CHAPTER-2

Propylphosphonic anhydride (T3P) mediated synthesis of novel 2-aryl quiolines and evaluation of their anticancer activity
Chapter 2

2.1. Section-A: Chemistry

2.1.1. Introduction:

N-Propanephosphonic acid anhydride, popularly known as T3P (Figure 4), is one such phosphorus-containing reagent among the many discovered in late years, it is phosphorus-based cyclic anhydride introduced by Wissmann et al. in 1980. It is also mentioned by other names like N-propylphosphonic cyclic acid anhydride (PPACA) and propane phosphonic acid anhydride (PPAA) but will be referred to throughout the thesis as T3P.

Propylphosphonic anhydride (T3P) is an excellent coupling reagent and water scavenger, which has drawn significant interest over the past few years. Its water scavenging and coupling capabilities have generated innovative uses for this reagent beyond peptide synthesis. Compared with other modern coupling agents T3P delivers outstanding advantages. T3P has low toxicity and produces an easily purified product with high yield and low epimerization. It was initially employed as peptide coupling agent and thereafter its utility had been successfully presented in a series of transitions, as easily as in industrial applications as a reagent for large-scale synthesis of natural products, heterocycles and drugs. Basavaprabhu and coworkers\(^1\) have reported a review article on applications of T3P as a reagent in organic synthesis.

![Figure-4 Structure of T3P](image)

Figure-4 Structure of T3P
2.1.2. T3P: A useful reagent in organic synthesis: A recent literature survey.

2.1.2.1. Synthesis of Heterocycles

2.1.2.1.1. Coumarin

John Kallikat Augustine and group\textsuperscript{2} have reported convenient and versatile method for the synthesis of coumarins via the Perkin condensation mediated by T3P. This strategy tolerates various 2-hydroxyarylcarbonyls and carboxylic acids giving access to distinctively substituted coumarins in good yields (Scheme 54).

\begin{center}
\includegraphics[width=0.8\textwidth]{Scheme-54.png}
\end{center}

\textbf{Scheme-54}

2.1.2.1.2. Quinazoline

Matthie Desroses and coworkers\textsuperscript{3} have reported T3P-mediated, convenient and efficient procedure for the synthesis of dihydroquinazolinones (Scheme 55). The most benefits of this protocol embrace its practical simplicity, short reaction times and particularly the ease with which products are isolated.

\begin{center}
\includegraphics[width=0.8\textwidth]{Scheme-55.png}
\end{center}

\textbf{Scheme-55}

2.1.2.1.3. Pyrimidines

Subba Poojari and coworkers\textsuperscript{4} have reported an efficient synthesis of thiopyrimidines with completely different substituents in position 2. A rapid, gentle and high yielding microwave-assisted one-pot cyclization of 5-substituted 2-amino thiophene-3-carboxamide derived from Gewald reaction with T3P and different acids provides the
corresponding thiopyrimidines (Scheme 56). The significant feature of this method includes less reaction time, high purity and reduced toxicity of the reaction.

![Scheme-56]

Franz L. Zumpe and coworkers\cite{5} have showed T3P as a unique promoter for the synthesis of dihydropyrimidinones by the three-component condensation of a β-ketoester or pentane-2,4-dione, aldehyde and urea or thiourea (Scheme 57). The method is applicable to substituted heterocyclic, aromatic, and aliphatic aldehydes.

![Scheme-57]

2.1.2.1.4. Pyrazolone

Matthieu Desroses and group\cite{6} have described a new, efficient, and easily reproducible T3P mediated microwave-assisted procedure to construct pyrazolone analogues (Scheme 58). With this simple and rapid method, a series of compounds were synthesized to examine the extent and limitations of this methodology. This approach provides a noteworthy alternative to the strongly acidic conditions that are more often than not applied to other synthetic methods.

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2.1.2.1.5. Quinolines

Quinolines and their derivatives are very significant structural motifs that occur widely in natural and synthetic products, frequently exhibiting a wide scope of biological action. Due to their wide range of applicability in medicinal, industrial, bioorganic, synthetic organic chemistry, there has been an increasing stake in developing efficient methods for quinoline synthesis in the field of synthetic organic chemistry.

Friedlander Quinoline synthesis

Mouhamad Jida and Benoit Deprez\(^7\) have reported a convenient, efficient and eco-friendly protocol for Friedlander synthesis of poly substituted quinolines and naphthyridines (Scheme 59). A extensive diversity of raw products were readily prepared in the presence of propylphosphonicanhydride (T3P) in short reaction times and excellent yields under mild conditions.

John Kallikat Augustine and coworkers\(^8\) have demonstrated the utilization of T3P as a promoter and water scavenger in the Friedlander annulation and thus introduced a highly efficient catalytic process to access carbocyclic and heterocyclic fused
quinolines (Scheme 60). The reaction conditions are sufficiently mild to tolerate the acid and base sensitive functional groups that can function as levers for further extension of the quinoline products.

![Scheme-60](image)

2.1.2.1.6. Oxa-, Thia- and Benzimidazoles

In continuation of our work\(^9\) on the development of useful synthetic methodologies, we have reported the use of T3P as an oxidizing and cyclodehydrating agent for the synthesis of benzimidazole and benzothiazole derivatives under mild conditions (Scheme 61). Short reaction time, broad functional group tolerance, low epimerization, easy and immediate isolation of the products, chemo selectivity and excellent yields are main advantages of this process.

![Scheme-61](image)

Xiaoan Wen and coworkers\(^{10}\) have showed that Propylphosphonic anhydride (T3P) promotes cyclization of o-aminobenzenethiol, o-aminophenol, and O-phenylenediamine with carboxylic acids under microwave irradiation to produce benzothiazoles, benzoazoles and benzimidazoles (Scheme 62). This one-pot procedure is efficient and allows short reaction times, easy work-up and good yields.
2.1.2.1.7. Imidazo pyridines

We have shown propyl phosphonic anhydride to be an effective and mild reagent for the single-pot synthesis of imidazo [1,2-a] pyridines from a mixture of alcohols.\textsuperscript{11} Alcohols are oxidized in situ to aldehydes under mild conditions, which in turn undergo a three-component reaction with various 2-aminopyridines and isocyanides to afford imidazo [1,2-a] pyridines in excellent yields (Scheme 63).

\[
\begin{align*}
\text{R-OH} + \text{R}_1\text{NHC}_2\text{H}_2\text{X} &\xrightarrow{T3P (in Ethyl acetate)} \text{R}_1\text{NHC}_2\text{X} - \text{R} \\
X &= \text{SH, NH}_2, \text{OH} & \text{X} &= \text{S, NH, O}
\end{align*}
\]

