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Original article

Synthesis and identification of β-aryloxyquinoline based diversely fluorine substituted *N*-aryl quinolone derivatives as a new class of antimicrobial, antituberculosis and antioxidant agents

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ABSTRACT

A new class of β-aryloxyquinoline based diversely fluorine substituted *N*-aryl quinolone derivatives **8a**–**x** have been synthesized *via* a one-pot multicomponent reaction. *In vitro* antimicrobial activity of the synthesized compounds was investigated against a representative panel of pathogenic strains. Compounds **8g**, **8h**, **8m**, **8q** and **8v** exhibited excellent antimicrobial activity compared with first line drugs. *In vitro* antituberculosis activity was evaluated against *Mycobacterium tuberculosis* H37Rv and compounds **8h** and **8q** emerged as the promising antimicrobial member with better antituberculosis activity. The brine shrimp bioassay was carried out to study the *in vitro* cytotoxic properties of the synthesized compounds. *In vitro* antioxidant activity was evaluated by ferric-reducing antioxidant power method. Compounds **8e**, **8k**, **8l**, **8s**, **8u** and **8w** showed highest antioxidant potency.

1. Introduction

Fluorine has played a pivotal role in novel drug discovery for modulating physical and biological properties of the molecule. Due to its higher electronegativity, incorporation of fluorine atom(s) within the molecule can enhance their biopotency, bioavailability, metabolic stability and lipophilicity [1]. Trifluoromethylation is one of the most significant strategies to improve pharmacological activities of the molecule due to its high lipophilicity, thereby enhancing *in vivo* uptake and transport of the candidate [2].

Emerging infectious diseases and the increasing number of multi-drug resistant microbial pathogens still make the treatment of infectious diseases an important and pressing global health problem. Therefore, there is a vital need to discover new antimicrobial agents to avert the emergence of resistance and ideally shorten the duration of therapy [3,4]. Furthermore, the ever-increasing drug resistance, toxicity and side effects of currently used antituberculosis drugs, and the absence of their bactericidal activity highlight the need for new, safer and more effective antimycobacterial compounds. So this spot of research is accorded an immense meaning and keeps on attracting much attention of growing number of medicinal chemists.

Nowadays free metals become more harmful which play an important role in the pathogenesis of many diseases, accounting for continuing interest in the identification and development of novel antioxidants that prevent metal-induced damages. Compounds with antioxidant activity have been found to posses anticancer, anti-cardiovascular, anti-inflammatory and many other activities [5].

Over the past few years, we have been principally engrossed in the synthesis of quinoline incorporating structures for biological evaluations [6–10] on the premise that the quinoline moiety is found in a large variety of naturally occurring compounds and also chemically useful synthons bearing diverse bioactivities [5,11–19]. It has been well-established that presence of aryl ring appended by ether linkage at 2nd position of quinoline moiety is highly active against bacterial pathogen and plays a pivotal role in development of new antimicrobial drugs [6] and in consequence, emerged as a validated molecular target.

On the other hand, since the discovery of nalidixic acid, numerous quinolone derivatives have been prepared in search of improved antibacterial activities [20–22]. In the past decade, the so-called new fluorine substituted quinolones such as norfloxacin,

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ofloxacin, ciprofloxacin, temafloxacin, defloxacin, sparfloxacin, delafloxacin, lomefloxacin etc. which possess potent antibacterial activity has been discovered and used clinically [23] (Fig. 1).

Although their structure–activity relationship (SAR) has been extensively studied [20–26], the SAR for *N*-1 and C-4 of quinolone ring has not reached a clear conclusion. Studies by Koga et al. indicated that the antibacterial activity of quinolones is greatly influenced by the steric bulk of the *N*-1 substituent [24], Later, Chu et al. discovered that quinolones with fluorophenyl at the *N*-1 position also possess excellent activity [25,26].

In this study, we introduce substituted β-aryloxyquinolines at the C-4 position and diversely fluorine substituted aromatic ring at *N*-1 position on 5-quinolone for probing biological activity. An attempt has been through to undertake the synthesis of *N*-aryl quinolone bearing β-aryloxyquinoline derivatives with the assumption that the incorporation of more than one bioactive moieties into a single scaffold may produce novel heterocycles with fascinating antimicrobial activity along with antitubercular and antioxidant activity.

2. Chemistry

The synthetic approach adopted to obtain the target compounds is depicted in Scheme 1. The starting material 2-chloro-3-formylquinolines **1a**–**d** were prepared according to literature procedure [27] by Vilsmeier–Haack reaction and conveniently converted to β-aryloxyquinoline-3-carbaldehydes **3a**–**d** by nucleophilic displacement of chloro group at C2 in **1a**–**d** with *p*-cyano phenol **2** in refluxing dimethylformamide using anhydrous potassium carbonate as a base. The required β-enaminones **6a**–**f** were prepared by the reaction of β-diketones **4a**, **b** with fluorinated aromatic amines **5a**–**c** in methanol under reflux in the presence of a catalytic amount of acetic acid [28]. Subsequently, the one-pot three component cyclocondensation of a series of β-aryloxyquinoline-3-carbaldehydes **3a**–**d**, malononitrile **7** and β-enaminones **6a**–**f** in ethanol containing a catalytic amount of piperidine afforded the target compounds **8a**–**x** (Scheme 1) in good to excellent yields (Supplementary Table 1).

3. Pharmacology

3.1. Antimicrobial activity

The antimicrobial activity of synthesized compounds was carried out by broth microdilution method according to National Committee for Clinical Laboratory Standards (NCCLS) [29]. Antibacterial activity was screened against three Gram positive (*B. subtilis* MTCC 441, *C. tetani* MTCC 449, *S. pneumonia* MTCC 1936) and three Gram negative (*E. coli* MTCC 443, *S. typhi* MTCC 98, *V. cholerae* MTCC 3906) bacteria by using ampicillin, ciprofloxacin, norfloxacin and chloramphenicol as a standard antibacterial drug. Antifungal activity was screened against two fungal species (*A. fumigatus* MTCC 3008 and *C. albicans* MTCC 227) where griseofulvin and nystatin used as standard antifungal drugs. The antimicrobial screening data are shown in Table 1. All MTCC cultures were collected from Institute of Microbial Technology, Chandigarh and tested against above mentioned known drugs. Mueller Hinton broth was used as nutrient medium to grow and dilute the drug suspension for the test.

3.2. Antituberculosis activity

In vitro antituberculosis activity of all the newly synthesized compounds against *Mycobacterium tuberculosis* H37Rv strain was

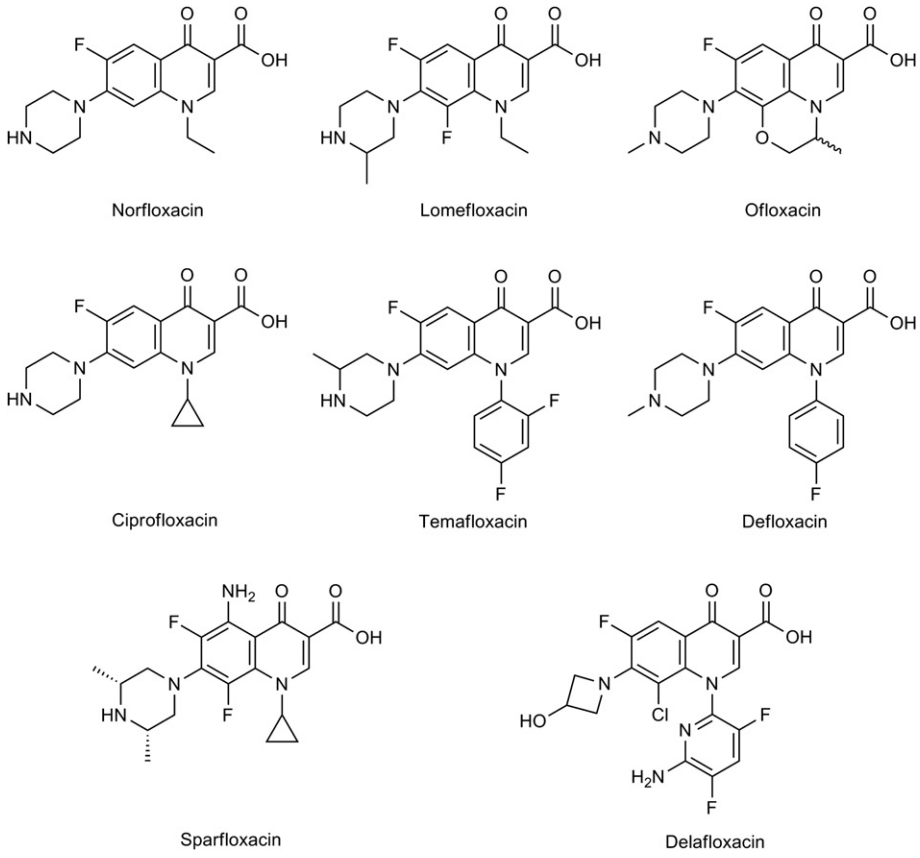
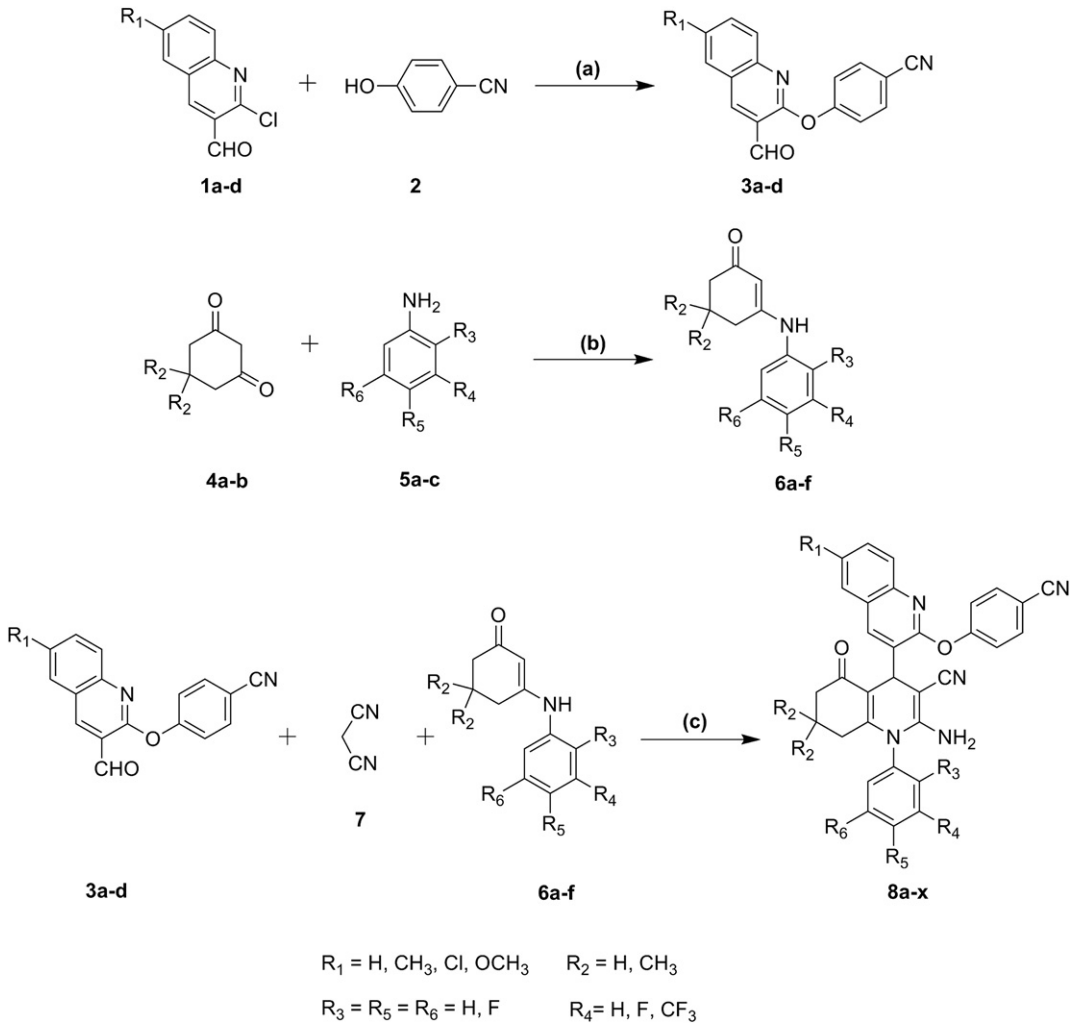


