Synthesis, characterization, antibacterial and antioxidant studies of oxetoquinolines

Abstract
A series of 2H-oxeto[2,3-b]quinolines have been synthesized involving the simple steps of oxidation, reduction and cyclization of 2-chloro-3-formyl quinolines. All the synthesized compounds were evaluated for their antibacterial screening and antioxidant studies. Most of the compounds showed moderate to good activity against almost all the tested pathogens. In particular, compound 1e showed good antibacterial and antioxidant activity.

Keywords: 2-chloro-3-formyl quinolines, cyclization, Oxetoquinolines, antibacterial, antioxidant studies

Introduction
Mounting infectious diseases and the developing number of multi-drug resistant microbial pathogens still make the treatment of particular diseases an important and serious global problem. Therefore, an extensive research for the discovery and synthesis of new classes of antimicrobial agents is needed. The quinoline derivatives have been known to possess wide spectrum of pharmacological properties. They are the backbone in numerous commercial products such as perfumes, dyes including pharmaceuticals. Quinoline derivatives viz., ofloxacin, norfloxacin, ciprofloxacin, chloroquine, etc., have been used as efficient drugs till date. The derivatives of quinoline exhibit diverse biological and physiological activities such as antimicrobial, antituberculosis, antimalarial, antiinflammatory, anticancer, antihypertensive and anti-HIV. Recently, quinoline has been employed in the study of bioorganic and bioorganometallic processes.

The chemistry of quinolines is well established in the field of pharmacological activity. This system has proved to be a very attractive scaffold for medicinal chemist in recent years. However, quinoline derivatives were extensively studied as bioactive compounds, such as pyrazoloquinolines, diazepinoquinolines, thiadiazepinoquinolines and known to possess remarkable biological activities. For many of the activities the molecular mode of action is known. Recent research efforts have also highlighted the ability of agents based on pyrazoloquinoline skeleton to modulate adenosine A3 receptors and the phosphodiesterase receptors. Literature shows the annulations of pyridines to Quinoline to create functionalized quinoline derivatives. Based on above facts and in search of newer drugs, the annulations of the oxeto ring onto a quinoline scaffold approach. To the best of our knowledge, it is new and literature reports show about oxetoquinolines are not yet in the field of quinoline derivatives. Herein, we report the synthesis and chemistry of oxetoquinoline derivatives via the Vilsmeier-Haack reaction and its antibacterial and antioxidant studies.

Results and discussion
Over the years, 2-chloroquinoline-3-carbaldehydes, prepared by a Vilsmeier Haack Reaction on acetanilides and their derivatives have attracted much attention due to their wide spectrum of biological and pharmacological activities such as antimicrobial, anti-inflammatory, antimalarial and antiviral activities. They occupy a prominent position in the intermediate category as they can be utilized for further functionalization of many heterocyclic ring systems and for various functional group interconversions. On continuation of our research on heterocyclic compounds and in search of newer derivatives of quinolines as potential biological molecules, we have realized the title compounds in a two step synthetic protocol.

The reaction of 2-chloroquinoline-3-carbaldehyde 2 with 4N hydrochloric acid afforded the oxo derivative of the quinoline aldehyde 3. Subsequently, the sodiumborohydride reduction of the oxo derivative in a co-solvent of Tetrahydrofuran and ethanol yielded the alcohol derivative 4. The compound 4 under reflux conditions with ethyl acetate, dried over sodium sulphate, concentrated and column purified (Figure 1).

Figure 1  $R_1=R_2=R_3=R_4=H$, 4h, 80% yield; b: $R_1=R_3=CH_3$, $R_2=R_4=H$, 3.5h, 83% yield; c: $R_1=OCH_3$, $R_2=R_3=R_4=H$, 3h, 77% yield; d: $R_1=R_2=CH_3$, $R_3=R_4=H$, 3h, 78% yield; e: $R_1=C_6H_5$, $R_2=R_3=R_4=H$, 4h, 72% yield