Scheme-62

2.1.2.1.8. Indole

Matthieu Desroses and coworkers\textsuperscript{12} have reported a rapid, mild, and high yielding protocol for the Fischer indolization of arylhydrazines with T3P under microwave irradiation. Significant features of this method include short reaction times and preparative ease (Scheme 64).

\[
\begin{align*}
\text{R-NHC}_2\text{H}_2\text{N}^+\text{R}_1\text{O} &\xrightarrow{T3P/DMSO, EtOAc} \text{R}_2\text{NHC}_2\text{R}_1 \\
\text{R}_2\text{OH}
\end{align*}
\]

Scheme-63

2.1.2.1.9. Oxa and thiadiazoles

John Kallikat Augustine and coworkers\textsuperscript{13} have demonstrated propyl phosphonic anhydride (T3P) to be an efficient and mild reagent for the one-pot synthesis of 1,2,4-
oxadiazoles, 1,3,4-oxadiazoles, and 1,3,4-thiadiazoles from carboxylic acids (Scheme 65).

![Scheme-65](image)

2.1.2.10. Naphthyridines and phenanthridines

John Kallikat Augustine and coworkers reported a new method\textsuperscript{14} for the selective synthesis of 5,6-dihydrophenanthridines, 5,6-dihydrobenzo[c][1,8]naphthyridines and their fully aromatized analogues via the Pictet–Spengler reaction mediated by propylphosphonic anhydride (T3P) (Scheme 66).

![Scheme-66](image)

2.1.2.11. Thiazolidinones

We have described Propylphosphonic anhydride to be an effective and mild reagent for the one-pot synthesis of 4-thioazolidinones from a variety of alcohols. Alcohols
are oxidized in situ to aldehydes under mild conditions, which in turn undergo a three-component reaction with various anilines and mercaptoacetic acid to afford 4-thioazolidinones\cite{15} in excellent yields (Scheme 67).

\[ R_1-\underset{\text{CH}}{\text{R}_2} + R_3-\text{NH}_2 + \underset{\text{SH}}{\text{CO}} \rightarrow \text{T3P/DMSO, Ethyl acetate} \rightarrow R_3-\underset{\text{R}_1}{\text{S}} \underset{\text{R}_2}{\text{N}} \]

Scheme-67

2.1.2.12. β-Lactums

Graeme Coulthard and group have developed a T3P-mediated\cite{16} synthesis of β-lactams from imines and aryl-substituted acetic acids. In the majority of cases investigated the reactions are high yielding and provide the trans-β-lactam as the major diastereoisomer. This is complementary to T3P-mediated methods using heteroatom substituted acetic acids which give predominantly cis-β-lactams (Scheme 68).

\[ \text{R}_1-\underset{\text{N}}{\text{R}_2} + \underset{\text{HO}}{\text{Ar}} \rightarrow \text{T3P, NEt(\text{Pr})2, CHCl}_3, 70^\circ\text{C, 20 h} \rightarrow \text{R}_1-\underset{\text{Ar}}{\text{N}} \underset{\text{O}}{\text{O}} \]

Scheme-68

2.1.2.13. Imidazoles

Manuel Lasalle and group\cite{17} describe here an efficient method to synthesize 5-amino-2-thioimidazole compounds by T3P-mediated microwave cyclodehydration of \( N \)-acetamidoisothiourea intermediates and the target imidazoles in good to excellent yields, compare to earlier reported methods, imidazoles can be obtained in one pot synthesis (Scheme 69).
2.1.2.14. 2, 3-disubstituted 3H-quinazolin-4-ones

We developed a T3P catalyzed\textsuperscript{[18]} novel and straightforward methodology for the synthesis of 2,3-disubstituted quinazolinones (Scheme 70) by one-pot and three component reaction using anthranilic acid. This method features short reaction time, broad functional group tolerance, easy isolation, high yield and simple procedure.

\textbf{Scheme-70}

2.1.2.15. 2-Azetidinones

Maaroof Zarei developed a series of 2-azetidinones\textsuperscript{[19]} by [2+2] ketene-imine cycloaddition of substituted acetic acids and imines using propylphosphonic anhydride (T3P) as acid activator under mild conditions (Scheme 71).

\textbf{Scheme-71}

2.1.2.2. Oxidation reaction

There are many reagents for the oxidation of alcohols, and the Swern oxidation is one such protocol that uses activated dimethyl sulfoxide as an oxidant. In Swern-type oxidations, the activation of dimethyl sulfoxide is violent and exothermic, and successful activation requires low temperatures, usually lower than \(-20^\circ\text{C}\) or even as
low as \(-60^\circ C\). To improve existing strategies, Meudt et al. developed a modified Swern oxidation in which T3P was used in combination with dimethyl sulfoxide as an oxidant (Scheme 72) and also they have showed that secondary alcohonic group in the side chain of Boc-Thr-OMe was oxidized to produce the corresponding ketone in the presence of T3P and dimethyl sulfoxide at 0 \(^\circ C\) to room temperature without loss of stereochemistry at the chiral center (Scheme 73).

![Scheme-72](image)

### 2.1.2.3. Rearrangement Reactions

#### 2.1.2.3.1. Lossen Rearrangement

Sureshbabu and co-workers\(^{[20]}\) employed T3P as an acid activator in acetonitrile to obtain hydroxamic acids under ultrasonication and then used as a promoter for the rearrangement of hydroxamic acids into isocyanate in the presence of NMM under reflux conditions. The formed isocyanate was treated with a variety of nucleophiles such as amines, alcohols and thiols to yield ureas, carbamates and thiocarbamates respectively (Scheme 74).

