CHAPTER III

Synthesis of Tetrahydroquinolines
1. Introduction

Quinoline is a nitrogen-based heterocyclic structural unit and it has the formula C₉H₇N and is a colourless hygroscopic liquid with a strong odour. Aged samples, if exposed to light, become yellow and later brown. Quinoline is only slightly soluble in cold water but dissolves readily in hot water and in most of the organic solvents.¹ Recently quinolines have attracted the attention of researchers because of their broad range of activities and applications. The most important sources of quinoline include wood preservation, coal processing, petroleum and shale oil. Quinoline nucleus is found to occur in many natural alkaloids, therapeutics and in other synthetic analogues.² In 1820, quinine (1) (Fig III.1) was isolated from the bark of the cinchona tree which replaced the crude bark extract for the treatment of malaria. Other quinoline derivatives (2-6) shown in Fig III.2 having various activities were also isolated from different plant species.³ In 1834, the quinoline was first extracted from coal tar by Friedlieb Ferdinand Runge.⁴ Quinolines show a wide spectrum of biological activities such as antiproliferative, antiplasmodial, antimalarial, antibacterial, and anticancer activities.⁵ Because of a variety of pharmacological properties, quinolines represent a large family of heterocyclic compounds which find application in the design of medicinally important compounds and also useful synthetic building blocks in organic chemistry.⁶-¹³

![Fig III.1: Natural Quinoline derivatives](image-url)
Figure III.2: Medicinally important quinoline derivatives
2. Synthesis of *quinolines*: A Short review

**Classical methods:**

1. Aniline heated with sulphuric acid, glycerol and an oxidizing agent - nitrobenzene to yield quinoline is named as the Skarup synthesis.  

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[Diagram of reaction]
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**Scheme III.1:** Skarup synthesis

2. Reaction of an aniline with \( \alpha,\beta \)-unsaturated carbonyl compounds to form quinolines is named as Doebner–Miller reaction.

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[Diagram of reaction]
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**Scheme III.2:** Doebner–Miller reaction

3. In 1888, the first Combes quinoline synthesis was reported by Combes, it involves the condensation of unsubstituted anilines with \( \beta \)-diketones to form substituted quinolines after an acid-catalyzed ring closure of an intermediate Schiff base.

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[Diagram of reaction]
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**Scheme III.3:** Combes quinoline synthesis
4. In 1887, Max Conrad and Leonhard Limpach reported the Conrad–Limpach quinoline synthesis\textsuperscript{10} in which the condensation of anilines with \( \beta \)-ketoesters gives 4-hydroxyquinolines via a Schiff base.

**Scheme III.4:** Conrad–Limpach quinoline synthesis

5. German chemist Paul Friedländer\textsuperscript{11} reported the reaction of 2-Aminobenzaldehydes with ketones to form quinoline derivatives. The reaction is named as Friedlander annulations. It is one of the most simple and straightforward methods for the synthesis of poly-substituted quinolines.

**Scheme III.5:** Friedlander quinoline synthesis

*Other reported methods for the synthesis of quinolines:*

6. Reddy *et al.*,\textsuperscript{12} described a three-component one-pot reaction between 3,4-dimethoxyaniline, aldehydes and ethyl-3,3-diethoxypropionate to get quinoline derivatives by using montmorillonite K-10 (Mont K-10) as a green catalyst by utilizing the oxygen from air and water.
Scheme III.6: Mont K-10 catalysed synthesis of quinolines

7. Ranuet et al.,\textsuperscript{13} developed a simple and efficient procedure for the synthesis of 4-alkylquinoline derivatives by a one-pot reaction of anilines with alkyl vinyl ketones on the surface of silica gel insemintated with indium(III) chloride under microwave irradiation without any solvent.

Scheme III.7: Synthesis of quinolines under microwave irradiation

8. Anvar and his co-workers\textsuperscript{14} demonstrated a method for the synthesis of quinoline derivatives involving a microwave-assisted, one pot-three-component reaction between aromatic amines, aromatic aldehydes and phenylacetylene in the presence of catalytic amounts of potassium dodecatungsto cobaltate trihydrate (K$_5$CoW$_{12}$O$_{40}$·3H$_2$O).

Scheme III. 8: Potassium dodecatungsto cobaltate trihydrate catalysed synthesis of quinoline derivatives
9. Naik et al.\textsuperscript{15} have reported a rapid and efficient method for the synthesis of various carbonitrile quinoline/benzo[h]quinoline derivatives by utilizing benzaldehyde, methylecyanoacetate and aromatic amines with nanostructured TiO\textsubscript{2} under solvent-free microwave irradiation.

\begin{center}
\begin{tabular}{c}
\begin{tikzpicture}
\node (a) at (0,0) {$\text{CHO}$};
\node (b) at (1.5,0) {$\text{NC}$};
\node (c) at (3,0) {$\text{O}$};
\node (d) at (4.5,0) {$\text{R}_1$};
\node (e) at (6,0) {$\text{NH}_2$};
\node (f) at (7.5,0) {$\text{R}_1$};
\node (g) at (9,0) {$\text{CN}$};
\node (h) at (10.5,0) {$\text{OH}$};
\node (i) at (12,0) {$\text{TiO}_2$};
\node (j) at (13.5,0) {No solvent};
\draw (a) -- (b) -- (c) -- (d) -- (e) -- (f) -- (g) -- (h);
\end{tikzpicture}
\end{tabular}
\end{center}