Its IR spectrum showed strong absorption peaks at 1643 & 1258cm$^{-1}$ for C=O and C-O stretching frequencies respectively. The
1H-NMR spectrum revealed a fine splitting pattern of the aromatic protons as δ 7.80 (s, 1H, C4-H), δ 7.58-7.60 (d, 1H, C2-H), δ 7.20-7.22 (d, 1H, C7-H), δ 7.37-7.41 (t, 1H, C6-H), δ 7.08-7.12 (t, 1H, C5-H). In addition, it showed a sharp singlet at δ 4.86 which accounts for two proton methylene group (−CH2−). It is worthy to note that there is a sharp downfield shift from δ 4.28 to δ 4.86 due to the cyclization reaction towards the formation of oxeto ring system. At the same time the peaks for the aromatic hydroxy and the primary hydroxyl groups are missing. The above details are further advocated by the mass spectra, which showed the prominent mass peak at (m/z) 158 [M]+1. All the above spectral details supported the compound (1a) as 2H-oxeto[2,3-b]quinoline. The reaction was also extended to its methyl, methoxy, dimethyl and chloro derivatives.

### Biological evaluation

#### Antibacterial activity

The newly synthesized 2H-oxeto[2,3-b]quinolines were screened for their in vitro antibacterial activities against the seven different types of pathogens namely, *Staphylococcus aureus*, *Aeromonas hydrophila*, *Escherichia coli*, *Klesiella pneumonia*, *Salmonella paratyphi*, *Salmonella typhi* and *Mycobacterium butyricum* by the agar well diffusion method using Ofloxacin as control drug. The pathogens were cultured on the nutrient agar medium and DMSO was used as solvent and as negative control. The diameter of zone of inhibition measured in mm is compared to the current antimicrobial drug Ofloxacin as shown in Table 1. The susceptibility was assessed on the basis of the diameter of zone of inhibition against the bacterial strains. The antibacterial activity results revealed that the majority of the synthesized compounds showed varying degrees of inhibition against the tested microorganisms. It was found that compounds 1c and 1e exhibited good inhibition towards almost all the pathogens and comparable to that of the standard Ofloxacin. Compounds 1a and 1b showed least activity whereas compound 1d showed moderate activity with the inhibition zone ranging between 2–6mg/disc with all the tested pathogens. This notable difference among the synthesized compounds towards the antibacterial activity may be due to substituent present at C6-position, which plays a key role in the efficacy of the compound.

#### Antioxidant activity using FRAP assay

FRAP assay usually determines the reduction of ferric ion Fe3+ to ferrous ion Fe2+ in the presence of antioxidants. The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity. It can be concluded from the Table 2 that the compound 1e shows the higher ferric reducing power than its other analogs 1(a-d). The reducing power of the compound 1e was comparable to that of the standard BHT. The other remaining compounds 1a-d, showed good to moderate antioxidant activity. From the values it is assumed that in this assay the substitutions at the C6-position in the quinoline moiety as well as the hetero atoms might play substantial role in suppressing the radicals.

### Table 1 In vitro antibacterial activity of 2H-oxeto[2,3-b]quinolines(1a–e) (mg/disc) by disc-diffusion assay

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>Diameter of zone of inhibition (mm)</th>
<th>1a (mg/disc)</th>
<th>1b (mg/disc)</th>
<th>1c (mg/disc)</th>
<th>1d (mg/disc)</th>
<th>1e (mg/disc)</th>
<th>A</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>50</td>
<td>100</td>
<td>10</td>
<td>50</td>
<td>100</td>
<td>10</td>
<td>50</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>-</td>
<td>-</td>
<td>1</td>
<td>-</td>
<td>2</td>
<td>-</td>
<td>2</td>
</tr>
<tr>
<td><em>Aeromonas hydrophila</em></td>
<td>1</td>
<td>3</td>
<td>5</td>
<td>1</td>
<td>2</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>2</td>
<td>-</td>
<td>2</td>
</tr>
<tr>
<td><em>Klesiella pneumoniae</em></td>
<td>-</td>
<td>-</td>
<td>1</td>
<td>-</td>
<td>2</td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td><em>Salmonella paratyphi</em></td>
<td>-</td>
<td>-</td>
<td>2</td>
<td>-</td>
<td>3</td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td><em>Salmonella typhi</em></td>
<td>-</td>
<td>1</td>
<td>3</td>
<td>1</td>
<td>2</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td><em>Mycobacterium butyricum</em></td>
<td>-</td>
<td>3</td>
<td>-</td>
<td>1</td>
<td>3</td>
<td>1</td>
<td>2</td>
</tr>
</tbody>
</table>