Fig. 1. Representative of fluorine substituted quinolone drugs.



Scheme 1. General synthetic route for the synthesis of title compounds **8a**–**x**.

determined by using Lowenstein-Jensen medium (conventional method) as described by Rattan [30] and the observed results are presented in Table 2 in the form of % inhibition, relative to that of standard antitubercular drugs isoniazid and rifampicin. Of the compounds studied, five compounds those exhibited highest % inhibition, were again screened to get their MIC values (Table 3).

3.3. Cytotoxicity (brine shrimp lethality bioassay)

The *in vitro* lethality test was carried out using brine shrimp eggs i.e. Artemia cysts. The Brine shrimp lethality bioassay is considered as a useful tool for preliminary toxicity assessment of bioactive compounds. Five compounds those exhibited highest % inhibition, were again screened for their cytotoxicity (Table 4) by using the protocol of Meyer et al. [31].

3.4. Antioxidant activity (FRAP assay)

Ferric reducing antioxidant power (FRAP) of newly synthesized compounds was measured by a modified method of Benzie and Strain [32]. The antioxidant potentials of the compounds **3a**–**d** and **8a**–**x** were estimated as their power to reduce the TPTZ-Fe(III) complex to TPTZ-Fe(II) complex. Absorbance of intensive blue colour [Fe(II)-TPTZ] complex was measured at 593 nm. The ascorbic acid was used as a standard antioxidant compound. The results were expressed as ascorbic equivalent (mmol/100 gm compound) and are listed in Table 5.

4. Results and discussion

4.1. Biological results

4.1.1. Antimicrobial activity

Upon investigation of antimicrobial activity data (Table 1), it has been observed that compounds **8g**, **8h** and **8q** showed excellent activity MIC = 62.5 μg/mL, 25 μg/mL and 62.5 μg/mL respectively against gram negative bacteria *V. cholerae* as compared to ampicillin MIC = 100 μg/mL, chloramphenicol 50 μg/mL and ciprofloxacin 25 μg/mL. In case of inhibiting *S. typhi*, compounds **8g** MIC = 50 μg/mL and **8m** 62.5 μg/mL were found to be exceedingly potent upon comparison with reference drugs. Towards *E. coli*, compounds **3b**, **8h** and **8v** MIC = 62.5 μg/mL have shown outstanding inhibitory effect as well as compound **8q** MIC = 62.5 μg/mL displayed strong inhibition against *S. pneumonia* as compared to the standard drugs. In case of inhibiting gram positive bacteria *B. subtilis* and *C. tetani*, all the compounds have shown good to excellent activity than that of standard drugs except **8y** and **8z**. Some of the compounds were found to be equipotent upon comparison with standard drugs.

Antifungal study revealed that all the synthesized β-arylox-yquinolines and their quinolyl quinoline derivatives have poor

Table 1
In vitro antimicrobial activity of β-aryloxyquinolines **3a**–**d** and their *N*-aryl quinolone derivatives **8a**–**x** (MICs, μg/mL).

Entry	Gram positive bacteria			Gram negative bacteria			Fungi	
	B.S. MTCC 441	C.T.MTCC 449	S.P. MTCC 1936	E.C. MTCC 443	S.T. MTCC 98	V.C. MTCC 3906	A.F. MTCC 3008	C.A. MTCC 227
3a	200	250	250	100	100	250	500	1000
3b	100	100	200	62.5	125	125	250	>1000
3c	125	125	125	250	200	200	250	>1000
3d	250	200	200	250	100	250	1000	1000
8a	100	125	250	100	250	200	250	>1000
8b	250	200	100	250	250	250	1000	500
8c	250	100	100	100	125	100	>1000	250
8d	200	100	200	125	200	100	500	500
8e	100	250	125	200	250	250	1000	1000
8f	250	200	200	250	200	200	1000	1000
8g	125	200	250	100	50	62.5	>1000	250
8h	200	100	100	62.5	200	25	>1000	>1000
8i	200	100	250	200	250	200	500	250
8j	250	250	200	200	250	250	1000	>1000
8k	200	125	250	250	250	100	>1000	250
8l	100	250	125	100	100	125	1000	>1000
8m	200	100	100	100	62.5	100	1000	250
8n	100	125	250	250	125	250	>1000	1000
8o	125	200	200	200	250	100	>1000	1000
8p	200	125	100	125	200	250	500	500
8q	200	200	62.5	250	200	62.5	1000	1000
8r	250	250	125	125	125	100	1000	>1000
8s	200	125	125	250	100	250	1000	1000
8t	250	200	250	200	250	200	>1000	>1000
8u	200	200	200	500	500	250	1000	1000
8v	250	250	250	62.5	200	100	>1000	>1000
8w	200	500	125	200	125	100	1000	500
8x	250	500	200	125	200	250	500	1000
Ampicillin	250	250	100	100	100	100	n.t.[a]	n.t.
Chloramphenicol	50	50	50	50	50	50	n.t.	n.t.
Ciprofloxacin	50	100	50	25	25	25	n.t.	n.t.
Norfloxacin	100	50	10	10	10	10	n.t.	n.t.
Griseofulvin	n.t.	n.t.	n.t.	n.t.	n. t.	n.t.	100	500
Nystatin	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	100	100

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

activity against *A. fumigatus*. In comparison with standard fungicidal griseofulvin MIC = 500 μg/mL, compounds **8c**, **8g**, **8i**, **8k** and **8m** MIC = 250 μg/mL were found to posses better inhibitory results towards fungal pathogen *C. albicans*.

The anti-microorganism tests with all four β-aryloxyquinolines and their twenty four fluorine substituted *N*-aryl quinolone derivatives (Table 1) have established some interesting structure-activity relationships. Upon cyclocondensation of compounds **3a**–**d** with malononitrile and various β-enaminones, resulted compounds **8a**–**x** have found to be increase antimicrobial activity up to two, three or four fold. The data indicate that a change in the substituent might also affect the antibacterial activity of title compounds **8a**–**x**. The parent compounds with R_2 = H showed strong inhibitory effect against most of the employed strains than $R_2 = CH_3$ i.e. **8c**, **8g**, **8i**, **8k** and **8m**. Compounds having $R_1 = CH_3$ on β-aryloxyquinoline and fluorine substitution at R_3 and R_6 position on *N*-aryl rings displayed highest antibacterial activity against gram negative bacteria i.e. **8g** and **8h**. It is interesting to point out that compound **8q** carrying electron negative groups on both β-aryloxyquinoline and *N*-aryl rings showed excellent antibacterial potency towards *S. pneumonia* and *V. cholerae*. This excellent inhibition could be attributed to the strong electron negativity combination of Cl and CF_3 groups. Surprisingly, replacement of methyl group by H at R_2 in compound **8g** (compound **8h**) showed superior inhibition against *C. tetani*, *E. coli* and *V. cholerae* but observed remarkable reduction in antimicrobial spectrum

Table 2
In vitro antituberculosis activity (% Inhibition) of β-aryloxyquinolines **3a**–**d** and their *N*-aryl quinolone derivatives **8a**–**x** against *M. tuberculosis* H37Rv (at concentration 250 μg/mL).

Entry	% Inhibition	Entry	% Inhibition
3a	42	**8l**	93
3b	26	**8m**	84
3c	61	**8n**	87
3d	11	**8o**	95
8a	10	**8p**	84
8b	21	**8q**	99
8c	75	**8r**	98
8d	64	**8s**	35
8e	52	**8t**	54
8f	45	**8u**	88
8g	80	**8v**	24
8h	99	**8w**	23
8i	27	**8x**	62
8j	32	Rifampicin	98
8k	38	Isoniazid	99

Table 3
In vitro antituberculosis activity of title compounds exhibiting higher % inhibition against *M. tuberculosis* H37Rv (MICs, μg/mL).