![Scheme-74](image)
2.1.2.3.2. Beckmann Rearrangement

John Kallikat Augustine and coworkers\textsuperscript{[21]} have reported an efficient method for the Beckmann rearrangement of ketoximes to amides mediated by a catalytic amount (15 mol \%) of propylphosphonic anhydride (T3P) (Scheme 75). Aldoximes underwent second order Beckmann rearrangement to provide the corresponding nitriles in excellent yields on reacting with T3P (15 mol \%) at room temperature.

\[
\begin{align*}
\text{N} & \quad \text{O} \\
\text{R} & \quad \text{R}_1 \\
\begin{array}{ccc}
\text{N} & \text{O} & \text{R} \\
\text{R} & \text{R}_1 & \text{N}
\end{array}
\end{align*}
\]

\[\text{Scheme-75}\]

2.1.2.3.3. Curtius Rearrangement

The Curtius rearrangement of acid azides is a widely employed route for the synthesis of ureas and carbamates.\textsuperscript{[22]} The usage of T3P enables the synthesis starting from acids to be carried out in a one-pot manner. T3P was used as a promoter for the formation of acid azides. Upon heating, the acid azide subsequently undergoes rearrangement to the isocyanate, which then couples with the alcohol to afford the carbamates (Scheme 76).

\[
\begin{align*}
\text{O} & \quad \text{N} \\
\text{R} & \quad \text{CH}_2 \text{R} \\
\begin{array}{ccc}
\text{O} & \text{N} & \text{R} \\
\text{R} & \text{CH}_2 & \text{R}
\end{array}
\end{align*}
\]

\[\text{Scheme-76}\]

2.1.2.4. Functional group transformation

Abdellah Ech-Chahad and coworkers\textsuperscript{[23]} have reported T3P mediated simple and one pot conversion of carboxylic acids into hydroxamic acids (Scheme 77). The method is especially suitable for labile substrates, whose activation via chloride is not trivial, for \(\alpha,\beta\)-unsaturated acids that do not tolerate large excesses of hydroxylamine, and for
hydroxyacids, whose oxyamidation with other protocols would require hydroxyl protection.

![Scheme-77]

Basavaprabhu and coworkers\textsuperscript{[24]} have described a general, mild, efficient, and environmentally benign protocol, which makes use of T3P as an acid activating agent for the direct synthesis of acid azides from carboxylic acids (Scheme 78). Further, the protocol is employed for the one-pot synthesis of $\alpha$-ureidopeptides starting from N-protected $\alpha$-amino acids (Scheme 79).

![Scheme-78]

2.1.2.5. T3P in peptide chemistry

Chilakapati Madhu and group developed alternative protocol for the direct conversion of Na-protected amino/peptide acids into thioacids by employing T3P/Na$_2$S system. A series of carboxylic acids including Na-Fmoc/Z/Boc amino acids have been converted into corresponding thio acids in excellent yields.\textsuperscript{[25]} Also the protocol can further be extended even to the large scale preparations as the byproducts released were innocuous, water soluble and the protocol is operationally simple (Scheme 80).
K. M. Sharnabai et al. assembled a simple and modular procedure for the preparation of a series of N-protected α-amino/peptide Weinreb amides by employing combination of T3P and DBU. The use of mild conditions enables the isolation (Scheme 81) of the products without racemization.\textsuperscript{[26]}

2.1.3. Results and Discussion

T3P-DMSO mediated synthesis of 2-aryl quinolines from alcohols

Recently, we have reported an one pot tandem approach for the synthesis of 4-thioazolidinones from alcohols using DMSO-propylphosphonic anhydride (T3P) media as an oxidizing as well as cyclodehydrating agent. In continuation of our work on the development of new synthetic methodologies towards pharmaceutically important heterocyclic compounds, we attempted T3P\textsuperscript{®}-DMSO mediated one pot three-component synthesis of 2-aryl quinolines directly from various alcohols. The present method is intended to produce aromatized quinoline ring system, involving oxidation, condensation followed by cyclization under mild reaction (Scheme 82) conditions and the results are presented in Table.1
Scheme-82 Synthesis of 2-aryl quinoline (F1).

Table-1 T3P®/DMSO mediated synthesis of F1 under different reaction condition.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Solvent&lt;sup&gt;a&lt;/sup&gt;</th>
<th>T3P&lt;sup&gt;b&lt;/sup&gt; (mmoles)</th>
<th>Temperature (°C)</th>
<th>Time (min)</th>
<th>Yield&lt;sup&gt;c&lt;/sup&gt; (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>EtOAc</td>
<td>1.0</td>
<td>0-25</td>
<td>8</td>
<td>20</td>
</tr>
<tr>
<td>2</td>
<td>EtOAc</td>
<td>1.5</td>
<td>0-25</td>
<td>8</td>
<td>55</td>
</tr>
<tr>
<td>3</td>
<td>EtOAc</td>
<td>2.0</td>
<td>0-25</td>
<td>4</td>
<td>93</td>
</tr>
<tr>
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<td>EtOAc</td>
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<td>0-25</td>
<td>4</td>
<td>89</td>
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<tr>
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<td>THF</td>
<td>2.0</td>
<td>0-25</td>
<td>4</td>
<td>70</td>
</tr>
<tr>
<td>6</td>
<td>Toluene</td>
<td>2.0</td>
<td>0-25</td>
<td>4</td>
<td>25</td>
</tr>
<tr>
<td>7</td>
<td>CH₂Cl₂</td>
<td>2.0</td>
<td>0-25</td>
<td>4</td>
<td>35</td>
</tr>
<tr>
<td>8</td>
<td>CHCl₃</td>
<td>2.0</td>
<td>0-25</td>
<td>4</td>
<td>43</td>
</tr>
<tr>
<td>9</td>
<td>Dioxane</td>
<td>2.0</td>
<td>0-25</td>
<td>4</td>
<td>40</td>
</tr>
<tr>
<td>10</td>
<td>CH₃CN</td>
<td>2.0</td>
<td>0-25</td>
<td>4</td>
<td>45</td>
</tr>
<tr>
<td>11</td>
<td>EtOAc</td>
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<td>40</td>
<td>3</td>
<td>81</td>
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<tr>
<td>12</td>
<td>EtoAc</td>
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<td>50</td>
<td>2.5</td>
<td>76</td>
</tr>
<tr>
<td>13</td>
<td>EtOAc</td>
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<td>60</td>
<td>2</td>
<td>72</td>
</tr>
<tr>
<td>14</td>
<td>EtOAc</td>
<td>2.0</td>
<td>70</td>
<td>1</td>
<td>68</td>
</tr>
</tbody>
</table>

<sup>a</sup> Solvents: DMSO 2:1 ratio.

<sup>b</sup> T3P® 50% solution in ethyl acetate.

