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Original article

Synthesis and identification of β-aryloxyquinolines and their pyrano[3,2-*c*] chromene derivatives as a new class of antimicrobial and antituberculosis agents

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ABSTRACT

A new class of β-aryloxyquinolines **3a**–**i** and their pyrano[3,2-*c*]chromene derivatives **6a**–**r** incorporating a validated molecular target has been synthesized *via* a nucleophilic displacement and a one-pot multicomponent reaction respectively. *In vitro* antimicrobial activity of the synthesized compounds were investigated against a representative panel of pathogenic strains specifically *Bacillus subtilis*, *Clostridium tetani*, *Streptococcus pneumoniae*, *Escherichia coli*, *Salmonella typhi*, *Vibrio cholera*, *Aspergillus fumigatus* and *Candida albicans*. Compounds **3c**, **3e**, **3g**, **6f**, **6l** and **6q** exhibited excellent antibacterial activity while compound **6p** exhibited more potent antifungal activity than that of first line standard drugs. *In vitro* antituberculosis activity was evaluated against *Mycobacterium tuberculosis* H37Rv and compound **6f** is emerged as the promising antimicrobial member with better antitubercular activity. Majority of the compounds appears to be better antimicrobials but poor antituberculars.

1. Introduction

An alarming increment in pathogenic resistance to existing drugs is a serious problem with antimicrobial therapy and necessitates continuing research into new classes of antimicrobials [1]. According to the World Health Organization, one third of human population is infected by *Mycobacterium tuberculosis* and approximately two million people die from tuberculosis annually. The cure of tuberculosis (isoniazid and rifampicin) [2] is remote from ideal due to its time-consuming nature. Tuberculosis coupled with HIV infection constructs a lethal combination. Tuberculosis is currently to blame for 13% of the number of deaths due to HIV infection [3]. On the core of these reports, the discovery of new therapeutical targets and the development of newer antimicrobial and antitubercular drugs are urgently looked-for. Consequently, this spot of research is carrying an immense significance and keeps on attracting much interest of contemporary medicinal chemistry.

Over the past few years, we have been principally engrossed in the synthesis of quinoline incorporating structures for antimicrobial evaluations [4–12] 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 like antimicrobial [13], antituberculosis [14], antimalarial [15,16], anti-inflammatory [17], anti-cancer [18], antihypertensive [19], tyrokinase PDGF-RTK inhibiting agents [20] and anti-HIV [21,22].

It has been well-established that presence of aryl ring appended by ether linkage at C-2 position of quinoline moiety is highly active against *M. tuberculosis* H37Rv and plays a pivotal role in development of new antituberculosis drugs [23] and in consequence, emerged as a validated molecular target. On the other hand, there are much sound reports expressing that pyrano[3,2-*c*]chromene is a class of vital heterocycles with a wide range of biological effects [24] such as spasmolytic, diuretic, anticoagulant, anti-cancer and anti-anaphylactic activity [25]. Further fused chromene derivatives have a relatively broad spectrum with high activity profile against various bacteria and fungi [26] along with antiproliferative [27], sexpheromonal [28], mutagenicitical [29], antitumoral [30], antiviral [31,32] and central nervous system activities [33]. Despite the immense pharmacological effects of β-aryloxyquinolines and pyrano[3,2-*c*]chromene derivatives, no large systematic study has been hitherto undertaken either to focus the synthesis of a heterocycle incorporating both of these biolabile nuclei or to optimize the fused chromene antimicrobials against MTB.

The molecular manipulation of promising lead compounds is still an organized and chief approach to widen the vicinity of medicine research. It involves an initiative to merge the separate pharmacophoric groups of analogous activity into one compound, thus making structural changes in the biological activity. An attempt has been

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through to undertake the synthesis of β-aryloxyquinolines and their dihydropyrano[3,2-*c*]chromene derivatives with the assumption that the assimilation of more than one bioactive moieties into a single scaffold may produce novel heterocycles with fascinating antimicrobial activities along with efficacious bioactivity to combat tuberculosis, a global health emergency.

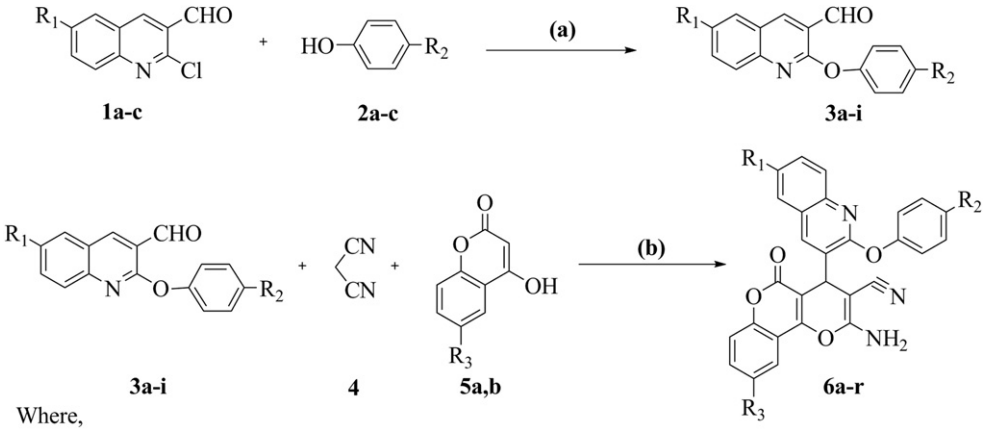
In the radiance of the aforementioned facts and as a prolongation of our investigations on the synthesis of biologically active heterocyclic compounds [4–12], we were provoked to synthesize new β-aryloxyquinolines and their pyrano[3,2-*c*]chromene derivatives incorporating a validated molecular target and evaluate them as antimicrobial and antitubercular agents. This communication additionally enable us to match up between antimicrobial and antimycobacterial activities of two structurally allied candidates specifically β-aryloxyquinolines and their dihydropyrano[3,2-*c*]chromene derivatives.

2. Chemistry

The reaction sequences employed for synthesis of title compounds **3a**–**i** and **6a**–**r** are depicted in Scheme 1. The starting material 2-chloro-3-formylquinolines **1a**–**c** were prepared according to literature procedure [34] by Vilsmeier–Haack reaction and conveniently converted to β-aryloxyquinoline-3-carbaldehydes **3a**–**i** by nucleophilic displacement of chloro group at C2 in **1a**–**c** with phenols **2a**–**c** in refluxing dimethylformamide using anhydrous potassium carbonate as a base. Subsequently, the one-pot three component cyclocondensation of a series of β-aryloxyquinoline-3-carbaldehydes **3a**–**i**, malononitrile **4** and 4-hydroxycoumarins **5a,b** in ethanol containing a catalytic amount of piperidine afforded the target compounds **6a**–**r** in good to excellent yields (Table 1). The formation of compounds **6a**–**r** may proceed *via* initial formation of an intermediate afforded by Knoevenagel condensation of aldehyde with malononitrile which would undergo intermolecular cyclization, driven through the nucleophilic attack of 4-hydroxycoumarin in basic reaction conditions [35].

