5.1 INTRODUCTION

This part of the chapter deals with synthesis of some new 4H-benzo[g]chromene derivatives bearing 2-aryloxyquinoline moiety and investigation of their antimicrobial and antimycobacterial activity. This synthetic study involves etherification reaction to obtain ether linked formylated derivatives i.e. 2-aryloxy-3-formylquinoline which upon one pot multicomponent reaction with malononitrile and 2-hydroxynaphthalene-1,4-dione affords 4H-benzo[g]chromene derivatives bearing ether linked quinoline. So, it is worthy to narrate brief introduction regarding synthetic and biological significance of the chromene derivatives.

5.1.1 CHROMENE

Chromene is a bicyclic organic compound that results from the fusion of a benzene ring to a heterocyclic pyran ring therefore it is also called benzopyran. There are two isomers of benzopyran depending on the orientation of the fusion of the two rings, resulting in 1-benzopyran (chromene) and 2-benzopyran (isochromene). Each of these structures has further isomers depending on the position of the carbon that is fully saturated. 1-Benzopyran thus gives rise to 2H-1-benzopyran (2H-chromene) and 4H-1-benzopyran (4H-chromene). 2H-Chromene has allyl ether type structure and it is also known as α-chromene or Δ³-chromene. 4H-Chromene has vinyl ether type structure and it is also known as a γ-chromene or Δ²-chromene. Analogously, 2-benzopyran gives rise to 1H-2-benzopyran (1H-isochromene) and 3H-2-benzopyran (3H-isochromene).

5.1.2 SYNTHETIC and BIOLOGICAL SIGNIFICANCE of CHROMENE

The syntheses of 4H-chromene and its derivatives have attracted great interest due to their biological and pharmacological activities. The 4H-chromene derivatives show various pharmacological properties such as spasmyloytic, diuretic, anti-coagulant, anti-cancer, anti-HIV, antitumor, anti-malarial activities, anti-alzheimer, anti-leukemic, antibacterial, emetic and anti-anaphylactic activities. Moreover, they can also be employed as cosmetics and pigments and utilized as potential biodegradable agrochemicals. The basic structural framework of chromenes is a common feature of many tannins and polyphenols found in tea,
fruits, vegetables and red wine and these compounds have become more important as a result of their health-promoting effects. Several substituted chromene derivatives have been reported with the aim of identifying new physiologically active compounds. Some of them are summarized below.

<table>
<thead>
<tr>
<th>Therapeutic agent</th>
<th>Pharmacology</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image1" alt="Chemical Structure 1" /></td>
<td>Antibacterial activity(^{15})</td>
</tr>
<tr>
<td><img src="image2" alt="Chemical Structure 2" /></td>
<td>Apoptosis inducers(^{16})</td>
</tr>
<tr>
<td><img src="image3" alt="Chemical Structure 3" /></td>
<td>Cytotoxicity for cancer cells(^{17})</td>
</tr>
<tr>
<td><img src="image4" alt="Chemical Structure 4" /></td>
<td>For treatment of immune system or cell proliferation diseases especially complications of diabetes(^{18})</td>
</tr>
<tr>
<td><img src="image5" alt="Chemical Structure 5" /></td>
<td>Anticancer(^{19})</td>
</tr>
<tr>
<td><img src="image6" alt="Chemical Structure 6" /></td>
<td>Antivascular and antitumor agent(^{20})</td>
</tr>
</tbody>
</table>
Chapter 5

Apoptosis inducers

Antibacterial activity

Apoptosis inducers

Apoptosis inducers

Apoptosis inducers

Apoptosis inducers

Apoptosis inducers

Apoptosis inducers

Apoptosis inducers

181
Several new and alternative protocols for the synthesis of chromene derivatives have been reported.

Ahmad Shaabani et al.\textsuperscript{29} have reported an efficient synthetic approach for the synthesis of the highly functionalized 4\textit{H}-benzo[\textit{g}]chromene and dihydropyranolo[2,3-\textit{g}]chromenes from readily available substrates in fairly good yields.

An efficient synthesis of 4\textit{H}-Benzo[\textit{g}]chromene-5,10-dione derivatives through Triethylbenzylammonium chloride (TEBA) catalyzed\textsuperscript{30} multicomponent reaction under solvent-free conditions have been reported.

J M Khurana et al.\textsuperscript{31} have reported DBU catalyzed one-pot synthesis of 3,4-dihydropyranolo[3,2-\textit{c}]chromenes, 2-amino-4\textit{H} benzo[\textit{h}]chromenes and 2-amino-4\textit{H}-benzo[\textit{g}]chromenes from aldehydes, active methylene compounds malononitrile/ethyl cyanocacetate, and 4-hydroxycoumarin/1-naphthol/2-hydroxynaphthalene-1,4-dione in water under reflux.
DBU = 1,8-diazabicyclo[5.4.0]undec-7-ene

A series of benzo[g]chromenes derivatives were synthesized via the reaction of arylidenemalononitrile and 2-hydroxynaphthalene-1,4-dione in the mixed solvent DMF and glacial HOAc under microwave irradiation\(^{32}\) without catalyst.

The three-component one-pot synthesis of 2-amino-5-oxo-dihydropyran[3,2-c]chromene derivatives by condensing 4-hydroxycoumarin, aldehydes and alkynitriles under several conditions is reported in the presence of piperidine\(^{33}\), K\(_2\)CO\(_3\)\(^{34}\), diammoniumhydrogenphosphate\(^{35}\), H\(_6\)[P\(_2\)W\(_{18}\)O\(_{62}\)]\(\cdot\)18H\(_2\)O\(^{36}\) and tetrabutylammonium bromide\(^{37}\).
A three-component synthesis of pyrano benzochromene spirooxindoles in aqueous medium is as well reported\textsuperscript{38}.

\[
\text{OH} \quad \begin{array}{c}
\text{R'} \quad \text{N} \quad \text{O} \quad \text{R''} \\
\text{O} \quad \text{CN} \quad \text{CN}
\end{array} \quad \text{OH} \quad \begin{array}{c}
\text{R'} \quad \text{N} \quad \text{O} \quad \text{R''} \\
\text{O} \quad \text{CN} \quad \text{CN}
\end{array} \quad \text{OH} \quad \begin{array}{c}
\text{R'} \quad \text{N} \quad \text{O} \quad \text{R''} \\
\text{O} \quad \text{CN} \quad \text{CN}
\end{array} \quad \text{OH} \quad \begin{array}{c}
\text{R'} \quad \text{N} \quad \text{O} \quad \text{R''} \\
\text{O} \quad \text{CN} \quad \text{CN}
\end{array}
\]

Parsaniya et al.\textsuperscript{39} have synthesized some new spiro derivatives of pyrano[3,2-\textit{c}]chromene in the presence of morpholine.

Benzochromenes are generally prepared by reaction of arylidenenitrile and naphthol. Various methods for preparing these compounds have been reported i.e. using sodium hydroxide\textsuperscript{40}, H\textsubscript{2}O\textsuperscript{41}, TEBA\textsuperscript{42}, piperidine/morpholine\textsuperscript{43} and chitosan\textsuperscript{44}.

The most straightforward synthesis of this heterocyclic system involves a three-component coupling of aldehyde, malononitrile and naphthol. A few diverse catalysts have been employed for this multicomponent reaction. Recently, Mg/Al hydrotalcite\textsuperscript{45}, \(N,N\)-dimethylaminoethylbenzyldimethylammonium chloride\textsuperscript{46}, thiourea\textsuperscript{47}, diazabicyclo[2.2.2]octane\textsuperscript{48}, methanesulfonic acid\textsuperscript{49}, I\textsubscript{2}/K\textsubscript{2}CO\textsubscript{3}/H\textsubscript{2}O\textsuperscript{50}, basic ionic liquids\textsuperscript{51}, magnesium oxide\textsuperscript{52}, Preyssler heteropolyacid \(\text{H}_{14}[\text{NaP}_{5}\text{W}_{30}\text{O}_{110}]\textsuperscript{53}\), InCl\textsubscript{3}\textsuperscript{54}, hexadecyltrimethylammonium bromide\textsuperscript{55}, TiCl\textsubscript{4}\textsuperscript{56}, cetyltrimethylammonium bromide coupled\textsuperscript{57} with ultrasound, basic alumina\textsuperscript{58}, KF/Al\textsubscript{2}O\textsubscript{3}\textsuperscript{59,64}, tetrabutylammonium bromide\textsuperscript{60,62}, triethylamine\textsuperscript{61}, cetyltrimethylammonium chloride\textsuperscript{63} and piperidine\textsuperscript{65} have been used as catalysts for this multicomponent reaction. This reaction is also reported under microwave irradiation\textsuperscript{66}.