\textbf{Scheme III.9:} Nano TiO\textsubscript{2} catalysed synthesis of quinolines

10. Quinoline derivatives were synthesized by employing 2-aminoacetophenone and phenylacetylene in the presence of Zn(OTf)\textsubscript{2} as an effective catalyst under microwave irradiation, as presented by Praveen and co-workers.\textsuperscript{16}

\begin{center}
\begin{tabular}{c}
\begin{tikzpicture}
\node (a) at (0,0) {$\text{O}$};
\node (b) at (1.5,0) {$\text{R}_2$};
\node (c) at (3,0) {$\text{N}_2$};
\node (d) at (4.5,0) {$\text{Ph}$};
\node (e) at (6,0) {$\text{NH}_2$};
\node (f) at (7.5,0) {$\text{Ph}$};
\node (g) at (9,0) {$\text{R}_1$};
\node (h) at (10.5,0) {450W};
\node (i) at (12,0) {$\text{Zn(OTf)}_2$};
\draw (a) -- (b) -- (c) -- (d) -- (e) -- (f) -- (g) -- (h) -- (i);
\end{tikzpicture}
\end{tabular}
\end{center}

\textbf{Scheme III.10:} Zn(OTf)\textsubscript{2} catalysed synthesis of quinolines

11. Ren et al.\textsuperscript{17} have developed a method in which 2-aminobenzyl alcohol reacts with ketones in toluene in the presence of a palladium catalyst along with KOH to give the corresponding quinoline derivatives in good yields.

\begin{center}
\begin{tabular}{c}
\begin{tikzpicture}
\node (a) at (0,0) {$\text{O}$};
\node (b) at (1.5,0) {$\text{OH}$};
\node (c) at (3,0) {$\text{NH}_2$};
\node (d) at (4.5,0) {$\text{Ph}$};
\node (e) at (6,0) {$\text{Pd}$};
\node (f) at (7.5,0) {$\text{KOH}$};
\node (g) at (9,0) {toluene};
\draw (a) -- (b) -- (c) -- (d) -- (e) -- (f) -- (g);
\end{tikzpicture}
\end{tabular}
\end{center}

\textbf{Scheme III.11:} Palladium catalysed synthesis of quinoline derivatives
12. A method for producing quinoline derivatives using ultrasound and an ionic liquid has been established by Kowsari et al.\textsuperscript{18} A two-component, one-pot condensation reaction of isatin with enolizable ketone yields the quinoline derivative in high yields.

\begin{center}
\begin{tikzpicture}
  \node (a) at (0,0) {\text{\textbf{Scheme III.12: Ionic liquid catalysed synthesis of quinolines}}};
\end{tikzpicture}
\end{center}

13. A. R. Sardarian and coworkers\textsuperscript{19} poly-substituted synthesis of quinolines from \(\beta\)-ketoesters and 2-Aminoacetophenone in the presence of catalytic amounts of dodecylphosphonic acid (DPA) in aqueous medium and under solvent-free condition.

\begin{center}
\begin{tikzpicture}
  \node (a) at (0,0) {\text{\textbf{Scheme III.13: DPA catalysed quinoline synthesis}}};
\end{tikzpicture}
\end{center}

14. An efficient microwave assisted, solvent-free synthesis of substituted quinoline derivatives from o-nitrobenzaldehyde and enolizable ketones using SnCl\(_2\)\(2\)H\(_2\)O as a catalyst has been introduced by Chaudhuri and co-workers.\textsuperscript{20} This method is relatively faster and it affords the desired products in respectable yields.

\begin{center}
\begin{tikzpicture}
  \node (a) at (0,0) {\text{\textbf{Scheme III.14: Quinoline synthesis from enolizable ketones}}};
\end{tikzpicture}
\end{center}
Highlights

Most of the developed methods suffer from harsh reaction conditions, low yields, high temperature, tedious work-up and use of stoichiometric and relatively expensive reagents/catalysts. We, herein, report a general and effective one-step procedure for the synthesis of polysubstituted quinolines and their biological activity.

3. Present Work

In continuation of development of methods for the synthesis of novel heterocyclic compounds using silica supported, heterogeneous catalysts, herein, we report an efficient synthesis of tetrahydroquinoline derivatives by a one-pot four-component reaction of ethyl acetoacetate, dimedone, ammonium acetate with suitable aromatic aldehydes in ethanol. Silica iodide (SiO$_2$-I) acts as an efficient heterogeneous catalyst to afford the products in excellent yields in short reaction duration. The process is simple and environmentally benign as it involves use of a heterogeneous recyclable catalyst. Some of the prepared quinoline derivatives showed potent anti-cancer activity against HepG2 and MCF-7 cell lines. Docking study was carried out to evaluate the binding affinity of the synthesized molecules and the standard drug doxorubicin with Estrogen Receptor (ER).

![Scheme III.15: Synthesis of 4-aryl-2,7,7-trimethyl-5-oxo-1,4,6,8-tetrahydroquinoline-3-carboxylic acid ethyl esters](image-url)
3.1 Research highlights

- To synthesize novel substituted 4-aryl-2,7,7-trimethyl-5-oxo-1,4,6,8-tetrahydroquinoline-3-carboxylic acid ethyl esters.
- To characterize the synthesized compounds by FT-IR, $^1$HNMR, $^{13}$CNMR. Micro elemental analysis and studies on the biological evaluation of the synthesized compounds.
- To evaluate the binding affinity of the synthesized molecules by the. *In-silico*: Molecular docking study.
- To publish the work in peer reviewed journal.