<sup>c</sup> Isolated yield.
Initially, a model reaction was conducted between benzyl alcohol (1a) (1.1 mmol), aniline (1.0 mmol) (2a) and ethyl vinyl ether (1.0 mmol) (3) in the presence of T3P® (1.0 mmol) in a mixture of solvents containing EtOAc: DMSO in 2:1 ratio at 25 °C for 8 h, which afforded 2-arylquinolines with 20% yield. Then we have monitored the reaction by increasing the equivalence of T3P® and we have observed that 2.0 mmol of T3P® gave maximum yield 93% of the desired 2-arylquinoline (F1) (Table 1, Entry 3). These results suggested that the equivalence of T3P® plays an important role in the progress of the reaction. Next we studied the effect of solvent on reaction. Initially the reaction was carried out in DMSO and we didn’t get the considerable yield of the required compound 2-arylquinoline, later we have tried the various solvent mixture like THF, Toluene, CH₂Cl₂, CHCl₃, Dioxane and CH₃CN with DMSO; all the solvent systems doesnot give significant yield (Table 1, Entries 5-10). Hence ethyl acetate with DMSO proved to be the best solvent mixture for this reaction. Optimization of temperature for the reaction was carried out at temperature of 40, 50, 60 and 70 °C (Table 1, Entries 11-14) it was observed that increase in temperature resulted in gradual decrease in yield and reaction time.

Hence room temperature was chosen as the optimum temperature for this reaction.

The scope of this method was extended by the study of the reaction of different substituted benzyl alcohols and anilines for the transformation. It was observed that diverse functional groups played significant roles in providing the product yields. Benzyl alcohols with electron-donating as well as electron-withdrawing groups at 4-position reacted well with aniline derivatives and gave almost equal product yields. In case of 2-substituted benzyl alcohols yields were less due to steric hindrance associated with the substituent (Table 2, Entries 4, 6, 10, 12). In case of 3-substituted anilines mixtures of 5 and 7 substituted quinoline regioisomers would be obtained as
products with considerable yield. It is noteworthy that both benzyl alcohol and aniline bearing various functionalities such as nitro, methyl, halogen, methoxy, ester, trifluoromethyl and hydroxyl groups survived the reaction (Scheme 83) and the relevant results are presented in Table 2.

Scheme 83 Synthesis of 2-aryl quinolines

Table 2 Reaction scope of T3P-DMSO media

<table>
<thead>
<tr>
<th>Sl no</th>
<th>Product 1</th>
<th>Product 2</th>
<th>Time (h)</th>
<th>Yield [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1a</td>
<td>2a</td>
<td>4</td>
<td>93[27]</td>
</tr>
<tr>
<td>2</td>
<td>1b</td>
<td>2b</td>
<td>5</td>
<td>80</td>
</tr>
<tr>
<td>3</td>
<td>1c</td>
<td>2c</td>
<td>5</td>
<td>75</td>
</tr>
<tr>
<td>4</td>
<td>1d</td>
<td>2d</td>
<td>4</td>
<td>87</td>
</tr>
</tbody>
</table>
Benzyl alcohols (1.1 mmol), Anilines (1.0 mmol), Vinyl ether (1.0 mmol), T3P® (2.0 mmol).

b Isolated yields.

c Literature reported compounds.

2.1.4 Conclusion

A convenient and versatile method has been developed and optimized for the synthesis of 2-arylquinolines mediated by T3P®-DMSO, a mild and low toxic peptide coupling agent. The method not only employs readily accessible T3P®, but also tolerates diverse benzyl alcohols and various anilines giving access to distinctively substituted 2-aryl quinolines in good yields. Further, an easy to handle reagent for bulk reactions and the reaction conditions are sufficiently mild that could tolerate sensitive functional groups and make the process more practical for 2-arylquinolines synthesis.

The possible mechanism involves the reaction of DMSO with T3P® followed by substitution reaction of alcohol and results in the cleavage of phosphoester bond to form intermediate 5. Elimination of dimethyl sulfide gives carbonyl compound 6 which undergoes condensation and cyclization by [4+2] Diels-Alder cycloaddition with vinyl ethyl ether through modified Povarov reaction to afford 2-arylquinolines 4 as shown in Scheme 84.
Scheme-84 Possible mechanism of the T3P°-DMSO mediated 2-arylquinolines synthesis.

2.1.5. Experimental section

2.1.5.1. General

Melting points were recorded (uncorrected) on a Buchi Melting Point B-545 instrument. Infrared (IR) spectra were recorded using a PerkinElmer Spectrum Version FTIR Spectrum Two- 94012 series. All reagents and solvents used were commercially procured and used as received. The \(^1\)H NMR spectra were measured on a VNMRS-400 "Agilent-NMR" at 400 MHz with TMS as internal standard. The \(^{13}\)C NMR spectra were measured on a VNMRS-400 "Agilent-NMR" at 100 MHz. The mass spectra were recorded on a Waters-US-SYNAPT- G2 mass spectrometer.

All solvents and reagents were purchased from standard firms which were used as such without further purification. Chromatographic purification was conducted by column chromatography over 60-120 mesh silica gel using hexane-EtOAc as eluent.
2.1.5.2. General procedure for one pot synthesis of 2-arylquinoline: To a suspension of benzyl alcohol (1.1 g, 0.0101 mol), aniline (0.94 g, 0.0101 mol), and ethyl vinyl ether (0.728 g, 0.0101 mol) in a mixture of EtOAc: DMSO (8.0: 2.0 mL) was added T3P® (2.0 mmol, 50% solution in ethyl acetate) at 0°C, and the resulting mixture was stirred at room temperature for 4–5 h. Progress of the reaction was monitored by TLC. The reaction mass was concentrated, the obtained residue was neutralized with 10% NaHCO₃ solution, and then extracted with ethyl acetate (2 x 20 mL), the combined organic phase was washed with water and brine solution, and dried over anhydrous sodium sulphate. The organic phase was evaporated and the crude product was purified by column chromatography using silica gel mesh 60-120 (15% EtOAc in hexanes).

2.1.5.3. Characterization data of isolated 2-arylquinoline compounds

2-Phenyl quinoline (F1) Pale yellow solid (1.8 g, 93 %); Mp: obs. 85-86 °C; lit.²²:

\[
\begin{align*}
\text{IR } \nu_{\text{max}} \text{ (KBr, cm}^{-1}\text{): } & 2962, 1593, 1498, 1250, 1027; \\
\text{¹H NMR (CDCl}_3\text{- 400 MHz) } \delta: & 8.23-8.15 \text{ (4H, m, Ar-H), } 7.89-7.81 \text{ (2H, m, Ar-H), } 7.74-7.70 \text{ (1H, m, Ar-H), } 7.54-7.46 \text{ (4H, m, Ar-H) ppm; } \\
\text{¹³C NMR (100 MHz, CDCl}_3\text{): } & 157.39, 146.11, 139.7, 129.7, 129.6, 129.3, 128.8, 127.5, 127.4, 127.2, 126.2, 119.0 \text{ ppm; } \\
\text{HRMS [M+H]} & \text{obs: 206.0973, (Mcal) 206.0970. }
\end{align*}
\]