I Yavari et al.\textsuperscript{67} have reported new route for the synthesis of benzochromene derivatives.
5.2 PRESENT STUDY

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\textsuperscript{68,69}. 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. 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.

It has been well-established that presence of aryl ring appended by ether linkage at 2\textsuperscript{nd} position of quinoline moiety is highly active against \textit{Mycobacterium tuberculosis} H37Rv and plays a pivotal role in development of new antituberculosis drugs\textsuperscript{70} and in consequence, emerged as a validated molecular target. Despite the immense pharmacological effects of 2-aryloxyquinolines and benzo[g]chromene derivatives, no 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 made to undertake the synthesis of benzo[g]chromene derivatives of 2-aryloxyquinolines 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, we were provoked to synthesize new benzo[g]chromene derivatives of 2-aryloxyquinolines incorporating a validated molecular target and evaluate them as antimicrobial and antitubercular agents.

The constitution of all the synthesized derivatives was identified using elemental analysis, FT-IR, $^1$H NMR, $^{13}$C NMR and selected compounds were confirmed by mass spectrometry and subjected to in vitro antimicrobial study against eight human pathogens, of which three Gram-positive bacterial pathogens; *Streptococcus pneumoniae*, *Clostridium tetani*, *Bacillus subtilis*, three Gram-negative bacterial pathogens; *Salmonella typhi*, *Vibrio cholerae*, *Escherichia coli* and two fungal pathogens; *Aspergillus fumigatus* and *Candida albicans*, using broth microdilution MIC method (discussed in chapter 1). All the compounds were also evaluated against *Mycobacterium tuberculosis* H37Rv (discussed in chapter 1).

### 5.2.1 EXPERIMENTAL

- All the reagents were obtained commercially and used with further purification. Solvents used were of analytical grade.
- All melting points were taken in open capillaries and are uncorrected.
- Thin-layer chromatography (TLC, on aluminium plates coated with silica gel 60 F$_{254}$, 0.25 mm thickness, Merck) was used for monitoring the progress of all reactions, purity and homogeneity of the synthesized compounds.
- Elemental analysis (% C, H, N) was carried out by Perkin-Elmer 2400 series-II elemental analyzer and all compounds are within ±0.4% of theory specified.
- The IR spectra were recorded on a Shimadzu FTIR 8401 spectrophotometer using KBr discs and only the characteristic peaks are reported in cm$^{-1}$.
- $^1$H NMR and $^{13}$C NMR spectra were recorded in DMSO-$d_6$ on a Bruker Avance 400 MHz spectrometer using solvent peak as internal standard. Chemical shifts are reported in parts per million (ppm).
- Mass spectra were scanned on a Shimadzu LCMS 2010 spectrometer. Mode of ionization employed was ESI (electrospray ionization).
The title compounds 6a-r were synthesized in following four steps:

1. Synthesis of 4-substituted acetanilide: Discussed in chapter 2 part I. (section 2.1.2.1)
2. Synthesis of 6-substituted-2-chloro-3-formylquinoline: Discussed in chapter 2 part I. (section 2.1.2.1)
3. Synthesis of 6-(un)-substituted-2-(4-(un)-substituted-aryloxy)quinoline-3-carbaldehyde [3a-r]:

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.
4. Synthesis of 2-Amino-4-(6-(un)-substituted-2-(4-(un)-substituted aryloxy)quinolin-3-yl)-5,10-dihydro-5,10-dioxo-4H-benzo[g]chromene-3-carbonitrile [6a-r]:

A 100 mL round bottomed flask, fitted with a reflux condenser, was charged with a mixture of 2-aryloxyquinoline-3-carbaldehyde 3a-i (1 mmol), malononitrile 4 (1 mmol), 2-hydroxynaphthalene-1,4-dione 5a (1 mmol), and a catalytic amount of piperidine 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 (5 mL) and purified by leaching in equal volume ratio of chloroform and methanol (10 mL) to obtain pure solid sample.
5.2.2 RESULTS AND DISCUSSION

A series of 2-Amino-4-(6-(un)-substituted-2-(4-(un)-substituted aryloxy)quinolin-3-yl)-5,10-dihydro-5,10-dioxo-4H-benzo[g]chromene-3-carbonitrile 6a-r have been synthesized substantially as illustrated in Reaction Scheme II through. 2-aryloxyquinoline-3-carbaldehydes 3a-r was achieved 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 2-aryloxyquinoline-3-carbaldehydes 3a-r, malononitrile 4 and 2-hydroxynaphthalene-1,4-dione 5a in ethanol containing a catalytic amount of piperidine afforded the target compounds 6a-r.

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 2-hydroxynaphthalene-1,4-dione in basic reaction conditions (Scheme 3).

The structures of newly synthesized compounds were elucidated by combined use of IR, ¹H and ¹³C NMR, mass spectral data and elemental analysis. The absorption bands for compounds 6a-r in IR-spectra were observed in the range of
2195-2205 cm\(^{-1}\) corresponding to C≡N. The NH\(_2\) stretching and C=O stretching vibrations for all the compounds were observed in range of 3300-3450 cm\(^{-1}\) and 1600-1670 cm\(^{-1}\) respectively. The \(^1\)H NMR spectrum of compounds 6a-r indicated the presence of one singlet in the range \(\delta\) 5.10-5.24 ppm of C4H proton. In the \(^13\)C NMR spectral data of the title compounds 6a-r, most characteristic signal around \(\delta\) 32.00 ppm indicated the formation of benzo[g]chromene ring and the carbonyl carbon was observed at around \(\delta\) 177.32-183.25 ppm. The signal at around \(\delta\) 56.00 ppm is assigned to carbon attached with carbonitrile while signals around \(\delta\) 106.58–160.12 ppm are attributed to all the aromatic carbons of compounds 6a-r.

The obtained elemental analysis values are in good agreement with theoretical data. The mass spectra of compounds 6a and 6i detected the expected molecular ion signals corresponding to respective molecular formula. i.e. mass spectra of compound 6a (R\(_1\)=H, R\(_2\)=H) and 6b (R\(_1\)=OCH\(_3\), R\(_2\)=CH\(_3\)) gave molecular ion peak at \(m/z\) 456 (M+1) and \(m/z\) 500 (M+1) corresponding to molecular formula C\(_{29}\)H\(_{17}\)N\(_3\)O\(_3\) and C\(_{31}\)H\(_{21}\)N\(_3\)O\(_4\) respectively.

Elemental analysis of all the derivatives is shown in Table 2.