Entry	% Inhibition	MIC
8h	99	62.5
8l	93	125
8o	95	100
8q	99	25
8r	98	62.5
Rifampicin	98	40
Isoniazid	99	0.20

Table 4
Effect of compounds on brine shrimp lethality bioassay.

Entry	Concentration ($\mu g\ mL^{-1}$)	Log (Conc.)	No. of nauplii taken	No. of nauplii dead	% of mortality	LC_{50} ($\mu g\ mL^{-1}$)
8h	5	0.699	10	0	0	27.50
	10	1	10	1	10	
	20	1.301	10	2	20	
	30	1.477	10	5	50	
	40	1.602	10	7	70	
	50	1.699	10	8	80	
8l	5	0.699	10	1	10	17.14
	10	1	10	3	30	
	20	1.301	10	5	50	
	30	1.477	10	7	70	
	40	1.602	10	8	80	
	50	1.699	10	9	90	
8o	5	0.699	10	2	20	14.10
	10	1	10	4	40	
	20	1.301	10	6	60	
	30	1.477	10	7	70	
	40	1.602	10	8	80	
	50	1.699	10	9	90	
8q	5	0.699	10	0	0	37.78
	10	1	10	1	10	
	20	1.301	10	2	20	
	30	1.477	10	4	40	
	40	1.602	10	5	50	
	50	1.699	10	7	70	
8r	5	0.699	10	1	10	23.34
	10	1	10	2	20	
	20	1.301	10	4	40	
	30	1.477	10	5	50	
	40	1.602	10	7	70	
	50	1.699	10	8	80	

towards *B. subtilis*, *S. pneumonia*, *S. typhi* and *C. albicans*. In addition, it should be noted that the fluorine substitution at R_3 and R_6 position on *N*-aryl ring are better than substitution at R_4 and R_5 i.e. **8g**, **8h**, **8m** and **8q**. This revealed that the position of fluorine substitutions on *N*-aryl ring has an impact on the antimicrobial activity. The Order of functional groups basis on activity (higher to lower), $R_1 = CH_3 > Cl > H > OCH_3$, $R_2 = H > CH_3$ and $R_3, R_6 = F > R_4 = CF_3 > R_4$, $R_5 = F$. A close examination of the structures of the active compounds depicted in Table 1 revealed that, their antimicrobial activity is strongly bound to the nature of the substituent on β-aryloxyquinoline, together with the substituent linked to the quinolone and *N*-aryl rings of the structure.

Table 5
In vitro antioxidant activity of the compounds **3a**–**d** and their **8a**–**x** derivatives.

Entry	OD (593 nm)	Frap value[a]	Entry	OD (593 nm)	Frap value[a]
3a	0.097	19.21	**8l**	1.712	339.19
3b	0.081	16.04	**8m**	0.506	100.25
3c	0.176	34.87	**8n**	0.011	2.17
3d	0.237	46.95	**8o**	0.015	2.97
8a	0.083	16.44	**8p**	0.017	3.36
8b	0.426	84.40	**8q**	0.026	5.15
8c	0.976	193.37	**8r**	0.036	7.13
8d	0.099	19.61	**8s**	1.401	277.57
8e	1.365	270.44	**8t**	0.128	25.36
8f	0.102	20.20	**8u**	1.864	369.31
8g	1.208	239.33	**8v**	0.772	152.95
8h	0.070	13.86	**8w**	1.310	259.54
8i	1.226	242.90	**8x**	0.072	14.26
8j	0.617	122.24	A.A.	2.511	–
8k	1.276	252.81			

A.A. = Ascorbic acid.
Concentration of compounds used = 200 μg/mL.
Concentration of Standard (A.A.) = 176 μg/mL.
[a] A.A. mm/100 gm sample.

4.1.2. Antituberculosis activity

The encouraging results from the antimicrobial studies impelled us to go for the preliminary screening of the synthesized compounds for their *in vitro* antituberculosis activity against *M. tuberculosis* H37Rv. Of the compounds screened for antituberculosis activity, compound **8q** displayed excellent activity against *M. tuberculosis* H37Rv. This excellent potency could be credited to the strong electron negativity combination of Cl and CF_3 groups. Whereas compounds **8h** and **8r** which are having inductively electron withdrawing but mesomerically electron releasing methyl groups on both quinoline and quinolone rings with fluorine substituent on *N*-aryl ring found to possess better activity against *M. tuberculosis* H37Rv. Antitubercular potency of compounds **3b** (26%) and **3c** (61%) were found to get intensified, upon cyclocondensation with malononitrile and β-enaminones which results compounds **8h** (99%), **8l** (93%), **8o** (95%), **8q** (99%) and **8r** (98%).

Compounds **8h** and **8q** have been emerged as the promising antimicrobial member along with better antitubercular activity (Tables 1–3) of this series of compounds. Unfortunately, majority of compounds showed poor inhibition of *M. tuberculosis* growth.

4.1.3. Cytotoxicity

The LC_{50} values obtained for the five compounds those exhibited highest % inhibition are shown in Table 4. As can be seen for the five compounds, there was no significant toxicity observed in case of compounds **8q**, **8h** and **8r** after 24 h incubation. Among the compounds tested, compounds **8l** and **8o** showed greater toxicity.

4.1.4. Antioxidant activity

The examination of the data (Table 5) revealed that compounds **8l** and **8u** showed relatively high antioxidant power while, compounds **8e**, **8k**, **8s** and **8w** found to have better ferric reducing power. Compounds **8c**, **8g** and **8i** displayed promising antioxidant potency.

From the ferric reducing power results it can be stated that, compounds carrying electron withdrawing Cl group at R_1 position exhibited very poor antioxidant activity, while compounds having electron donating CH_3 and OCH_3 groups at R_1 position exhibited excellent ferric reducing power. In addition, it should be noted that the compounds with unsubstituted quinolone ring ($R_2 = H$) gives better results than methyl substituted quinolone ring ($R_2 = CH_3$) i.e. compounds **8c**, **8e**, **8g**, **8i**, **8k**, **8s**, **8u** and **8w** except **8l.** In case of compound **8l**, CH_3 group at R_2 position improves the antioxidant power, it may be due to the combination effect of $R_1 = CH_3$, $R_2 = CH_3$ and $R_3 = CF_3$.

5. Conclusion

In conclusion, the aim of the present investigation was to design a new series of quinolone derivatives by introduction of substituted β-aryloxyquinolines at the C-4 position and diversely fluorine substituted aromatic ring at *N*-1 position for probing antimicrobial, antituberculosis and antioxidant activity. Modification of substituents on both β-aryloxyquinolines ring and *N*-aryl quinolone ring with various electron releasing and electron withdrawing groups improved the activity. Compounds **8g**, **8h**, **8m**, **8q** and **8v** exhibited excellent antimicrobial inhibition. While, compounds **8e**, **8k**, **8l**, **8s**, **8u** and **8w** showed highest ferric reducing power. Compounds **8h** and **8q** emerged as the promising antimicrobial member with better antitubercular activity and lower toxicity. Consequently, such type of compounds would represent a fertile matrix for further development of more biologically potent agents that deserve further investigation and derivatization in order to discover the scope and limitation of its biological activities.

6. Experimental

6.1. Chemistry

All reactions were performed with commercially available reagents and they were used without further purification. Organic solvents were purified by standard methods [33] and stored over molecular sieves. All reactions were monitored by thin-layer chromatography (TLC, on aluminium plates coated with silica gel 60 F_{254}, 0.25 mm thickness, Merck) carried on fluorescent coated plates and detection of the components was made by exposure to iodine vapours or UV light. Melting points were determined in open capillaries and the declared values are not corrected. Infrared 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}. ^1H and ^{13}C Nuclear Magnetic Resonance spectra were recorded in DMSO-d_6 on a Bruker Avance 400F (MHz) spectrometer (Bruker Scientific Corporation Ltd., Switzerland) using residual solvent signal as an internal standard at 400 MHz and 100 MHz respectively. Chemical shifts are reported in parts per million (ppm). Splitting patterns are designated as s, for singlet; d, for doublet; m, for multiplet. UV spectra were recorded on Shimadzu Type 160-A spectrometer. The ESI mass spectra were scanned on a Shimadzu LCMS 2010 spectrometer (Shimadzu, Tokyo, Japan) at Oxygen Healthcare Research Pvt. Ltd., Ahmedabad. Elemental analyses were performed by Perkin–Elmer 2400 series-II elemental analyzer (Perkin–Elmer, USA) at Sophisticated Instrumentation Centre for Applied Research and Training (SICART), Vallabh Vidyanagar and all compounds are within ±0.4% of the theoretical compositions. Yields are not optimized. Ampicillin, ciprofloxacin, norfloxacin, chloramphenicol, griseofulvin, nystatin, isoniazid, rifampicin and L-ascorbic acid were commercial.

6.1.1. General procedure for the synthesis of 4-(6-(un)substituted-3-formylquinolin-2-yloxy)benzonitrile **3a–d**

A 100 mL round bottomed flask, fitted with a reflux condenser, was charged with a mixture of 2-chloro-3-formylquinoline **1a–d** (1 mmol), *p*-cyano phenol **2** (1 mmol) and anhydrous potassium carbonate (2 mmol) in dimethylformamide (10 mL). The mixture was heated at 90 °C for 3.0 h and the progress of the reaction was monitored by TLC. After the completion of reaction (as evidenced by TLC), the reaction mixture was cooled to room temperature and then poured into chilled water (100 mL) with continuous stirring followed by neutralization with 1.5 N HCl until pH 7 resulted. The solid mass separated was collected by filtration, washed well with water, dried and crystallized from ethyl acetate.

6.1.2. General procedure for the synthesis of 2′-amino-6-(un) substituted-2-(4-cyanophenoxy)-1′-(2,5/3,4/4-substitutedphenyl)-7′,7′-(un)substituted-5′-oxo-1′,4′,5′,6′,7′,8′-hexahydro-3,4′-biquinoline-3′-carbonitrile **8a–x**

A 100 mL round bottomed flask, fitted with a reflux condenser, was charged with a mixture of β-aryloxyquinoline-3-carbaldehydes **3a–d** (1 mmol), malononitrile **7** (1 mmol), β-enaminone **6a,b** (1 mmol) and a catalytic amount of piperidine (0.2 mmol) in ethanol (10 mL). The reaction mixture was heated under reflux for 1–1.5 h and the progress of the reaction was monitored by TLC. After the completion of reaction (as evidenced by TLC), the reaction mixture was cooled to room temperature and stirred magnetically for further 20 min, the solid mass separated was collected by filtration, washed well with ethanol (15 mL) and purified by leaching in equal volume ratio of chloroform and methanol (15 mL) to obtain pure solid sample.