3. Pharmacology

The MICs of synthesized compounds were carried out by broth microdilution method according to National Committee for



Where,

Compound	R_1	R_2	R_3
1a	H	-	-
1b	CH_3	-	-
1c	OCH_3	-	-
2a	H	-	-
2b	CH_3	-	-
2c	OCH_3	-	-
3a	H	H	-
3b	H	CH_3	-
3c	H	Cl	-
3d	CH_3	H	-
3e	CH_3	CH_3	-
3f	CH_3	Cl	-
3g	OCH_3	H	-
3h	OCH_3	CH_3	-
3i	OCH_3	Cl	-
5a	-	-	H
5b	-	-	CH_3

Compound	R_1	R_2	R_3
6a	H	H	H
6b	H	CH_3	H
6c	H	Cl	H
6d	CH_3	H	H
6e	CH_3	CH_3	H
6f	CH_3	Cl	H
6g	OCH_3	H	H
6h	OCH_3	CH_3	H
6i	OCH_3	Cl	H
6j	H	H	CH_3
6k	H	CH_3	CH_3
6l	H	Cl	CH_3
6m	CH_3	H	CH_3
6n	CH_3	CH_3	CH_3
6o	CH_3	Cl	CH_3
6p	OCH_3	H	CH_3
6q	OCH_3	CH_3	CH_3
6r	OCH_3	Cl	CH_3

Scheme 1. Synthesis of 6-(un)-substituted-2-(4-(un)-substituted-aryloxy)quinoline-3-carbaldehude **3a-i** and 2-amino-5-oxo-9-(un)-substituted-4-(6-(un)-substituted-2-2(4-(un)-substituted aryloxy)quinolin-3-yl)-4,5-dihydropyrano[3,2-c]chromene-3-carbonitrile **6a-r**. Reagents and reaction conditions. (a) DMF.K_2CO_3. Reflux, 3.5 h (b) Piperidine, Ethanol, Reflux, 1.5 h.

Table 1
Physicochemical characteristics of β–aryloxyquinolines **3a**–**i** and their dihydropyrano[3,2-*c*]chromene derivatives **6a**–**r**.

Compd.	R_1	R_2	R_3	Formula (Mw)	Mp (°C)	Yield[a]%	Analysis (%) calc./found		
							C	H	N
3a	H	H	–	$C_{16}H_{11}NO_2$ (249.08)	126	77	77.10	4.45	5.62
							77.39	4.52	5.68
3b	H	CH_3	–	$C_{17}H_{13}NO_2$ (263.09)	137–139	62	77.55	4.98	5.32
							77.82	5.24	5.04
3c	H	Cl	–	$C_{16}H_{10}ClNO_2$ (283.04)	165	74	67.74	3.55	4.94
							67.52	3.76	4.68
3d	CH_3	H	–	$C_{17}H_{13}NO_2$ (263.09)	154	69	77.55	4.98	5.32
							77.81	5.26	5.66
3e	CH_3	CH_3	–	$C_{18}H_{15}NO_2$ (277.11)	179	80	77.96	5.45	5.05
							78.15	5.24	5.29
3f	CH_3	Cl	–	$C_{17}H_{12}ClNO_2$ (297.06)	154	67	68.58	4.06	4.70
							66.83	3.96	4.86
3g	OCH_3	H	–	$C_{17}H_{13}NO_3$ (279.09)	182–184	78	73.11	4.69	5.02
							73.00	4.90	5.35
3h	OCH_3	CH_3	–	$C_{18}H_{15}NO_3$ (293.11)	146–148	62	73.71	5.15	4.78
							73.90	5.22	4.94
3i	OCH_3	Cl	–	$C_{17}H_{12}ClNO_3$ (313.05)	180	65	65.08	3.86	4.46
							65.34	4.02	4.78
6a	H	H	H	$C_{28}H_{17}N_3O_4$ (459.12)	234	74	73.20	3.73	9.15
							73.42	3.59	8.95
6b	H	CH_3	H	$C_{29}H_{19}N_3O_4$ (473.14)	225–227	77	73.56	4.04	8.87
							73.45	4.28	8.78
6c	H	Cl	H	$C_{28}H_{16}ClN_3O_4$ (493.08)	263	80	68.09	3.27	8.51
							67.99	3.02	8.84
6d	CH_3	H	H	$C_{29}H_{19}N_3O_4$ (473.14)	249	81	73.56	4.04	8.87
							73.69	4.25	8.96
6e	CH_3	CH_3	H	$C_{30}H_{21}N_3O_4$ (487.15)	281–283	73	73.91	4.34	8.62
							73.88	4.12	8.46
6f	CH_3	Cl	H	$C_{29}H_{18}ClN_3O_4$ (507.10)	264	82	68.58	3.57	8.27
							68.69	4.77	8.53
6g	OCH_3	H	H	$C_{29}H_{19}N_3O_5$ (489.13)	237	65	71.16	3.91	8.58
							71.38	3.80	8.37
6h	OCH_3	CH_3	H	$C_{30}H_{21}N_3O_5$ (503.15)	269–272	71	71.56	4.20	8.35
							71.82	3.95	8.48
6i	OCH_3	Cl	H	$C_{29}H_{18}ClN_3O_5$ (523.09)	284	79	66.48	3.46	8.02
							66.22	3.68	7.84
6j	H	H	CH_3	$C_{29}H_{19}N_3O_4$ (473.14)	289	63	73.56	4.04	8.87
							73.69	4.20	8.60
6k	H	CH_3	CH_3	$C_{30}H_{21}N_3O_4$ (487.15)	265	79	73.91	4.34	8.62
							74.21	4.29	8.44
6l	H	Cl	CH_3	$C_{29}H_{18}ClN_3O_4$ (507.10)	256–258	68	68.58	3.57	8.27
							68.89	3.68	8.11
6m	CH_3	H	CH_3	$C_{30}H_{21}N_3O_4$ (487.15)	227	79	73.91	4.34	8.62
							74.12	4.65	3.69
6n	CH_3	CH_3	CH_3	$C_{31}H_{23}N_3O_4$ (501.17)	256	83	74.24	4.62	8.38
							74.19	4.82	8.01
6o	CH_3	Cl	CH_3	$C_{30}H_{20}ClN_3O_4$ (521.11)	269	80	69.03	3.86	8.05
							68.85	3.69	7.83
6p	OCH_3	H	CH_3	$C_{30}H_{21}N_3O_5$ (503.15)	266–268	74	71.56	4.20	8.35
							71.84	4.15	8.09
6q	OCH_3	CH_3	CH_3	$C_{31}H_{23}N_3O_5$ (517.16)	281	68	71.94	4.48	8.12
							71.79	4.20	8.38
6r	OCH_3	Cl	CH_3	$C_{30}H_{20}ClN_3O_5$ (537.11)	239	78	66.98	3.75	7.81
							67.23	4.02	7.65

[a] All the yields are on isolated basis.

Clinical Laboratory Standards (NCCLS) [36]. Antibacterial activity was screened against three Gram positive (*Bacillus subtilis* MTCC 441, *Clostridium tetani* MTCC 449, *Streptococcus pneumoniae* MTCC 1936) and three Gram negative (*Escherichia coli* MTCC 443, *Salmonella typhi* MTCC 98, *Vibrio cholerae* MTCC 3906) bacteria by using ampicillin as a standard antibacterial drug. Antifungal activity was screened against two fungal species (*Aspergillus fumigatus* MTCC 3008 and *Candida albicans* MTCC 227) where griseofulvin and nystatin used as standard antifungal drugs. The antimicrobial screening data are shown in Table 2. 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. Inoculum's size for test strain was adjusted to 10^8 CFU (Colony Forming Unit) per milliliter by comparing the turbidity. DMSO was used as diluents to get desired concentration of compounds to test upon standard bacterial strains.