A, Ofloxacin, _—‘ no inhibition.
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Table 2 Anti-oxidant Activity of 2H-oxeto[2,3-b]quinolines (1a–e) - The ferric reducing/antioxidant power (FRAP)

<table>
<thead>
<tr>
<th>Compound</th>
<th>The ferric reducing/antioxidant power (mg/mL)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>1mg/mL</td>
</tr>
<tr>
<td>la</td>
<td>0.015</td>
</tr>
<tr>
<td>lb</td>
<td>0.018</td>
</tr>
<tr>
<td>lc</td>
<td>0.02</td>
</tr>
<tr>
<td>ld</td>
<td>0.01</td>
</tr>
<tr>
<td>le</td>
<td>0.197</td>
</tr>
<tr>
<td>BHT</td>
<td>0.243</td>
</tr>
</tbody>
</table>

Experimental section

General

Thin layer chromatography was used to access the reactions and the purity of products. Melting points were determined on a Boetius Micro heating Table (Japan) and were uncorrected. IR spectra were recorded with a Shimadzu–8201FT instrument (Japan) in KBr discs and only noteworthy absorption levels (reciprocal centimeter) are recorded with a Shimadzu–8201FT instrument (Japan) in KBr discs.

Synthesis of 3-(hydroxymethyl)-6-methylquinolin-2-ol (4a):

It was prepared similar to 4a. m.p. 265°C; IR (KBr): 1660 (C=O), 1588 (C=N) and 3280 (-OH) cm⁻¹; ¹H-NMR (400MHz, CDCl₃); δ 11.72 (bs, 1H, -Ar-OH), 7.68 (s, 1H, C₆-H), 7.56-7.61 (d, 1H, C₅-H), 7.24-7.26 (d, 1H, C₇-H), 5.04-5.08 (t, 1H, -CH₂-OH), 4.22-4.24 (d, 2H, -CH₂-OH), 2.46 (s, 3H, -CH₃). ¹³C-NMR (100MHz, CDCl₃); δ 121.8, 123.7, 124.6, 126.8, 136.1, 144.9, 163.6 ppm. Anal. calcd. for C₄₂H₄₀N₂O₂: C, 72.86; H, 6.48; N, 6.92.

Synthesis of 3-(hydroxymethyl)-6,8-dimethylquinolin-2-ol (4d):

It was prepared similar to 4a. m.p. 226°C; IR (KBr):1660 (C=O), 1600 (C=N), 3291 (-OH) cm⁻¹; ¹H-NMR (400MHz, CDCl₃); δ 11.81 (bs, 1H, -Ar-OH), 7.79 (s, 1H, C₆-H), 7.60-7.62 (d, 1H, C₅-H), 7.24-7.26 (d, 1H, C₇-H), 5.02-5.06 (t, 1H, -CH₂-OH), 4.23-4.26 (d, 2H, -CH₂-OH), 2.42 (s, 3H, -CH₃) ppm; ¹³C-NMR (100MHz, CDCl₃); δ 121.8, 123.6, 124.4, 126.8, 136.5, 144.9, 163.6 ppm. Anal. calcd. for C₄₂H₄₀N₂O₂: C, 72.86; H, 6.48; N, 6.92.

Synthesis of 3-(hydroxymethyl)-6-methoxyquinolin-2-ol (4c):

It was prepared similar to 4a. m.p. 290°C; IR (KBr): 1650 (C=O), 1590 (C=N) and 3284 (-OH) cm⁻¹; ¹H-NMR (400MHz, CDCl₃); δ 11.79 (bs, 1H, -Ar-OH), 7.77 (s, 1H, C₆-H), 7.59-7.61 (d, 1H, C₅-H), 7.22-7.24 (d, 1H, C₇-H), 7.35-7.40 (t, 1H, CH₄-C=O), 5.15-5.19 (t, 1H, -CH₂-OH), 4.31-4.34 (d, 2H, -CH₂-OH), 3.91 (s, 3H, -OH) ppm; ¹³C-NMR (100MHz, CDCl₃); δ 53.9, 58.5, 121.8, 122.3, 124.7, 134.7, 141.5, 154.8, 164.4 ppm. Anal. calcd. for C₄₂H₄₀N₂O₂: C, 64.38; H, 5.40; N, 6.83; found: C, 64.31; H, 5.45; N, 6.85.