<table>
<thead>
<tr>
<th>Compd.</th>
<th>R(_1)</th>
<th>R(_2)</th>
<th>Formula (Mw)</th>
<th>Analysis (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>H</td>
<td>H</td>
<td>C(<em>{29})H(</em>{17})N(_3)O(_3) (455.46)</td>
<td>76.47 (76.60)</td>
</tr>
<tr>
<td>H</td>
<td>CH(_3)</td>
<td></td>
<td>C(<em>{30})H(</em>{19})N(_3)O(_3) (469.49)</td>
<td>76.75 (76.52)</td>
</tr>
<tr>
<td>H</td>
<td>Cl</td>
<td></td>
<td>C(<em>{29})H(</em>{18})ClN(_3)O(_3) (489.91)</td>
<td>71.10 (71.25)</td>
</tr>
<tr>
<td>H</td>
<td>OCH(_3)</td>
<td></td>
<td>C(<em>{30})H(</em>{19})N(_3)O(_4) (485.49)</td>
<td>74.22 (74.10)</td>
</tr>
<tr>
<td>H</td>
<td>Br</td>
<td></td>
<td>C(<em>{29})H(</em>{16})BrN(_3)O(_3) (534.36)</td>
<td>65.18 (65.03)</td>
</tr>
<tr>
<td>H</td>
<td>F</td>
<td></td>
<td>C(<em>{29})H(</em>{16})FN(_3)O(_3) (473.45)</td>
<td>73.57 (73.71)</td>
</tr>
<tr>
<td>CH(_3)</td>
<td>H</td>
<td></td>
<td>C(<em>{30})H(</em>{19})N(_3)O(_3) (469.49)</td>
<td>76.75 (76.92)</td>
</tr>
<tr>
<td>CH(_3)</td>
<td>CH(_3)</td>
<td></td>
<td>C(<em>{31})H(</em>{21})N(_3)O(_3) (483.52)</td>
<td>77.00 (76.89)</td>
</tr>
<tr>
<td>CH(_3)</td>
<td>Cl</td>
<td></td>
<td>C(<em>{30})H(</em>{18})ClN(_3)O(_3) (503.94)</td>
<td>71.50 (71.71)</td>
</tr>
<tr>
<td>CH(_3)</td>
<td>OCH(_3)</td>
<td></td>
<td>C(<em>{31})H(</em>{21})N(_3)O(_4) (499.52)</td>
<td>74.54 (74.23)</td>
</tr>
<tr>
<td>6a</td>
<td>CH₃</td>
<td>Br</td>
<td>C₃₀H₁₈BrN₃O₃</td>
<td>65.71</td>
</tr>
<tr>
<td>------</td>
<td>------</td>
<td>------</td>
<td>--------------</td>
<td>-------</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(548.39)</td>
<td>(65.58)</td>
</tr>
<tr>
<td>6l</td>
<td>CH₃</td>
<td>F</td>
<td>C₃₀H₁₈FN₃O₃</td>
<td>73.91</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(487.48)</td>
<td>(74.15)</td>
</tr>
<tr>
<td>6m</td>
<td>OCH₃</td>
<td>H</td>
<td>C₃₀H₁₉N₂O₄</td>
<td>74.22</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(485.49)</td>
<td>(74.36)</td>
</tr>
<tr>
<td>6n</td>
<td>OCH₃</td>
<td>CH₃</td>
<td>C₃₁H₂₁N₃O₄</td>
<td>74.54</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(499.52)</td>
<td>(74.69)</td>
</tr>
<tr>
<td>6o</td>
<td>OCH₃</td>
<td>Cl</td>
<td>C₃₀H₁₈ClN₃O₄</td>
<td>69.30</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(519.93)</td>
<td>(69.51)</td>
</tr>
<tr>
<td>6p</td>
<td>OCH₃</td>
<td>OCH₃</td>
<td>C₃₁H₂₁N₃O₅</td>
<td>72.23</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(515.52)</td>
<td>(72.01)</td>
</tr>
<tr>
<td>6q</td>
<td>OCH₃</td>
<td>Br</td>
<td>C₃₀H₁₈BrN₃O₄</td>
<td>63.84</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(564.39)</td>
<td>(64.01)</td>
</tr>
<tr>
<td>6r</td>
<td>OCH₃</td>
<td>F</td>
<td>C₃₀H₁₈FN₃O₄</td>
<td>71.57</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(503.48)</td>
<td>(71.46)</td>
</tr>
</tbody>
</table>

[6a]

2-Amino-4-(2-phenylquinolin-3-yl)-5,10-dihydro-5,10-dioxo-4H-benzo[g]chromene-3-carbonitrile

**Yield:** 84 %  
**m.p.:** 226-228 °C

**IR (KBr, ν max, cm⁻¹):** 3350 and 3300 (asym. and sym. str. of -NH₂), 2200 (-C≡N str.), 1670 (C=O str.), 1600 (C=O str.), 1235 cm⁻¹ (C-O-C ether stretching)

**MS:** (M+1) 456

**¹H NMR (400 MHz, DMSO-d₆):** δ 5.20 (s, 1H, CH), 7.19-8.46 (m, 16H, Ar-H & NH₂)

**¹³C NMR (100 MHz, DMSO-d₆):** δ: 32.58 (C4), 56.00 (C-C≡N), 119.85, 121.34, 121.96, 125.14, 125.55, 126.35, 126.56, 127.06, 128.10, 129.97, 130.35, 130.99, 131.53, 134.64, 135.08, 139.34, 145.12, 150.24, 154.01, 159.44, 159.57 (21C, Ar-C), 177.38 (C=O), 183.17 (C=O)
IR Spectrum of Compound 6a

H NMR spectrum of compound 6a

^1H NMR spectrum of compound 6a
Chapter 5

\[
\text{\textsuperscript{13}C NMR spectrum of compound 6a}
\]

[6b]

2-Amino-4-(2-(4-methylphenyl)quinolin-3-yl)-5,10-dihydro-5,10-dioxo-4H-benzo[g]chromene-3-carbonitrile

Yield: 80 %  \hspace{1cm} \text{m.p.: 270-272 °C}

IR (KBr, \(v_{\text{max}}, \text{ cm}^{-1}\)): 3405 and 3345 (asym. and sym. str. of -NH\(_2\)), 2205 (-C≡N str.), 1665 (C=O str.), 1605 (C=O str.), 1230 cm\(^{-1}\) (C-O-C ether stretching)

MS: (M+1) 474

\(\text{\textsuperscript{1}H NMR (400 MHz, DMSO-}\text{d}_6\)): \(\delta\) 2.31 (s, 3H, Ar-CH\(_3\)), 5.18 (s, 1H, CH), 7.24-8.30 (m, 15H, Ar-H & NH\(_2\))

\(\text{\textsuperscript{13}C NMR (100 MHz, DMSO-}\text{d}_6\) \(\delta\): 20.84 (Ar-CH\(_3\)), 32.44 (C4), 56.06 (C-C≡N), 119.84, 121.27, 121.88, 125.21, 125.66, 126.39, 126.72, 127.30, 128.85, 129.44, 130.25, 130.79, 131.50, 134.41, 135.83, 138.14, 145.41, 148.21, 154.12, 159.46, 159.67 (21C, Ar-C), 177.38 (C=O), 183.15 (C=O)
[6c]

2-Amino-4-(2-(4-chlorophenyl)quinolin-3-yl)-5,10-dihydro-5,10-dioxo-4H-benzo[g]chromene-3-carbonitrile

Yield: 79%  
m.p.: 251-253 °C

IR (KBr, v_{max}, cm^{-1}): 3405 and 3350 (asym. and sym. str. of -NH₂), 2200 (-C≡N str.), 1670 (C=O str.), 1610 (C=O str.), 1225 cm^{-1} (C-O-C ether stretching)

¹H NMR (400 MHz, DMSO-d₆): δ 5.21 (s, 1H, CH), 7.46-8.49 (m, 15H, Ar-H & NH₂)

¹³C NMR (100 MHz, DMSO-d₆): δ: 32.48 (C4), 56.03 (C-C≡N), 119.77, 121.20, 121.83, 124.94, 125.84, 126.34, 126.67, 127.12, 128.61, 129.00, 130.10, 130.57, 131.29, 134.44, 135.26, 138.51, 144.25, 150.20, 154.15, 159.17, 159.63 (21C, Ar-C), 177.32 (C=O), 183.25 (C=O)

[6d]

2-Amino-4-(2-(4-methoxyphenyl)quinolin-3-yl)-5,10-dihydro-5,10-dioxo-4H-benzo[g]chromene-3-carbonitrile

Yield: 76%  
m.p.: 233-235 °C

IR (KBr, v_{max}, cm^{-1}): 3435 and 3340 (asym. and sym. str. of -NH₂), 2200 (-C≡N str.), 1670 (C=O str.), 1605 (C=O str.), 56.05 (C-O-C ether stretching)

¹H NMR (400 MHz, DMSO-d₆): δ 3.84 (s, 3H, Ar-OCH₃), 5.20 (s, 1H, CH), 7.10-8.27 (m, 15H, Ar-H & NH₂)