2-(2-Chlorophenyl)-7-methoxyquinoline (F2): White solid, Mp: obs. 133-135 °C;

\[
\begin{align*}
\text{IR } \nu_{\text{max}} \text{ (KBr, cm}^{-1}\text{): } & 2944, 1533, 1520, 1213, 1111, 835,754; \\
\text{¹H NMR (CDCl}_3\text{- 400 MHz) } \delta: & 8.05-7.95 \text{ (4H, m, Ar-H), } 7.72-7.69 \text{ (1H, q, } J = 7.6 \text{ Hz, } J = 3.2 \text{ Hz, Ar-H), } 7.32-7.3 \text{ (1H, t, } J = 8.2 \text{ Hz, Ar-H), } 7.29-7.02 \text{ (3H, m, Ar-H), } 3.87 \text{ (3H, s, -;}
\end{align*}
\]
OCH₃) ppm; ¹³C NMR (100 MHz, CDCl₃): 160.6, 156.2, 145.6, 135.6, 131.1, 129.1, 129, 122.4, 118.8, 115.8, 115.5, 55.5 ppm; HRMS [M+H] obs: 270.0688, (M+2) 272.0692, (Mcal) 270.0686.

7-Bromo-8-methyl-2-(2-nitrophenyl)quinoline (F3) Brown solid, Mp: obs. 122-124 °C; IR ν max (KBr, cm⁻¹): 2863, 1543, 1255, 1133, 943, 835; ¹H NMR (CDCl₃- 400 MHz) δ: 8.22-8.17 (2H, m, Ar-H), 7.79-7.65 (2H, m, Ar-H), 7.59-7.41 (2H, m, Ar-H), 7.33-7.31 (2H, d, J = 7.2 Hz, Ar-H), 2.57 (3H, s, -CH₃) ppm; ¹³C NMR (100 MHz, CDCl₃): 158.3, 153.6, 145.2, 138.7, 137.1, 133.1, 132.2, 129.7, 128.8, 127.4, 125.3, 123.2, 118.1, 17.7 ppm; HRMS [M+H] obs: 343.1743, (Mcal) 343.1740.

7-Fluoro-2-(2,4-dimethylphenyl)quinoline (F4) White solid. Mp: obs. 142-140 °C; IR ν max (KBr, cm⁻¹): 2873, 1533, 1271, 1223, 912, 809; ¹H NMR (CDCl₃- 400 MHz) δ: 7.95-7.88 (4H, m, Ar-H), 7.54-7.5 (1H, m, Ar-H), 7.33-7.31 (1H, d, J = 7.4 Hz, Ar-H), 7.07-7.0 (2H, m, Ar-H), 2.53 (6H, s, CH₃) ppm; ¹³C NMR (100 MHz, CDCl₃): 165.4, 159.9, 143.1, 137.8, 136.3, 133.7, 130.2, 129.4, 127.3, 125.3, 123.6, 119.4, 17.9, 19.5 ppm; HRMS [M+H] obs: 252.1188, (Mcal) 252.1189.

7-Methoxy-2-(4-methoxyphenyl)quinoline (F5) White solid, Mp: obs. 134-132 °C; IR ν max (KBr, cm⁻¹): 2865, 1505, 1243, 1019, 829; ¹H NMR (CDCl₃-400 MHz) δ: 8.04-8.20 (2H, d, J =8.1 Hz, Ar-H), 7.85-7.77 (2H, m, Ar-H), 7.46-7.4 (2H, m, Ar-H), 7.29-7.27 (1H, d, J = 7.4 Hz, Ar- H), 7.07-7.05 (1H, d, J = 7.8 Hz, Ar-H), 3.96 (6H, s, -OCH₃) ppm; ¹³C NMR (100 MHz, CDCl₃): 163.2, 160.5, 157.1,
149.9, 143.5, 136.2, 130.1, 129.3, 128.7, 127.5, 123.1, 118.2, 117.4, 55.5 ppm; 

HRMS [M+H] obs: 266.1180, (Mcal) 266.1181.

2-(4-Fluoro-2-methoxyphenyl)-8-methoxyquinoline (F6) Pale yellow solid, Mp: obs. 144-142 °C; IR ν max (KBr, cm⁻¹): 2878, 1538, 1257, 1082, 822; ¹H NMR (CDCl₃- 400 MHz) δ: 8.09-8.07 (1H, d, J = 8.0 Hz, Ar- H), 7.80-7.75 (1H, m, Ar-H), 7.56-7.54 (1H, d, J = 7.4 Hz, Ar- H), 7.44-7.37 (2H, m, Ar-H), 7.17-7.15 (1H, d, J = 7.2 Hz, Ar-H), 7.0-6.85 (2H, m, Ar-H), 3.95 (6H, s, -OCH₃) ppm; ¹³C NMR (100 MHz, CDCl₃): 163.5, 160.7, 158.1, 157.9, 142.1, 138.3, 133.2, 129.6, 127.2, 125.1, 123.4, 120.9, 119.7, 56.3, 55.8 ppm; HRMS [M+H] obs: 284.1109, (Mcal) 284.1105.

Methyl 4-(benzo[g]quinolin-2-yl)-3-methoxybenzoate (F7) White solid, Mp: obs. 96- 98 °C; IR ν max (KBr, cm⁻¹): 2875, 1755, 1628, 1232, 1056, 801; ¹H NMR (CDCl₃- 400 MHz) δ: 8.18-8.16 (1H, d, J = 7.8 Hz, Ar-H), 8.07-8.01 (1H, m, Ar-H), 7.86-7.79 (1H, m, Ar-H), 7.69-7.58 (4H, m, Ar-H), 7.56-7.54 (1H, d, J = 7.4 Hz, Ar-H), 7.47-7.38 (3H, m, Ar-H), 4.05 (3H, s, -COOCH₃), 3.87 (3H, s, -OCH₃) ppm; ¹³C NMR (100 MHz, CDCl₃): 167.7, 159.3, 158.6, 147.9, 139.3, 137.7, 131.1, 129.4, 128.9, 128.2, 127.6, 126.8, 125.9, 124.3, 122.7, 120.1, 56.4, 48.7 ppm; HRMS [M+H] obs: 344.1309, (Mcal) 344.1306.