3.2 Results and Discussion

We started the work by examining the reaction of 4-methoxybenzaldehyde with dimedone, ethyl acetoacetate and ammonium acetate to get 4-(4'-methoxyphenyl)-2,7,7-trimethyl-5-oxo-1,4,6,8-tetrahydroquinoline-3-carboxylic acid ethyl ester (4c) in EtOH in the presence of catalytic SiO$_2$-I as a heterogeneous catalyst at reflux after 2–3 h in 90 % yield. (Table III.1)

<table>
<thead>
<tr>
<th>Entry</th>
<th>Catalyst</th>
<th>Time (h)</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Na$_2$CO$_3$</td>
<td>6</td>
<td>30</td>
</tr>
<tr>
<td>2</td>
<td>Ba(OH)$_2$</td>
<td>6</td>
<td>60</td>
</tr>
<tr>
<td>3</td>
<td>SnCl$_2$</td>
<td>8</td>
<td>50</td>
</tr>
<tr>
<td>4</td>
<td>Acidic Molecular sieves 4A</td>
<td>9</td>
<td>40</td>
</tr>
<tr>
<td>5</td>
<td>Amberlite IR120H</td>
<td>13</td>
<td>20</td>
</tr>
<tr>
<td>6</td>
<td>CeCl$_3$</td>
<td>12</td>
<td>30</td>
</tr>
<tr>
<td>7</td>
<td>SiO$_2$</td>
<td>4</td>
<td>60</td>
</tr>
<tr>
<td>8</td>
<td>SiO$_2$-Cl$^c$</td>
<td>3.5</td>
<td>70</td>
</tr>
<tr>
<td>9</td>
<td>SiO$_2$-I$^c$</td>
<td>2.5</td>
<td>90</td>
</tr>
</tbody>
</table>

*Table III.1: Effect of various catalysts on the synthesis of 4c*

$^a$Reaction condition: 4-methoxybenzaldehyde (1 mmol), dimedone (1 mmol), ethyl acetoacetate (1 mmol), ammonium acetate (1.5 mmol) and EtOH (5 mL); $^b$10 mol%; $^c$0.1 g; $^d$Isolated yield.
To understand the importance of this method, we performed all the further reactions using SiO$_2$-I (0.1 g) in EtOH at reflux, and it was found that, SiO$_2$-I can efficiently catalyze the reaction between dimedone, ethyl acetoacetate, ammonium acetate, and different aromatic aldehydes to give excellent yield of the desired products within 2–3 h as shown in the (Table III.2) From the data presented in this Table, it is clear that, the method is effective for both electron withdrawing and electron donating aromatic aldehydes. Then, we carried out the reaction using aliphatic aldehydes (Table III.2, entries 10 and 11), and it was found that, there was no product formation even after 15 h.

**Table III.2: Synthesis of Tetrahydroquinolines (4a–k)**

<table>
<thead>
<tr>
<th>Entry</th>
<th>Aldehydes</th>
<th>Product</th>
<th>Time (h)</th>
<th>Yield$^a$ (%)</th>
<th>Found</th>
<th>Reported</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2,3,4-MeOC$_6$H$_2$CHO</td>
<td>4a</td>
<td>2.5</td>
<td>90</td>
<td>180–182$^\dagger$</td>
<td>–</td>
</tr>
<tr>
<td>2</td>
<td>3-Br,4-MeOC$_6$H$_2$CHO</td>
<td>4b</td>
<td>2.5</td>
<td>87</td>
<td>250–251$^\dagger$</td>
<td>–</td>
</tr>
<tr>
<td>3</td>
<td>4-MeOC$_6$H$_2$CHO</td>
<td>4c</td>
<td>2.5</td>
<td>90</td>
<td>145–147</td>
<td>145–147$^{22}$</td>
</tr>
<tr>
<td>4</td>
<td>2-IC$_6$H$_4$CHO</td>
<td>4d</td>
<td>3.0</td>
<td>86</td>
<td>180–182$^\dagger$</td>
<td>–</td>
</tr>
<tr>
<td>5</td>
<td>3-HO,4-MeOC$_6$H$_2$CHO</td>
<td>4e</td>
<td>2.3</td>
<td>85</td>
<td>199–201</td>
<td>200–202$^{23}$</td>
</tr>
<tr>
<td>6</td>
<td>3,5-BrC$_6$H$_5$CHO</td>
<td>4f</td>
<td>2.4</td>
<td>82</td>
<td>255–257$^\dagger$</td>
<td>–</td>
</tr>
<tr>
<td>7</td>
<td>3,4-ClC$_6$H$_3$CHO</td>
<td>4g</td>
<td>2.8</td>
<td>80</td>
<td>216–218</td>
<td>216–218$^{23}$</td>
</tr>
<tr>
<td>8</td>
<td>3-F,4-ClC$_6$H$_3$CHO</td>
<td>4h</td>
<td>2.6</td>
<td>89</td>
<td>220–222$^\dagger$</td>
<td>–</td>
</tr>
<tr>
<td>9</td>
<td>4-HOC$_6$H$_4$CHO</td>
<td>4i</td>
<td>3.0</td>
<td>87</td>
<td>238–240</td>
<td>238–240$^{23}$</td>
</tr>
<tr>
<td>10</td>
<td>HCHO</td>
<td>4j</td>
<td>15.0</td>
<td>ND</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>11</td>
<td>CH$_3$CHO</td>
<td>4k</td>
<td>15.0</td>
<td>ND</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