6.1.2.1. 2′-Amino-2-(4-cyanophenoxy)-1′-(2,5-difluorophenyl)-5′-oxo-1′,4′,5′,6′,7′,8′-hexahydro-3,4′-biquinoline-3′-carbonitrile (**8a**).

IR (KBr, ν_{max}, cm^{-1}): 3445 and 3350 (asym. and sym. str. of $-NH_2$), 2175 (C≡N str.), 1650 (C=O str.), 1225 (C–O–C ether str.). ^1H NMR (400 MHz, DMSO-d_6): δ 1.78–1.85 (m, 2H, CH_2–CH_2), 1.97–2.04 (m, 2H, CH_2–CO), 2.11–2.30 (m, 2H, CH_2–CH_2–CO), 5.04 (s, 1H, CH), 5.71 (s, 2H, NH_2), 7.29–8.21 (m, 12H, Ar–H). ^{13}C NMR (100 MHz, DMSO–d_6) δ: 21.12 (CH_2), 28.31 (CH_2), 32.45 (C4), 36.49 (CH_2–CO), 59.01 (C–CN), 107.45, 111.21, 119.01, 119.17, 119.92, 120.10, 120.72, 121.98, 122.75, 123.18, 125.88, 127.17, 128.35, 130.31, 130.92, 133.22, 134.61, 138.43, 144.61, 152.12, 154.19, 158.33, 159.29, 162.77 (24C, Ar–C), 195.48 (C=O); MS (*m/z*): 545.7 $[M + 1]^+$.

6.1.2.2. 2′-Amino-2-(4-cyanophenoxy)-1′-(2,5-difluorophenyl)-7′,7′-dimethyl-5′-oxo-1′,4′,5′,6′,7′,8′-hexahydro-3,4′-biquinoline-3′-carbonitrile (**8b**).

IR (KBr, ν_{max}, cm^{-1}): 3460 and 3355 (asym. and sym. str. of $-NH_2$), 2165 (C≡N str.), 1670 (C=O str.), 1230 (C–O–C ether str.). ^1H NMR (400 MHz, DMSO-d_6): δ 0.91 (s, 3H, CH_3), 0.93 (s, 3H, CH_3), 1.81 (d, 1H, J = 16.0 Hz, CH_2), 2.01 (d, 1H, J = 16.0 Hz, CH_2), 2.10–2.24 (m, 2H, CH_2–CO), 5.01 (s, 1H, CH), 5.69 (s, 2H, NH_2), 7.35–8.21 (m, 12H, Ar–H). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 27.19 (CH_3), 29.09 (CH_3), 32.61 (C4), 34.10 (C(CH_3)$_2$), 41.12 (CH_2), 49.68 (CH_2–CO), 58.89 (C–CN), 106.98, 110.93, 119.15, 119.47, 119.81, 120.11, 120.82, 121.85, 122.68, 123.43, 125.51, 127.15, 128.12, 130.35, 130.65, 133.29, 135.01, 138.30, 144.62, 151.93, 154.15, 158.39, 159.40, 161.64 (24C, Ar–C), 195.29 (C=O); MS (*m/z*): 573.9 $[M+1]^+$.

6.1.2.3. 2′-Amino-2-(4-cyanophenoxy)-1′-(3,4-difluorophenyl)-5′-oxo-1′,4′,5′,6′,7′,8′-hexahydro-3,4′-biquinoline-3′-carbonitrile (**8c**).

IR (KBr, ν_{max}, cm^{-1}): 3470 and 3350 (asym. and sym. str. of $-NH_2$), 2175 (C≡N str.), 1675 (C=O str.), 1245 (C–O–C ether str.). ^1H NMR (400 MHz, DMSO-d_6): δ 1.79–1.88 (m, 2H, CH_2–CH_2), 1.98–2.03 (m, 2H, CH_2–CO), 2.17–2.32 (m, 2H, CH_2–CH_2–CO), 5.06 (s, 1H, CH), 5.66 (s, 2H, NH_2), 7.18–8.25 (m, 12H, Ar–H). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 21.19 (CH_2), 28.37 (CH_2), 32.44 (C4), 36.44 (CH_2–CO), 58.75 (C–CN), 107.69, 111.18, 118.79, 118.98, 119.17, 119.43, 120.60, 121.92, 122.89, 123.36, 125.69, 127.02, 128.29, 130.07, 130.87, 133.04, 134.67, 138.24, 144.58, 152.06, 154.08, 158.27, 159.28, 162.61 (24C, Ar–C), 195.59 (C=O); MS (*m/z*): 545.8 $[M + 1]^+$.

6.1.2.4. 2′-Amino-2-(4-cyanophenoxy)-1′-(3,4-difluorophenyl)-7′,7′-dimethyl-5′-oxo-1′,4′,5′,6′,7′,8′-hexahydro-3,4′-biquinoline-3′-carbonitrile (**8d**).

IR (KBr, ν_{max}, cm^{-1}): 3435 and 3360 (asym. and sym. str. of $-NH_2$), 2185 (C≡N str.), 1660 (C=O str.), 1220 (C–O–C ether str.). ^1H NMR (400 MHz, DMSO-d_6): δ 0.89 (s, 3H, CH_3), 0.92 (s, 3H, CH_3), 1.77 (d, 1H, J = 16.2 Hz, CH_2), 1.98 (d, 1H, J = 16.2 Hz, CH_2), 2.08–2.26 (m, 2H, CH_2–CO), 5.08 (s, 1H, CH), 5.81 (s, 2H, NH_2), 7.31–8.26 (m, 12H, Ar–H). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 27.21 (CH_3), 29.13 (CH_3), 32.29 (C4), 34.09 (C(CH_3)$_2$), 41.30 (CH_2), 49.78 (CH_2–CO), 58.71 (C–CN), 107.37, 111.35, 118.62, 118.90, 119.38, 119.62, 120.25, 121.63, 122.38, 123.40, 125.75, 127.09, 128.46, 130.12, 130.91, 133.25, 134.69, 137.90, 144.65, 152.12, 154.45, 158.35, 159.31, 162.54 (24C, Ar–C), 195.63 (C=O); MS (*m/z*): 574.0 $[M + 1]^+$.

6.1.2.5. 2′-Amino-2-(4-cyanophenoxy)-5′-oxo-1′-[3-(trifluoromethyl)phenyl]-1′,4′,5′,6′,7′,8′-hexahydro-3,4′-biquinoline-3′-carbonitrile (**8e**).

IR (KBr, ν_{max}, cm^{-1}): 3430 and 3345 (asym. and sym. str. of $-NH_2$), 2160 (C≡N str.), 1655 (C=O str.), 1235 (C–O–C ether str.). ^1H NMR (400 MHz, DMSO-d_6): δ 1.76–1.84 (m, 2H, CH_2–CH_2), 1.95–2.07 (m, 2H, CH_2–CO), 2.15–2.35 (m, 2H, CH_2–CH_2–CO), 5.02 (s, 1H, CH), 5.79 (s, 2H, NH_2), 7.26–8.24 (m, 12H, Ar–H). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 21.31 (CH_2), 28.42 (CH_2), 32.35 (C4), 36.38 (CH_2–CO), 59.11 (C–CN), 106.83, 111.04, 118.43, 118,77, 119.25, 119.63, 120.33, 121.81, 122.78, 123.45, 125.66, 127.20, 128.47, 130.35, 130.98, 131.28, 133.09, 134.42, 138.11, 144.53, 152.25, 154.63,

158.44, 159.19, 160.48 (25C, Ar–C), 195.51 (C=O); MS (*m/z*): 578.1 $[M + 1]^+$.

*6.1.2.6. 2′-Amino-2-(4-cyanophenoxy)-7′,7′-dimethyl-5′-oxo-1′-[3-(trifluoromethyl)phenyl]-1′,4′,5′,6′,7′,8′-hexahydro-3,4′-biquinoline-3′-carbonitrile (**8f**).* IR (KBr, ν_{max}, cm^{-1}): 3450 and 3330 (asym. and sym. str. of $-NH_2$), 2175 (C≡N str.), 1650 (C=O str.), 1240 (C–O–C ether str.). ^1H NMR (400 MHz, DMSO-d_6): δ 0.93 (s, 3H, CH_3), 0.96 (s, 3H, CH_3), 1.75 (d, 1H, $J = 16.0$ Hz, CH_2), 1.99 (d, 1H, $J = 16.0$ Hz, CH_2), 2.12–2.25 (m, 2H, CH_2–CO), 5.05 (s, 1H, CH), 5.72 (s, 2H, NH_2), 7.38–8.29 (m, 12H, Ar–H). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 27.15 (CH_3), 29.18 (CH_3), 32.40 (C4), 33.87 (C(CH_3)$_2$), 41.19 (CH_2), 49.81 (CH_2–CO), 58.86 (C–CN), 107.26, 111.31, 118.44, 118.85, 119.37, 115.43, 120.44, 121.70, 122.93, 123.39, 125.78, 127.26, 128.53, 130.47, 131.07, 131.62, 133.12, 134.47, 138.27, 144.62, 152.38, 154.73, 158.40, 159.36, 162.70 (25C, Ar–C), 195.42 (C=O); MS (*m/z*): 605.9 $[M + 1]^+$.