Synthesis of 3-(hydroxymethyl)-6,8-dimethylquinolin-2-ol (4b):

It was prepared similar to 4a. m.p. 219°C; IR (KBr):1649 (C=O), 1588 (C=N) and 3280 (-OH) cm⁻¹; ¹H-NMR (400MHz, CDCl₃); δ 11.72 (bs, 1H, -Ar-OH), 7.68 (s, 1H, C₆-H), 7.56-7.61 (d, 1H, C₅-H), 7.28-7.33 (t, 1H, C₇-H), 5.02-5.06 (t, 1H, -CH₂-OH), 4.22-4.24 (d, 2H, -CH₂-OH), 2.46 (s, 3H, -CH₃). ¹³C-NMR (100MHz, CDCl₃); δ 121.8, 123.6, 124.4, 126.8, 136.5, 144.9, 163.6 ppm. Anal. calcd. for C₄₂H₄₀N₂O₂: C, 70.92; H, 6.45; N, 6.89; found: C, 70.86; H, 6.48; N, 6.92.

Synthesis of 6-chloro-3-(hydroxymethyl)quinolin-2-ol (4e):

It was prepared similar to 4a. m.p. 226°C; IR (KBr):1660 (C=O), 1600 (C=N), 3291 (-OH) cm⁻¹; ¹H-NMR (400MHz, CDCl₃); δ 11.81 (bs, 1H, -Ar-OH), 7.79 (s, 1H, C₆-H), 7.60-7.62 (d, 1H, C₅-H), 7.24-7.26 (d, 1H, C₇-H), 5.16-5.20 (t, 1H, -CH₂-OH), 4.32-4.34 (d, 2H, -CH₂-OH) ppm; ¹³C-NMR (100MHz, CDCl₃); δ 58.3, 122.4, 124.6, 128.7, 130.8, 131.8, 135.1, 142.2, 166.3 ppm. Anal. calcd. for C₄₂H₄₀ClN₂O₂: C, 75.30; H, 3.85; N, 6.68; found: C, 75.27; H, 3.87; N, 6.69.

Synthesis of 2H-oxeto[2,3-b]quinoline (1a):

It was prepared similar to 4a. 2H-oxeto[2,3-b]quinoline was refluxed in glacial acetic acid (10ml) for 4 hours. The completion of the reaction was checked by TLC. The reaction mixture was concentrated and recrystallized from methanol.

Figure 2 ¹H-NMR spectrum of compound (1a)
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It was prepared similar to 1a. m.p. 184°C; IR (KBr): 1640 (C=N), 1250 (C-O) cm⁻¹; ¹H-NMR (400MHz, CDCl₃): δ 7.14 (s, 1H), 4.80 (s, 2H), 2.5 (s, 3H), 2.2 (s, 3H); ¹³C-NMR (100MHz, CDCl₃): δ 22.7, 22.3, 33.9 (CH₃), 117.9, 119.6, 125.9, 132.0, 133.9, 137.9, 142.5, 144.0, 161.9; LC-MS (m/z)=186 [M⁺+1]. Anal. calcd. for C₉H₇NO: C, 77.81; H, 5.99; N, 7.56; found: C, 77.75; H, 6.03; N, 7.59.

**Synthesis of 5-chloro-2H-oxeto[2,3-b]quinoline (1e)**

It was prepared similar to 1a. m.p. 183°C; IR (KBr): 1640 (C=N), 1252 (C-O); 691 (C-Cl) cm⁻¹; ¹H-NMR (400MHz, CDCl₃) δ 8.5 (s, 1H), 7.61 (s, 1H), 7.25-7.27 (d, 1H), 7.13-7.16 (d, 1H), 4.92 (s, 2H); ¹³C-NMR (100MHz, CDCl₃): δ 34.08 (CH₃), 118.6, 120.5, 126.9, 132.9, 134.7, 138.7, 143.3, 144.8, 162.8ppm; LC-MS (m/z)=192 [M⁺+1], 194 [M⁺+2]. Anal. calcd. for C₁₀H₈ClNO: C, 62.68; H, 3.16; N, 7.31; found: C, 62.66; H, 3.17; N, 7.32.