In vitro antituberculosis activity of all the newly synthesized compounds against *M. tuberculosis* H37Rv strain was determined by using Lowenstein–Jensen medium (conventional method) as described by Rattan [37] and the observed results are presented in Table 3 in the form of % inhibition, relative to that of standard antitubercular drug isoniazid. Of the compounds studied, three compounds those exhibited highest % inhibition, were again screened to get their MIC values (Table 4).

Table 2
In vitro antimicrobial activity of β–aryloxyquinolines **3a**–**i** and their dihydropyrano [3,2-*c*]chromene derivatives **6a**–**r** MICs, μg/mL.

Compd.	Gram positive bacteria			Gram negative bacteria			Fungi	
	B.S.	C.T.	S.P.	E.C.	S.T.	V.C.	A.F.	C.A.
	MTCC	MTCC	MTCC	MTCC	MTCC	MTCC	MTCC	MTCC
	441	449	1936	443	98	3906	3008	227
3a	1000	500	500	500	500	500	>1000	>1000
3b	500	250	250	100	100	250	500	500
3c	200	500	62.5	100	200	500	>1000	250
3d	250	200	500	200	200	250	500	250
3e	250	200	200	50	100	200	1000	500
3f	500	500	250	250	100	500	1000	500
3g	100	250	200	62.5	100	200	>1000	1000
3h	250	500	500	250	250	500	1000	250
3i	200	200	250	200	200	200	1000	500
6a	200	250	250	200	250	250	500	250
6b	200	250	250	200	200	250	500	500
6c	100	200	500	250	250	250	500	250
6d	200	250	250	100	200	200	1000	500
6e	100	200	200	200	200	200	1000	500
6f	200	250	200	250	100	62.5	1000	1000
6g	100	200	200	100	100	250	500	500
6h	200	500	200	250	500	500	>1000	1000
6i	500	500	500	250	250	500	>1000	1000
6j	500	250	500	200	500	250	>1000	>1000
6k	250	500	250	250	250	250	500	500
6l	250	500	200	100	50	500	500	500
6m	200	200	250	100	200	250	>1000	>1000
6n	1000	500	1000	500	500	500	500	500
6o	500	250	500	200	250	200	500	250
6p	100	500	250	500	500	250	500	100
6q	250	250	250	62.5	200	500	500	200
6r	250	500	500	250	250	500	500	500
Ampi.	250	250	100	100	100	100	n. t.[a]	n. t.
Grise.	n. t.	n. t.	n. t.	n. t.	n. t.	n. t.	100	500
Nyst.	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, Ampi.: Ampicillin, Grise.: Griseofulvin, Nyst.: Nystatin.
[a] n. t. not tested.

4. Results and discussion

4.1. Analytical results

A series of two allied candidates 6-(un)-substituted-2-(4-(un)-substituted-aryloxy)quinoline-3-carbaldehyde **3a**–**i** and 2-amino-5-oxo-9-(un)-substituted-4-(6-(un)-substituted-2-(4-(un)-substituted aryloxy)quinolin-3-yl)-4,5-dihydropyrano[3,2-*c*]chromene-3-carbonitrile **6a**–**r** have been synthesized substantially through the synthetic route as illustrated in Scheme 1. The structures of all the newly synthesized compounds were confirmed by FTIR, ^{1}H NMR, ^{13}C NMR, mass and elemental analysis. The IR spectrum of compound **3a**–**i** exhibited characteristic absorption band in the range 1230–1240 cm^{-1} is mainly attributed to the presence of ether linkage. Absorption band around 1710–1725 cm^{-1} confirms the presence of aldehyde functionality as well as absorption band around 3000–3035 cm^{-1} is due to aromatic C–H stretching. The ^{1}H NMR spectra of compound **3a**–**i** exhibited the presence of the aldehyde proton as a singlet around δ 10.50 ppm and aromatic protons resonate as multiplets at around δ 7.11–8.96 ppm. The ^{13}C NMR spectrum is in well agreement with the structure assigned. In the ^{13}C NMR spectra, signals around δ 120.20–161.47 ppm are attributed to aromatic carbons and the carbonyl carbon was observed at about δ 190.49 ppm. The IR spectrum of title compounds **6a**–**r** revealed the presence of lactone, cyano and amino groups due to the appearance of absorption bands at around 1730, 2210 and 3450 & 3350 cm^{-1} respectively. Its ^{1}H NMR spectrum indicated the presence of one singlet in the range δ 4.86–4.96 ppm of –CH proton and the disappearance of a singlet from δ 10.50 ppm of –CHO clearly confirmed the cyclization of Knoevenagel intermediate. Moreover, singlet in the range δ 6.80–7.07 ppm and multiplets in the range δ 6.84–8.49 ppm appeared for amine and aromatic protons respectively. In the ^{13}C NMR spectral data of the title compounds **6a**–**r**, most characteristic signal around δ 34.00 ppm indicated the formation of pyrano[3,2-*c*] chromene ring and the carbonyl carbon was observed at around δ 160.00 ppm. The signal at around δ 56.00 ppm is assigned to carbon attached with carbonitrile while signals around δ 102.11–159.86 ppm are attributed to all the aromatic carbons of compounds **6a**–**r**.

The obtained elemental analysis values are in good agreement with theoretical data. Mass spectra of all the title compounds gave expected M^+ peak corresponding with proposed molecular mass. Physicochemical properties of all the derivatives are shown in Table 1.

Table 3
In vitro antituberculosis activity (% Inhibition) of β-aryloxyquinolines **3a**–**i** and their dihydropyrano[3,2-*c*]chromene derivatives **6a**–**r** against *M. tuberculosis* H37Rv (at concentration 250 μg/mL).

Compd.	% Inhibition	Compd.	% Inhibition
3a	95	**6f**	98
3b	74	**6g**	11
3c	64	**6h**	24
3d	62	**6i**	32
3e	84	**6j**	91
3f	93	**6k**	45
3g	42	**6l**	23
3h	23	**6m**	10
3i	54	**6n**	24
6a	21	**6o**	38
6b	10	**6p**	45
6c	32	**6q**	61
6d	52	**6r**	27
6e	74	**Isoniazid**	99

Table 4
In vitro antituberculosis activity of title compounds exhibiting higher % inhibition against *M. tuberculosis* H37Rv (MICs, μg/mL).

Compd.	% Inhibition	MIC
3a	95	260
3f	93	310
6f	98	250
Isoniazid	99	0.20

4.2. Biological results

Reviewing of the antibacterial activities of β-aryloxyquinolines and their dihydropyrano[3,2-*c*]chromene derivatives (Table 2) indicate that compound **3e** (R_1 = CH_3, R_2 = CH_3) and **6l** (R_1 = H, R_2 = Cl, R_3 = CH_3) showed highest activity i.e. 50 μg/mL against *E. coli* and *S. typhi* respectively. Interestingly, compound **3c** (R_1 = H, R_2 = Cl) showed MIC 200 μg/mL and 500 μg/mL against *B. subtilis* and *C. tetani* respectively and upon cyclocondensation with malononitrile and 4-hydroxycoumarin resulted compound **6c** (R_1 = H, R_2 = Cl, R_3 = H) which have been found to possess increased potency i.e. 100 μg/mL against *B. subtilis* and 200 μg/mL against *C. tetani*. In case of inhibiting Gram negative bacteria *E. coli*, compound **3g** (R_1 = OCH_3, R_2 = H) and **6q** (R_1 = OCH_3, R_2 = CH_3, R_3 = CH_3) displayed better activity i.e. 62.5 μg/mL while compound **6f** (R_1 = CH_3, R_2 = Cl, R_3 = H) displayed better activity against Gram

negative bacteria *V. cholerae*. The majority of compounds are found to possess higher potency as compared to standard bactericidal ampicillin against Gram positive bacteria *B. subtilis*.