*6.1.2.7. 2′-Amino-2-(4-cyanophenoxy)-1′-(2,5-difluorophenyl)-6-methyl-5′-oxo-1′,4′,5′,6′,7′,8′-hexahydro-3,4′-biquinoline-3′-carbonitrile (**8g**).* IR (KBr, ν_{max}, cm^{-1}): 3445 and 3360 (asym. and sym. str. of $-NH_2$), 2160 (C≡N str.), 1670 (C=O str.), 1235 (C–O–C ether str.). ^1H NMR (400 MHz, DMSO-d_6): δ 1.80–1.88 (m, 2H, CH_2–CH_2), 1.94–2.07 (m, 2H, CH_2–CO), 2.17–2.32 (m, 2H, CH_2–CH_2–CO), 2.49 (s, 3H, Ar–CH_3), 5.05 (s, 1H, CH), 5.77 (s, 2H, NH_2), 7.33–8.25 (m, 11H, Ar–H). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 21.35 (CH_2), 21.42 (Ar–CH_3), 28.37 (CH_2), 32.41 (C4), 36.45 (CH_2–CO), 59.25 (C–CN), 107.34, 111.02, 111.81, 119.28, 119.73, 120.46, 120.90, 121.49, 123.15, 123.46, 126.52, 126.89, 127.12, 130.66, 131.50, 132.25, 134.48, 134.68, 135.23, 136.82, 142.16, 152.15, 153.28, 159.38 (24C, Ar–C), 195.47 (C=O); MS (*m/z*): 560.3 $[M + 1]^+$.

*6.1.2.8. 2′-Amino-2-(4-cyanophenoxy)-1′-(2,5-difluorophenyl)-6,7′,7′-trimethyl-5′-oxo-1′,4′,5′,6′,7′,8′-hexahydro-3,4′-biquinoline-3′-carbonitrile (**8h**).* IR (KBr, ν_{max}, cm^{-1}): 3465 and 3365 (asym. and sym. str. of $-NH_2$), 2180 (C≡N str.), 1660 (C=O str.), 1220 (C–O–C ether str.). ^1H NMR (400 MHz, DMSO-d_6): δ 0.92 (s, 3H, CH_3), 0.98 (s, 3H, CH_3), 1.86 (d, 1H, $J = 16.2$ Hz, CH_2), 2.00 (d, 1H, $J = 16.2$ Hz, CH_2), 2.09–2.26 (m, 2H, CH_2–CO), 2.45 (s, 3H, Ar–CH_3), 5.08 (s, 1H, CH), 5.80 (s, 2H, NH_2), 7.43–8.20 (m, 11H, Ar–H). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 21.35 (Ar–CH_3), 27.16 (CH_3), 29.41 (CH_3), 32.46 (C4), 34.28 (C(CH_3)$_2$), 40.91 (CH_2), 49.78 (CH_2–CO), 60.37 (C–CN), 107.53, 110.83, 111.78, 119.23, 119.52, 120.64, 120.89, 121.58, 123.26, 123.37, 126.36, 126.79, 127.03, 130.71, 131.47, 132.12, 134.49, 134.69, 135.12, 137.06, 142.96, 151.09, 151.78, 158.26 (24C, Ar–C), 195.40 (C=O); MS (*m/z*): 587.9 $[M + 1]^+$.

*6.1.2.9. 2′-Amino-2-(4-cyanophenoxy)-1′-(3,4-difluorophenyl)-6-methyl-5′-oxo-1′,4′,5′,6′,7′,8′-hexahydro-3,4′-biquinoline-3′-carbonitrile (**8i**).* IR (KBr, ν_{max}, cm^{-1}): 3470 and 3360 (asym. and sym. str. of $-NH_2$), 2175 (C≡N str.), 1640 (C=O str.), 1215 (C–O–C ether str.). ^1H NMR (400 MHz, DMSO-d_6): δ 1.72–1.83 (m, 2H, CH_2–CH_2), 1.91–2.02 (m, 2H, CH_2–CO), 2.12–2.31 (m, 2H, CH_2–CH_2–CO), 2.42 (s, 3H, Ar–CH_3), 5.00 (s, 1H, CH), 5.61 (s, 2H, NH_2), 7.42–8.21 (m, 11H, Ar–H). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 21.18 (CH_2), 21.39 (Ar–CH_3), 28.12 (CH_2), 32.29 (C4), 36.51 (CH_2–CO), 59.74 (C–CN), 106.84, 111.18, 119.44, 121.67, 123.65, 125.34, 126.77, 126.89, 127.12, 127.52, 130.44, 131.60, 131.94, 132.4, 134.67, 135.58, 135.96, 137.55, 138.51, 143.12, 151.62, 158.07, 158.74, 161.07 (24C, Ar–C), 195.39 (C=O); MS (*m/z*): 560.0 $[M + 1]^+$.

*6.1.2.10. 2′-Amino-2-(4-cyanophenoxy)-1′-(3,4-difluorophenyl)-6,7′,7′-trimethyl-5′-oxo-1′,4′,5′,6′,7′,8′-hexahydro-3,4′-biquinoline-3′-carbonitrile (**8j**).* IR (KBr, ν_{max}, cm^{-1}): 3465 and 3355 (asym. and sym. str. of $-NH_2$), 2155 (C≡N str.), 1645 (C=O str.), 1220 (C–O–C ether str.). ^1H NMR (400 MHz, DMSO-d_6): δ 0.90 (s, 3H, CH_3), 0.93 (s, 3H, CH_3), 1.82 (d, 1H, $J = 16.2$ Hz, CH_2), 2.03 (d, 1H, $J = 16.2$ Hz, CH_2), 2.13–2.27 (m, 2H, CH_2–CO), 2.50 (s, 3H, Ar–CH_3), 5.05 (s, 1H, CH), 5.65 (s, 2H, NH_2), 7.26–8.28 (m, 11H, Ar–H). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 21.41 (Ar–CH_3), 27.09 (CH_3), 29.20 (CH_3), 32.38 (C4), 34.13 (C(CH_3)$_2$), 41.22 (CH_2), 49.66 (CH_2–CO), 59.61 (C–CN), 107.43, 110.84, 111.67, 119.10, 119.57, 120.69, 120.77, 121.86, 123.47, 123.59, 126.48, 126.90, 127.15, 130.78, 131.53, 132.25, 134.55, 134.70, 135.07, 137.24, 142.86, 151.16, 151.92, 160.18 (24C, Ar–C), 195.61 (C=O); MS (*m/z*): 588.0 $[M + 1]^+$.

*6.1.2.11. 2′-Amino-2-(4-cyanophenoxy)-6-methyl-5′-oxo-1′-[3-(trifluoromethyl)phenyl]-1′,4′,5′,6′,7′,8′-hexahydro-3,4′-biquinoline-3′-carbonitrile (**8k**).* IR (KBr, ν_{max}, cm^{-1}): 3455 and 3325 (asym. and sym. str. of $-NH_2$), 2180 (C≡N str.), 1655 (C=O str.), 1235 (C–O–C ether str.). ^1H NMR (400 MHz, DMSO-d_6): δ 1.75–1.86 (m, 2H, CH_2–CH_2), 1.93–2.06 (m, 2H, CH_2–CO), 2.15–2.33 (m, 2H, CH_2–CH_2–CO), 2.44 (s, 3H, Ar–CH_3), 5.09 (s, 1H, CH), 5.73 (s, 2H, NH_2), 7.38–8.27 (m, 11H, Ar–H). ^{13}C NMR (100 MHz, DMSO–d_6) δ: 21.25 (CH_2), 21.33 (Ar–CH_3), 28.38 (CH_2), 32.35 (C4), 36.40 (CH_2–CO), 58.39 (C–CN), 107.51, 110.82, 116.52, 119.30, 119.68, 120.12, 120.75, 121.92, 123.09, 123.41, 126.39, 126.85, 127.11, 127.54, 130.78, 131.67, 132.24, 134.63, 134.70, 135.04, 137.15, 142.81, 151.12, 151.93, 159.08 (25C, Ar–C), 195.42 (C=O); MS (*m/z*): 592.1 $[M + 1]^+$.

*6.1.2.12. 2′-Amino-2-(4-cyanophenoxy)-6,7′,7′-trimethyl-5′-oxo-1′-[3-(trifluoromethyl)phenyl]-1′,4′,5′,6′,7′,8′-hexahydro-3,4′-biquinoline-3′-carbonitrile (**8l**).* IR (KBr, ν_{max}, cm^{-1}): 3475 and 3330 (asym. and sym. str. of $-NH_2$), 2160 (C≡N str.), 1670 (C=O str.), 1240 (C–O–C ether str.). ^1H NMR (400 MHz, DMSO-d_6): δ 0.88 (s, 3H, CH_3), 0.90 (s, 3H, CH_3), 1.70 (d, 1H, $J = 16.2$ Hz, CH_2), 2.02 (d, 1H, $J = 16.2$ Hz, CH_2), 2.13–2.22 (m, 2H, CH_2–CO), 2.47 (s, 3H, Ar–CH_3), 5.01 (s, 1H, CH), 5.56 (s, 2H, NH_2), 7.40–8.20 (m, 11H, Ar–H). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 21.40 (Ar–CH_3), 27.13 (CH_3), 29.33 (CH_3), 32.50 (C4), 33.96 (C(CH_3)$_2$), 41.74 (CH_2), 49.71 (CH_2–CO), 59.04 (C–CN), 107.82, 110.23, 119.13, 121.85, 123.42, 125.32, 126.75, 126.84, 126.99, 127.12, 127.37, 130.07, 131.27, 131.77, 132.12, 134.75, 135.06, 135.17, 137.19, 138.24, 143.03, 151.49, 152.15, 158.29, 159.10 (25C, Ar–C), 195.47 (C=O); MS (*m/z*): 619.8 $[M + 1]^+$.

*6.1.2.13. 2′-Amino-6-chloro-2-(4-cyanophenoxy)-1′-(2,5-difluorophenyl)-5′-oxo-1′,4′,5′,6′,7′,8′-hexahydro-3,4′-biquinoline-3′-carbonitrile (**8m**).* IR (KBr, ν_{max}, cm^{-1}): 3470 and 3345 (asym. and sym. str. of $-NH_2$), 2175 (C≡N str.), 1675 (C=O str.), 1225 (C–O–C ether str.). ^1H NMR (400 MHz, DMSO-d_6): δ 1.78–1.90 (m, 2H, CH_2–CH_2), 1.98–2.08 (m, 2H, CH_2–CO), 2.19–2.36 (m, 2H, CH_2–CH_2–CO), 5.07 (s, 1H, CH), 5.76 (s, 2H, NH_2), 7.31–8.22 (m, 11H, Ar–H). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 21.28 (CH_2), 28.41 (CH_2), 32.60 (C4), 36.47 (CH_2–CO), 59.12 (C–CN), 107.91, 110.90, 118.68, 119.25, 120.92, 121.63, 122.47, 123.52, 126.91, 127.78, 128.44, 129.31, 129.82, 130.47, 132.21, 132.79, 134.78, 137.55, 143.10, 149.78, 151.97, 152.44, 158.20, 159.91 (24C, Ar–C), 195.53 (C=O); MS (*m/z*): 580.1 $[M + 1]^+$, 581.1 [M + 2].

