H, *J* = 8.4 Hz), 11.47 (s, −NH, 1H). ^{13}C NMR (75 MHz, DMSO-d_6) δ; 33.4, 96.2, 108.6, 110.2, 119.1, 120.0, 122.7, 125.6, 126.2, 126.4, 129.2, 129.5, 131.9, 139.3, 151.8, 152.3, 156.6. LC−MS (ESI) *m*/*z* 387 (M + 1). Anal. Calcd for $C_{17}H_{12}F_6N_4$; Calc: C, 52.86; H, 3.13; N, 14.50; found: C, 52.89; H, 3.20; N, 14.56.

*6.5.15. (E)-1-(2,8-Bis(trifluoromethyl)quinolin-4-yl)-2-((benzo[c][1,2,5]oxadiazol-5-yl)methylene)hydrazine (**4o**)*

Compound **4o** was obtained as yellow solid. M.P 263–264 °C. Yield 85%. ^{1}H NMR (300 MHz, DMSO-d_6) δ; 7.79–7.84 (m, −CH, 2H), 8.11 (d, −CH, 1H, *J* = 9.3 Hz), 8.24–8.33 (m, −CH, 3H), 8.59 (s, −N=CH−, 1H), 8.74 (d, −CH, 1H, *J* = 8.7 Hz), 12.07 (s, −NH, 1H). ^{13}C NMR (75 MHz, DMSO-d_6) δ; 97.3, 111.8, 117.9, 119.7, 120.2, 122.3, 123.6, 125.1, 125.4, 126.1, 126.6, 127.0, 127.3, 129.6, 129.8, 130.9, 134.6, 144.3, 145.6, 145.8, 147.9, 148.4, 149.6. LC−MS (ESI) *m*/*z* 426 (M + 1). Anal. Calcd for $C_{18}H_9F_6N_5O$; Calc: C, 50.83; H, 2.13; N, 16.47; found: C, 50.88; H, 2.19; N, 16.55.

*6.5.16. (E)-1-(2,8-Bis(trifluoromethyl)quinolin-4-yl)-2-((1-methyl-1H-benzo[d][1,2,3]triazol-5-yl)methylene)hydrazine (**4p**)*

Compound **4p** was obtained as pale yellow solid. M.P. >300 °C. Yield 82%. ^{1}H NMR (300 MHz, DMSO-d_6) δ; 4.33 (s, −NCH_3, 3H), 7.74–7.79 (m, −CH, 2H), 7.91 (d, −CH, 1H, *J* = 8.7 Hz), 8.16 (d, −CH, 1H, *J* = 9 Hz), 8.22 (d, −CH, 1H, *J* = 7.2 Hz), 8.35 (s, −CH, 1H), 8.62 (s,

−N=CH−, 1H), 8.71 (d, −CH, 1H, $J = 8.7$ Hz), 11.77 (s, −NH, 1H). ^{13}C NMR (75 MHz, DMSO-d_6) δ; 34.7, 97.2, 111.2, 117.2, 119.1, 120.2, 122.5, 123.8, 125.0, 125.6, 126.1, 126.6, 127.0, 127.3, 129.7, 129.8, 130.9, 134.6, 144.3, 145.8, 145.9, 147.9, 148.4, 149.7. LC−MS (ESI) *m*/*z* 439 (M + 1). Anal. Calcd for $C_{19}H_{12}F_6N_6$; Calc: C, 52.06; H, 2.76; N, 19.17; found: C, 52.12; H, 2.80; N, 19.23.

*6.5.17. (E)-1-(2,8-Bis(trifluoromethyl)quinolin-4-yl)-2-((7-methyl-1H-indol-3-yl)methylene)hydrazine (**4q**)*