¹³C NMR (100 MHz, DMSO-d₆): δ: 32.48 (C4), 55.86 (Ar-OCH₃), 56.05 (C-C≡N), 113.25, 119.85, 121.09, 121.52, 124.85, 125.92, 126.47, 126.81, 127.70, 128.35, 130.64, 130.78, 131.41, 134.52, 135.28, 139.02, 144.88, 150.24, 154.29, 159.33, 159.60 (21C, Ar-C), 177.40 (C=O), 183.14 (C=O)
[6e]

2-Amino-4-(2-(4-bromophenyl)quinolin-3-yl)-5,10-dihydro-5,10-dioxo-4H-benzo[g]chromene-3-carbonitrile

Yield: 84%  
m.p.: 226-228 °C

IR (KBr, ν_max, cm⁻¹): 3400 and 3330 (asym. and sym. str. of -NH₂), 2200 (-C≡N str.), 1670 (C=O str.), 1600 (C=O str.), 1225 cm⁻¹ (C-O-C ether stretching)

¹H NMR (400 MHz, DMSO-d₆): δ 5.24 (s, 1H, CH), 7.58-8.42 (m, 15H, Ar-H & NH₂)

¹³C NMR (100 MHz, DMSO-d₆) δ: 32.50 (C4), 56.01 (C-C=N), 119.85, 121.23, 121.65, 124.02, 125.58, 126.41, 126.69, 127.75, 128.88, 129.25, 130.26, 130.61, 131.73, 134.47, 135.08, 138.55, 143.76, 151.15, 154.10, 159.48, 159.62 (21C, Ar-C), 177.33 (C=O), 183.13 (C=O)

[6f]

2-Amino-4-(2-(4-fluorophenyl)quinolin-3-yl)-5,10-dihydro-5,10-dioxo-4H-benzo[g]chromene-3-carbonitrile

Yield: 81%  
m.p.: 270-272 °C

IR (KBr, ν_max, cm⁻¹): 3405 and 3325 (asym. and sym. str. of -NH₂), 2195 (-C≡N str.), 1670 (C=O str.), 1610 (C=O str.), 1225 cm⁻¹ (C-O-C ether stretching)

¹H NMR (400 MHz, DMSO-d₆): δ 5.23 (s, 1H, CH), 7.28-8.54 (m, 15H, Ar-H & NH₂)

¹³C NMR (100 MHz, DMSO-d₆) δ: 32.50 (C4), 56.00 (C-C=N), 117.02, 119.81, 121.17, 121.65, 125.10, 125.67, 126.13, 126.70, 127.71, 128.25, 129.20, 130.41, 130.90, 131.33, 134.55, 135.28, 138.25, 144.57, 153.97, 159.40, 159.55 (21C, Ar-C), 177.35 (C=O), 183.16 (C=O)
[6g]

2-Amino-4-(6-methyl-2-phenylquinolin-3-yl)-5,10-dihydro-5,10-dioxo-4H-benzo[g]chromene-3-carbonitrile

Yield: 72 %  
M.p.: 262-264 °C

IR (KBr, \(v_{\text{max}}\) cm\(^{-1}\)): 3425 and 3350 (asym. and sym. str. of -NH\(_2\)), 2200 (-C≡N str.), 1665 (C=O str.), 1605 (C=O str.), 1235 cm\(^{-1}\) (C-O-C ether stretching)

\(^1\)H NMR (400 MHz, DMSO-\(d_6\)): \(\delta\) 2.47 (s, 3H, Ar-CH\(_3\)), 5.21 (s, 1H, CH), 7.40-8.14 (m, 15H, Ar-H & NH\(_2\))

\(^13\)C NMR (100 MHz, DMSO-\(d_6\)): \(\delta\): 20.58 (Ar-CH\(_3\)), 32.48 (C4), 56.04 (C-C≡N), 119.80, 121.20, 124.60, 125.44, 126.31, 126.76, 127.05, 127.82, 128.56, 129.12, 130.25, 130.66, 131.48, 134.63, 135.82, 138.43, 141.00, 150.74, 154.25, 159.40, 159.67 (21C, Ar-C), 177.36 (C=O), 183.13 (C=O)

[6h]

2-Amino-4-(6-methyl-2-(4-methylphenyl)quinolin-3-yl)-5,10-dihydro-5,10-dioxo-4H-benzo[g]chromene-3-carbonitrile

Yield: 77 %  
M.p.: 240-242 °C

IR (KBr, \(v_{\text{max}}\) cm\(^{-1}\)): 3420 and 3325 (asym. and sym. str. of -NH\(_2\)), 2200 (-C≡N str.), 1670 (C=O str.), 1605 (C=O str.), 1240 cm\(^{-1}\) (C-O-C ether stretching)

\(^1\)H NMR (400 MHz, DMSO-\(d_6\)): \(\delta\) 2.32 (s, 3H, Ar-CH\(_3\)), 2.46 (s, 3H, Ar-CH\(_3\)), 5.18 (s, 1H, CH), 7.30-8.38 (m, 15H, Ar-H & NH\(_2\))

\(^13\)C NMR (100 MHz, DMSO-\(d_6\)): \(\delta\): 20.87 (Ar-CH\(_3\)), 21.57 (Ar-CH\(_3\)), 32.47 (C4), 56.07 (C-C≡N), 119.84, 121.42, 124.17, 125.81, 126.33, 127.02, 127.63, 128.29, 129.93, 130.15, 130.56, 131.55, 132.21, 134.26, 135.30, 138.83, 141.66, 149.42, 154.30, 159.45, 159.62 (21C, Ar-C), 177.34 (C=O), 183.15 (C=O)
[61]

2-Amino-4-(6-methyl-2-(4-chlorophenyl)quinolin-3-yl)-5,10-dihydro-5,10-dioxo-4H-benzo[g]chromene-3-carbonitrile

Yield: 83%  
m.p.: 278-280°C

IR (KBr, \(v_{\text{max}}, \text{cm}^{-1}\)): 3450 and 3350 (asym. and sym. str. of -NH\(_2\)), 2200 (-C=N str.), 1665 (C=O str.), 1600 (C=O str.), 1230 cm\(^{-1}\) (C-O-C ether stretching)

\(^1\)H NMR (400 MHz, DMSO-\(d_6\)): \(\delta\) 2.32 (s, 3H, Ar-CH\(_3\)), 5.18 (s, 1H, CH), 7.41-8.57 (m, 14H, Ar-H & NH\(_2\))

\(^13\)C NMR (100 MHz, DMSO-\(d_6\)): \(\delta\) 21.56 (Ar-CH\(_3\)), 32.50 (C4), 56.01 (C-C=O), 119.88, 121.24, 124.83, 125.37, 126.35, 126.81, 127.86, 128.43, 129.10, 129.77, 130.19, 130.76, 131.52, 134.58, 135.48, 136.27, 138.50, 142.53, 156.78, 158.52, 159.60 (21C, Ar-C), 177.34 (C=O), 183.14 (C=O)

[62]

2-Amino-4-(6-methyl-2-(4-methoxyphenyl)quinolin-3-yl)-5,10-dihydro-5,10-dioxo-4H-benzo[g]chromene-3-carbonitrile

Yield: 77%  
m.p.: 210-212°C

IR (KBr, \(v_{\text{max}}, \text{cm}^{-1}\)): 3400 and 3325 (asym. and sym. str. of -NH\(_2\)), 2200 (-C=N str.), 1670 (C=O str.), 1610 (C=O str.), 1235 cm\(^{-1}\) (C-O-C ether stretching)

\(^1\)H NMR (400 MHz, DMSO-\(d_6\)): \(\delta\) 2.61 (s, 3H, Ar-CH\(_3\)), 3.85 (s, 3H, Ar-OCH\(_3\)), 5.19 (s, 1H, CH), 7.04-8.38 (m, 14H, Ar-H & NH\(_2\))

\(^13\)C NMR (100 MHz, DMSO-\(d_6\)): \(\delta\) 21.57 (Ar-CH\(_3\)), 32.49 (C4), 55.83 (Ar-OCH\(_3\)), 56.00 (C-C=O), 115.06, 119.85, 121.34, 124.20, 125.61, 126.25, 127.54, 127.88, 128.42, 128.97, 129.07, 130.11, 130.55, 131.26, 134.37, 135.72, 138.14, 141.05, 156.38, 158.49, 159.58 (21C, Ar-C), 177.32 (C=O), 183.14 (C=O)
[6k]

2-Amino-4-(6-methyl-2-(4-bromophenyl)quinolin-3-yl)-5,10-dihydro-5,10-dioxo-4H-benzo[g]chromene-3-carbonitrile

Yield: 71%  m.p.: 275-277 °C

**IR (KBr, ν_{max}, cm^{-1}):** 3420 and 3325 (asym. and sym. str. of -NH$_2$), 2205 (-C≡N str.), 1660 (C=O str.), 1600 (C=O str.), 1225 cm$^{-1}$ (C-O-C ether stretching)

**$^1$H NMR (400 MHz, DMSO-$d_6$):** δ 2.33 (s, 3H, Ar-CH$_3$), 5.20 (s, 1H, CH), 7.41-8.45 (m, 14H, Ar-H & NH$_2$), 2205 (-C≡N str.), 1660 (C=O str.), 1600 (C=O str.)