**Antibacterial activity by Agar well diffusion method**

The antibacterial activity of the synthesized compounds 4 (a-e) was performed on agar well diffusion method. Each chemical compound was mixed with the equal amount of dimethyl sulfoxide (DMSO) i.e., 1 µl of DMSO contains 1 mg of chemical compound and considered as the stock solution. The synthesized active chemical compounds were then subjected to the antimicrobial activity against the human pathogenic bacteria isolated from Hospital clinical samples which was collected by the Rhizosphere Biology Laboratory, Bharathidasan University, Tiruchirappalli, Tamil Nadu, India. The procured pathogenic bacteria namely Staphylococcus aureus, Aeromonas hydrophila, Escherichia coli, Klebsiella pneumoniae, Salmonella paratyphi, Salmonella typhi and Mycobacterium butyricum (MTCC 940) were cultured in nutrient agar medium and incubated as well as maintained at 37°C for at 24h. The medium was further used for the antimicrobial activity studies with the synthesized chemical compounds by the agar well diffusion method. Each compound 4 (a-e) were poured and checked for their antibacterial activity against the seven collected strains with different concentrations like 10g/10µl, 50mg/50µl and 100mg/100µl. The zone of inhibition was found after the incubation period of 24h at 37°C and the measurements were reported as diameters in millimeters.

The higher zone of inhibition was considered as significant activity of the synthesized chemical compound against the virulent human pathogenic bacteria (Figure 5) (Figure 6).²⁷⁻²⁹

![Figure 3 Mass spectrums (LC-MS) of compound (1a)](image)

![Figure 4 IR-spectrum of compound (1a)](image)

![Figure 5 ¹H-NMR spectrum of compound (4a)](image)

Antioxidant activity using FRAP assay

The total antioxidant potential of a sample was determined using the ferric reducing ability of plasma FRAP assay as a measure of antioxidant potential. A potential antioxidant will reduce the ferric ion (Fe^{3+}) to the ferrous ion (Fe^{2+}); the latter forms a blue complex (Fe^{2+}/TPTZ), which increases the absorption at 593nm. Briefly, FRAP reagent was prepared by mixing acetate buffer (300M, pH 3.6), a solution of 10µM TPTZ in 40µM HCl and 20µM FeCl₃ at 10:1:1 (v/v/v). The reagent (300µl) and sample solutions (10µl) were added to each well and mixed thoroughly. The absorbance was taken at 593nm after 10min. Standard curve was prepared using different concentrations of trolox. All the solutions were used on the day of preparation and the results were corrected for dilution and expressed in µM trolox per 100g dry weight (dw) and all determinations were performed in triplicates. The stock solutions of the each synthesized chemical compound individually mixed with double distilled water about 1mg/mL and variant concentration about 10mg/mL was prepared and proceeded the above procedure to react with the FRAP reagents and taken the Optical density level at 593nm in UV visible spectroscopy. The Optical density was noted and the strength meant its antioxidant potential to control the free radical scavenging activity through standard curve.

Conclusion

In summary some suitable and elegant methods have been chosen for the synthesis of novel heterocyclic compounds such as—Hydrogenated oxetoquinolines though the annulation of the oxeto ring onto a quinoline scaffolds methodology. The key intermediate, 2-chloro-3-formyl quinolines were synthesized by the Vilsmeier Haack and further explored toward the synthesis of oxetoquinolines. The novel 2H-oxeto[2,3-b]quinoline and its derivatives were studied for its anti bacterial and antioxidant studies. All the compounds were found to be active against all the pathogens though they did not reach the effectiveness of the standards. In addition compound 1e showed good anti oxidant activity compared to other synthesized compounds.

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Conflicts of interest

Authors declare that there is no conflict of interest.

References