Compound **4q** was obtained as yellow solid. M.P. 283−284 °C. Yield 73%. ^{1}H NMR (300 MHz, DMSO-d_6) δ; 2.51 (s, $-CH_3$, 3H), 7.06 (d, −CH, 1H, $J = 7.2$ Hz), 7.15 (t, −CH, 1H, $J = 7.8$ Hz), 7.68 (s, −CH, 1H), 7.76 (t, −CH, 1H, $J = 8.1$ Hz), 7.96 (m, −CH, 1H), 8.08 (d, −CH, 1H, $J = 7.8$ Hz), 8.22 (d, −CH, 1H, $J = 7.2$ Hz), 8.71−8.76 (m, −CH, −N=CH−, 2H), 11.50 (s, −NH, 1H), 11.70 (brs, indole −NH, 1H). ^{13}C NMR (75 MHz, DMSO-d_6) δ; 17.2, 96.1, 112.3, 117.9, 119.2, 120.3, 121.4, 121.8, 123.8, 124.0, 124.3, 125.1, 126.6, 127.0, 127.4, 129.6, 131.1, 137.1, 144.2, 144.6, 148.0, 148.4, 149.7. LC−MS (ESI) *m*/*z* 437 (M + 1). Anal. Calcd for $C_{21}H_{14}F_6N_4$; Calc: C, 57.80; H, 3.23; N, 12.84; found: C, 57.85; H, 3.30; N, 12.93.

*6.5.18. (E)-1-(2,8-Bis(trifluoromethyl)quinolin-4-yl)-2-((5-(acetoxymethyl)furan-2-yl)methylene)hydrazine (**4r**)*

Compound **4r** was obtained as white solid. M.P 220−221 °C. Yield 70%. ^{1}H NMR (300 MHz, DMSO-d_6) δ; 2.08 (s, $-CH_3$, 3H), 5.14 (s, $-CH_2$, 2H), 6.70 (d, −CH, 1H, $J = 3$ Hz), 7.03 (d, −CH, 1H, $J = 3.3$ Hz), 7.62 (s, −CH, 1H), 7.80 (t, −CH, 1H, $J = 7.8$ Hz), 8.26 (d, −CH, 1H, $J = 7.2$ Hz), 8.34 (s, −N=CH−, 1H), 8.69 (d, −CH, 1H, $J = 8.4$ Hz), 11.73 (s, −CH, 1H). ^{13}C NMR (75 MHz, DMSO-d_6) δ; 21.0, 58.0, 97.0, 113.5, 115.1, 118.0, 125.8, 127.3, 129.9, 130.0, 135.8, 144.4, 147.9, 148.3, 149.6, 150.0, 152.0, 170.4 (−C=O). LC−MS (ESI) *m*/*z* 446 (M + 1). Anal. Calcd for $C_{19}H_{13}F_6N_3O_3$; Calc: C, 51.25; H, 2.94; N, 9.44; found: C, 51.33; H, 2.99; N, 9.53.

*6.5.19. (E)-1-(2,8-Bis(trifluoromethyl)quinolin-4-yl)-2-((4-methyl-1H-imidazol-5-yl)methylene)hydrazine (**4s**)*

Compound **4s** was obtained as yellow solid. M.P. 266−268 °C. Yield 65%. ^{1}H NMR (300 MHz, DMSO-d_6) δ; 2.43 (s, $-CH_3$, 3H), 7.70−7.77 (m, −CH, 3H), 8.21 (d, −CH, 1H, $J = 7.2$ Hz), 8.46 (s, −N=CH−, 1H), 8.66 (d, −CH, 1H, $J = 8.4$ Hz), 11.46 (s, −NH, 1H), 12.34 (brs, imidazole −NH, 2H). LC−MS (ESI) *m*/*z* 388 (M + 1). Anal. Calcd for $C_{16}H_{11}F_6N_5$; Calc: C, 49.62; H, 2.86; N, 18.08; found: C, 49.69; H, 2.93; N, 18.14.

*6.5.20. (E)-1-(2,8-Bis(trifluoromethyl)quinolin-4-yl)-2-((5-methoxy-1H-indol-3-yl)methylene)hydrazine (**4t**)*

Compound **4t** was obtained as yellow solid. M.P. 238−240 °C. Yield 72%. ^{1}H NMR (300 MHz, DMSO-d_6) δ; 3.89 (s, $-OCH_3$, 3H), 6.89 (m, −CH, 1H), 7.40 (d, −CH, 1H, $J = 8.7$ Hz), 7.72−7.91 (m, −CH, −N=CH−, 4H), 8.22 (d, −CH, 1H, $J = 7.5$ Hz), 8.70−8.74 (m, −CH, 2H), 11.53 (s, −NH, 1H), 11.59 (brs, indole −NH, 1H). ^{13}C NMR (75 MHz, DMSO-d_6) δ; 55.9, 95.6, 101.3, 109.6, 111.3, 117.6, 119.0, 119.8, 123.2, 125.6, 126.1, 126.3, 127.1, 127.8, 129.2, 129.3, 130.9, 143.1, 146.5, 148.7, 149.9, 156.6. LC−MS (ESI) *m*/*z* 452 (M + 1). Anal. Calcd for $C_{21}H_{14}F_6N_4O$; Calc: C, 55.76; H, 3.12; N, 12.39;foundC, 55.82; H, 3.19; N, 12.32.