[6l]

2-Amino-4-(6-methyl-2-(4-fluorophenyl)quinolin-3-yl)-5,10-dihydro-5,10-dioxo-4H-benzo[g]chromene-3-carbonitrile

Yield: 75%  m.p.: 258-260 °C

**IR (KBr, ν_{max}, cm^{-1}):** 3400 and 3330 (asym. and sym. str. of -NH$_2$), 2205 (-C≡N str.), 1665 (C=O str.), 1610 (C=O str.), 1230 cm$^{-1}$ (C-O-C ether stretching)

**$^1$H NMR (400 MHz, DMSO-$d_6$):** δ 2.32 (s, 3H, Ar-CH$_3$), 5.16 (s, 1H, CH), 7.36-8.68 (m, 14H, Ar-H & NH$_2$), 21.57 (Ar-CH$_3$), 32.46 (C4), 56.05 (C-C=N), 119.82, 121.25, 121.55, 125.63, 126.34, 127.15, 127.80, 128.20, 128.64, 129.41, 130.16, 130.49, 131.57, 132.26, 134.74, 135.08, 138.40, 141.22, 157.01, 158.55, 159.63 (21C, Ar-C), 177.38 (C=O), 183.16 (C=O)
[6m]

2-Amino-4-(6-methoxy-2-phenylquinolin-3-yl)-5,10-dihydro-5,10-dioxo-4H-benzo[g]chromene-3-carbonitrile

Yield: 80%  
m.p.: 240-242 °C

IR (KBr, \(v_{\text{max}} \text{ cm}^{-1}\)): 3400 and 3340 (asym. and sym. str. of NH\(_2\)), 2205 (-C≡N str.), 1665 (C=O str.), 1600 (C=O str.), 1230 cm\(^{-1}\) (C-O-C ether stretching)

\(^1\)H NMR (400 MHz, DMSO-\(d_6\)): \(\delta\) 3.83 (s, 3H, Ar-OCH\(_3\)), 5.21 (s, 1H, CH), 7.04-8.30 (m, 15H, Ar-H & NH\(_2\))

\(^13\)C NMR (100 MHz, DMSO-\(d_6\)) \(\delta\): 32.40 (C4), 55.83 (Ar-OCH\(_3\)), 56.10 (C-C≡N), 106.60, 119.82, 121.24, 121.47, 125.88, 126.13, 126.74, 127.54, 128.15, 129.64, 130.28, 130.59, 131.27, 134.39, 135.68, 138.31, 140.22, 152.11, 156.77, 158.20, 159.50 (21C, Ar-C), 177.40 (C=O), 183.15 (C=O)

[6n]

2-Amino-4-(6-methoxy-2-(4-methylphenyl)quinolin-3-yl)-5,10-dihydro-5,10-dioxo-4H-benzo[g]chromene-3-carbonitrile

Yield: 74%  
m.p.: 204-206 °C

IR (KBr, \(v_{\text{max}} \text{ cm}^{-1}\)): 3400 and 3330 (asym. and sym. str. of NH\(_2\)), 2200 (-C≡N str.), 1665 (C=O str.), 1605 (C=O str.), 1235 cm\(^{-1}\) (C-O-C ether stretching)

MS: (M+1) 500

\(^1\)H NMR (400 MHz, DMSO-\(d_6\)): \(\delta\) 2.31 (s, 3H, Ar-CH\(_3\)), 3.82 (s, 3H, Ar-OCH\(_3\)), 5.16 (s, 1H, CH), 7.03-8.33 (m, 14H, Ar-H & NH\(_2\))

\(^13\)C NMR (100 MHz, DMSO-\(d_6\)) \(\delta\): 20.87 (Ar-CH\(_3\)), 32.41 (C4), 55.82 (Ar-OCH\(_3\)), 56.14 (C-C≡N), 106.61, 119.83, 121.48, 121.53, 122.10, 126.35, 127.24, 128.38, 130.29, 130.98, 131.52, 133.87, 134.64, 135.09, 138.22, 140.58, 150.20, 152.05, 156.72, 158.03, 159.49 (21C, Ar-C), 177.38 (C=O), 183.14 (C=O)
IR Spectrum of Compound 6n

'H NMR spectrum of compound 6n
2-Amino-4-(6-methoxy-2-(4-chlorophenyl)quinolin-3-yl)-5,10-dihydro-5,10-dioxo-4H-benzo[g]chromene-3-carbonitrile

Yield: 80%

m.p.: 234-236 °C

IR (KBr, \( \nu_{\text{max}} \), \( \text{cm}^{-1} \)): 3405 and 3325 (asym. and sym. str. of \(-\text{NH}_2\)), 2200 (-C\(=\)N str.), 1670 (C=O str.), 1600 (C=O str.), 1235 cm\(^{-1}\) (C-O-C ether stretching)

\(^1\)H NMR (400 MHz, DMSO-\(d_6\)): \( \delta \) 3.81 (s, 3H, Ar-\(\text{OCH}_3\)), 5.14 (s, 1H, CH), 7.03-8.50 (m, 14H, Ar-H & \(-\text{NH}_2\))

\(^{13}\)C NMR (100 MHz, DMSO-\(d_6\)) \( \delta \): 32.45 (C4), 55.83 (Ar-\(\text{OCH}_3\)), 56.13 (C-C\(=\)N), 106.62, 121.12, 121.53, 125.63, 126.31, 127.27, 128.89, 129.58, 129.77, 130.25, 131.63, 132.12, 133.57, 135.48, 138.42, 140.44, 150.42, 153.01, 156.44, 158.25, 159.42, (21C, Ar-C), 177.45 (C=O), 183.20 (C=O)

\(13\)C NMR spectrum of compound 6n
[6p]
2-Amino-4-(6-methoxy-2-(4-methoxyphenyl)quinolin-3-yl)-5,10-dihydro-5,10-
dioxo-4H-benzo[g]chromene-3-carbonitrile

Yield: 84 %  m.p.: 220-222 °C

IR (KBr, \(v_{\text{max}}, \text{ cm}^{-1}\)): 3415 and 3330 (asym. and sym. str. of -NH\(_2\)), 2200 (-C≡N str.), 1670 (C=O str.), 1610 (C=O str.), 1235 cm\(^{-1}\) (C-O-C ether stretching)

\(^1\text{H NMR (400 MHz, DMSO-}d_6\)): \(\delta\) 3.82 (s, 3H, Ar-OCH\(_3\)), 3.87 (s, 3H, Ar-OCH\(_3\)), 5.15 (s, 1H, CH), 7.04-8.31 (m, 14H, Ar-H & NH\(_2\))

\(^13\text{C NMR (100 MHz, DMSO-}d_6\) \(\delta\): 32.44 (C4), 55.82 (Ar-OCH\(_3\)), 55.83 (Ar-OCH\(_3\)), 56.12 (C=C=N), 106.58, 115.25, 119.89, 121.23, 121.85, 125.55, 126.74, 127.88, 128.57, 129.41, 130.25, 130.59, 131.84, 134.50, 135.12, 138.46, 140.44, 152.27, 156.54, 158.08, 159.57 (21C, Ar-C), 177.40 (C=O), 183.22 (C=O)