$^a$Isolated yield; $^\dagger$Novel compound

4. Experimental section

4.1 Materials and methods

All chemicals were commercially available and used without further purification, except liquid aldehydes which were distilled before use. All yields refer to the yields of the isolated products after purification. All the products were characterized by the IR, $^1$HNMR, $^{13}$CNMR,
Mass spectral and CHN analyses. Melting points were determined on a Raaga, INDIAN make melting point apparatus. Nuclear magnetic resonance spectra were obtained on a Bruker AMX instruments in CDCl₃ using TMS as an internal standard [400 MHz and 100 MHz for ¹H NMR for ¹³C NMR respectively]. ESI-MS analysis was carried out using ESI-Q TOF instrument. For biological assays, the HepG2 and MCF-7 cell lines were obtained from National Centre for Cell Sciences (NCCS), Pune, INDIA.

4.2 General procedure for the synthesis of tetrahydroquinolines

A mixture of aldehyde (1 mmol), dimesione (1 mmol), ethyl acetacetate (1 mmol) and ammonium acetate (1.5 mmol) and SiO₂-I (0.1 g) in EtOH (5 mL) was refluxed for 2–3 h. After the completion of the reaction (TLC), the mixture was cooled and poured onto crushed ice. The solid product was dissolved in ethanol, and filtered to separate the catalyst. The product after the removal of the solvent under vacuum was crystallized from ethanol, and the catalyst was kept aside for further use. The spectral and analytical data for some of the prepared compounds is presented in the Table III.2.

5. Spectral and analytical data

2,7,7-Trimethyl-5-oxo-4-(2',3',4' trimethoxyphenyl)-1,4,6,8-tetrahydroquinoline-3-carboxylic acid ethyl ester (4a):

Colorless solid, Mp: 180–182 °C; IR (KBr): v 3350 (N-H), 1712 (C=O); 1700, 1642, 1611, 1499, 1422, 1305, 1214, 1053, 1011 cm⁻¹; ¹H NMR (400 MHz, CDCl₃): δ 0.95 (s, 3H, CH₃,C(11) H), 1.06 (s, 3H, CH₃,C(12)H), 1.95 (t, J = 6.8Hz, 3H,C(21) H), 2.21(m, 4H, 2CH₂, C(6) C(8) H), 2.35 (s, 3H,CH₃, C(1) H), 3.76–3.89 (s, 9H, 3 × OCH₃, C(22)C(23)C(24) H), 4.04–4.06 (q, J = 2.4 Hz, 2H,C(20) H), 5.11 (s, 1H, CH,C(4) H), 6.52 (d, J = 8.8 Hz, 1H,C(14) H), 6.97 (d, J = 6.8 Hz, 1H,C(15) H), 10.24 (s, 1H, N-H) ppm; ¹³C NMR (100MHz, CDCl₃): δ 14.30 (C-21), 19.42(C-1), 27.03(C-11, C-12), 29.54 (C-4), 32.62 (C-7), 50.95 (C-8), 55.84 (C-6,C-24), 59.73 (C-22), 60.41 (C-24), 60.50 (C-23),100.10 (C-10) 105.50 (C-3), 106.28 (C-15), 111.12 (C-13), 125.70 (C-14), 132.46 (C-17), 142.10 (C-2), 142.94 (C-9),149.44 (C-16), 152.11 (C-18), 168.10 (C-19,C=O), 195.72 (C-5, C=O) ppm; ESI-MS: [M] 429.5; Anal. Calcd. C₂₄H₃₃NO₅(%): C, 67.11; H, 7.27; N, 3.26; Found: C, 66.01; H, 7.07; N, 3.21.
4-(3'-Bromo-4'-methoxyphenyl)-2,7,7-trimethyl-5-oxo-1,4,6,8-tetrahydroquinoline-3-carboxylic acid ethyl ester (4b):

Colorless solid, Mp: 250–251 °C; IR (KBr): ν 3346 (N-H), 1716 (C=O); 1708, 1609, 1521, 1489, 1410, 1300, 1207, 1063, 1020 cm\(^{-1}\); \(^1\)HNMR (400 MHz, CDCl\(_3\)): δ 0.96 (s, 3H, CH\(_3\), C(11) H), 1.07 (s, 3H, CH\(_3\), C(12) H), 1.21 (t, J = 7.2 Hz, 3H, C(21) H), 2.36–2.44 (m, 4H, 2CH\(_2\), C(6) C(8) H), 2.70 (s, 3H, CH\(_3\), C(1) H), 3.80 (s, 3H, OCH\(_3\), C(22) H), 4.05–4.10 (q, J = 7.2 Hz, 2H, C(20) H), 4.96 (s, 1H, CH, C(4) H), 6.66 (d, J = 8.0 Hz, 3H, C(14) H), 6.78 (d, J = 8.0 Hz, 1H, C(15) H), 10.28 (s, 1H, NH) ppm; \(^13\)CNMR (100MHz, CDCl\(_3\)): δ 14.25 (C-21), 17.09 (C-7), 18.90 (C-1), 26.80 (C-11,C-12), 29.83 (C-4), 48.15 (C-8), 54.17 (C-22), 58.07 (C-6), 59.37 (C-20), 85.82 (C-10), 108.08 (C-17), 122.67 (C-3), 123.52 (C-15), 128.08 (C-14), 131.43 (C-13), 137.19 (C-18), 145.35 (C-2), 148.98 (C-9), 156.92 (C-16), 164.25 (C-19,C=O), 197.64 (C-5,C=O) ppm. ESI-MS: [M+1]448.1; Anal.Caled. C\(_{22}\)H\(_{24}\)BrNO\(_4\) : C, 58.94; H, 5.85; N, 3.12. Found: C, 57.33; H, 5.05; N, 3.10.