*6.6. General procedure for the synthesis of title compounds (**5a**−**e**, **6a**−**c**)*

To a suspension of **3** (2 mmol) in dry toluene (4 mL) equimolar quantity of substituted iso(thio)cyanate (2 mmol) was added slowly and the reaction mixture was heated at 110 °C for 30 min. The completion of the reaction was monitored by TLC. The solid obtained on cooling was filtered and washed with *n*-hexane (50 mL).

*6.6.1. 1-(2,8-Bis(trifluoromethyl)quinolin-4-yl)-4-(4-fluorophenyl) semicarbazide (**5a**)*

Compound **5a** was obtained as white solid. M.P. 234−236 °C. Yield 88%. ^{1}H NMR (300 MHz, DMSO-d_6) δ; 7.08−7.13 (m, −CH, 3H), 7.47−7.52 (m, −CH, 2H), 7.79 (t, −CH, 1H, $J = 8.1$ Hz), 8.26 (d, −CH, 1H, $J = 7.5$ Hz), 8.65 (d, −CH, 1H, $J = 8.4$ Hz), 8.80 (s, −NH, 1H), 9.13 (s, −NH, 1H), 10.00 (s, −CONH, 1H). ^{13}C NMR (75 MHz, DMSO-d_6) δ; 95.6, 115.4, 115.7, 118.5, 120.1, 121.4, 122.5, 123.8, 125.7, 126.1, 126.6, 127.0, 127.4, 127.7, 128.6, 129.3, 129.8, 129.9, 136.1, 144.1, 148.0, 148.4, 154.6, 156.0, 156.5, 159.6. LC−MS (ESI) *m*/*z* 433 (M + 1). Anal. Calcd for $C_{18}H_{11}F_7N_4O$; Calc: C, 50.01; H, 2.56; N, 12.96; found: C, 50.05; H, 2.63; N, 12.88.

*6.6.2. 1-(2,8-Bis(trifluoromethyl)quinolin-4-yl)-4-(3-cyanophenyl) semicarbazide (**5b**)*

Compound **5b** was obtained as white solid. M.P. 242−245 °C. Yield 82%. ^{1}H NMR (300 MHz, DMSO-d_6) δ; 7.09 (s, −CH, 1H), 7.42−7.51 (m, −CH, 2H), 7.80 (m, −CH, 1H), 7.98 (s, −CH, 1H), 8.27 (d, −CH, 1H, $J = 7.2$ Hz), 8.65 (d, −CH, 1H, $J = 8.7$ Hz), 9.03 (s, −NH, 1H), 9.42 (brs, −NH, 1H), 10.05 (s, −CONH, 1H). ^{13}C NMR (75 MHz, DMSO-d_6) δ; 95.7, 112.1, 118.5, 119.2, 120.1, 122.1, 123.6, 124.0, 125.7, 126.1, 126.7, 127.1, 128.6, 129.3, 130.4, 130.6, 137.7, 140.8, 144.1, 148.1, 154.5, 155.8. LC−MS (ESI) *m*/*z* 440 (M + 1). Anal. Calcd for $C_{19}H_{11}F_6N_5O$; Calc: C, 51.95; H, 2.52; N, 15.94; found: C, 52.03; H, 2.59; N, 15.98.

*6.6.3. 1-(2,8-Bis(trifluoromethyl)quinolin-4-yl)-4-(2-methoxyphenyl)semicarbazide (**5c**)*