[6q]
2-Amino-4-(6-methoxy-2-(4-bromophenyl)quinolin-3-yl)-5,10-dihydro-5,10-
dioxo-4H-benzo[g]chromene-3-carbonitrile

Yield: 70 %  m.p.: 261-263 °C

IR (KBr, \(v_{\text{max}}, \text{ cm}^{-1}\)): 3420 and 3330 (asym. and sym. str. of -NH\(_2\)), 2200 (-C≡N str.), 1665 (C=O str.), 1600 (C=O str.), 1230 cm\(^{-1}\) (C-O-C ether stretching)

\(^1\text{H NMR (400 MHz, DMSO-}d_6\)): \(\delta\) 3.83 (s, 3H, Ar-OCH\(_3\)), 3.87 (s, 3H, Ar-OCH\(_3\)), 5.15 (s, 1H, CH), 7.06-8.44 (m, 14H, Ar-H & NH\(_2\))

\(^13\text{C NMR (100 MHz, DMSO-}d_6\) \(\delta\): 32.48 (C4), 55.83 (Ar-OCH\(_3\)), 56.05 (C=C=N), 106.60, 119.86, 121.24, 121.56, 122.57, 125.58, 126.12, 127.34, 128.16, 129.44, 130.06, 130.85, 131.35, 133.47, 135.49, 138.59, 140.21, 154.20, 156.23, 158.21, 158.35, (21C, Ar-C), 177.45 (C=O), 183.20 (C=O)
[6r]

2-Amino-4-((6-methoxy-2-(4-fluorophenyl)quinolin-3-yl)-5,10-dihydro-5,10-dioxo-4H-benzo[g]chromene-3-carbonitrile

Yield: 75 %  m.p.: 242-244 °C

IR (KBr, $v_{\text{max}}$ cm$^{-1}$): 3405 and 3325 (asym. and sym. str. of -NH$_2$), 2200 (-C≡N str.), 1670 (C=O str.), 1600 (C=O str.), 1225 cm$^{-1}$ (C-O-C ether stretching)

$^1$H NMR (400 MHz, DMSO-$d_6$): $\delta$ 3.82 (s, 3H, Ar-OCH$_3$), 5.12 (s, 1H, CH), 7.05-8.59 (m, 14H, Ar-H & NH$_2$)

$^{13}$C NMR (100 MHz, DMSO-$d_6$) $\delta$: 32.45 (C4), 55.81 (Ar-OCH$_3$), 56.11 (C=C=N), 106.65, 114.25, 119.82, 121.15, 121.58, 125.85, 126.36, 128.54, 129.46, 130.25, 130.61, 131.44, 133.50, 135.26, 136.15, 140.41, 152.43, 156.58, 158.00, 159.12, 159.92 (21C, Ar-C), 177.42 (C=O), 183.19 (C=O)

5.2.3 ANTIMICROBIAL and ANTIMYCOBACTERIAL ACTIVITY

The MICs of synthesized compounds were carried out by broth microdilution method according to National Committee for Clinical Laboratory Standards (NCCLS)$^{71}$ as described in Chapter 1. Antibacterial activity was screened against three Gram positive (B. subtilis MTCC 441, C. tetani MTCC 449, S. pneumoniae MTCC 1936) and three Gram negative (E. coli MTCC 443, S. typhi MTCC 98, V. cholerae MTCC 3906) bacteria by using ampicillin, ciprofloxacin and norfloxacin as a standard antibacterial drug. Antifungal activity was screened against two fungal species (A. fumigats MTCC 3008 and C. albicans MTCC 227) where griseofulvin and nystatin used as standard antifungal drugs. The antimicrobial screening data are shown in Table 3.

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. In vitro antituberculosis activity of all the newly synthesized compounds against Mycobacterium tuberculosis H37Rv strain was determined by using Lowenstein-Jensen medium (conventional method) as described by Rattan$^{72}$ and the observed results are presented in Table 4 in the form of %
inhibition, relative to that of standard antitubercular drug isoniazid. Compounds effecting < 90% inhibition in the primary screen were not evaluated further. Compounds demonstrating at least 90% inhibition in the primary were re-tested at lower concentration (MIC) in a Lowenstein-Jensen medium.

**Biological Results:**

<table>
<thead>
<tr>
<th>Compound</th>
<th>R₁</th>
<th>R₂</th>
<th>Compound</th>
<th>R₁</th>
<th>R₂</th>
</tr>
</thead>
<tbody>
<tr>
<td>3a or 6a</td>
<td>H</td>
<td>H</td>
<td>3j or 6j</td>
<td>CH₃</td>
<td>OCH₃</td>
</tr>
<tr>
<td>3b or 6b</td>
<td>H</td>
<td>CH₃</td>
<td>3k or 6k</td>
<td>CH₃</td>
<td>Br</td>
</tr>
<tr>
<td>3c or 6c</td>
<td>H</td>
<td>Cl</td>
<td>3l or 6l</td>
<td>CH₃</td>
<td>F</td>
</tr>
<tr>
<td>3d or 6d</td>
<td>H</td>
<td>OCH₃</td>
<td>3m or 6m</td>
<td>OCH₃</td>
<td>H</td>
</tr>
<tr>
<td>3e or 6e</td>
<td>H</td>
<td>Br</td>
<td>3n or 6n</td>
<td>OCH₃</td>
<td>CH₃</td>
</tr>
<tr>
<td>3f or 6f</td>
<td>H</td>
<td>F</td>
<td>3o or 6o</td>
<td>OCH₃</td>
<td>Cl</td>
</tr>
<tr>
<td>3g or 6g</td>
<td>CH₃</td>
<td>H</td>
<td>3p or 6p</td>
<td>OCH₃</td>
<td>OCH₃</td>
</tr>
<tr>
<td>3h or 6h</td>
<td>CH₃</td>
<td>CH₃</td>
<td>3q or 6q</td>
<td>OCH₃</td>
<td>Br</td>
</tr>
<tr>
<td>3i or 6i</td>
<td>CH₃</td>
<td>Cl</td>
<td>3r or 6r</td>
<td>OCH₃</td>
<td>F</td>
</tr>
</tbody>
</table>

The antimicrobial activity of some of the 2-aryloxyquinoline-3-carbaldehyde 3a-c, 3g-i and 3m-o has already been reported by our group.\(^7\)

Reviewing the antibacterial activity of 2-aryloxyquinoline-3-carbaldehyde 3d-f, 3j-l and 3p-r assay indicated that compounds displayed good inhibitory effects on the growth of the tested Gram positive bacterial strains compared to Gram negative bacterial strains. Compounds 3d and 3j-l (MIC < 250 µg/mL) displayed excellent activity as compared to ampicillin (MIC = 250 µg/mL) against *Bacillus subtilis* also
compounds 3e and 3q (MIC = 250 µg/mL) are found equipotent to ampicillin against the same organism.

The analogs 3f and 3j (MIC = 250 µg/mL) were equally active and compounds 3d, 3k and 3p-q (MIC < 250 µg/mL) are found more potent as compared to standard ampicillin (MIC = 250 µg/mL) against Clostridium tetani. Compound 3k and 3p (MIC = 100 µg/mL) are also equally active as compared to standard ciprofloxacin against the same organism. With regard to activity against Strepotococcus pneumonia, compound 3j (MIC = 62.5 µg/mL) showed excellent activity where as 3p (MIC = 100 µg/mL) is found equipotent to standard ampicillin (MIC = 1000 µg/mL). Against Escherichia coli analogs 3f and 3l are equally active compared to standard ampicillin (MIC = 100 µg/mL). The analog 3p (MIC = 100 µg/mL) is found eqipotent and 3k (MIC = 50 µg/mL) displayed excellent activity against Salmonella typhi compared to ampicillin. In case of inhibiting Vibrio cholera two compounds 3d and 3r displayed equal activity as ampicillin. Concerning the antifungal activity of tested compounds 3d, 3f-j, 3l and 3p exhibited good growth inhibitory action on Candida albicans.