Antifungal study revealed that all the synthesized β-aryloxyquinolines and their dihydropyrano[3,2-*c*]chromene derivatives have poor activity against *Aspergillus fumigatus*. In comparison with standard fungicidal griseofulvin, among β-aryloxyquinolines, compound **3c** (R_1 = H, R_2 = Cl), **3d** (R_1 = CH_3, R_2 = H), **3h** (R_1 = OCH_3, R_2 = CH_3) and among dihydropyrano[3,2-*c*]chromenes, compound **6a** (R_1 = H, R_2 = H, R_3 = H), **6c** (R_1 = H, R_2 = Cl, R_3 = H), **6o** (R_1 = CH_3, R_2 = Cl, R_3 = CH_3), **6p** (R_1 = OCH_3, R_2 = H, R_3 = CH_3) and **6q** (R_1 = OCH_3, R_2 = CH_3, R_3 = CH_3) displayed better activity against *C. albicans*.

The encouraging results from the antimicrobial studies prompted us to go for the preliminary screening of the title compounds for their *in vitro* antituberculosis activity against *M. tuberculosis* H37Rv. Of the compounds screened for antituberculosis activity, compound **3a** (R_1 = H, R_2 = H) and compound **6f** (R_1 = CH_3, R_2 = Cl, R_3 = H) found to possess better activity against *M. tuberculosis* H37Rv. Antitubercular potency of compound **3f** (R_1 = CH_3, R_2 = Cl) i.e. 93% was found to get intensified to 98%, upon cyclocondensation with malononitrile and unsubstituted 4-hydroxycoumarin which results compound **6f** (R_1 = CH_3, R_2 = Cl, R_3 = H). MIC values of three compounds those exhibited highest % inhibition, are found to be 260 μg/mL for compound **3a** (R_1 = H, R_2 = H), 310 μg/mL for **3f** (R_1 = CH_3, R_2 = Cl) and 250 μg/mL for **6f** (R_1 = CH_3, R_2 = Cl, R_3 = H).

Compound **6f** (R_1 = CH_3, R_2 = Cl, R_3 = H) has been emerged as the promising antimicrobial member along with better antitubercular activity (Table 2,3 and 4) of this series of compounds. Unfortunately, majority of compounds showed poor inhibition of *M. tuberculosis* growth.

5. Conclusion

In this paper, we report the synthesis, antimicrobial and antituberculosis activity of two structurally correlated heterocyclic candidates i.e. β-aryloxyquinolines **3a**–**i** and their pyrano[3,2-*c*]chromene derivatives **6a**–**r**. The engaged synthetic strategy efficiently involves the multicomponent reaction (MCR) approach and allows the construction of relatively complicated nitrogen and oxygen carrying heterocyclic system as well as the introduction of various substituted β-aryloxyquinolines, the validated molecular target at 4- position of pyrano[3,2-*c*]chromene. Reviewing and comparing the activity data (Table 2,3 and 4), it is worthy to mention that the biological activity of the target compounds depends not only on the bicyclic heteroaromatic pharmacophore appended through ether linked aryl ring but also on the nature of the peripheral substituents and may also upon their spatial relationship and positional changes. Of the compounds studied, we identified the most effective antimicrobial member possessing better antituberculosis activity proved to be having methyl substituted quinoline, ether linked 4-chloro substituted phenyl ring and unsubstituted pyrano[3,2-*c*]chromene ring. These studies provide insight to defining the ample scope and boundaries of both allied candidates for further detailed preclinical investigations.

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 [38] 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 vapors 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 & ^{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). 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 & Training (SICART), Vallabh Vidyanagar and all compounds are within ±0.4% of the theoretical compositions. Yields are not optimized. Ampicillin, griseofulvin, nystatin and isoniazid were commercial.

6.1.1. General procedure for the synthesis of 6-(un)-substituted-2-(4-(un)-substituted-aryloxy)quinoline-3-carbaldehyde **3a**–**i**

A 100 mL round bottomed flask, fitted with a reflux condenser, was charged with a mixture of 2-chloro-3-formylquinoline **1a**–**c** (5 mmol), appropriate phenol **2a**–**c** (5 mmol), anhydrous potassium carbonate (10 mmol) in dimethylformamide (5 mL). The mixture was heated under reflux for 3.5 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 then poured into chilled water (50 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.

*6.1.1.1. 2-Phenoxyquinoline-3-carbaldehyde (**3a**).* IR (KBr, ν_{max}, cm^{-1}): 3030 (ArC–H), 1685 (C=O stretching), 1235 cm^{-1} (C–O–C ether stretching). ^1H NMR (400 MHz, DMSO-d_6): δ 7.29–8.93 (m, 10H, Ar–H), 10.51 (s, 1H, CHO). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 120.61, 122.43, 125.36, 125.66, 126.28, 127.35, 130.09, 130.56, 133.52, 141.79, 148.06, 153.49, 160.54 (13C, Ar–C), 189.33 (CHO); MS (*m/z*): 249 (M^+).

*6.1.1.2. 2-(4-Methylphenoxy)quinoline-3-carbaldehyde (**3b**).* IR (KBr, ν_{max}, cm^{-1}): 3020 (ArC–H), 1710 (C=O stretching), 1230 cm^{-1} (C–O–C ether stretching). ^1H NMR (400 MHz, DMSO-d_6): δ 2.50 (s, 3H, CH_3), 7.26–8.90 (m, 9H, Ar–H), 10.57 (s, 1H, CHO). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 21.31 (CH_3), 120.23, 122.47, 124.82, 125.72, 126.32, 127.30, 130.40, 130.86, 132.41, 140.65, 148.45, 153.96, 160.12 (13C, Ar–C), 190.87 (CHO); MS (*m/z*): 263 (M^+).

*6.1.1.3. 2-(4-Chlorophenoxy)quinoline-3-carbaldehyde (**3c**).* IR (KBr, ν_{max}, cm^{-1}): 3025 (ArC–H), 1720 (C=O stretching), 1230 cm^{-1} (C–O–C ether stretching). ^1H NMR (400 MHz, DMSO-d_6): δ 7.22–8.96 (m, 9H, Ar–H), 10.59 (s, 1H, CHO). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 120.21, 122.52, 124.21, 125.70, 126.41, 127.34, 130.92, 130.99, 132.56, 140.41, 148.82, 154.25, 160.00 (13C, Ar–C), 189.42 (CHO); MS (*m/z*): 283 (M^+), 285 (M+2).