Compound **5c** was obtained as white solid. M.P 225−227 °C. Yield 84%. ^{1}H NMR (300 MHz, DMSO-d_6) δ; 3.84 (s, OCH_3, 3H), 6.85−6.91 (m, −CH, 1H), 6.96−7.04 (m, −CH, 2H), 7.09 (s, −CH, 1H), 7.79 (m, −CH, 1H, $J = 8.1$), 7.97 (d, −CH, 1H, $J = 7.5$ Hz), 8.26 (d, −CH, 1H, $J = 7.2$ Hz), 8.39 (s, −NH, 1H), 8.64 (d, −CH, 1H, $J = 8.4$ Hz), 9.07 (s, −NH, 1H), 10.00 (brs, −CONH, 1H). ^{13}C NMR (75 MHz, DMSO-d_6) δ; 56.2, 95.7, 111.3, 118.3, 119.5, 120.1, 121.0, 122.5, 123.1, 123.7, 125.9, 126.1, 126.7, 127.1, 127.3, 128.4, 129.9, 144.1, 148.1, 148.5, 148.8, 154.7, 155.4. LC−MS (ESI) *m*/*z* 445 (M + 1). Anal. Calcd for $C_{19}H_{14}F_6N_4O_2$; Calc: C, 51.36; H, 3.18; N, 12.61; found: C, 51.44; H, 3.11; N, 12.67.

*6.6.4. 1-(2,8-Bis(trifluoromethyl)quinolin-4-yl)-4-(3,5-dimethylphenyl)semicarbazide (**5d**)*

Compound **5d** was obtained as white solid. M.P 252−255 °C. Yield 89%. ^{1}H NMR (300 MHz, DMSO-d_6) δ; 2.20 (s, $-(CH_3)_2$, 6H), 6.62 (s, −CH, 1H), 7.07−7.12 (m, −CH, 3H), 7.78 (m, −CH, 1H), 8.26 (d, −CH, 1H, $J = 7.2$ Hz), 8.65 (d, −CH, 1H, $J = 8.4$ Hz), 8.72 (s, −NH, 1H), 8.91 (s, −NH, 1H), 9.98 (brs, −CONH, 1H). ^{13}C NMR (75 MHz, DMSO-d_6) δ; 21.5, 95.6, 117.3, 118.5, 120.1, 122.5, 123.8, 124.3, 125.6, 126.1, 126.6, 127.0, 127.7, 129.8, 129.9, 137.9, 139.6, 144.1, 148.0, 148.4, 154.7, 155.8. LC−MS (ESI) *m*/*z* 443 (M + 1). Anal. Calcd for $C_{20}H_{16}F_6N_4O$; Calc: C, 54.30; H, 3.65; N, 12.67; found: C, 54.38; H, 3.69; N, 12.73.

*6.6.5. 1-(2,8-Bis(trifluoromethyl)quinolin-4-yl)-4-pentylsemicarbazide (**5e**)*

Compound **5e** was obtained as white solid. M.P 181−182 °C. Yield 62%. ^{1}H NMR (300 MHz, DMSO-d_6) δ; 0.82 (t, $-CH_3$, 3H, $J = 6.9$ Hz), 1.21−1.25 (m, $-(CH_2)_2$, 4H), 1.35−1.39 (m, $-CH_2$, 2H), 3.01−3.03 (m, $-NCH_2$, 2H), 6.86 (m, −CH, 1H), 6.97 (s, −CH, 1H), 7.74 (t, −CH, 1H, $J = 7.8$ Hz), 8.23 (d, −CH, 1H, $J = 6.9$ Hz), 8.37 (s, −NH, 1H), 8.62 (d, −CH, 1H, $J = 8.4$ Hz), 9.84 (brs, −CONH, 1H). ^{13}C NMR (75 MHz, DMSO-d_6) δ; 14.2, 22.3, 28.8, 29.9, 40.4, 95.4, 118.5, 120.3, 122.2, 123.8, 126.2, 126.3, 125.4, 127.8, 129.7, 129.9, 144.1, 148.4, 154.7, 158.2. LC−MS (ESI) *m*/*z* 409 (M + 1). Anal. Calcd for $C_{17}H_{18}F_6N_4O$; Calc: C, 50.00; H, 4.44; N, 13.72; found: C, 50.12; H, 4.52; N, 13.79.

