Chapter 1

Green synthesis and *in vitro* anticancer activity of some new 5,6,7,8-tetrahydroquinoline-3-carbonitrile derivatives

*Abstract*

Today multi-component reactions (MCRs) have emerged as a powerful tool in organic synthesis that allows quick access to the diversity based library of small organic molecules for the medicinal and pharmaceutical applications. On the other hand, developing new MCRs with the environmentally benign concept has attracted particular attention because of the possibility that these reactions may comply with the green chemistry protocol. Herein we reported a green, efficient and simple procedure for one-pot synthesis of a series of 5,6,7,8-tetrahydroquinoline-3-carbonitrile derivatives (4a–r) via three-component coupling of 2,6-bis(3/4-substituted benzylidene) cyclohexanone (1), arylamines/ substituted-2-aminothiazoles (2) and malononitrile (3) by using a catalytic amount of Bleaching earth clay pH 12.5 (BEC) (10 wt%) as a promoter in PEG-400 as a green reaction medium. All the newly synthesized compounds were characterized and screened for their potential *in vitro* anticancer activity against MDA-MB-468 human breast cancer cell line, using Adriamycin (ADR) as a reference drug. Most of the synthesized compounds exhibited intensive anticancer activity.

*Keywords*: BEC (pH-12.5); PEG-400; Tetrahydroquinoline-3-carbonitrile; *In vitro*, Anticancer activity, Breast cancer cell line.
1.1 Introduction and Review of Literature

Cancer is enduring to be a major health issue in developing as well as undeveloped countries\textsuperscript{1,2}. Breast cancer is the most persistent cancer worldwide and a second leading cause of cancer related death among females in the world. The global concern of breast cancer exceeds all the other cancers and the incidence rates of breast cancer are increasing. Breast cancer in women has been a gloom issue in the medical history for decades\textsuperscript{3}. Breast cancer is a malignant tumor that initiate in the breast cells. A malignant tumor is a clusters of cancer cells that be capable of grow into (invade) surrounding tissues or spread (metastasize) to other distant areas of the body\textsuperscript{4}. For this reason, there is sustaining concern regarding the development of new and advantageous anti-breast cancer drugs.

The multi-functionalized quinolines as a pharmacophore imitates a class of heterocyclic compounds with compelling pharmacological proficiency, engrossing antiviral, antimalarial, antiasthmatic, anti-inflammatory, antibacterial, antifungal and anticancer\textsuperscript{5}. Fig 1.1 depicted structures of several literature anticancer agents bearing quinoline moiety\textsuperscript{6-8}. Quinoline derivatives have also been used in the synthesis of fungicides, virucides, biocides, alkaloids, rubber chemicals and flavoring agents\textsuperscript{9}. The relevance as substructures in a wide area of products, significant attempts continue to be assisted into the boosting of new quinoline based structures.

Encompassing the quinoline derivatives, tetrahydroquinoline scaffold is deliberated as one of the privileged structure in the field of drug discovery, as compounds enclosing this scaffold validates vast range of biological and pharmaceutical activities\textsuperscript{10a-i}, comprising anti-HIV\textsuperscript{11,12}, anticancer\textsuperscript{13}, antimalarial\textsuperscript{14}, cholesteryl ester transfer protein inhibitors\textsuperscript{15}, anti-diabetic\textsuperscript{16}, antifungal\textsuperscript{17}, C\textsubscript{5a} receptor antagonists agents\textsuperscript{18}, RET tyrosine kinase inhibitors\textsuperscript{19} etc.
In consideration of their remarkable significances, discrete synthetic methodologies for preparing tetrahydroquinoline derivatives have been proclaimed like, microwave supported, $\text{Sc(OTf)}_3$ (Lewis acid) catalyzed, KOH and Na metal catalyzed, metal-based and organo-catalyzed, catalyst free in $\text{H}_2\text{O}$. Most of these reported methods comprises some obstacles, such as adopting non-green catalytic systems, toxic reagents, pricey ligands, partial transformation of starting materials, lengthy reaction period and laborious work-up operations with scarce yields.