*6.1.1.4. 6-Methyl-2-phenoxyquinoline-3-carbaldehyde (**3d**).* IR (KBr, ν_{max}, cm^{-1}): 3015 (ArC–H), 1715 (C=O stretching), 1240 cm^{-1} (C–O–C ether stretching). ^1H NMR (400 MHz, DMSO-d_6): δ 2.53 (s, 3H, CH_3), 7.19–8.94 (m, 9H, Ar–H), 10.50 (s, 1H, CHO). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 21.48 (CH_3), 120.20, 122.63, 124.08, 125.26,

126.32, 127.76, 130.44, 130.88, 132.00, 140.61, 147.06, 153.81, 160.16 (13C, Ar–C), 191.09 (CHO); MS (*m/z*): 263 (M^+).

*6.1.1.5. 6-Methyl-2-(4-methylphenoxy)quinoline-3-carbaldehyde (**3e**).* IR (KBr, ν_{max}, cm^{-1}): 3030 (ArC–H), 1710 (C=O stretching), 1225 cm^{-1} (C–O–C ether stretching). ^1H NMR (400 MHz, DMSO-d_6): δ 2.39 (s, 3H, CH_3), 2.57 (s, 3H, CH_3), 7.11–8.90 (m, 8H, Ar–H), 10.48 (s, 1H, CHO). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 20.09 (CH_3), 21.42 (CH_3), 120.26, 122.47, 124.98, 125.66, 126.00, 127.18, 130.04, 130.76, 132.07, 140.43, 146.14, 153.42, 161.47 (13C, Ar–C), 190.49 (CHO); MS (*m/z*): 277 (M^+).

*6.1.1.6. 2-(4-Chlorophenoxy)-6-methylquinoline-3-carbaldehyde (**3f**).* IR (KBr, ν_{max}, cm^{-1}): 3000 (ArC–H), 1695 (C=O stretching), 1230 cm^{-1} (C–O–C ether stretching). ^1H NMR (400 MHz, DMSO-d_6): δ 2.50 (s, 3H, CH_3), 7.11–8.91 (m, 8H, Ar–H), 10.55 (s, 1H, CHO). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 21.55 (CH_3), 121.06, 122.35, 124.48, 125.86, 126.37, 127.00, 131.42, 131.89, 132.88, 140.60, 147.35, 153.36, 161.39 (13C, Ar–C), 190.26 (CHO); MS (*m/z*): 297 (M^+), 299 (M+2).

*6.1.1.7. 6-Methoxy-2-phenoxyquinoline-3-carbaldehyde (**3g**).* IR (KBr, ν_{max}, cm^{-1}): 3010 (ArC–H), 1715 (C=O stretching), 1235 cm^{-1} (C–O–C ether stretching). ^1H NMR (400 MHz, DMSO-d_6): δ 3.97 (s, 3H, OCH_3), 7.14–8.96 (m, 9H, Ar–H), 10.58 (s, 1H, CHO). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 56.31 (OCH_3), 120.63, 122.00, 124.16, 125.65, 126.82, 127.70, 130.34, 130.49, 132.07, 140.92, 147.22, 153.36, 161.22 (13C, Ar–C), 190.84 (CHO); MS (*m/z*): 279 (M^+).

*6.1.1.8. 6-Methoxy-2-(4-methylphenoxy)quinoline-3-carbaldehyde (**3h**).* IR (KBr, ν_{max}, cm^{-1}): 3035 (ArC–H), 1710 (C=O stretching), 1230 cm^{-1} (C–O–C ether stretching). ^1H NMR (400 MHz, DMSO-d_6): δ 2.39 (s, 3H, CH_3), 3.90 (s, 3H, OCH_3), 7.14–8.92 (m, 8H, Ar–H), 10.42 (s, 1H, CHO). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 20.09 (CH_3), 54.92 (OCH_3), 120.20, 122.33, 124.90, 125.67, 126.52, 128.09, 130.16, 130.96, 132.35, 140.77, 146.10, 153.57, 160.09 (13C, Ar–C), 191.08 (CHO); MS (*m/z*): 293 (M^+).

*6.1.1.9. 2-(4-Chlorophenoxy)-6-methoxyquinoline-3-carbaldehyde (**3i**).* IR (KBr, ν_{max}, cm^{-1}): 3015 (ArC–H), 1725 (C=O stretching), 1235 cm^{-1} (C–O–C ether stretching). ^1H NMR (400 MHz, DMSO-d_6): δ 3.76 (s, 3H, OCH_3), 7.16–8.94 (m, 8H, Ar–H), 10.51 (s, 1H, CHO). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 56.14 (OCH_3), 121.74, 122.59, 124.36, 125.92, 126.37, 127.07, 131.47, 131.99, 132.45, 140.95, 146.36, 153.95, 160.56 (13C, Ar–C), 191.20 (CHO); MS (*m/z*): 313 (M^+), 315 (M+2).

*6.1.2. General procedure for the synthesis of 2-amino-5-oxo-9-(un)-substituted-4-(6-(un)-substituted-2-(4-(un)-substituted aryloxy)quinolin-3-yl)-4,5-dihydropyrano [3,2-c]chromene-3-carbonitrile **6a-r***

A 100 mL round bottomed flask, fitted with a reflux condenser, was charged with a mixture of β-aryloxyquinoline-3-carbaldehyde **3a–i** (5 mmol), malononitrile **4** (5 mmol), 4-hydroxycoumarin **5a,b** (5 mmol), and a catalytic amount of piperidine (1 mmol) in ethanol (15 mL). The mixture was heated under reflux for 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 (20 mL) to obtain pure solid sample.

*6.1.2.1. 2-Amino-5-oxo-4-(2-phenoxyquinolin-3-yl)-4,5-dihydropyrano [3,2-c]chromene-3-carbonitrile (**6a**).* IR (KBr, ν_{max}, cm^{-1}): 3450 & 3350 (asym. & sym. stretching of $-NH_2$), 3030 (ArC–H), 2210 (C≡N stretching), 1730 (C=O lactone stretching), 1235 cm^{-1} (C–O–C ether stretching). ^1H NMR (400 MHz, DMSO-d_6): δ 4.92 (s, 1H, chromene H4), 6.91 (s, 2H, NH_2), 7.03–8.43 (m, 14H, Ar–H). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 34.01 (chromene C4), 56.15 (C–CN), 102.67, 113.10, 118.12, 120.40, 121.91, 122.00, 125.88, 125.96, 126.70, 127.00, 127.76, 128.28, 130.55, 133.36, 134.56, 136.94, 139.52, 145.70, 151.94, 152.12, 154.60, 158.59, 159.37 (23C, Ar–C), 160.39 (C=O); MS (*m/z*): 459 (M^+).

*6.1.2.2. 2-Amino-5-oxo-4-(2-(4-methylphenoxy)quinolin-3-yl)-4,5-dihydropyrano [3,2-c]chromene-3-carbonitrile (**6b**).* IR (KBr, ν_{max}, cm^{-1}): 3440 & 3355 (asym. & sym. stretching of $-NH_2$), 3025 (ArC–H), 2215 (C≡N stretching), 1725 (C=O lactone stretching), 1230 cm^{-1} (C–O–C ether stretching). ^1H NMR (400 MHz, DMSO-d_6): δ 2.29 (s, 3H, CH_3), 4.98 (s, 1H, chromene H4), 6.94 (s, 2H, NH_2), 6.96–8.37 (m, 13H, Ar–H). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 20.83 (CH_3), 33.84 (chromene C4), 56.19 (C–CN), 102.78, 113.45, 117.08, 119.76, 121.44, 122.86, 125.12, 125.48, 126.25, 127.00, 127.11, 128.16, 130.25, 133.39, 134.04, 136.45, 139.43, 145.27, 151.74, 152.72, 154.82, 159.34, 159.86 (23C, Ar–C), 160.22 (C=O); MS (*m/z*): 473 (M^+).