*6.1.2.14. 2′-Amino-6-chloro-2-(4-cyanophenoxy)-1′-(2,5-difluorophenyl)-7′,7′-dimethyl-5′-oxo-1′,4′,5′,6′,7′,8′-hexahydro-3,4′-biquinoline-3′-carbonitrile (**8n**).* IR (KBr, ν_{max}, cm^{-1}): 3445 and 3360 (asym. and sym. str. of $-NH_2$), 2185 (C≡N str.), 1660 (C=O str.), 1240 (C–O–C ether str.). ^1H NMR (400 MHz, DMSO-d_6): δ 0.91 (s, 3H, CH_3), 0.94 (s, 3H, CH_3), 1.85 (d, 1H, $J = 16.0$ Hz, CH_2), 2.01 (d, 1H, $J = 16.0$ Hz, CH_2), 2.14–2.27 (m, 2H, CH_2–CO), 5.06 (s, 1H, CH), 5.58 (s, 2H, NH_2), 7.36–8.25 (m, 11H, Ar–H). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 27.29 (CH_3), 29.25 (CH_3), 32.56 (C4), 34.18 (C(CH_3)$_2$), 41.43 (CH_2), 49.61 (CH_2–CO), 59.36 (C–CN), 106.83, 111.07, 118.72, 119.11, 120.82, 121.25, 122.51, 123.24, 126.47, 127.90, 128.46, 129.14, 129.78, 130.51, 132.27, 132.90, 134.81, 137.46, 143.37, 149.59, 151.82, 152.47,

158.34, 158.28 (24C, Ar–C), 195.60 (C=O); MS (*m/z*): 608.1 $[M + 1]^+$, 609.0 [M + 2].

*6.1.2.15. 2′-Amino-6-chloro-2-(4-cyanophenoxy)-1′-(3,4-difluorophenyl)-5′-oxo-1′,4′,5′,6′,7′,8′-hexahydro-3,4′-biquinoline-3′-carbonitrile (**8o**).* IR (KBr, ν_{max}, cm^{-1}): 3465 and 3330 (asym. and sym. str. of $-NH_2$), 2195 (C≡N str.), 1640 (C=O str.), 1235 (C–O–C ether str.). ^1H NMR (400 MHz, DMSO-d_6): δ 1.81–1.90 (m, 2H, CH_2–CH_2), 1.99–2.08 (m, 2H, CH_2–CO), 2.17–2.30 (m, 2H, CH_2–CH_2–CO), 5.08 (s, 1H, CH), 5.62 (s, 2H, NH_2), 7.23–8.30 (m, 11H, Ar–H). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 21.16 (CH_2), 28.19 (CH_2), 32.42 (C4), 36.52 (CH_2–CO), 60.09 (C–CN), 107.64, 111.28, 117.45, 119.34, 120.77, 121.27, 122.35, 123.51, 126.40, 127.88, 128.54, 129.17, 129.92, 130.64, 132.21, 132.77, 134.55, 137.05, 143.46, 149.62, 151.83, 152.54, 157.87, 162.25 (24C, Ar–C), 195.41 (C=O); MS (*m/z*): 579.7 $[M + 1]^+$, 580.7 [M + 2].

*6.1.2.16. 2′-Amino-6-chloro-2-(4-cyanophenoxy)-1′-(3,4-difluorophenyl)-7′,7′-dimethyl-5′-oxo-1′,4′,5′,6′,7′,8′-hexahydro-3,4′-biquinoline-3′-carbonitrile (**8p**).* IR (KBr, ν_{max}, cm^{-1}): 3460 and 3335 (asym. and sym. str. of $-NH_2$), 2200 (C≡N str.), 1645 (C=O str.), 1220 (C–O–C ether str.). ^1H NMR (400 MHz, DMSO-d_6): δ 0.90 (s, 3H, CH_3), 0.93 (s, 3H, CH_3), 1.88 (d, 1H, $J = 16.2$ Hz, CH_2), 2.03 (d, 1H, $J = 16.0$ Hz, CH_2), 2.18–2.26 (m, 2H, CH_2–CO), 5.06 (s, 1H, CH), 5.69 (s, 2H, NH_2), 7.22–8.26 (m, 11H, Ar–H). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 27.23 (CH_3), 29.27 (CH_3), 32.53 (C4), 32.72 (C(CH_3)$_2$), 41.48 (CH_2), 49.77 (CH_2–CO), 58.59 (C–CN), 108.05, 110.31, 118.96, 119.10, 120.81, 121.81, 122.09, 123.47, 126.89, 127.77, 128.25, 129.15, 129.96, 130.43, 132.38, 132.88, 134.76, 137.82, 143.07, 149.73, 151.89, 152.20, 157.97, 159.56 (24C, Ar–C), 195.44 (C=O); MS (*m/z*): 608.2 $[M + 1]^+$, 609.2 [M + 2].,

*6.1.2.17. . 2′-Amino-6-chloro-2-(4-cyanophenoxy)-5′-oxo-1′-[3-(trifluoromethyl)phenyl]-1′,4′,5′,6′,7′,8′-hexahydro-3,4′-biquinoline-3′-carbonitrile (**8q**).* IR (KBr, ν_{max}, cm^{-1}): 3445 and 3340 (asym. and sym. str. of $-NH_2$), 2190 (C≡N str.), 1680 (C=O str.), 1245 (C–O–C ether str.). ^1H NMR (400 MHz, DMSO-d_6): δ 1.73–1.85 (m, 2H, CH_2–CH_2), 1.95–2.07 (m, 2H, CH_2–CO), 2.12–2.29 (m, 2H, CH_2–CH_2–CO), 5.02 (s, 1H, CH), 5.59 (s, 2H, NH_2), 7.19–8.25 (m, 11H, Ar–H). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 21.34 (CH_2), 28.37 (CH_2), 32.81 (C4), 36.43 (CH_2–CO), 59.43 (C–CN), 107.25, 110.98, 116.28, 118.45, 119.15, 120.77, 121.80, 122.70, 123.48, 126.90, 127.81, 128.54, 129.35, 129.71, 130.49, 132.82, 133.04, 134.68, 137.88, 143.15, 149.79, 151.60, 152.27, 158.12, 160.75 (25C, Ar–C), 195.48 (C=O); MS (*m/z*): 611.9 $[M + 1]^+$, 612.9 [M + 2].

*6.1.2.18. 2′-Amino-6-chloro-2-(4-cyanophenoxy)-7′,7′-dimethyl-5′-oxo-1′-[3-(trifluoromethyl)phenyl]-1′,4′,5′,6′,7′,8′-hexahydro-3,4′-biquinoline-3′-carbonitrile (**8r**).* IR (KBr, ν_{max}, cm^{-1}): 3440 and 3370 (asym. and sym. str. of $-NH_2$), 2175 (C≡N str.), 1675 (C=O str.), 1230 (C–O–C ether str.). ^1H NMR (400 MHz, DMSO-d_6): δ 0.88 (s, 3H, CH_3), 0.92 (s, 3H, CH_3), 1.80 (d, 1H, $J = 16.0$ Hz, CH_2), 1.98 (d, 1H, $J = 16.0$ Hz, CH_2), 2.15–2.23 (m, 2H, CH_2–CO), 5.03 (s, 1H, CH), 5.64 (s, 2H, NH_2), 7.30–8.23 (m, 11H, Ar–H). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 27.20 (CH_3), 29.15 (CH_3), 32.70 (C4), 33.95 (C(CH_3)$_2$), 41.53 (CH_2), 49.75 (CH_2–CO), 59.71 (C–CN), 108.01, 111.22, 116.37, 118.40, 119.09, 120.45, 121.91, 122.77, 123.53, 126.91, 127.75, 128.67, 129.31, 129.65, 130.54, 132.88, 133.45, 134.71, 137.93, 143.27, 149.56, 151.82, 152.34, 158.28, 160.18 (25C, Ar–C), 195.52 (C=O); MS (*m/z*): 640.1 $[M+1]^+$, 641.1 [M+2].

*6.1.2.19. 2′-Amino-2-(4-cyanophenoxy)-1′-(2,5-difluorophenyl)-6-methoxy-5′-oxo-1′,4′,5′,6′,7′,8′-hexahydro-3,4′-biquinoline-3′-carbonitrile (**8s**).* IR (KBr, ν_{max}, cm^{-1}): 3435 and 3355 (asym. and sym. str. of $-NH_2$), 2180 (C≡N str.), 1660 (C=O str.), 1240 (C–O–C ether str.). ^1H NMR (400 MHz, DMSO-d_6): δ 1.76–1.87 (m, 2H, CH_2–CH_2), 1.97–2.09 (m, 2H, CH_2–CO), 2.17–2.35 (m, 2H, CH_2–CH_2–CO), 3.86 (s, 3H, Ar–OCH_3), 5.07 (s, 1H, CH), 5.73 (s, 2H, NH_2), 7.28–8.21 (m, 11H, Ar–H). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 21.28 (CH_2), 28.44 (CH_2), 32.31 (C4), 36.39 (CH_2–CO), 55.88 (Ar–OCH_3), 59.67 (C–CN), 106.68, 111.17, 118.77, 119.10, 120.68, 121.36, 122.48, 123.44, 126.51, 127.92, 128.30, 129.09, 129.80, 130.55, 132.44, 132.81, 134.78, 137.41, 143.64, 149.65, 151.84, 152.56, 158.80, 159.94 (24C, Ar–C), 195.44 (C=O); MS (*m/z*): 576.2 $[M + 1]^+$.