6.6.6. *1-(2,8-Bis(trifluoromethyl)quinolin-4-yl)-4-(2-fluorophenyl) thiosemicarbazide (**6a**)*

Compound **6a** was obtained as white solid. M.P 199–201 °C. Yield 54%. ^{1}H NMR (300 MHz, DMSO-d_6) δ; 7.00 (s, −CH, 1H), 7.15–7.31 (m, −CH, 4H), 7.79 (m, −CH, 1H), 8.27 (d, −CH, 1H, $J = 7.2$ Hz), 8.62 (d, −CH, 1H, $J = 8.1$ Hz), 9.96 (brs, −CSNH, 1H), 10.38 (brs, −NH, 1H), 10.41 (brs, −NH, 1H). ^{13}C NMR (75 MHz, DMSO-d_6) δ; 96.0, 116.1, 116.4, 118.8, 120.1, 122.5, 123.7, 124.5, 125.7, 126.1, 126.6, 127.0, 127.3, 128.2, 129.1, 129.9, 130.9, 144.0, 148.0, 148.5, 153.3, 156.3, 183.1. LC–MS (ESI) *m/z* 449 (M + 1). Anal. Calcd for $C_{18}H_{11}F_7N_4S$; Calc: C, 48.22; H, 2.47; N, 12.50; S, 7.15; found: C, 48.29; H, 2.59; N, 12.58; S, 7.21.

6.6.7. *1-(2,8-Bis(trifluoromethyl)quinolin-4-yl)-4-(2-methoxyethyl) thiosemicarbazide (**6b**)*

Compound **6b** was obtained as white solid. M.P 226–227 °C. Yield 45%. ^{1}H NMR (300 MHz, DMSO-d_6) δ; 3.18 (s, $-OCH_3$, 3H), 3.43 (t, $-CH_2$, 2H, $J = 5.4$ Hz), 3.59 (t, $-CH_2$, 2H, $J = 5.4$ Hz), 6.84 (s, −CH, 1H), 7.78 (t, −CH, 1H, $J = 7.8$ Hz), 8.26 (d, −CH, 1H, $J = 7.2$ Hz), 8.54 (brs, −CSNH, 1H), 8.57 (d, −CH, 1H, $J = 8.4$ Hz), 9.87(brs, −NH, 1H), 10.14 (brs, −NH, 1H). ^{13}C NMR (75 MHz, DMSO-d_6) δ; 43.7, 58.3, 70.4, 95.8, 118.7, 120.0, 122.5, 123.7, 125.6, 126.1, 126.6, 127.0, 128.1, 129.8, 144.0, 148.0, 148.4, 153.5, 182.2. LC–MS (ESI) *m/z* 413 (M + 1). Anal. Calcd for $C_{15}H_{14}F_6N_4OS$; Calc: C, 43.69; H, 3.42; N, 13.59; S, 7.78; found: C, 43.75; H, 3.48; N, 13.52; S, 7.83.

6.6.8. *1-(2,8-Bis(trifluoromethyl)quinolin-4-yl)-4-(3−CHlorophenyl)semicarbazide (**6c**)*

Compound **6c** was obtained as yellow solid. M.P 158–161 °C. Yield 36%. ^{1}H NMR (300 MHz, DMSO-d_6) δ; 7.17–7.21 (m, −CH, 2H), 7.30 (s, −CH, 1H), 7.36 (d, −CH, 1H, $J = 7.5$), 7.59–7.68 (m, −CH, 1H), 8.09–8.15 (m, −CH, 1H), 8.33 (d, −CH, 1H, $J = 7.2$ Hz), 8.68 (d, −CH, 1H, $J = 8.1$ Hz), 9.89 (brs, −CSNH, 1H), 10.38 (brs, −NH, 1H), 10.40 (brs, −NH, 1H). LC–MS (ESI) *m/z* 465 (M + 1). Anal. Calcd for $C_{18}H_{11}ClF_6N_4S$; Calc: C, 46.51; H, 2.39; 12.05; S, 6.90; found: C, 46.57; H, 2.46; 12.09; S, 6.97.

6.7. *General procedure for the synthesis of title compounds (**7a–d**)*

To a suspension of **3** (2 mmol) in ethanol (10 mL) was added substituted acetoacetate (2 mmol). The reaction mixture was stirred for 30 min, to it was added sodium ethoxide (2 mmol) slowly and the reaction mixture was heated at 80 °C for 30 min. The completion of the reaction was monitored by TLC. Reaction mixture was concentrated and the brown residue obtained was purified by column chromatography using pet ether/ethyl acetate (2:1) as the eluent.