To beaten these complexities recently, further consideration has been paid to the heterogeneous catalysis umpired reactions as an economic and environmental point of view. An ease of handling, recyclability, low erosion, minimal execution time, waste minimization, easy transport, and catalyst disposal are the several important applications of heterogeneous catalysts in organic synthesis.
Amongst the heterogeneous catalysts, naturally occurring clay has unique physical and chemical properties like shape selectivity, acidic or basic nature and thermal stability. Bleaching earth clay (pH 12.5) is very skillful, easy to separate, environmental-friendly, commercial, non-toxic, safe, and recyclable green catalyst that has been used for several base-catalyzed organic conversions\(^{30}\). The minute (5 micron) particle size of the clay imparts a magnificent surface area compared with other solid-supported catalysts. BEC (pH 12.5) acts as a superior heterogeneous catalyst by providing an enormously clean reaction with high yield and without any byproducts. Meanwhile reactions in PEG-400 have acquired substantial momentum due to its exclusive properties\(^{31-33}\) turn our diligence to use PEG-400 as one of green solvent in the present study.

1.2 Results and Discussion

1.2.1 Chemistry

In this chapter we reported a green, efficient and simple procedure for the one-pot synthesis of 8-(3/4-substituted benzylidene)-4-(4-substituted phenyl)-2-((3/4-substituted phenyl)amino)-5,6,7,8-tetrahydroquinoline-3-carbonitrile derivatives (4a-r) via three component coupling of 2,6-bis(3/4-substituted benzylidene) cyclohexanone (1), arylamines/substituted-2-amino thiazole (2) and malononitrile (3) by using catalytic amount of BEC (pH 12.5, 10 wt\%) as a promoter, in PEG-400 as a green reaction media worked very well (Scheme 1.1).

In the preliminary experimentation we firstly attempted the three component coupling of 2,6-bis(4-chlorobenzylidene) cyclohexanone (1) with an equimolar amount of 3-chloroaniline (2) and malononitrile (3) was preferred as a model reaction under catalyst free condition. Under catalyst free condition no reaction was scrutinized at room temperature, even at higher temperature or prolonged heating (Table 1.1, entry 1, 2). Further we studied the effect of several basic catalysts viz. triethyl amine (TEA), piperidine and morpholine in
PEG-400 were screened for their conversion; surprisingly, none of the catalyst gave satisfactory yield (40-50%, Table 1.1, entries 3-5). Subsequently we attempted BEC (pH-12.5) as a heterogeneous catalyst for achieving the targeted products (4a-r) in PEG-400.

Scheme 1.1 Synthetic route of 5,6,7,8-tetrahydroquinoline-3-carbonitrile derivatives (4a–r).

The observed results were rather interesting gives pronounced increment in the yields of products (70-93%) within shorter reaction time about 20-30 min only. At the moment we repeated the reaction in the presence of BEC (pH-12.5) and evaluated the amount of catalyst required for this transformation using 1 wt%, 5 wt % and 10 wt% of catalyst and it observed that the increase in amount of catalyst increases the yield 70%, 89%, and 93% respectively (Table 1.1, entries 6-8). Accordingly 10 wt% of BEC (pH-12.5) was found to be sufficient for successful completion of reaction gives maximum yield (93%). Any further addition of catalyst loading does not touch on the yield (Table 1.1, entry 9). The used catalyst can be recycled, and reused as such for the next reaction without any further purification. The catalytic activity of catalyst was examined by using the BEC for 4-5 runs and it has been...
observed that catalyst shown good catalytic power up to five runs with negligible loss of its activity (Table 1.2).

<table>
<thead>
<tr>
<th>Entry</th>
<th>Catalyst (mol/wt%)</th>
<th>Temp (°C)</th>
<th>Time (min)</th>
<th>Yield of 4b (%)</th>
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<tbody>
<tr>
<td>1</td>
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<td>RT</td>
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<td>0</td>
</tr>
<tr>
<td>2</td>
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<td>Triethyl Amine (mol%)</td>
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</tr>
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<td>4</td>
<td>Piperidine (mol%)</td>
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<tr>
<td>5</td>
<td>Morpholine (mol%)</td>
<td>70</td>
<td>&gt;60</td>
<td>40</td>
</tr>
<tr>
<td>6</td>
<td>BEC (1 wt%)</td>
<td>80</td>
<td>30</td>
<td>70</td>
</tr>
<tr>
<td>7</td>
<td>BEC (5 wt%)</td>
<td>80</td>
<td>25</td>
<td>89</td>
</tr>
<tr>
<td>8</td>
<td>BEC (10 wt%)</td>
<td>80</td>
<td>20</td>
<td>93</td>
</tr>
<tr>
<td>9</td>
<td>BEC (20 wt%)</td>
<td>80</td>
<td>30</td>
<td>75</td>
</tr>
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</table>

*a Reaction progress monitored by TLC; *b Yields refer to isolated yield.