6-Fluoro-2-(3-fluorophenyl)quinoline (F8) White solid, Mp: obs. 123-125 °C; IR ν max (KBr, cm⁻¹): 2902, 1548, 1223, 1051, 810; ¹H NMR (CDCl₃- 400 MHz) δ: 8.11-8.09 (1H, d, J = 7.8 Hz, Ar-H), 7.84-7.75 (3H, m, Ar-H), 7.46-7.38 (3H, m, Ar-H), 7.3-7.17 (2H, m, Ar-H); ¹³C NMR (100 MHz, CDCl₃): 165.2, 160.9, 157.3,
146.4, 139.1, 138.8, 133.4, 131.6, 127.1, 126.1, 125.4, 123.7, 122.2, 121.5, 119.9 ppm; HRMS [M+H] obs: 242.0781, (Mcal) 242.0781.

2-(4-(Trifluoromethyl)phenyl)-6,7-dimethoxyquinoline (F9) White solid, Mp: obs. 156-154 °C; IR ν max (KBr, cm⁻¹): 2919, 1643, 1379, 1220, 818; ¹H NMR (CDCl₃- 400 MHz) δ:

8.24-8.20 (2H, d, J = 7.8 Hz, Ar-H), 8.1-8.08 (1H, d, J = 8.0 Hz, Ar-H), 7.76-7.73 (3H, q, J = 7.4 Hz, Ar-H), 7.49-7.47 (1H, d, J = 7.2 Hz, Ar-H), 7.09-7.07 (1H, d, J = 8.0 Hz, Ar-H), 4.0 (6H, s, -OCH₃) ppm; ¹³C NMR (100 MHz, CDCl₃): 166.7, 163.1, 157.6, 154.2, 143.5, 137.1, 135.7, 133.7, 129.4, 126.9, 125.7, 123.8, 119.2, 55.8 ppm; HRMS [M+H] obs: 334.1056, (Mcal) 334.1055.

2-(2-Chlorophenyl)-6, 7-dimethoxyquinoline (F10) White solid, Mp: obs. 162-160 °C; IR ν max (KBr, cm⁻¹): 2874, 1503, 1212, 1043, 919, 798; ¹H NMR (CDCl₃- 400 MHz) δ: 7.98-7.96 (1H, d, J = 7.6 Hz, Ar-H), 7.6-7.42 (2H, m, Ar-H), 7.31-7.18 (4H, m, Ar-H), 7.1-7.08 (1H, d, J = 7.0 Hz, Ar-H), 3.96 (6H, s, -OCH₃) ppm; ¹³C NMR (100 MHz, CDCl₃): 155.1, 152.6, 150.1, 145.1, 139.8, 133.8, 132.3, 131.5, 130, 129.4, 127.1, 122.7, 120.9, 108.2, 104.9, 56.1, 56.0 ppm; HRMS [M+H] obs: 300.0793, (M+2) 302.0798 (Mcal) 300.0791.

6,7-dimethoxy-2-(naphthalen-2-yl)quinoline(F11):Yellowsolid Mp obs 146-148 °C; IR ν max (KBr, cm⁻¹): 2928.96, 1628.84, 1591.7, 1554.4; ¹H NMR (CDCl₃- 400 MHz) δ: 8.57 (S,1H), 8.30-8.28 (d, J=8.4 Hz, 1H), 8.08-8.06 (d, J=8.4Hz, 1H), 7.98-7.96 (d, J=8 Hz, 2H), 7.87-7.85 (m, 2H), 7.56 (s, 1H), 7.52-7.50
(t, J=4.4Hz, 2H), 7.06 (s, 1H), 4.07 (s, 3H), 4.02 (s, 3H) ppm; $^{13}$C NMR (100 MHz, CDCl$_3$): 155.13, 152.72, 145.32, 137.19, 134.95, 133.66, 133.57, 128.71, 128.42, 127.66, 126.59, 126.42, 126.21, 124.96, 122.78, 117.4, 108.38, 105.0, 56.16, 56.06 ppm; HRMS [M+H] obs: 316.1284, (Mcal) 316.1293.

2-(2,4-dichlorophenyl)-6,7-dimethoxyquinoline (F12) Off white solid Mp obs 151-153 °C; IR ν max (KBr, cm$^{-1}$): 2926.98, 1625.72, 1590.83, 1553.34; $^1$H NMR (CDCl$_3$- 400 MHz) δ: 8.52-8.23 (d, J=8.4Hz, 1H), 7.75-7.44 (d, J=2 Hz, 1H), 7.58-7.53 (m, 2H), 7.39-7.38 (m, 2H), 3.91 (s, 3H), 3.90 (s, 3H) ppm; $^{13}$C NMR (100 MHz, CDCl$_3$): 153.4, 152.9, 150.34, 144.9, 138.86, 134.74, 134.11, 133.49, 132.74, 129.71, 127.98, 122.97, 120.73, 108.11, 105.84, 56.11 ppm; HRMS [M+H] obs: 334.0711, (M+2) 336.0663, (M+4) 337.0702, (Mcal) 334.073.

6,7-dimethoxy-2-(pyridin-4-yl)quinoline (F13) Pale yellow solid Mp obs 81-83 °C; IR ν max (KBr, cm$^{-1}$): 3241.28, 2924.60, 2850.8, 1666.8, 1623.5, 1592.4; $^1$H NMR (CDCl$_3$- 400 MHz) δ: 8.68-8.67 (d, J= 4.8 Hz, 2H), 8.06-8.04 (d, J= 8.8 Hz, 1H), 8.02-8.0 (m, 2H), 7.72-7.7 (dd, J= 1.2 Hz, 1H), 7.44 (s, 1H), 7.02 (s, 1H), 3.97 (s, 6H) ppm; $^{13}$C NMR (100 MHz, CDCl$_3$): 153.08, 151.98, 150.56, 149.71, 147.45, 145.43, 139.39, 135.20, 123.76, 123.59, 121.51, 116.76, 108.36, 104.8, 56.14, 56.07 ppm; HRMS [M+H] obs: 267.1088, (Mcal) 267.1086.