4-(2'-Iodophenyl)-2,7,7-trimethyl-5-oxo-1,4,6,8-tetrahydroquinoline-3-carboxylic acid ethyl ester (4d):

Colorless solid, Mp: 180–182 °C; IR (KBr): ν 3339 (N-H), 1723 (C=O); 1709, 1600, 1545, 1408, 1400, 1299, 1207, 1083, 1030 cm\(^{-1}\); \(^1\)HNMR (400 MHz, CDCl\(_3\)): δ 0.93 (s, 3H, CH\(_3\), C(11) H), 1.06 (s, 3H, CH\(_3\), C(12) H), 1.18 (t, J = 6.4 Hz, 3H, C(21) H), 2.18–2.22 (m, 4H, 2CH\(_2\), C(6)C(8) H), 2.38 (s, 3H, CH\(_3\), C(1) H), 4.03–4.08 (q, J = 6.8 Hz, 2H, C(20) H), 4.97 (s, 1H, CH, C(4) H), 6.71 (d, J = 8.0 Hz, 2H, Ar-H, C(18) H), 7.18 (d, J = 8.0 Hz, 2H,Ar-H, C(15) H), 9.51 (s, 1H, NH) ppm; \(^13\)CNMR (100MHz, CDCl\(_3\)): δ 14.71 (C-21), 19.71 (C-1), 27.57(C-11,C-12), 29.96(C-4), 50.99(C-6,C-8),60.26 (C-20), 98.74 (C-14), 102.20 (C-3),106.92 (C-10),112.1(C-17),113.6(C-16), 129.4(C-18), 140.0(C-2,C-15), 143.9(C-9), 150.4(C-13), 168.0 (C-19, C=O), 196.3(C-5, C=O) ppm; ESI-MS: [M+1] 466.08; Anal.Caled. C\(_{21}\)H\(_{24}\)INO\(_3\) : C, 54.20; H, 5.20; N, 3.01. Found: C, 53.22; H, 5.12; N, 2.73.
4-(3′-Hydroxy-4′-methoxy-phenyl)-2,7,7-trimethyl-5-oxo-1,4,6,8-tetrahydroquinoline-3-carboxylic acid ethyl ester (4c):

Colorless solid, Mp: 199–201°C; IR (KBr): v 3310 (N-H), 1746 (C=O); 1699, 1602, 1525, 1412, 1401, 1229, 1203, 1023, 998 cm⁻¹; ¹H NMR (400 MHz, CDCl₃): δ 0.96 (s, 3H, CH₃, C(11) H), 1.07 (s, 3H, CH₃, C(12) H), 1.21 (t, J = 7.2 Hz, 3H, C(21) H), 2.31 (m, 4H, 2CH₂, C(6) C(8) H), 2.40 (s, 3H, CH₃, C(1) H), 2.62 (s, 1H, C(15) OH), 3.80 (s, 3H, OCH₃, C(22) H), 4.05–4.10 (q, J = 7.2 Hz, 2H, C(20) H), 4.95 (s, 1H, CH, C(4) H), 6.66–6.80 (m, 3H, Ar-H, C(14,17,18) H), 8.50 (s, 1H, NH) ppm; ¹³C NMR (100MHz, CDCl₃): δ 13.90 (C-21), 17.29 (C-7), 18.20 (C-1), 27.81 (C-11, C-12), 29.72 (C-4), 47.51 (C-8), 51.19 (C-6), 55.54 (C-22), 59.64 (C-20), 100.23 (C-3), 108.23 (C-10), 113.90, 114.08 (C-17), 116.23 (C-14), 128.23 (C-18), 130.26 (C-13), 136.91 (C-9), 142.50 (C-2,C-15), 145.02 (C-16), 160.26 (C-19,C=O), 196.94 (C-5, C=O) ppm; ESI-MS: [M+1] 386.19; Anal. Calcd. C₂₂H₂₇NO₅: C, 68.55; H, 7.06; N, 3.63. Found: C, 67.91; H, 6.78; N, 3.61.