*6.1.2.3. 2-Amino-5-oxo-4-(2-(4-chlorophenoxy)quinolin-3-yl)-4,5-dihydropyrano [3,2-c]chromene-3-carbonitrile (**6c**).* IR (KBr, ν_{max}, cm^{-1}): 3435 & 3365 (asym. & sym. stretching of $-NH_2$), 3020 (ArC–H), 2225 (C≡N stretching), 1710 (C=O lactone stretching), 1225 cm^{-1} (C–O–C ether stretching). ^1H NMR (400 MHz, DMSO-d_6): δ 4.90 (s, 1H, chromene H4), 6.83 (s, 2H, NH_2), 6.99–8.35 (m, 13H, Ar–H). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 34.66 (chromene C4), 56.26 (C–CN), 103.12, 112.50, 117.52, 118.78, 121.52, 122.84, 125.77, 125.98, 126.76, 127.11, 127.54, 128.38, 129.21, 133.30, 134.14, 136.64, 139.25, 145.44, 150.72, 153.71, 154.00, 158.01, 159.12 (23C, Ar–C), 160.92 (C=O); MS (*m/z*): 493 (M^+), 495 (M+2).

*6.1.2.4. 2-Amino-5-oxo-4-(6-methyl-2-phenoxyquinolin-3-yl)-4,5-dihydropyrano [3,2-c]chromene-3-carbonitrile (**6d**).* IR (KBr, ν_{max}, cm^{-1}): 3430 & 3365 (asym. & sym. stretching of $-NH_2$), 3020 (ArC–H), 2230 (C≡N stretching), 1735 (C=O lactone stretching), 1225 cm^{-1} (C–O–C ether stretching). ^1H NMR (400 MHz, DMSO-d_6): δ 2.38 (s, 3H, CH_3), 4.86 (s, 1H, chromene H4), 6.90 (s, 2H, NH_2), 6.91–8.39 (m, 13H, Ar–H). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 21.74 (CH_3), 34.53 (chromene C4), 56.20 (C–CN), 102.40, 112.00, 116.88, 119.55, 121.40, 122.87, 125.23, 125.85, 126.03, 127.74, 127.99, 128.56, 129.20, 133.39, 134.65, 136.12, 138.47, 144.20, 151.65, 152.36, 154.80, 158.35, 159.12 (23C, Ar–C), 161.27 (C=O); MS (*m/z*): 473 (M^+).

*6.1.2.5. 2-Amino-5-oxo-4-(6-methyl-2-(4-methylphenoxy)quinolin-3-yl)-4,5-dihydropyrano [3,2-c]chromene-3-carbonitrile (**6e**).* IR (KBr, ν_{max}, cm^{-1}): 3400 & 3370 (asym. & sym. stretching of $-NH_2$), 3010 (ArC–H), 2220 (C≡N stretching), 1730 (C=O lactone stretching), 1225 cm^{-1} (C–O–C ether stretching). ^1H NMR (400 MHz, DMSO-d_6): δ 2.28 (s, 3H, CH_3), 2.40 (s, 3H, CH_3), 4.90 (s, 1H, chromene H4), 6.84 (s, 2H, NH_2), 6.92–8.41 (m, 12H, Ar–H). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 20.91 (CH_3), 21.33 (CH_3), 34.04 (chromene C4), 56.59 (C–CN), 102.16, 112.14, 117.64, 119.70, 120.40, 122.46, 125.65, 125.94, 126.25, 127.07, 127.17, 128.96, 131.20, 133.55, 134.65, 136.18, 139.73, 145.27, 151.43, 152.00, 154.98, 159.08, 159.80 (23C, Ar–C), 161.52 (C=O); MS (*m/z*): 487 (M^+).

*6.1.2.6. 2-Amino-5-oxo-4-(2-(4-chlorophenoxy)-6-methylquinolin-3-yl)-4,5-dihydropyrano [3,2-c]chromene-3-carbonitrile (**6f**).* IR (KBr, ν_{max}, cm^{-1}): 3405 & 3360 (asym. & sym. stretching of $-NH_2$), 3020 (ArC–H), 2220 (C≡N stretching), 1715 (C=O lactone stretching), 1225 cm^{-1} (C–O–C ether stretching). ^1H NMR (400 MHz, DMSO-d_6): δ 2.20 (s, 3H, CH_3), 4.92 (s, 1H, chromene H4), 6.90 (s, 2H, NH_2), 6.97–8.46 (m, 12H, Ar–H). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 20.92

(CH_3), 34.04 (chromene C4), 56.59 (C—CN), 102.18, 112.20, 117.64, 119.32, 120.41, 122.40, 125.91, 125.94, 126.00, 127.04, 127.23, 128.90, 131.12, 134.50, 134.66, 137.69, 139.70, 145.20, 150.52, 152.01, 155.08, 159.18, 159.74 (23C, Ar—C), 161.50 (C═O); MS (*m/z*): 507 (M^+), 509 (M+2).

*6.1.2.7. 2-Amino-5-oxo-4-(6-methoxy-2-phenoxyquinolin-3-yl)-4,5-dihydropyrano [3,2-c]chromene-3-carbonitrile (**6g**).* IR (KBr, ν_{max}, cm^{-1}): 3430 & 3365 (asym. & sym. stretching of $-NH_2$), 3010 (ArC—H), 2235 (C≡N stretching), 1735 (C═O lactone stretching), 1235 cm^{-1} (C—O—C ether stretching). ^1H NMR (400 MHz, DMSO-d_6): δ 3.84 (s, 3H, OCH_3), 4.86 (s, 1H, chromene H4), 6.86 (s, 2H, NH_2), 6.98—8.42 (m, 13H, Ar—H). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 34.53 (chromene C4), 56.20 (C—CN), 56.70 (OCH_3), 102.17, 112.74, 116.00, 119.36, 121.47, 122.93, 125.23, 125.48, 126.00, 127.65, 127.76, 128.50, 129.21, 133.30, 134.01, 136.66, 138.40, 145.04, 151.25, 152.25, 154.98, 158.30, 159.17 (23C, Ar—C), 161.21 (C═O); MS (*m/z*): 489 (M^+).

*6.1.2.8. 2-Amino-5-oxo-4-(6-methoxy-2-(4-methylphenoxy)quinolin-3-yl)-4,5-dihydropyrano [3,2-c]chromene-3-carbonitrile (**6h**).* IR (KBr, ν_{max}, cm^{-1}): 3405 & 3355 (asym. & sym. stretching of $-NH_2$), 3010 (ArC—H), 2230 (C≡N stretching), 1735 (C═O lactone stretching), 1240 cm^{-1} (C—O—C ether stretching). ^1H NMR (400 MHz, DMSO-d_6): δ 2.24 (s, 3H, CH_3), 2.80 (s, 3H, OCH_3), 4.94 (s, 1H, chromene H4), 6.80 (s, 2H, NH_2), 6.92—8.49 (m, 12H, Ar—H). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 20.86 (CH_3), 34.04 (chromene C4), 56.53 (OCH_3), 56.59 (C—CN), 102.25, 112.10, 117.90, 119.77, 120.65, 123.00, 125.08, 125.64, 126.20, 127.17, 127.28, 128.65, 131.20, 133.16, 134.65, 136.89, 139.70, 145.21, 151.36, 152.07, 154.65, 159.16, 159.74 (23C, Ar—C), 161.44 (C═O); MS (*m/z*): 503 (M^+).