6.7.1. *1-(2,8-Bis(trifluoromethyl)quinolin-4-yl)-3-methyl-1H-pyrazol-5(4H)-one (**7a**)*

Compound **7a** was obtained as off white solid. M.P 124–126 °C. Yield 58%. ^{1}H NMR (300 MHz, $CDCl_3$) δ; 2.32 (s, $-CH_3$, 3H), 3.59 (s, $-CH_2$, 2H), 7.71 (m, $-CH_2$, 2H), 8.00 (s, −CH, 1H), 8.21 (d, −CH, 1H, $J = 6.9$ Hz), 8.36 (d, −CH, 1H, $J = 8.7$ Hz). ^{13}C NMR (75 MHz, $CDCl_3$) δ; 17.2, 42.0, 110.0, 112.0, 119.1, 121.6, 122.7, 123.4, 125.2, 126.6, 128.7, 129.1, 129.2, 129.4, 143.2, 145.3, 148.3, 148.8, 158.5, 171.5. LC–MS (ESI) *m/z* 362 (M + 1). Anal. Calcd for $C_{15}H_9F_6N_3O$; Calc: C, 49.87; H, 2.51; N, 11.63; found: C, 49.81; H, 2.57; N, 11.69.

6.7.2. *1-(2,8-Bis(trifluoromethyl)quinolin-4-yl)-3-(2-(benzo[d][1,3] dioxol-6-yl)ethyl)-1H-pyrazol-5(4H)-one (**7b**)*

Compound **7b** was obtained as brown liquid. Yield 47%. ^{1}H NMR (300 MHz, $CDCl_3$) δ; 2.88–3.01 (m, $-(CH_2)_2$, 4H), 3.49 (s, $-CH_2$, 2H), 5.98 (s, $-CH_2$, 2H), 6.69–6.81 (m, −CH, 3H), 7.71 (t, −CH, 1H, $J = 8.1$ Hz), 7.96 (s, −CH, 1H), 8.23 (m, −CH, 2H). ^{13} C NMR (75 MHz, $CDCl_3$) δ; 32.4, 37.1, 41.8, 101.2, 112.8, 115.2, 119.0, 119.6, 121.4, 125.3, 126.1, 126.4, 129.2, 129.3, 129.6, 146.1, 148.7, 148.7, 152.6, 155.6, 156.4, 172.6. LC–MS (ESI) *m/z* 496 (M + 1). Anal. Calcd for $C_{23}H_{15}F_6N_3O_3$; Calc: C, 55.77; H, 3.05; N, 8.48; found: C, 55.82; H, 3.09; N, 8.52.

6.7.3. *1-(2,8-Bis(trifluoromethyl)quinolin-4-yl)-3-phenyl-1H-pyrazol-5(4H)-one (**7c**)*

Compound **7c** was obtained as off white solid. M.P 196–198 °C. Yield 63%. ^{1}H NMR (300 MHz, $CDCl_3$) δ; 4.02 (s, $-CH_2$, 2H), 7.48–7.57 (m, −CH, 3H), 7.71–7.82 (m, −CH, 3H), 8.09 (s, −CH, 1H), 8.24 (d, −CH, 1H, $J = 7.2$ Hz), 8.47 (d, −CH, 1H, $J = 8.7$ Hz). LC–MS (ESI) *m/z* 424 (M + 1). Anal. Calcd for $C_{20}H_{11}F_6N_3O$; Calc: C, 56.75; H, 2.62; N, 9.93; found: C, 56.79; H, 2.68; N, 9.97.

6.7.4. *1-(2,8-Bis(trifluo romethyl)quinolin-4-yl)-3-(pyridin-4-yl)-1H-pyrazol-5(4H)-one (**7d**)*

Compound **7d** was obtained as pale yellow solid. M.P 268–271 °C. Yield 40%. ^{1}H NMR (300 MHz, $CDCl_3$) δ; 6.36 (s, −CH, 1H), 7.87 (m, −CH, 2H), 8.02 (t, −CH, 1H, $J = 7.8$ Hz), 8.38 (s, −CH, 1H), 8.46 (d, −CH, 1H, $J = 7.2$ Hz), 8.66–8.58 (m, −CH, 3H), 12.75 (brs, −OH, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ; 86.1, 101.2, 114.8, 119.2, 120.3, 123.1, 124.7, 125.2, 126.6, 129.0, 130.7, 131.0, 133.3, 140.7, 144.8, 150.3, 150.6, 156.7. LC–MS (ESI) *m/z* 425 (M + 1). Anal. Calcd for $C_{19}H_{10}F_6N_4O$; Calc: C, 53.78; H, 2.38; N, 13.20; found: C, 53.87; H, 2.46; N, 13.27.