4-(3′,5′-Dibromo-phenyl)-2,7,7-trimethyl-5-oxo-1,4,6,8-tetrahydroquinoline-3-carboxylic acid ethyl ester (4f):

Colorless solid, Mp: 255–257 °C; IR (KBr): v 3300 (N-H), 1796 (C=O); 1599, 1501, 1435, 1400, 1399, 1209, 1200, 1013, 910 cm⁻¹; ¹H NMR (400 MHz, CDCl₃): δ 0.96 (s, 6H, 2 CH₃, C(11)(12) H), 1.08 (t, J = 7.6 Hz, 3H, CH₃, C(21) H), 2.23 (m, 4H, 2CH₂, C(6)C(8) H), 2.33 (s, 3H, CH₃, C(1) H), 4.06–4.08 (q, J = 4.0 Hz, 2H, C(20) H), 4.96 (s, 1H, CH, C(4) H), 6.72–6.75 (d, J = 12.0 Hz, 2H, Ar-H, C(16)C(14) H), 7.39 (s, 1H, Ar-H, C(18) H) 9.69 (s, 1H, NH) ppm; ¹³C NMR (100MHz, CDCl₃): δ 13.30 (C-21), 17.70 (C-7), 18.90 (C-1), 26.80 (C-11,C-12), 30.91 (C-4), 55.82 (C-6), 59.95 (C-20), 100.10 (C-3), 108.02 (C-10), 122.47 (C-15), 128.45 (C-18), 128.52 (C-17), 129.75 (C-16), 130.14 (C-14), 137.51 (C-13), 145.66 (C-9), 153.06 (C-2), 160.15 (C-19, C=O), 194.24 (C-5, C=O) ppm; ESI-MS: [M] 495.00; Anal. Calcd. C₂₁H₂₃Br₂NO₅: C, 50.73; H, 4.66; N, 2.82. Found: C, 50.32; H, 4.02; N, 2.32.
4-(3',4'-Dichloro-phenyl)-2,7,7-trimethyl-5-oxo-1,4,6,8-tetrahydroquinoline-3-carboxylic acid ethyl ester (4g):

Colorless solid, Mp: 216–218 °C; IR (KBr): ν 3386 (N-H), 1711 (C=O), 1704, 1662, 1615, 1509, 1438, 1365, 1234, 1153, 1044 cm⁻¹; ¹H NMR (400 MHz, CDCl₃): δ 0.91 (s, 6H, 2CH₃ C(11) C(12) H), 1.02 (t, J = 7.6 Hz, 3H, CH₃ C(21) H), 2.16 (m, 4H, 2CH₂ C(6)C(8) H), 2.25 (s, 3H, CH₃ C(1) H), 3.99–4.01 (q, J = 6.8Hz, 2H, C(20) H), 5.05 (s, 1H, CH, C(4) H), 6.94–7.26 (m, 3H, Ar-H, C(14)C(17)C(18) H), 10.49 (s,1H,NH) ppm; ¹³C NMR (100MHz, CDCl₃): δ 14.25 (C-21), 16.08 (C-7), 19.48 (C-1), 27.65 (C-11, C-12), 29.28 (C-4), 50.90 (C-8), 54.20 (C-6), 59.76 (C-20), 102.61 (C-3), 109.08 (C-10), 127.29 (C-18), 130.10 (C-17), 132.16 (C-16, C-14), 138.11 (C-13, C-15) ppm, 139.32 (C-2), 145.10 (C-9), 167.55 (C-19, C=O), 195.64 (C-5, C=O) ppm. ESI-MS: [M+H]⁺ 407.1; Anal. Calcd for C₂₁H₂₃Cl₂NO₃: C, 61.77; H, 5.68; N, 3.43. Found: C, 60.32; H, 5.02; N, 3.32.

4-(4'-Chloro-3'-fluoro-phenyl)-2,7,7-trimethyl-5-oxo-1,4,6,8-tetrahydroquinoline-3-carboxylic acid ethyl ester (4h):

Colorless solid, Mp: 220–222 °C; IR (KBr): ν 3429 (N-H), 1709 (C=O), 1649, 1644, 1408, 1336, 1287, 1129, 1010,810 cm⁻¹; ¹H NMR (400 MHz, CDCl₃): δ 0.93 (s, 3H, CH₃ C(11) H), 1.08 (s, 3H, CH₃ C(12) H), 1.17 (t, J = 7.2 Hz, 3H, C(21) H), 2.20–2.23 (m, 4H, 2CH₂, C(6)C(8) H), 2.40 (s, 3H, CH₃,C(1) H), 3.31 (s, 1H, NH), 4.05–4.07 (q, J = 7.2 Hz, 2H, C(20) H), 5.02 (s, 1H, CH, C(4) H), 7.04–7.07 (m, 2H, Ar-H, C(14) H), 7.21–7.23 (m, 2H, Ar-H, C(17) C(18) H) ppm; ¹³C NMR (100MHz, CDCl₃): δ 14.32 (C-21),17.04 (C-7), 19.48 (C-1), 27.24 (C-11, C-12), 29.48 (C-4), 50.76 (C-8), 54.71 (C-6), 60.10 (C-20), 105.28 (C-3), 111.49 (C-10), 116.32 (C-16), 118.18 (C-14), 124.70 (C-18), 129.85 (C-17), 144.27 (C-2), 148.25 (C-13), 148.69 (C-9), 156.71 (C-15), 167.18 (C-19, C=O), 195.62 (C-5, C=O) ppm; ESI-MS: [M+H]⁺ 391.2; Anal. Calcd for C₂₁H₂₃ClFNO₃: C, 64.37; H, 5.92; N, 3.57. Found: C, 63.21; H, 5.31; N,3.20.
6. Biological evaluation

6.1 MTT assay\textsuperscript{24, 25}

Cell lines used: HepG2 (Hepatocellular carcinoma cells) and MCF-7 (Human breast adenocarcinoma cells). The anti-cancer activity was tested against the two cell lines - HepG2 and MCF-7. Minimum essential medium (MEM) growth medium augmented with 10% heat inactivated fetal bovine serum (FBS), penicillin (100 IU/mL), streptomycin (100 μg/mL) and amphotericin-B (5 μg/mL) was used to grow the cells in a humidified atmosphere of 5% CO\textsubscript{2} at 37 °C until confluent. The cells were trypsinized with TPVG solution (0.2% trypsin, 0.02% EDTA, 0.05% glucose in PBS). The stock cultures were maintained in 25 mL flat bottles.