*6.1.2.20. 2′-Amino-2-(4-cyanophenoxy)-1′-(2,5-difluorophenyl)-6-methoxy-7′,7′-dimethyl-5′-oxo-1′,4′,5′,6′,7′,8′-hexahydro-3,4′-biquinoline-3′-carbonitrile (**8t**).* IR (KBr, ν_{max}, cm^{-1}): 3450 and 3345 (asym. and sym. str. of $-NH_2$), 2190 (C≡N str.), 1655 (C=O str.), 1235 (C–O–C ether str.). ^1H NMR (400 MHz, DMSO-d_6): δ 0.93 (s, 3H, CH_3), 0.97 (s, 3H, CH_3), 1.86 (d, 1H, $J = 16.2$ Hz, CH_2), 2.00 (d, 1H, $J = 16.2$ Hz, CH_2), 2.16–2.25 (m, 2H, CH_2–CO), 3.81 (s, 3H, Ar–OCH_3), 5.01 (s, 1H, CH), 5.60 (s, 2H, NH_2), 7.20–8.20 (m, 11H, Ar–H). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 27.31 (CH_3), 29.08 (CH_3), 32.85 (C4), 34.17 (C(CH_3)$_2$), 41.38 (CH_2), 49.76 (CH_2–CO), 55.91 (Ar–OCH_3), 58.92 (C–CN), 107.34, 109.55, 11.48, 119.27, 121.68, 122.45, 122.80, 123.07, 126.40, 127.18, 127.45, 127.88, 128.13, 130.17, 131.62, 134.75, 135.44, 137.35, 137.68, 140.15, 152.11, 157.15, 158.17, 159.28 (24C, Ar–C), 195.45 (C=O); MS (*m/z*): 604.0 $[M + 1]^+$.

*6.1.2.21. 2′-Amino-2-(4-cyanophenoxy)-1′-(3,4-difluorophenyl)-6-methoxy-5′-oxo-1′,4′,5′,6′,7′,8′-hexahydro-3,4′-biquinoline-3′-carbonitrile (**8u**).* IR (KBr, ν_{max}, cm^{-1}): 3465 and 3360 (asym. and sym. str. of $-NH_2$), 2165 (C≡N str.), 1675 (C=O str.), 1225 (C–O–C ether str.). ^1H NMR (400 MHz, DMSO-d_6): δ 1.77–1.86 (m, 2H, CH_2–CH_2), 1.96–2.07 (m, 2H, CH_2–CO), 2.13–2.29 (m, 2H, CH_2–CH_2–CO), 3.79 (s, 3H, Ar–OCH_3), 5.09 (s, 1H, CH), 5.68 (s, 2H, NH_2), 7.25–8.27 (m, 11H, Ar–H). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 21.16 (CH_2), 28.51 (CH_2), 32.45 (C4), 36.52 (CH_2–CO), 55.82 (Ar–OCH_3), 59.12 (C–CN), 108.01, 110.12, 111.49, 119.14, 121.85, 122.49, 122.91, 123.28, 126.56, 127.47, 127.49, 127.72, 128.30, 130.28, 131.75, 134.82, 135.47, 137.48, 137.92, 140.17, 152.24, 157.43, 158.09, 161.26 (24C, Ar–C), 195.56 (C=O); MS (*m/z*): 576.2 $[M + 1]^+$.

*6.1.2.22. 2′-Amino-2-(4-cyanophenoxy)-1′-(3,4-difluorophenyl)-6-methoxy-7′,7′-dimethyl-5′-oxo-1′,4′,5′,6′,7′,8′-hexahydro-3,4′-biquinoline-3′-carbonitrile (**8v**).* IR (KBr, ν_{max}, cm^{-1}): 3455 and 3355 (asym. and sym. str. of $-NH_2$), 2160 (C≡N str.), 1650 (C=O str.), 1220 (C–O–C ether str.). ^1H NMR (400 MHz, DMSO-d_6): δ 0.90 (s, 3H, CH_3), 0.94 (s, 3H, CH_3), 1.74 (d, 1H, $J = 16.0$ Hz, CH_2), 1.99 (d, 1H, $J = 16.0$ Hz, CH_2), 2.14–2.24 (m, 2H, CH_2–CO), 3.83 (s, 3H, Ar–OCH_3), 5.05 (s, 1H, CH), 5.64 (s, 2H, NH_2), 7.34–8.28 (m, 11H, Ar–H). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 27.24 (CH_3), 29.14 (CH_3), 32.46 (C4), 33.88 (C(CH_3)$_2$), 41.68 (CH_2), 49.69 (CH_2–CO), 55.97 (Ar–OCH_3), 59.75 (C–CN), 107.41, 111.13, 119.98, 119.24, 121.79, 122.35, 122.80, 123.40, 126.55, 127.56, 127.52, 127.80, 128.37, 130.42, 131.88, 135.04, 135.58, 137.63, 137.89, 140.35, 152.34, 157.56, 158.12, 162.10 (24C, Ar–C), 195.62 (C=O); MS (*m/z*): 604.5 $[M + 1]^+$.

*6.1.2.23. 2′-Amino-2-(4-cyanophenoxy)-6-methoxy-5′-oxo-1′-[3-(trifluoromethyl)phenyl]-1′,4′,5′,6′,7′,8′-hexahydro-3,4′-biquinoline-3′-carbonitrile (**8w**).* IR (KBr, ν_{max}, cm^{-1}): 3450 and 3325 (asym. and sym. str. of $-NH_2$), 2175 (C≡N str.), 1640 (C=O str.), 1235 (C–O–C ether str.). ^1H NMR (400 MHz, DMSO-d_6): δ 1.79–1.88 (m, 2H, CH_2–CH_2), 1.98–2.08 (m, 2H, CH_2–CO), 2.15–2.31 (m, 2H, CH_2–CH_2–CO), 3.89 (s, 3H, Ar–OCH_3), 5.02 (s, 1H, CH), 5.58 (s, 2H, NH_2), 7.26–8.20 (m, 11H, Ar–H). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 21.17 (CH_2), 28.56 (CH_2), 33.16 (C4), 36.40 (CH_2–CO), 55.96 (Ar–OCH_3), 59.16 (C–CN), 106.78, 107.49, 111.14, 119.21, 121.93, 122.67, 122.88, 123.15, 126.53, 127.05, 127.56, 127.85, 128.40, 130.54, 131.60, 134.71, 135.13, 137.27, 137.51, 140.10, 152.05, 153.85, 157.04, 157.94, 158.57 (25C, Ar–C), 195.61 (C=O); MS (*m/z*): 608.2 $[M + 1]^+$.

*6.1.2.24. 2′-Amino-2-(4-cyanophenoxy)-6-methoxy-7′,7′-dimethyl-5′-oxo-1′-[3-(trifluoromethyl)phenyl]-1′,4′,5′,6′,7′,8′-hexahydro-3,4′-biquinoline-3′-carbonitrile (**8x**).* IR (KBr, ν_{max}, cm^{-1}): 3460 and 3330 (asym. and sym. str. of $-NH_2$), 2180 (C≡N str.), 1655 (C═O str.), 1230 (C–O–C ether str.). ^1H NMR (400 MHz, DMSO-d_6): δ 0.93 (s, 3H, CH_3), 0.97 (s, 3H, CH_3), 1.81 (d, 1H, J = 16.0 Hz, CH_2), 2.02 (d, 1H, J = 16.0 Hz, CH_2), 2.15–2.26 (m, 2H, CH_2–CO), 3.85 (s, 3H, Ar–OCH_3), 5.04 (s, 1H, CH), 5.74 (s, 2H, NH_2), 7.24–8.19 (m, 11H, Ar–H). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 27.30 (CH_3), 29.17 (CH_3), 32.94 (C4), 33.98 (C(CH_3)$_2$), 41.52 (CH_2), 49.79 (CH_2–CO), 55.83 (Ar–OCH_3), 59.40 (C–CN), 107.52, 107.62, 111.17, 119.43, 121.94, 122.61, 123.10, 123.62, 126.64, 127.24, 127.71, 127.91, 128.47, 130.62, 131.77, 132.18, 134.88, 135.25, 137.81, 140.26, 152.27, 153.91, 157.45, 157.81, 159.88 (25C, Ar–C), 195.44 (C═O); MS (m/z): 636.4 $[M + 1]^+$.

6.2. Biological assay

6.2.1. In vitro evaluation of antimicrobial activity

The MICs of synthesized compounds were carried out by broth microdilution method. DMSO was used as diluents to get desired concentration of compounds to test upon standard bacterial strains. Serial dilutions were prepared in primary and secondary screening. The control tube containing no antibiotic was immediately subcultured (before inoculation) by spreading a loopful evenly over a quarter of plate of medium suitable for the growth of the test organism and put for incubation at 37 °C overnight. The tubes were then incubated overnight. The MIC of the control organism was read to check the accuracy of the compound concentrations. The MIC was defined as the lowest concentration of the antibiotic or test sample allowing no visible growth. All the tubes not showing visible growth (in the same manner as control tube described above) was subcultured and incubated overnight at 37 °C. The amount of growth from the control tube before incubation (which represents the original inoculum) was compared. Subcultures might show: similar number of colonies indicating bacteriostatic; a reduced number of colonies indicating a partial or slow bactericidal activity and no growth if the whole inoculum has been killed. The test must include a second set of the same dilutions inoculated with an organism of known sensitivity. Each synthesized compound was diluted obtaining 2000 μg/mL concentration as a stock solution. In primary screening 500, 250 and 200 μg/mL concentrations of the synthesized compounds were taken. The active synthesized compounds found in this primary screening were further tested in a second set of dilution against all microorganisms. The compounds found active in primary screening were similarly diluted to obtain 100, 62.5, 50, 25 and 12.5 μg/mL concentrations. The highest dilution showing at least 99% inhibition is taken as MIC.