6.8. *Antibacterial testing by serial plate dilution method*

Serial dilutions of the drug in Muller–Hinton broth were taken in tubes and their pH was adjusted to 5.0 using phosphate buffer. A standardized suspension of the test bacterium was inoculated and incubated for 16–18 h at 37 °C. The minimum inhibitory concentration (MIC) was noted by seeing the lowest concentration of the drug at which there was no visible growth. A number of antimicrobial discs are placed on the agar for the sole purpose of producing zones of inhibition in the bacterial lawn. Twenty milliliters of agar media was poured into each Petri dish. Excess of suspension was decanted and plates were dried by placing in an incubator at 37 °C for 1 h. Using an agar punch, wells were made on these seeded agar plates and minimum inhibitory concentrations of the test compounds in dimethylsulfoxide (DMSO) were added into each labeled well. A control was also prepared for the plates in the same way using solvent DMSO. The Petri dishes were prepared in triplicate and maintained at 37 °C for 3–4 days. Antibacterial activity was determined by measuring the diameter of inhibition zone. Activity of each compound was compared with ciprofloxacin as standard [27,28].

6.9. *Antituberculosis testing by broth microdilution assay method*

The anti-TB activity of the compounds was tested by resazurin micro plate assay (REMA) as per Martin et al., with slight modification. Resazurin, a redox dye, is blue in its oxidized state. In the presence of viable cells it is reduced into resorufin, which is pink in color. *M. tuberculosis* H37Rv was grown in Middlebrook 7H9 broth (Difco BBL, Sparks, MD, USA) supplemented with 10% OADC (Becton Dickinson, Sparks, MD, USA) and 0.5% glycerol. The optical density of the bacterial culture was adjusted to McFarland 1.0 unit and 50 μL from this suspension was used as the inoculum. Stock solutions of the test compounds were prepared in dimethyl formamide (DMF) and were added to fresh medium in the wells of a 96-well micro plate to which 50 μl inoculum was added making the total assay volume 200 μL. The final concentrations of the test molecules were 1.56, 3.12, 6.25, 12.5, 25 and 50 μM. Growth control wells contained medium and *M. tuberculosis* alone. Rifampicin (0.5 μM) and isoniazid (1.5 μM) served as positive control for inhibition of growth. Negative control wells contained the highest volume of DMF used in test wells without any compound. After incubation at 37 °C for 7 days, 15 μl of 0.01% resazurin (Sigma, St. Louis. MO, USA) solution in sterile water was added to the first growth control wells and incubated for 24 h. Once the first set of growth controls turned pink, the dye solution was added to the second set of growth controls and the test wells, and incubated for 24 h at 37 °C. Blue color in the wells containing the test compounds would indicate inhibition of growth and pink would indicate lack of inhibition of growth of *M. tuberculosis*.

Acknowledgments

Authors are thankful to Dr. Ganesh Sambhasivam, CEO, Anthem biosciences, Bangalore, India, for his invaluable support and allocation of resources for this work. They are thankful to Dr. Suchetha Shetty, Department of Biochemistry, Justice K.S. Hegde Medical Academy, Deralakatte, India, for carrying out antibacterial screening. They are also grateful to Dr. Guru Row, Solid State and Structural Chemistry Unit (SSCU), II.Sc, Bangalore, India, for carrying out single crystal X-Ray diffraction studies.

References

[1] WHO. Global tuberculosis control report (2008).

[2] R. Jain, B. Vaitilingam, A. Nayyar, P.B. Palde, Bioorg. Med. Chem. Lett. 13 (2003) 1051–1054.

[3] WHO. Weekly epidemiological record No. 15. 78 (2003) 121–128.

[4] M. Zignol, M.S. Hosseini, A. Wright, C.L. Weezenbeek, P. Nunn, C.J. Watt, C. G. Williams, C. Dye, J. Infect. Dis. 194 (2006) 479–485.

[5] A. Lilienkampf, J. Mao, B. Wan, Y. Wang, S.G. Franzblau, A.P. Kozikowski, J. Med. Chem. 52 (2009) 2109–2118.

[6] P. Nasveld, S. Kitchener, Trans. R. Soc. Trop. Med. Hyg. 99 (2005) 2–5.

[7] P.A. Leatham, H.A. Bird, V. Wright, D. Seymour, A. Gordon, Eur. J. Rheumatol. Inflamm. 6 (1983) 209–211.