Table 1.2 Catalytic activity of BEC (pH-12.5)

<table>
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<tr>
<th>Run</th>
<th>1st</th>
<th>2nd</th>
<th>3rd</th>
<th>4th</th>
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<td>20</td>
<td>20</td>
<td>20</td>
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<td>95</td>
<td>93</td>
<td>93</td>
<td>92</td>
<td>88</td>
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</table>

On the basis of optimized conditions we then tested the generality and scope of the present MCRs. Interestingly, a wide variety of 2,6-bis(substituted benzylidene) cyclohexanones such as 4-chlorobenzylidene, 4-florobenzylidene, 3-nitrobenzylidene, 4-nitrobenzylidene, 4-methylbenzylidene and 4-methoxybenzylidene cyclohexanones underwent smooth coupling with different aroylamines and malononitrile (Table 1.3).
### Table 1.3 Synthesis of compounds (4a-r)

<table>
<thead>
<tr>
<th>Entry</th>
<th>Products&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Time (min)&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Yield (%)&lt;sup&gt;c&lt;/sup&gt;</th>
<th>M.P. (°C)</th>
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<td><img src="image1" alt="Product Image" /></td>
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<td>285-288</td>
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<tr>
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<td><img src="image2" alt="Product Image" /></td>
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<td>95</td>
<td>276-279</td>
</tr>
<tr>
<td>4c</td>
<td><img src="image3" alt="Product Image" /></td>
<td>20</td>
<td>93</td>
<td>273-276</td>
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<tr>
<td>4d</td>
<td><img src="image4" alt="Product Image" /></td>
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<td>92</td>
<td>279-282</td>
</tr>
<tr>
<td>4e</td>
<td><img src="image5" alt="Product Image" /></td>
<td>20</td>
<td>94</td>
<td>281-284</td>
</tr>
<tr>
<td>4f</td>
<td><img src="image6" alt="Product Image" /></td>
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<td>91</td>
<td>274-277</td>
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<td>4g</td>
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<td>90</td>
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<td>4i</td>
<td><img src="image9" alt="Product Image" /></td>
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<td>4m</td>
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<td><img src="image18" alt="Product Image" /></td>
<td>23</td>
<td>90</td>
<td>293-296</td>
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<sup>a</sup>Products were characterized by IR, <sup>1</sup>H NMR, <sup>13</sup>C NMR and MASS spectroscopy; <sup>b</sup>Reaction progress monitored by TLC; <sup>c</sup>Yield refers to pure products after column chromatography.
The arylamines involves 3-chloroanilines, 4-bromoanilines, 3-nitroanilines (Table 1.3) also underwent coupling with 2, 6-bis(substituted benzylidene) cyclohexanones and malononitrile to give the corresponding 5,6,7,8-tetrahydroquinoline-3-carbonitrile derivatives in good yields. To expand the scope of arylamines, we also used substituted 2-aminothiazoles (Table 1.3; 4o-r). All the synthesized compounds were well characterized by spectral analysis (IR, $^1$H NMR, $^{13}$C NMR and MASS).

The results (Scheme 1.1) indicate that substrates 1 bearing both electron-donating groups (such as alkoxy and methyl) and electron-withdrawing groups (such as nitro and halides) can be involved in these MCRs to afford the desired products (4a–r) with high yields. The above observations revealed that the electronic nature of the substituents had no significant effect on the present reaction.

Scheme 1.2 Possible mechanism of BEC (pH-12.5) catalyzed reaction
The suggested mechanism for the reaction is outlined in Scheme 1.2. The reaction is initiated by the BEC (pH 12.5) basic catalyst between arylamines and malononitrile to form the acrylonitrile derivatives. The 1,4-addition of acrylonitrile derivatives with 2,6-bis(substituted benzylidene) cyclohexanones followed by cyclization, dehydration and subsequent aromatization gives rise the substituted 5,6,7,8-tetrahydroquinoline-3-carbonitrile derivatives in good to excellent yields.