*6.1.2.9. 2-Amino-5-oxo-4-(2-(4-chlorophenoxy)-6-methoxyquinolin-3-yl)-4,5-dihydropyrano [3,2-c]chromene-3-carbonitrile (**6i**).* IR (KBr, ν_{max}, cm^{-1}): 3400 & 3350 (asym. & sym. stretching of $-NH_2$), 3025 (ArC—H), 2200 (C≡N stretching), 1730 (C═O lactone stretching), 1230 cm^{-1} (C—O—C ether stretching). ^1H NMR (400 MHz, DMSO-d_6): δ 3.84 (s, 3H, OCH_3), 4.97 (s, 1H, chromene H4), 7.07 (s, 2H, NH_2), 7.10—8.30 (m, 12H, Ar—H). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 33.41 (chromene C4), 55.88 (C—CN), 56.33 (OCH_3), 102.83, 106.71, 113.40, 117.06, 119.78, 122.31, 122.83, 123.14, 125.13, 127.43, 127.61, 128.45, 128.71, 129.80, 133.40, 138.67, 140.58, 152.70, 153.23, 154.80, 156.99, 157.84, 159.21 (23C, Ar—C), 160.21 (C═O); MS (*m/z*): 523 (M^+), 525 (M+2).

*6.1.2.10. 2-Amino-5-oxo-9-methyl-4-(2-phenoxyquinolin-3-yl)-4,5-dihydropyrano [3,2-c]chromene-3-carbonitrile (**6j**).* IR (KBr, ν_{max}, cm^{-1}): 3415 & 3370 (asym. & sym. stretching of $-NH_2$), 3010 (ArC—H), 2225 (C≡N stretching), 1730 (C═O lactone stretching), 1235 cm^{-1} (C—O—C ether stretching). ^1H NMR (400 MHz, DMSO-d_6): δ 2.29 (s, 3H, CH_3), 4.94 (s, 1H, chromene H4), 6.93 (s, 2H, NH_2), 6.96—8.29 (m, 13H, Ar—H). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 20.84 (CH_3), 34.09 (chromene C4), 56.22 (C—CN), 102.11, 113.36, 116.80, 119.84, 121.63, 122.30, 126.10, 126.57, 126.96, 127.36, 130.20, 132.27, 133.93, 134.20, 134.47, 134.81, 138.80, 142.12, 150.74, 151.06, 154.04, 159.30, 159.48 (23C, Ar—C), 160.19 (C═O); MS (*m/z*): 473 (M^+).

*6.1.2.11. 2-Amino-5-oxo-9-methyl-4-(2-(4-methylphenoxy)quinolin-3-yl)-4,5-dihydropyrano [3,2-c]chromene-3-carbonitrile (**6k**).* IR (KBr, ν_{max}, cm^{-1}): 3400 & 3355 (asym. & sym. stretching of $-NH_2$), 3010 (ArC—H), 2225 (C≡N stretching), 1720 (C═O lactone stretching), 1225 cm^{-1} (C—O—C ether stretching). ^1H NMR (400 MHz, DMSO-d_6): δ 2.29 (s, 3H, CH_3), 2.48 (s, 3H, CH_3), 4.97 (s, 1H, chromene H4), 6.88 (s, 2H, NH_2), 6.93—8.25 (m, 12H, Ar—H). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 20.86 (CH_3), 20.95 (CH_3), 34.11 (chromene C4), 56.26 (C—CN), 102.60, 113.25, 116.85, 119.56, 121.40, 122.39, 126.19, 126.23, 126.96, 127.02, 130.24, 132.16, 133.90, 134.25, 134.44, 134.70, 138.80, 143.63, 150.92, 151.80, 154.06, 159.12, 159.44 (23C, Ar—C), 161.03 (C═O); MS (*m/z*): 487 (M^+).

*6.1.2.12. 2-Amino-5-oxo-9-methyl-4-(2-(4-chlorophenoxy)quinolin-3-yl)-4,5-dihydropyrano [3,2-c]chromene-3-carbonitrile (**6l**).* IR (KBr, ν_{max}, cm^{-1}): 3425 & 3365 (asym. & sym. stretching of $-NH_2$), 3005 (ArC—H), 2220 (C≡N stretching), 1730 (C═O lactone stretching), 1230 cm^{-1} (C—O—C ether stretching). ^1H NMR (400 MHz, DMSO-d_6): δ 2.33 (s, 3H, CH_3), 4.90 (s, 1H, chromene H4), 6.90 (s, 2H, NH_2), 6.93—8.21 (m, 12H, Ar—H). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 20.80 (CH_3), 34.16 (chromene C4), 56.41 (C—CN), 103.04, 113.14, 116.09, 119.84, 121.16, 122.30, 126.25, 126.46, 126.90, 127.11, 130.20, 132.52, 133.90, 134.17, 134.44, 134.73, 138.86, 143.98, 150.54, 151.80, 154.74, 159.30, 159.56 (23C, Ar—C), 160.71 (C═O); MS (*m/z*): 507 (M^+), 509 (M+2).

*6.1.2.13. 2-Amino-5-oxo-9-methyl-4-(6-methyl-2-phenoxyquinolin-3-yl)-4,5-dihydropyrano [3,2-c]chromene-3-carbonitrile (**6m**).* IR (KBr, ν_{max}, cm^{-1}): 3400 & 3375 (asym. & sym. stretching of $-NH_2$), 3010 (ArC—H), 2220 (C≡N stretching), 1745 (C═O lactone stretching), 1230 cm^{-1} (C—O—C ether stretching). ^1H NMR (400 MHz, DMSO-d_6): δ 2.25 (s, 3H, CH_3), 2.28 (s, 3H, CH_3), 4.93 (s, 1H, chromene H4), 6.90 (s, 2H, NH_2), 6.93—8.27 (m, 12H, Ar—H). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 20.81 (CH_3), 20.93 (CH_3), 34.13 (chromene C4), 56.26 (C—CN), 102.43, 113.11, 116.45, 119.72, 121.05, 122.30, 126.12, 126.87, 126.96, 127.14, 130.65, 132.22, 133.90, 134.44, 134.48, 134.86, 138.36, 143.63, 150.87, 151.12, 154.74, 159.30, 159.46 (23C, Ar—C), 160.12 (C═O); MS (*m/z*): 487 (M^+).

*6.1.2.14. 2-Amino-5-oxo-9-methyl-4-(6-methyl-2-(4-methylphenoxy) quinolin-3-yl)-4,5 dihydropyrano [3,2-c]chromene-3-carbonitrile (**6n**).* IR (KBr, ν_{max}, cm^{-1}): 3405 & 3360 (asym. & sym. stretching of $-NH_2$), 3010 (ArC—H), 2200 (C≡N stretching), 1730 (C═O lactone stretching), 1235 cm^{-1} (C—O—C ether stretching). ^1H NMR (400 MHz, DMSO-d_6): δ 2.28 (s, 3H, CH_3), 2.40 (s, 3H, CH_3), 2.42 (s, 3H, CH_3), 4.92 (s, 1H, chromene H4), 6.91 (s, 2H, NH_2), 6.94—8.24 (m, 11H, Ar—H). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 20.82 (CH_3), 20.96 (CH_3), 21.34 (CH_3), 34.01 (chromene C4), 56.15 (C—CN), 102.67, 113.10, 116.85, 119.80, 121.45, 122.38, 126.19, 126.77, 126.96, 127.06, 130.24, 132.20, 133.93, 134.18, 134.44, 134.77, 138.86, 143.63, 150.90, 151.86, 154.74, 159.37, 159.40 (23C, Ar—C), 160.33 (C═O); MS (*m/z*): 501 (M^+).