[8] W.A. Denny, W.R Wilson, D.C Ware, G.J. Atwell, J.B Milbank, R.J Stevenson. U.S Patent 7064117(2006).

[9] A. Mahamoud, J. Chevalier, A. Davin-Regli, J. Barbe, Jean-Marie Pages, Curr. Drug Targ. 7 (2006) 843–847.

[10] N. Muruganantham, R. Sivakumar, N. Anbalagan, V. Gunasekaran, J.T. Leonard, Biol. Pharm. Bull. 27 (2004) 1683–1687.

[11] M.P. Maguire, K.R. Sheets, K. McVety, A.P. Spada, A. Zilberstein, J. Med. Chem. 37 (1994) 2129–2137.

[12] W.D. Wilson, M. Zhao, S.E. Patterson, R.L. Wydra, L. Janda, L. Strekowski, Med. Chem. Res. 2 (1992) 102–110.

[13] L. Strekowski, J.L. Mokrosz, V.A. Honkan, A. Czarny, M.T. Cegla, S.E. Patterson, R.L. Wydra, R.F. Schinazi, J. Med. Chem. 34 (1991) 1739–1746.

[14] Z. Murai, B. Baran, J. Tolna, E. Szily, G. Gazdag, Orv. Hetil. 146 (2005) 133–136.

[15] C.M. Kunin, W.Y. Ellis, Antimicrob. Agents Chemother. 44 (2000) 848–852.

[16] J. Mao, H. Yuan, Y. Wang, B. Wan, M. Pieroni, Q. Huang, R.B. Breemen, A. P. Kozikowski, S.G. Franzblau, J. Med. Chem. 52 (2009) 6966–6978.

[17] L.E. Bermudez, P. Kolonoski, L.E. Seitz, M. Petrofsky, R. Reynolds, M. Wu, L. S. Young, Antimicrob. Agents Chemother. 48 (2004) 3556–3558.

[18] S. Jayaprakash, Y. Iso, B. Wan, S.G. Franzblau, A.P. Kozikowski, Chem. Med. Chem. 1 (2006) 593–597.

[19] K. Andries, P. Verhasselt, J. Guillemont, H.W.H. Gohlmann, J.M. Neefs, H. Winkler, J. Van Gestel, P. Timmerman, M. Zhu, E. Lee, P. Williams, D. de Chaffoy, E. Huitric, S. Hoffner, E. Cambau, C. Truffot-Pernot, N. Lounis, V. Jarlier, Science 307 (2005) 223–227.

[20] S. Eswaran, A.V. Adhikari, N.S. Shetty, Eur. J. Med. Chem. 44 (2009) 4637–4647.

[21] S. Eswaran, A.V. Adhikari, R.A Kumar, Eur. J. Med. Chem. 45 (2009) 957–966.

[22] S. Eswaran, A.V. Adhikari, I.H. Chowdhury, Bioorg. Med. Chem. Lett. 20 (2010) 1040–1044.

[23] A.L. Barry, Procedure for testing antimicrobial agents in agar media. in: V. L. Corian (Ed.), Antibiotics in Laboratory Medicine. Williams and Wilkins, Baltimore, MD, 1980, pp. 1–23.

[24] D. James, Mac Lowry, J.M. Jaqua, T.S. Sally, Appl. Microbiol. 20 (1970) 46–53.

[25] A. Martin, M. Camacho, F. Portaels, J.C. Palomino, Resazurin microtiter assay plate testing of *Mycobacterium tuberculosis* susceptibilities to second-line drugs: rapid, simple, and inexpensive method. Antimicrob. Agents Chemother. 47 (2003) 3616–3619.

[26] A.Y. Coban, C.C. Cekic, K. Bilgin, M. Uzun, A. Akgunes, E. Cetinkaya, B. Durupinar, Rapid susceptibility test for *Mycobacterium tuberculosis* to isoniazid and rifampin with resazurin method in screw-cap tubes. J. Chemother. 18 (2006) 140–143.

[27] C.H. Fenlon, M.H. Cynamon, Antimicrob. Agents Chemother. 29 (1986) 386–388.

[28] R. Davis, A. Markham, J.A. Balfour, Ciprofloxacin: an updated review of its pharmacology, therapeutic efficacy and tolerability. Drugs 51 (1996) 1019–1074.