6,7-dimethoxy-2-(pyridin-3-yl)quinoline (F14) Pale yellow solid Mp obs 80-82 °C; IR ν max (KBr, cm$^{-1}$): 2924.63, 2850.61, 1666.2, 1622.8, 1592.33; $^1$H NMR (CDCl$_3$- 400 MHz) δ: 9.45 (s, 1H), 8.82-8.80 (d, J=4.8 Hz, 1H), 8.61-8.59 (d, J=6.4 Hz, 1H), 8.25-8.23 (d, J= 8Hz, 1H), 7.89-7.87 (dd, J=1Hz, 1H), 7.64-7.58
6,7-dimethoxy-2-(m-tolyl)quinoline (F15) Yellow solid Mp obs 120-122 °C; IR ν max (KBr, cm⁻¹): 2924.96, 1624.66, 1604.23, 1554.24; ¹H NMR (CDCl₃- 400 MHz) δ: 8.06-8.04 (d, J=8.4 Hz, 1H), 7.94 (s, 1H), 7.867-7.85 (d, J= 7.6 Hz, 1H), 7.72-7.70 (d, J= 8Hz, 1H), 7.55 (s, 1H), 7.40-7.36 (t, J= 7.6 Hz, 1H), 7.25-7.24 (m, 1H), 7.05 (s, 1H), 4.05 (s, 3H), 4.02 (s, 3H), 2.46 ( s, 3H) ppm; ¹³C NMR (100 MHz, CDCl₃): 152.86, 152.38, 150.07, 149.50, 148.36, 145.4, 135.45, 135.17, 134.67, 123.63, 123.06, 116.78, 108.21, 104.86, 56.12, 56.06 ppm; HRMS [M+H] obs: 267.1088, (Mcal) 267.1086.

2-(3-chlorophenyl)-6,7-dimethoxyquinoline (F16) Brown solid Mp 80 °C,IR ν max (KBr, cm⁻¹): 2925.94, 1623.66, 1608.42; ¹H NMR (CDCl₃- 400 MHz) δ: 8.48 (s, 1H), 8.06- 8.04 (d, J=8Hz, 1H), 7.64-7.62 (d, J=7.4Hz, 1H), 7.54-7.52(d, J=8Hz,1H), 7.48-7.44(t, J=7.6Hz, 1H), 7.28 (s, 1H), 7.23-7.21(m,1H), 7.04 (s,1H), 4.04(s, 3H), 4.01 (s, 1H) ppm; ¹³C NMR (100 MHz, CDCl₃): 153.9, 135.29, 134.33, 132.49, 129.77, 129.64, 129.37, 129.18, 127.63, 127.5, 127.29, 126.62, 122.16, 119.62, 106.66, 105.02, 56.23, 56.02 ppm; HRMS [M+H] obs: 301.0682, 303.0702 (Mcal) 301.0684.

4-(6,7-dimethoxyquinolin-2-yl)benzonitrile (F 17) Brown solid Mp 110-112 °C; IR ν max (KBr, cm⁻¹): 2923.48, 2225.35, 1712.81, 1622.33, 1605.95, 1502.43; ¹H NMR (CDCl₃- 400 MHz) δ: 8.25-8.23 (d, J=8Hz, 2H), 8.12-8.10 (d, J= 8.8Hz, 1H), 7.8-7.73 (m, 3H), 7.5 (s, 1H), 7.08
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(s, 1H), 4.07 (s, 3H), 4.04 (s, 3H) ppm; $^{13}$C NMR (100 MHz, CDCl$_3$): 152.81, 145.42, 144.06, 135.17, 132.51, 136.8, 129.97, 127.68, 127.05, 123.34, 118.87, 117.3, 116.92, 112.19, 108.31, 104.83, 56.17, 56.07 ppm; HRMS [M+H] obs: 291.1339, (Mcal) 291.1335.

2.2. Section-B: Biological studies

2.2.1. Biological significance of 2-aryl quinolines as anticancer agents.

Chih-Hua Tseng and group were synthesized number of 2,3-diarylquinoline derivatives and evaluated for antiproliferative activities against the growth of HepG2, Hep 3B, A549, H1299, MCF-7, MDA-MB-231 cancer cell lines.$^{[28]}$ Among these C-6 substituted 2,3-diarylquinoline derivatives show good activity, compound I was one of the most active against the growth of Hep 3B, H1299, and MDA-MB-231 with a GI$_{50}$ value of 0.71, 1.46, and 0.72 mm respectively. Compound I was selected as a new lead for potential anticancer drug candidates.

Zhenfeng Duan et al. reported (2-(4-Methoxyphenyl)-4-quinolinyl) (2-piperidinyl) methanol (NSC23925) and its four isomers, compound II incontestable the foremost potent activity.$^{[29]}$ II reversed multidrug resistance (MDR) in several drug-resistant cell lines expressing Pgp, including colon, uterine, ovarian, breast, and sarcoma cancer. II significantly enhanced in vivo antitumor activity of paclitaxel in MDR xenograft models, while not increasing the level of paclitaxel toxicity. II and derivatives of this compound might hold therapeutic value in the treatment of MDR-dependent cancers.
Eun Jeong Koh reported a new series of 4-aryl-8-amino(acetamido)quinolines.\textsuperscript{[30]} The target compounds were evaluated for antiproliferative activities against ten human melanoma cell lines. Compounds possessing amide linker and 2,3-dihydrobenzo[b][1,4]dioxine terminal moiety showed the very best potency against A375P cell line with IC\textsubscript{50} values in sub-micromolar scale. This study led to discovery of two lead compounds, III and IV, their high potencies over the ten tested melanoma cell lines, and repressive activity of compound IV over ERK kinase create this compound promising leads for future development of antiproliferative agents targeting ERK pathway for treatment of skin cancer.

Juan R. Rodrigues and group represented a series of seven new quinolinyl acrylate derivatives and evaluated against human prostate cancer cells PC-3 and LNCaP \textit{in vitro} and \textit{in vivo}.\textsuperscript{[31]} The most effective compound V reduced the viability in each cell lines in a time and dose-dependent manner. Repressing effects were conjointly noted on the migration, invasion, and adhesion of the prostate cancer cells as easily as on the neo angiogenesis, clonogenic and MMP-9 activity. The effect \textit{in vivo} was studied in PC-3 xenografts in nude mice. The study suggests the multi-target efficacy of the
quinolinyl derivates against human prostate cancer cells and supports its potential therapeutic usefulness.

M. S. Shahabuddin et al. reported two molecules with identical chemical backbone but side chains were different, namely 8-methoxy pyrimido[4′,5′:4,5]thieno (2,3-b)quinoline-4(3H)-one (MPTQ) and 4-morpholino pyrimido[4′,5′:4,5]thieno(2,3-b)quinoline (morpho-PTQ) at the 8th and 4th position, respectively.\[^{32}\] IC\(_{50}\) values of MPTQ was estimated between 2–15 µM among the leukemic cells studied, whereas it absolutely was quite 200 µM once morpho-PTQ was used. Cell cycle analysis shows a rise in sub-G1 phase, with none specific cell cycle arrest. So the observed low IC\(_{50}\) value of MPTQ makes it a promising cancer chemotherapeutic agent.