*6.1.2.15. 2-Amino-5-oxo-9-methyl-4-(2-(4-chlorophenoxy)-6-methyquinolin-3-yl)-4,5-dihydropyrano [3,2-c]chromene-3-carbonitrile (**6o**).* IR (KBr, ν_{max}, cm^{-1}): 3410 & 3385 (asym. & sym. stretching of $-NH_2$), 3015 (ArC—H), 2225 (C≡N stretching), 1735 (C═O lactone stretching), 1235 cm^{-1} (C—O—C ether stretching). ^1H NMR (400 MHz, DMSO-d_6): δ 2.25 (s, 3H, CH_3), 2.31 (s, 3H, CH_3), 4.98 (s, 1H, chromene H4), 6.91 (s, 2H, NH_2), 6.94—8.22 (m, 11H, Ar—H). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 20.82 (CH_3), 20.91 (CH_3), 34.16 (chromene C4), 56.20 (C—CN), 102.47, 113.11, 116.40, 119.77, 121.94, 122.35, 126.12, 126.74, 126.90, 127.22, 130.60, 132.21, 133.96, 134.45, 134.49, 134.96, 138.30, 143.52, 149.56, 151.14, 154.04, 159.37, 159.78 (23C, Ar—C), 161.05 (C═O); MS (*m/z*): 521 (M^+), 523 (M+2).

*6.1.2.16. 2-Amino-5-oxo-9-methyl-4-(6-methoxy-2-phenoxyquinolin-3-yl)-4,5-dihydropyrano [3,2-c]chromene-3-carbonitrile (**6p**).* IR (KBr, ν_{max}, cm^{-1}): 3405 & 3350 (asym. & sym. stretching of $-NH_2$), 3010 (ArC—H), 2235 (C≡N stretching), 1715 (C═O lactone stretching), 1235 cm^{-1} (C—O—C ether stretching). ^1H NMR (400 MHz, DMSO-d_6): δ 2.25 (s, 3H, CH_3), 2.84 (s, 3H, OCH_3), 4.91 (s, 1H, chromene H4), 6.87 (s, 2H, NH_2), 6.90—8.41 (m, 12H, Ar—H). ^{13}C NMR (100 MHz, DMSO-

d_6) δ: 20.39 (CH_3), 34.95 (chromene C4), 56.48 (OCH_3), 56.68 (C–CN), 103.83, 112.25, 117.65, 119.89, 120.60, 123.36, 125.00, 125.64, 126.37, 127.08, 127.65, 128.60, 131.24, 133.54, 134.67, 136.80, 139.73, 145.21, 151.30, 152.77, 154.49, 159.45, 159.82 (23C, Ar–C), 160.36 (C=O); MS (*m/z*): 503 (M^+).

*6.1.2.17. 2-Amino-5-oxo-9-methyl-4-(6-methoxy-2-(4-methylphenoxy) quinolin-3-yl)-4,5-dihydropyrano [3,2-c]chromene-3-carbonitrile (**6q**).* IR (KBr, ν_{max}, cm^{-1}): 3400 & 3350 (asym. & sym. stretching of $-NH_2$), 3000 (ArC–H), 2230 (C≡N stretching), 1740 (C=O lactone stretching), 1230 cm^{-1} (C–O–C ether stretching). ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.27 (s, 3H, CH_3), 2.34 (s, 3H, CH_3), 2.82 (s, 3H, OCH_3), 4.97 (s, 1H, chromene H4), 6.84 (s, 2H, NH_2), 6.90–8.43 (m, 11H, Ar–H). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 20.84 (CH_3), 20.93 (CH_3), 34.04 (chromene C4), 56.51 (OCH_3), 56.57 (C–CN), 102.27, 112.90, 117.84, 119.52, 120.64, 123.48, 125.16, 125.53, 126.32, 127.87, 127.98, 128.60, 131.54, 133.16, 134.04, 136.80, 139.57, 145.20, 151.30, 152.56, 154.75, 159.65, 159.86 (23C, Ar–C), 160.36 (C=O); MS (*m/z*): 517 (M^+).

*6.1.2.18. 2-Amino-5-oxo-9-methyl-4-(2-(4-chlorophenoxy)-6-methoxyquinolin-3-yl)-4,5-dihydropyrano [3,2-c]chromene-3-carbonitrile (**6r**).* IR (KBr, ν_{max}, cm^{-1}): 3415 & 3365 (asym. & sym. stretching of $-NH_2$), 3000 (ArC–H), 2215 (C≡N stretching), 1725 (C=O lactone stretching), 1225 cm^{-1} (C–O–C ether stretching). ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.26 (s, 3H, CH_3), 2.85 (s, 3H, OCH_3), 4.90 (s, 1H, chromene H4), 6.84 (s, 2H, NH_2), 6.90–8.47 (m, 11H, Ar–H). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 20.80 (CH_3), 34.01 (chromene C4), 56.53 (OCH_3), 56.61 (C–CN), 102.21, 112.78, 117.33, 119.77, 120.60, 123.00, 125.74, 125.64, 126.42, 127.14, 127.36, 128.61, 131.27, 133.35, 134.41, 136.92, 139.71, 145.75, 151.36, 152.37, 154.67, 159.27, 159.78 (23C, Ar–C), 160.62 (C=O); MS (*m/z*): 537 (M^+), 539 (M+2).

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 showing no visible growth (same as control tube) were 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 and 25 μg/mL concentrations. The highest dilution showing at least 99% inhibition is taken as MIC.

6.2.2. In vitro evaluation of antituberculosis activity

An antituberculosis activity of the title compounds against *M. tuberculosis* H37Rv was performed by Lowenstein–Jensen method [37] with minor modification where 250 μg/mL dilution of each test compound was added to Lowenstein–Jensen medium and then media was sterilized by inspissation method. A culture of *M. tuberculosis* H37Rv growing on Lowenstein–Jensen medium was harvested in 0.85% saline in bijou bottle. Each test compound was diluted to 250 μg/mL concentration using DMSO as diluents. These tubes were then incubated at 37 °C for 24 h followed by streaking of *M. tuberculosis* H37Rv (5 × 104 bacilli per tube). The growth of bacilli was seen after 28 days of incubation. The tubes having the compounds were compared with control tubes where medium alone was incubated with *M. tuberculosis* H37Rv. The antituberculosis activity of β-aryloxyquinolines **3a**–**i** and their pyrano[3,2-*c*] chromene derivatives **6a**–**r** is expressed as % inhibition (Table 3), relative to that of standard drug isoniazid. The concentration at which no development of colonies occurred or < 20 colonies was taken as MIC of test compound. MIC values of three compounds with highest % inhibition i.e. **3a**, **3f** and **6f** are shown in Table 4. The standard strain *M. tuberculosis* H37Rv was tested with standard drug isoniazid for comparison purpose.

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