Reviewing the antibacterial activity of 4H-benzo[g]chromene derivatives 6a-r, assay indicated that compounds displayed variable inhibitory effects on the growth of the tested Gram positive and Gram negative bacterial strains. Among the Gram positive bacteria tested, two strains namely; Bacillus subtilis and Clostridium tetani showed relative high sensitivity towards the tested compounds. In this view, compounds 6a, 6b, 6d, 6g-j and 6l-n (MIC < 250 µg/mL) exhibited excellent activity as compared to ampicillin (MIC = 250 µg/mL) against Bacillus subtilis, whereas the analogs 6e, 6f, 6p and 6r (MIC = 250 µg/mL) are found equipotent to ampicillin. Moreover, compounds 6a, 6b, 6g and 6j (MIC = 100 µg/mL) also found equipotent to norfloxacin (MIC = 100 µg/mL) against the same organism. With regard to activity against Clostridium tetani, the best activity was displayed by compounds 6a, 6d, 6e, 6g, 6j, 6n, 6p and 6q (MIC < 250 µg/mL), whereas compounds 6b, 6c, 6h, 6l and 6o (MIC = 250 µg/mL) showes antibacterial activity comparable to ampicillin (MIC = 250 µg/mL). In case of inhibiting Gram positive bacteria Streptococcus pneumoniae, compound 6g (MIC = 25 µg/mL) have shown pronounced activity as compared to standard ampicillin (MIC = 100 µg/mL), chloramphenicol (MIC = 50 µg/mL) and ciprofloxacin (MIC = 50 µg/mL). The analogs 6b, 6i, 6o and 6p are equally active and 6n is more active than ampicillin (MIC = 100 µg/mL) against the same organism.
On the other hand, investigation of antibacterial activity of the active compounds against the three tested Gram negative strains revealed that two analogs namely 6b (MIC = 62.5 µg/mL) and 6i (MIC = 12.5 µg/mL) are able to produce excellent growth inhibitory activity against *Escherichia coli* compared to ampicillin (MIC = 100 µg/mL). Whereas, compounds 6c, 6g, 6h, 6k and 6n (MIC = 100 µg/mL) exhibited inhibitory effects as that of ampicillin against the same organism. Against *Salmonella typhi*, compounds 6b (MIC = 50 µg/mL) and 6h (MIC = 12.5 µg/mL) possess increased potency than ampicillin (MIC = 100 µg/mL), chloramphenicol (MIC = 50 µg/mL) and ciprofloxacin (MIC = 25 µg/mL). The analogs 6a, 6g, 6j and 6o (MIC = 100 µg/mL) are found equally active to ampicillin against the same organism. With regard to activity against *Vibrio cholerae* two compounds 6g (MIC = 12.5 µg/mL) and 6i (MIC = 50 µg/mL) have shown chief activity compared to ampicillin (MIC=100 µg/mL), chloramphenicol (MIC = 50 µg/mL) and ciprofloxacin (MIC = 50 µg/mL), compounds 6b, 6f and 6q (MIC = 100 µg/mL) are found equipotent to ampicillin towards the same organism.

Concerning the antifungal activity of tested compounds, only one organism namely; *Candida albicans* showed certain sensitivity against some of the tested compounds, whereas another fungal strain was almost insensitive to the some compounds. Seven compounds namely 6b, 6e, 6i, 6k, 6m, 6n and 6r exhibited good growth inhibitory action on *Candida albicans* with MIC range of 100-250 µg/mL, when compared with griseofulvin (MIC = 100 µg/mL) and nystatin (MIC = 100 µg/mL), the standard antifungal agents utilized in this assay. Only one compound 6n is able to inhibit the growth of *Aspergillus fumigatus* of MIC 100 µg/mL.
### Table 3
**In vitro** antimicrobial activity of benzo[g]chromene derivatives 6a-r MICs, µg/mL.

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>3d</td>
<td>100</td>
<td>200</td>
<td>500</td>
<td>125</td>
<td>250</td>
<td>100</td>
<td>500</td>
</tr>
<tr>
<td>3e</td>
<td>250</td>
<td>500</td>
<td>250</td>
<td>200</td>
<td>200</td>
<td>500</td>
<td>1000</td>
</tr>
<tr>
<td>3f</td>
<td>500</td>
<td>250</td>
<td>500</td>
<td>100</td>
<td>500</td>
<td>125</td>
<td>500</td>
</tr>
<tr>
<td>3j</td>
<td>200</td>
<td>250</td>
<td><strong>62.5</strong></td>
<td>250</td>
<td>125</td>
<td>200</td>
<td>1000</td>
</tr>
<tr>
<td>3k</td>
<td>125</td>
<td>100</td>
<td>250</td>
<td>500</td>
<td><strong>50</strong></td>
<td>200</td>
<td><strong>&gt;1000</strong></td>
</tr>
<tr>
<td>3l</td>
<td>100</td>
<td>500</td>
<td>1000</td>
<td>100</td>
<td>250</td>
<td>500</td>
<td><strong>&gt;1000</strong></td>
</tr>
<tr>
<td>3p</td>
<td>500</td>
<td>100</td>
<td>100</td>
<td>125</td>
<td><strong>100</strong></td>
<td>250</td>
<td><strong>&gt;1000</strong></td>
</tr>
<tr>
<td>3q</td>
<td>250</td>
<td>200</td>
<td>200</td>
<td>200</td>
<td>500</td>
<td>125</td>
<td>500</td>
</tr>
<tr>
<td>3r</td>
<td>500</td>
<td>500</td>
<td>200</td>
<td>500</td>
<td>250</td>
<td><strong>100</strong></td>
<td><strong>&gt;1000</strong></td>
</tr>
<tr>
<td>6a</td>
<td>100</td>
<td>200</td>
<td>250</td>
<td>125</td>
<td><strong>100</strong></td>
<td>200</td>
<td><strong>&gt;1000</strong></td>
</tr>
<tr>
<td>6b</td>
<td>100</td>
<td>250</td>
<td><strong>100</strong></td>
<td><strong>62.5</strong></td>
<td><strong>50</strong></td>
<td><strong>100</strong></td>
<td>500</td>
</tr>
<tr>
<td>6c</td>
<td>500</td>
<td>250</td>
<td>200</td>
<td>100</td>
<td>250</td>
<td>200</td>
<td><strong>&gt;1000</strong></td>
</tr>
<tr>
<td>6d</td>
<td>200</td>
<td>100</td>
<td>500</td>
<td>250</td>
<td>500</td>
<td>200</td>
<td><strong>&gt;1000</strong></td>
</tr>
<tr>
<td>6e</td>
<td>250</td>
<td>200</td>
<td>250</td>
<td>200</td>
<td>200</td>
<td>500</td>
<td><strong>&gt;1000</strong></td>
</tr>
<tr>
<td>6f</td>
<td>250</td>
<td>500</td>
<td>200</td>
<td>500</td>
<td>125</td>
<td><strong>100</strong></td>
<td><strong>&gt;1000</strong></td>
</tr>
<tr>
<td>6g</td>
<td>100</td>
<td>100</td>
<td>25</td>
<td><strong>100</strong></td>
<td><strong>100</strong></td>
<td><strong>12.5</strong></td>
<td>250</td>
</tr>
<tr>
<td>6h</td>
<td>200</td>
<td>250</td>
<td>125</td>
<td><strong>100</strong></td>
<td><strong>62.5</strong></td>
<td>200</td>
<td>500</td>
</tr>
<tr>
<td>6i</td>
<td>125</td>
<td>500</td>
<td><strong>100</strong></td>
<td><strong>12.5</strong></td>
<td>200</td>
<td><strong>50</strong></td>
<td>250</td>
</tr>
<tr>
<td>6j</td>
<td>100</td>
<td>100</td>
<td>250</td>
<td>125</td>
<td><strong>100</strong></td>
<td>125</td>
<td><strong>&gt;1000</strong></td>
</tr>
<tr>
<td>6k</td>
<td>500</td>
<td>500</td>
<td>200</td>
<td><strong>100</strong></td>
<td>250</td>
<td>200</td>
<td>100</td>
</tr>
<tr>
<td>6l</td>
<td>200</td>
<td>250</td>
<td>500</td>
<td>250</td>
<td>500</td>
<td>250</td>
<td>1000</td>
</tr>
<tr>
<td>6m</td>
<td>125</td>
<td>500</td>
<td>500</td>
<td>250</td>
<td>200</td>
<td>250</td>
<td><strong>&gt;1000</strong></td>
</tr>
<tr>
<td>6n</td>
<td>200</td>
<td>100</td>
<td><strong>62.5</strong></td>
<td><strong>100</strong></td>
<td>125</td>
<td>250</td>
<td><strong>100</strong></td>
</tr>
<tr>
<td>6o</td>
<td>500</td>
<td>250</td>
<td><strong>100</strong></td>
<td>500</td>
<td><strong>100</strong></td>
<td>200</td>
<td><strong>&gt;1000</strong></td>
</tr>
<tr>
<td>6p</td>
<td>250</td>
<td>100</td>
<td><strong>100</strong></td>
<td>125</td>
<td>200</td>
<td>500</td>
<td>200</td>
</tr>
<tr>
<td>6q</td>
<td>500</td>
<td>200</td>
<td>200</td>
<td>500</td>
<td><strong>100</strong></td>
<td>500</td>
<td><strong>&gt;1000</strong></td>
</tr>
<tr>
<td>6r</td>
<td>250</td>
<td>500</td>
<td>200</td>
<td>250</td>
<td>500</td>
<td>200</td>
<td><strong>&gt;1000</strong></td>
</tr>
<tr>
<td>Ampi.</td>
<td>250</td>
<td>250</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>n. t.</td>
<td>n. t.</td>
</tr>
<tr>
<td>Cipro</td>
<td>50</td>
<td>100</td>
<td>50</td>
<td>25</td>
<td>25</td>
<td>25</td>
<td>n. t.</td>
</tr>
<tr>
<td>Norfl</td>
<td>100</td>
<td>50</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>n. t.</td>
</tr>
<tr>
<td>Grise.</td>
<td>n. t.</td>
<td>n. t.</td>
<td>n. t.</td>
<td>n. t.</td>
<td>n. t.</td>
<td>n. t.</td>
<td>100</td>
</tr>
<tr>
<td>Nyst.</td>
<td>n. t.</td>
<td>n. t.</td>
<td>n. t.</td>
<td>n. t.</td>
<td>n. t.</td>
<td>n. t.</td>
<td>100</td>
</tr>
</tbody>
</table>