M. S. Shahabuddin and group reported novel intercalating compounds of pyrimido[4′,5′:4,5]selenolo(2,3-b)quinoline derivatives having a butylamino or piperazino group at fourth position (BPSQ & PPSQ,) are studied.\[^{33}\] Cell cycle analysis and tritiated thymidine assay revealed that BPSQ affects the cell cycle progression by arresting at S phase. Further, changes in the expression levels of BCL2/BAD confirmed the activation of apoptosis. Hence, the identified novel compound VII which might have clinical relevancy in cancer chemotherapeutics.
Charles M. Keyari and group reported a series of 7-amino- and 7-acetamidoquinoline-5,8-diones with aryl substituents at the 2-position were synthesized, and evaluated as potential NAD(P)H: quinone oxido reductase (NQO1) -directed antitumor agents.\textsuperscript{34} Surprisingly, quinoline containing compound, 7-acetamido-2-(8'-quinolinyl) quinoline-5,8-dione \textbf{VIII}, showed selective cytotoxicity toward the NQO1-expressing MDA468-NQ16 breast cancer cells versus the NQO1-null MDA468-WT cells. Compound \textbf{IX} showed potent activity against human breast cancer cells expressing or not expressing NQO1.

Alain Fournet and group synthesized several quinolines and were evaluated against HTLV-1 infected cells. Some of them were able to inhibit HTLV-1 cell-growth at 10 mM. Some structure–activity relationships were observed,\textsuperscript{35} used at concentrations of 50 and 10 mM. HUT-102, MT-2, C8166 and C91/PL are HTLV-1 transformed cell lines, and MOLT4 and Jurkat are leukemic cells that are not infected by HTLV-1. From this comparative study, it is possible to draw some structure–activity relationships. Compound with a shorter alkyl chain \textbf{X} and \textbf{XI} show a stronger activity compare to other derivatives.
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2.2.2.2 Results and discussion

2.2.2.1. 2-Aryl quinolines (F11-F17) induces cytotoxicity on human cancer cell lines.

We first investigated the antiproliferative activity of novel 2-aryl quinoline derivatives (F11-F17) on HCT116 cells using an MTT assay. Among the newly synthesized quinolines, compound F16 displayed most potent cytotoxic effect compared to other structural analogues of the series with an IC$_{50}$ of 25.5 µM. Compound F13, F15 and F17 did not display cytotoxicity up to 50 µM and the IC$_{50}$ values of remaining compounds of the series ranges between 44 µM to 50 µM. Further, we evaluated the effect of lead compound against HCT116 and A549 at indicated dose and time points. Compound F16 significantly reduce the viable cells in both the cell lines in time and dose dependent manner (Figure 5). Further investigation on the effect of compound F16 on normal cells (Vero, Monkey kidney epithelial cells) revealed that, compound F16 induce minimal cytotoxicity on Vero cells up to 72 h at 50 µM. Paclitaxel was used as the positive control for in vitro cytotoxicity assay.

![Figure-5](image)

**Figure-5** Compound F16 elicit an antiproliferative effect against HCT116 and A549 cells in a time and dose dependent manner.
2.2.2.2. **Compound F16 interferes with cell cycle and triggers cell cycle arrest at G2/M phase in HCT116 cells.**

Initial identification of lead compound prompted us for further evaluation. We next treated the HCT116 cells with compound **F16** at 20 µM up to 36 h, thereafter, cells were harvested, processed, stained with propidium iodide and analysed for cell cycle distribution using flow cytometry. The treatment of HCT116 cells with compound **F16** gradually increased the G2/M population with decrease in G1 cells in time dependent manner as compared to the diluent control cells (Figure 6). The results demonstrate that compound **F16** arrests cell cycle progression at G2/M phase and contribute to cell death.

![Figure-6](image_url) **Figure-6** Compound **F16** interferes with cell cycle and triggers cell cycle arrest at G2/M phase in HCT116 cells.

**2.2.3. Conclusion**

In summary, the cytotoxicity assays were used for preliminary screening of 2-aryl-quinolines. Among all the screened derivatives, compound **F16** was found to be most potent against human cancer cell lines at lower concentrations. Further we carried cell cycle analysis results demonstrate that compound **F16** arrests cell cycle progression at G2/M phase and contribute to cell death. Hence the 2-(3-chlorophenyl)-6,7 dimethoxyquinoline (**F16**) is more potent with IC$_{50}$ of 25.5 µM on HCT116 cells respectively.
2.2.4. Experimental Section

*In vitro cytotoxicity assay*

Cancer cells were cultured in Dulbecco’s Modified Eagle Medium (DMEM) containing 1X antibiotic-antimycotic solution with 10% FBS. The cytotoxic effect of newly synthesized quinoline derivatives were evaluated against HCT116 (Colon cancer) and A549 (Lung cancer) cell lines by the MTT dye uptake method.\[^{36}\] Briefly, cells (2.5 X 10\(^4\)/ml) were incubated in triplicate in a 96-well plate in the presence or absence of different concentrations of our compounds in a final volume of 0.2 ml for indicated time intervals at 37 \(^\circ\)C. Thereafter, 20 μl MTT solution (5 mg/ml in PBS) was added to each well. After a 2 h incubation at 37 \(^\circ\)C, 0.1 ml lysis buffer (20% SDS, 50% dimethyl-formamide) was added; incubation was continued overnight at 37 \(^\circ\)C; and then the optical density (OD) at 570 nm was measured by Varioskan plate reader.\[^{37}\]

*Flow cytometric analysis*

The effect of lead cytotoxic compound on cell cycle distribution of HCT116 cells was analysed as described previously.\[^{38}\] Briefly, HCT116 (1 X 10\(^5\)) cells were seeded into each well of a 6-well plate and treated with compound F16 at 20 μM for 24 h and 36 h. Thereafter, cells were harvested, washed, fixed with 70% ethanol overnight at 37 \(^\circ\)C with 0.1 % RNase A in PBS. Cells were then washed again, resuspended and stained in PBS containing 0.5 μg/ml propidium iodide for 30 min at room temperature. Samples was analyzed using flow cytometry, histogram was plotted using flowing software.
2.3. References and notes


Appendices
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$^1$H NMR spectrum of compound F12
HRMS spectrum of compound F12
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$^{13}$C NMR spectrum of compound F12

IR spectrum of compound F12
$^1$H NMR spectrum of compound F14
LCMS spectrum of compound F14
$^{13}$C NMR spectrum of compound F14

IR spectrum of compound F14
$^1$H NMR spectrum of compound F17
HRMS spectrum of compound F17

IR spectrum of compound F17
$^{13}$C NMR spectrum of compound F17