To determine the antiproliferative effect of the test sample, the cells were seeded in 96-well flat bottom microtitre plates with a density of 1 × 104 cells per well and incubated for 24 h at 37 °C in 5% CO\textsubscript{2} atmosphere to allow cell adhesion. After 24 h, the medium was removed when partial monolayer was formed. The cells were treated with different concentrations of standard drug (Doxorubicin) and sample compounds for 48 h. Microscopic examination was carried out and observations were recorded at every 24 h. After the treatment, the solutions in the wells were discarded. The wells were then treated with 50 μL of freshly prepared MTT reagent (2 mg/mL prepared in PBS). The plates were shaken gently and incubated for 3 h at 37 °C in 5% CO\textsubscript{2} atmosphere. After 3 h, the supernatant liquid was removed to get the formazan crystals in the wells. Addition of 50 μL of iso-propanol to each well dissolved the formazan crystals. Finally, the optical density was recorded at a wavelength of 540 nm using a Micro-plate reader (Bio-Tek, ELX-800 MS).

The percentage growth was calculated using the following formula:

\[ \text{% growth inhibition} = \frac{\text{test absorbance-blank absorbance}}{\text{control absorbance-blank absorbance}} \times 100 \]

The % growth concentration from IC\textsubscript{50} (Concentration of drug required to kill 50% of cells in exponentially growing cultures after a 48 h exposure to the drug) values are shown in the Table III.3 for the HepG2, and MCF-7 cell lines. In this study the standard drug was (Doxorubicin) and the values obtained are comparable with the values of the compounds 4e and 4i which showed considerable activity.
Table III.3: In vitro anticancer activity of quinolines 4a–4i on HepG2 and MCF-7 human cancer cell lines.

<table>
<thead>
<tr>
<th>Drug/Product</th>
<th>IC$_{50}$ value (µg/mL) on HepG2 cells</th>
<th>Drug/Product</th>
<th>IC$_{50}$ value (µg/mL) on MCF-7 cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Doxorubicin</td>
<td>1.21 ± 0.05</td>
<td>Doxorubicin</td>
<td>1.09 ± 0.03</td>
</tr>
<tr>
<td>4a</td>
<td>4.40 ± 0.22</td>
<td>4a</td>
<td>4.80 ± 0.16</td>
</tr>
<tr>
<td>4b</td>
<td>4.80 ± 0.12</td>
<td>4b$^+$</td>
<td>3.40 ± 0.17</td>
</tr>
<tr>
<td>4c</td>
<td>4.60 ± 0.25</td>
<td>4c$^+$</td>
<td>3.20 ± 0.14</td>
</tr>
<tr>
<td>4d</td>
<td>12.50 ± 0.28</td>
<td>4d</td>
<td>10.20 ± 0.34</td>
</tr>
<tr>
<td>4e$^+$</td>
<td>3.40 ± 0.21</td>
<td>4e$^+$</td>
<td>2.80 ± 0.07</td>
</tr>
<tr>
<td>4f$^+$</td>
<td>3.20 ± 0.11</td>
<td>4f$^+$</td>
<td>3.60 ± 0.21</td>
</tr>
<tr>
<td>4g</td>
<td>5.50 ± 0.24</td>
<td>4g</td>
<td>4.60 ± 0.22</td>
</tr>
<tr>
<td>4h</td>
<td>10.50 ± 0.35</td>
<td>4h</td>
<td>7.20 ± 0.80</td>
</tr>
<tr>
<td>4i$^+$</td>
<td>3.80 ± 0.09</td>
<td>4i$^+$</td>
<td>2.60 ± 0.07</td>
</tr>
</tbody>
</table>

$^+$active

7. Molecular docking study

7.1 Protein preparation

X-ray crystal structure of ER (PDBID:2IOK) was retrieved from the RCSB protein data bank. Atomic overlaps from the X-ray structure were removed. Auto dock tools were used to prepare the ER by removing ligand, water molecules, non standard residues and alternate residues. Later polar hydrogens were added to the ER in standard orientation without optimization and Gasteiger charges were added using MGL tools (http://mglttools.scripps.edu/).

7.2 Ligand preparation

All the 3D structures were drawn by Chem Draw software. Drug doxorubicin was retrieved from the complex PDBID 111E. Using Argus lab $^{27}$, all the drawn structures including doxorubicin were subjected to geometric cleaning and geometry optimization. Gasteiger charges were added and nonpolar hydrogens were merged and rotatable bonds were determined based on the nature of the ligand by using MGL tools.
7.3 Grid map generation

The grid maps were generated; spacing was adjusted to 0.500Å to enable ligand binding. Grid dimension was adjusted to 42×64×64 points. AUTODOCK interaction maps were used for docking protocol. Prior to the actual docking run, these maps were calculated by AUTOGRID. For each ligand atom type, the interaction energy between the ligand atom and the receptor was calculated for the entire binding site which is discretized through a grid.\textsuperscript{28} The protein was embedded in a 3D grid and a probe was placed at each grid point. Interaction energy of the protein was assigned at each grid point and the affinity grid and electrostatic potential for every atom of the ligand was calculated. Electrostatic interactions were evaluated by interpolation.\textsuperscript{29}

7.4 Docking

Autodock Vina, a docking program\textsuperscript{30} was used to evaluate binding affinity of synthesized molecules and doxorubicin with ER. Vina was used to dock the receptor and ligand molecules. Binding energy of docked complex ER-ligands were evaluated by using empirical free-energy functions and Lamarckian genetic algorithm. The calculated binding free energy (ΔG) is based on the electrostatic, van der Waal’s forces, hydrogen bonding and desolvation effects. Finally Vina results were analyzed using the MGL tools.