A.F.: *Aspergillus fumigatus*, C.A.: *Candida albicans*

Ampi.: Ampicillin, Cipro.: Ciprofloxacin, Norfl.: Norfloxacin
Grise.: Griseofulvin, Nyst.: Nystatin

*a* n. t. not tested.
Table 4: Antimycobacterial activity of the compounds 6a-r.

<table>
<thead>
<tr>
<th>Compd.</th>
<th>Primary screen (6.25 µg/mL)</th>
<th>Actual MIC µg/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>3d</td>
<td>60</td>
<td>-</td>
</tr>
<tr>
<td>3e</td>
<td>79</td>
<td>-</td>
</tr>
<tr>
<td>3f</td>
<td>52</td>
<td>-</td>
</tr>
<tr>
<td>3j</td>
<td>93</td>
<td>100</td>
</tr>
<tr>
<td>3k</td>
<td>84</td>
<td>-</td>
</tr>
<tr>
<td>3l</td>
<td>33</td>
<td>-</td>
</tr>
<tr>
<td>3p</td>
<td>65</td>
<td>-</td>
</tr>
<tr>
<td>3q</td>
<td>90</td>
<td>200</td>
</tr>
<tr>
<td>3r</td>
<td>21</td>
<td>-</td>
</tr>
<tr>
<td>6a</td>
<td>22</td>
<td>-</td>
</tr>
<tr>
<td>6b</td>
<td>87</td>
<td>-</td>
</tr>
<tr>
<td>6c</td>
<td>08</td>
<td>-</td>
</tr>
<tr>
<td>6d</td>
<td>95</td>
<td>62.5</td>
</tr>
<tr>
<td>6e</td>
<td>97</td>
<td>50</td>
</tr>
<tr>
<td>6f</td>
<td>66</td>
<td>-</td>
</tr>
<tr>
<td>6g</td>
<td>90</td>
<td>250</td>
</tr>
<tr>
<td>6h</td>
<td>74</td>
<td>-</td>
</tr>
<tr>
<td>6i</td>
<td>90</td>
<td>250</td>
</tr>
<tr>
<td>6j</td>
<td>44</td>
<td>-</td>
</tr>
<tr>
<td>6k</td>
<td>12</td>
<td>-</td>
</tr>
<tr>
<td>6l</td>
<td>98</td>
<td>25</td>
</tr>
<tr>
<td>6m</td>
<td>80</td>
<td>-</td>
</tr>
<tr>
<td>6n</td>
<td>95</td>
<td>50</td>
</tr>
<tr>
<td>6o</td>
<td>60</td>
<td>-</td>
</tr>
<tr>
<td>6p</td>
<td>79</td>
<td>-</td>
</tr>
<tr>
<td>6q</td>
<td>52</td>
<td>-</td>
</tr>
<tr>
<td>Isoniazide</td>
<td>99</td>
<td>0.2</td>
</tr>
<tr>
<td>Rifampicin</td>
<td>98</td>
<td>40</td>
</tr>
</tbody>
</table>

A close examination of the structures of the active compounds in Table 3 revealed that, their antimicrobial activity is strongly bound to the nature of the substituent at the quinoline-C₆, together with the the substituent linked to the aryloxy part of the structure. In general, it could be clearly recognized that potential antibacterial activity was encountered without a substituent in the quinoline ring and with methyl substituent on aryloxy ring 6b also its counterpart 6g with methyl substituent in the quinoline ring and without a substituent on aryloxy ring exhibited significant activity. Different substitution (halo, methyl, methoxy) on quinoline and aryloxy ring affect antibacterial activity of synthesized compounds drastically and
substitution of methyl group on either of the ring revealed to be crucial for antibacterial activity. From the antifungal activity data it was concluded that compounds 6k, 6n and 6m are the most active among all the synthesized compounds. Moreover, good antifungal activity of analogs 6a, 6b, 6d, 6m-p and 6r indicates that the functional groups at R₁ = H or OMe position interferes in the antifungal potency of the title compounds.

Concerning the antimycobacteral activity of tested compounds, of the entire 2-aryloxyquinoline-3-carbaldehyde and benzo[g]chromene derivatives 6a-r, compounds 3j, 3q, 6d, 6f, 6h, 6j, 6m, 6o exhibited significant % inhibition in the primary screen (MIC = 6.25 µg/mL) and are chosen for secondary screening. The compound 6m (MIC = 25 µg/mL) have shown pronounced activity as compared to standard rifampicin (MIC = 40 µg/mL). Analogs 6d and 6f also exhibited good activity with MIC = 62.5 µg/mL and MIC = 50 µg/mL respectively.

6.3 CONCLUSION

In conclusion, a new library of 4H-benzo[g]chromene derivatives has been synthesized efficiently via one-pot multicomponent reaction approach. The engaged synthetic strategy allows the construction of relatively complicated nitrogen and oxygen carrying heterocyclic system as well as introduction of various substituted 2-aryloxyquinolines, the validated molecular target at 4- position of benzo[g]chromene. It is worth to mention that the biological activity of target compounds depends not only on the bicyclic heteroaromatic pharmacophore appended through ether linked aryl ring but also on the nature of peripheral substituents and may also upon their spatial relationship and positional changes. Of the compounds studied, analogs 6b and 6g emerged as most potential antimicrobial agents. Analogs 6d, 6f, 6h, 6j, 6m and 6o were considered lead compounds worthy of further structural optimization and development as potential antituberculosis agents. Therefore, it was concluded that there exists ample scope in this class of compounds for both antimicrobial as well as antitubercular activity.
REFERENCES:
32. Xing-Han Wang, Xiao-Hong Zhang, Shu-Jiang Tu, Feng Shi, Xiang Zou, Shu Yan, Zheng-Guo Han, Wen-Juan Hao, Xu-Dong Cao, Shan-Shan Wu J Het Chem 46 (2009) 832.