6.2.2. In vitro evaluation of antituberculosis activity

Drug susceptibility and determination of antituberculosis activity of the test compounds against *M. tuberculosis* H37Rv were performed by Lowenstein–Jensen method [30] with slight modification where 250 μg/mL dilution of each test compound were added liquid Lowenstein-Jensen Medium and then media were sterilized by inspissation method. A culture of *M. tuberculosis* H37Rv growing on Lowenstein-Jensen medium was harvested in 0.85% saline in bijou bottles. All test compound make solution of 250 μg/mL concentration of compounds was prepared in DMSO. These tubes were then incubated at 37 °C for 24 h followed by streaking of *M. tuberculosis* H37Rv (5×10^4 bacilli per tube). These tubes were then incubated at 37 °C. Growth of bacilli was seen after 12 days, 22 days and finally 28 days of incubation. Tubes having the compounds were compared with control tubes where medium alone was incubated with *M. tuberculosis* H37Rv. The concentration at which no development of colonies occurred or < 20 colonies was taken as MIC concentration of test compound. The screening results are summarized as % inhibition (Table 2) relative to standard drugs isoniazid and rifampicin. MIC values of five compounds with highest % inhibition i.e. **8h**, **8l**, **8o**, **8q** and **8r** are shown in Table 3. The standard strain *M. tuberculosis* H37Rv was tested with standard drugs isoniazid and rifampicin for comparison purpose.

6.2.3. Cytotoxicity – brine shrimp lethality bioassay

Brine shrimp lethality bioassay technique was applied for the determination of general toxic property of compounds. The *in vitro* lethality test has been carried out using brine shrimp eggs i.e. Artemia cysts. Brine shrimp eggs were hatched in a shallow rectangular plastic dish (22 × 32 cm), filled with artificial seawater, which was prepared with commercial salt mixture and double distilled water. An unequal partition was made in the plastic dish with the help of a perforated device. Approximately 50 mg of eggs were sprinkled into the large compartment, which was darkened while the minor compartment was opened to ordinary light. After two days nauplii were collected by a pipette from the lighter side. A stock solution of the test complex was prepared in DMSO. From this stock solution, solutions were transferred to the vials to make final concentration 5, 10, 20, 30, 40, 50 μg/mL (dilutions were used in triplicate for each test sample and LC_{50} is the mean of three values) and three vial was kept as control having of DMSO only. After two days, when the of nauplii were ready, 1 mL of seawater and 10 of nauplii were added to each vial and the volume was adjusted with seawater to 2.5 mL per vial [31]. After 24 h each vial was observed using a magnifying glass and the number of survivors in each vial was counted and noted. Data were analysed by simple logit method to determine the LC_{50} values, in which log of dose concentration of samples were plotted against percent of mortality of nauplii [34].

6.2.4. In vitro evaluation of antioxidant activity

FRAP assay was employed to measure total antioxidant capacity of the compounds. It measure a reduction power of the compounds, converting ferric tripyridyl triazine (Fe (III)-TPTZ) complex into a blue colour ferrous tripyridyl triazine (Fe (II)-TPTZ) complex at low pH, measurable at 593 nm [32].

Reagents:

Buffer solution: 0.187 gm sodium acetate and 1.6 mL acetic acid dissolved in double distilled water to make 100 mL.

TPTZ: 0.155 gm TPTZ was dissolved in 100 mL 40 mM HCl.

$FeCl_3$ solution: 0.324 gm $FeCl_3$ was dissolved in 100 mL distilled water.

Standard Ascorbic acid: 0.176 gm of standard ascorbic acid was dissolved in 100 mL distilled water.

Fe(II)-TPTZ(2,4,6-tripyridyl-s-triazine) reagent was prepared by mixing a 10.0 mL TPTZ solution, 10 mL $FeCl_36H_2O$ solution and 100 mL acetate buffer at pH 3.6. A mixture of 200.0 μL sample solution and 3 mL of Fe(II)TPTZ reagent was incubated at 37 °C for 15 min. The absorbance of colour complex Fe(II)TPTZ was measured at 593 nm using ascorbic acid as standard. The results were expressed as ascorbic equivalent (mmol/100 gm compound).

Ascorbic acid taken = 1.99×10^{-4} mm.

Sample taken = 0.04 mg.

The Ferric Reducing Antioxidant Power (FRAP) can be calculated using the following equation:

$$\underset{(mmA.A./100gm\ \ sample)}{FRAPvalue} = \frac{\Delta OD_{593nm}\ \ of\ \ test\ \ sample}{\Delta OD_{593nm}\ \ of\ \ standard} \times \frac{standard\ \ (mm)}{sample\ \ (mg)} \times 10^5$$

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2013.03.017.

References

[1] A. Saeed, U. Shaheen, A. Hameed, F. Kazmi, J. Fluorine Chem. 131 (2010) 333–339.
[2] L. Zhu, Z. Miao, C. Sheng, W. Guo, J. Yao, W. Liu, X. Che, W. Wang, P. Cheng, W. Zhang, Eur. J. Med. Chem. 45 (2010) 2726–2732.
[3] A.K. Zafer, T.Z. Gulhan, O. Ahmet, R. Gilbert, Eur. J. Med. Chem. 43 (2008) 155–159.
[4] T.P. Tim Cushnie, A.J. Lamb, Int. J. Antimicrob. Agents 26 (2005) 343–356.
[5] M. Sankaran, C. Kumarasamy, U. Chokkalingam, P.S. Mohan, Bioorg. Med. Chem. Lett. 20 (2010) 7147–7151.
[6] D.C. Mungra, M.P. Patel, D.P. Rajani, R.G. Patel, Eur. J. Med. Chem. 46 (2011) 4192–4200.
[7] N.M. Shah, M.P. Patel, R.G. Patel, Eur. J. Med. Chem. 54 (2012) 239–247.
[8] H.G. Kathrotiya, N.A. Patel, M.P. Patel, R.G. Patel, Chin. Chem. Lett. 23 (2012) 273–276.
[9] J.A. Makawana, D.C. Mungra, M.P. Patel, R.G. Patel, Bioorg. Med. Chem. Lett. 21 (2011) 6166–6169.
[10] H.H. Jardosh, M.P. Patel, Med. Chem. Res. 22 (2013) 905–915.
[11] R.V. Patel, P. Kumari, D.P. Rajani, K.H. Chikhalia, Eur. J. Med. Chem. 46 (2011) 4354–4365.
[12] K.D. Thomas, A.V. Adhikari, S. Telkar, I.H. Chowdhury, R. Mahmoode, N.K. Pal, G. Rowd, E. Sumesh, Eur. J. Med. Chem. 46 (2011) 5283–5292.
[13] K. Starcevic, D. Pesic, A. Toplak, G. Landek, S. Alihodzic, E. Herreros, S. Ferrer, R. Spaventi, M. Peric, Eur. J. Med. Chem. 49 (2012) 365–378.
[14] P.A. Leatham, H.A. Bird, V. Wright, D. Seymour, A. Gordon, Eur. J. Rheumatol. Inflammation. 6 (1983) 209–211.
[15] H.S. Abbas, H.N. Hafez, Abdel-R.B.A. El-Gazzar, Eur. J. Med. Chem. 46 (2011) 21–30.
[16] N. Muruganantham, R. Sivakumar, N. Anbalagan, V. Gunasekaran, J.T. Leonard, Biol. Pharm. Bull. 27 (2004) 1683–1687.
[17] M.P. Maguire, K.R. Sheets, K. McVety, A.P. Spada, A. Zilberstein, J. Med. Chem. 37 (1994) 2129–2137.
[18] W.D. Wilson, M. Zhao, S.E. Patterson, R.L. Wydra, L. Janda, L. Strekowski, Med. Chem. Res. 2 (1992) 102–110.
[19] A. Zieba, A. Sochanik, A. Szurko, M. Rams, A. Mrozek, P. Cmoch, Eur. J. Med. Chem. 45 (2010) 4733–4739.
[20] Q. Guo, L. Feng, M. Liu, Y. Zhang, Y. Chai, K. Lv, H. Guo, L. Han, Eur. J. Med. Chem. 45 (2010) 5498–5506.
[21] Y. Chai, Z. Wan, B. Wang, H. Guo, M. Liu, Eur. J. Med. Chem. 44 (2009) 4063–4069.
[22] S. Srinivasan, R.M.B. Shafreen, P. Nithyanand, P. Manisankar, S.K. Pandian, Eur. J. Med. Chem. 45 (2010) 6101–6105.
[23] C. Zhi, Z. Long, A. Manikowski, J. Comstock, W. Xu, N.C. Brown, et al., J. Med. Chem. 49 (2006) 1455–1465.
[24] H. Koga, A. Itoh, S. Murayama, S. Suzue, T. Irikura, J. Med. Chem. 23 (1980) 1358–1363.
[25] D.T.W. Chu, P.B. Fernandes, A.K. Claiborne, E. Pihuleac, C.W. Nordeen, R.E. Maleczka, A.G. Pernet, J. Med. Chem. 223 (1985) 1558–1564.
[26] D.T.W. Chu, P.B. Femandes, R.E. Malenka, C.W. Nordeen, A.G. Pernet, J. Med. Chem. 30 (1987) 504–509.
[27] O. Meth-Cohn, N.A. Bramha, Tetrahedron Lett. 23 (1978) 2045–2048.
[28] N.J. Thumar, M.P. Patel, Arch. Pharm. Chem. Life Sci. 2 (2011) 91–101.
[29] NCCLS (National Committee for Clinical Laboratory Standards), Performance, Standards for Antimicrobial Susceptibility Testing: Twelfth Informational Supplement (2002), ISBN 1-56238-454-6. M100-S12 (M7).
[30] A. Rattan, Antimicrobials in Laboratory Medicine, Churchill B. I., Livingstone, New Delhi, 2000, pp. 85–108.
[31] B.N. Meyer, N.R. Ferrigni, J.E. Putnam, J.B. Jacobsen, D.E. Nicholsand, J.L. Mclaughlin, Planta. Med. 45 (1982) 31–34.
[32] I.F.F. Benzie, J.J. Strain, Anal. Biochem. 239 (1996) 70–76.
[33] B.S. Furniss, A.J. Hannaford, P.W.G. Smith, A.R. Tatchell, Vogel's Textbook of Practical Organic Chemistry, fifth ed., Longman, Harlow, 2004.
[34] Md.R. Islam, S.M.R. Islam, A.S.M. Noma, J.A. Khanam, S.M.M. Ali, S. Alam, M.W. Lee, Mycobiology 35 (2007) 25–29.