7.5 Results

In order to know inhibition interaction of ER by the prepared compounds, the docking tools were used through Autodock Vina. The binding free energy (ΔG) concept is used to evaluate the binding affinity of protein-ligand complex using docking studies. The negative or low value of binding free energy (ΔG) indicates the strong binding affinity between protein-ligand complex and the ligand in the docking complex is in the most favorable conformation\textsuperscript{31, 32} In the present study, we compared binding affinity between prepared structures and the standard drug using binding free energy (ΔG) in Kcal/mol. Lowest binding free energy (ΔG) docked complex, ER-4e is shown in Fig.III.3 and ER-doxorubicin complex is shown in Fig.III.4. Interaction of ER with 4e is shown in Fig.III.5 and Fig.III. 6. Results of binding free energy (ΔG) are presented in the Table III.4, which reflects the binding affinity of the standard and the prepared compound 4e with ER. ER-4e and ER-doxorubicin complexes show binding free energy (ΔG) of −7.9 and −7.3 Kcal/mol respectively. Docking interactions revealed that, doxorubicin interacts with Gln 506 through a hydrogen bond, other
amino acids present in the docking site are: Asn 439, Gln 441, Glu 444, Ala 493, Leu 495 and Arg 503 which play a vital role in binding.

Ligand 4e interacts with Thr 347 through hydrogen bond, other amino acids present in the docking site for 4e are: Leu 346, Trp 383, Leu 384, Leu 387, Phe 404, Met 421, Leu 525 and His 524. The analyzed results predict that, 4e has greater binding affinity among the seven prepared structures and is found to be greater than the drug doxorubicin (entries 1 and 5).

Table III. 4: Docking results of the synthesized compounds and doxorubicin drug with ER binding free energy (ΔG) in Kcal/mol.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Complex</th>
<th>Binding free energy (ΔG) Kcal/mol</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Doxorubicin</td>
<td>-7.3</td>
</tr>
<tr>
<td>2</td>
<td>4a</td>
<td>-6.1</td>
</tr>
<tr>
<td>3</td>
<td>4b</td>
<td>-6.4</td>
</tr>
<tr>
<td>4</td>
<td>4d</td>
<td>-6.3</td>
</tr>
<tr>
<td>5</td>
<td>4e</td>
<td>-7.9</td>
</tr>
<tr>
<td>6</td>
<td>4f</td>
<td>-6.5</td>
</tr>
<tr>
<td>7</td>
<td>4g</td>
<td>-6.5</td>
</tr>
<tr>
<td>8</td>
<td>4h</td>
<td>-6.9</td>
</tr>
</tbody>
</table>
**Fig. III.3**: Stick and wire model of docked complex; 4e ligand binding at ER docking site.

**Fig. III.4**: Stick model showing the hydrogen interaction of doxorubicin with ER.

**Fig. III.5**: Interaction of 4e with threonine which is present at the docking site ER. Wire: ER; Stick: 4e.

**Fig. III.6**: Ribbon model of ER-4e docked complex showing 4e present at the docking site of ER. Red ribbon: ER; Magenta stick: 4e
8. Conclusions

In conclusion, we have developed a new green protocol for the synthesis of 4-aryl-2,7,7-trimethyl-5-oxo-1,4,6,8-tetrahydroquinoline-3-carboxylic acid ethyl esters by a one-pot four component cyclization reaction of aromatic aldehydes, dimedone, ethyl acetoacetate and ammonium acetate in the presence of a heterogeneous catalyst: silica iodide in ethanol. The reaction is environmentally benign, efficient and mild as it involves the use of a recyclable heterogeneous catalyst. From the wet lab studies, it is found that, quinoline derivatives 4e, 4f and 4i showed excellent in vitro anticancer activity towards HepG2 cell lines and 4b, 4c, 4e, 4f and 4i showed reasonably good activity towards MCF-7 human cancer cell lines, which can be used as lead compounds for developing new potential class of anticancer drugs. Docking results revealed that, 4e has more binding affinity towards ER among seven prepared molecules which is greater than the standard drug: doxorubicin. The obtained in vitro results are correlated with the docking studies.
9. References

1. H:\\unw2\Quinoline - Wikipedia, the free encyclopedia htm.
(xv), 1-8.


Spectra
$^1$H NMR spectrum of compound 4d
$^{13}$C NMR spectrum of compound 4d
ESI-MS of compound 4d
$^1$H NMR spectrum of compound 4g
$^{13}$C NMR spectrum of compound 4g
ESI-MS of compound 4g
$^1$H NMR spectrum of compound 4h
$^{13}$C NMR spectrum of compound 4h
ESI-MS of compound 4h
Elemental analysis of the products