1.2.2 Biology

1.2.2.1 Anticancer Activity

In the present study, we investigated the in vitro anti-breast cancer properties of 5,6,7,8-tetrahydroquinoline-3-carbonitrile derivatives (4a–r) by using Sulforhodamine B (SRB) assay with respect to the standard Adriamycin (ADR) and a spectrometric assay as well. The data was plotted as concentration of synthesized compounds versus percentage of cell growth and the experimental sets are compared with positive control i.e. Adriamycin (ADR) as shown in Fig 1.2. Results of the study indicate that (Table 1.4), amongst the representative compounds, the 4a (AN-1) and 4c (AN-3) were found to have good in vitro anticancer activity against the Human Breast Cancer Cell Line (MDA-MB-468) at the concentration of 10⁻⁴M. As the compounds significantly inhibited the cell growth at this concentration as compared to positive control Adriamycin (ADR). While the compounds 4b (AN-2), 4d (AN-4) and 4e (AN-5) demonstrated the good to moderate in vitro anticancer activity at 10⁻⁴M concentrations as compared to positive control Adriamycin (ADR) (Fig 1.3). The susceptibility of cells towards the various concentrations of synthesized compounds was illustrated by GI50 value i.e. concentration of drugs causing 50% inhibition of cell growth (Table 1.5).
### Table 1.4 Anticancer screening of some representative derivatives (4a-e)

<table>
<thead>
<tr>
<th>Comp code</th>
<th>Experiment 1</th>
<th>Experiment 2</th>
<th>Experiment 3</th>
<th>Average Values</th>
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<tr>
<td>10^{-7}M</td>
<td>10^{-5}M</td>
<td>10^{-4}M</td>
<td>10^{-3}M</td>
<td>10^{-2}M</td>
</tr>
<tr>
<td>4a (AN-1)</td>
<td>100.0</td>
<td>100.0</td>
<td>100.0</td>
<td>-67.5</td>
</tr>
<tr>
<td>4b (AN-2)</td>
<td>100.0</td>
<td>100.0</td>
<td>100.0</td>
<td>-28.0</td>
</tr>
<tr>
<td>4c (AN-3)</td>
<td>100.0</td>
<td>100.0</td>
<td>98.6</td>
<td>-68.0</td>
</tr>
<tr>
<td>4d (AN-4)</td>
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<td>100.0</td>
<td>-28.5</td>
</tr>
<tr>
<td>4e (AN-5)</td>
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<td>100.0</td>
<td>100.0</td>
<td>-33.3</td>
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<tr>
<td>ADR</td>
<td>-1.9</td>
<td>-29.6</td>
<td>-75.4</td>
<td>-79.7</td>
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</table>
**Fig 1.2** Graphical representation of anticancer screening of representative compounds

**Table 1.5** Molar drug concentrations calculated from graph

<table>
<thead>
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<th>LC50</th>
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<th>G150*</th>
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<td>4a (AN-1)</td>
<td>92.96</td>
<td>63.3</td>
<td>33.6</td>
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<tr>
<td>4b (AN-2)</td>
<td>&gt;100</td>
<td>82.1</td>
<td>41.3</td>
</tr>
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<td>4c (AN-3)</td>
<td>94.7</td>
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<td>33.1</td>
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<td>4d (AN-4)</td>
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<td>79.2</td>
<td>38.3</td>
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<td>ADR</td>
<td>&lt;0.1</td>
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<td>&lt;0.1</td>
</tr>
</tbody>
</table>

**Fig 1.3** The cellular morphological changes of MDA-MB-468 cells treated with representative compounds (4a-e)
1.3 Material and Methods

1.3.1 Chemistry

All the melting points were uncorrected and determined in an open capillary tube. The chemicals and solvents used were of laboratory grade and were purified. Completion of the reaction was monitored by thin layer chromatography on precoated sheets of silica gel-G (Merck, Germany) using iodine vapors for detection. BEC (pH 12.5) was gifted from Supreme silicone Pune Pvt. Limited. IR spectra were recorded in KBr pellets on an FTIR Schimadzu spectrophotometer. \(^1\)H NMR (300 MHz) and \(^{13}\)C NMR (100 MHz) spectra were recorded in (DMSO)-d\(_6\) with an Avance spectrometer (Bruker, Germany) at a 300-MHz frequency using TMS as an internal standard; chemical shifts are reported in parts per million. Multiplicities are reported as follows: s (singlet), d (doublet), t (triplet), m (multiplet). Mass spectra were recorded on an EI-Shimadzu QP 2010 PLUS GC-MS system (Shimadzu, Japan). Elemental analysis was performed on a Carlo Erba 106 Perkin-Elmer model 240 analyzer (Perkin-Elmer, USA).

1.3.2 General procedure for the synthesis of 8-(3/4-substituted benzylidene)-4-(4-substituted phenyl)-2-((3/4-substituted phenyl)amino)-5,6,7,8-tetrahydroquinoline-3-carbonitrile derivatives (4a-r)

A mixture of 2,6-bis(3/4-substituted benzylidene)cyclohexanone (1.00 mmol), arylamines/ substituted 2-aminothiazole (1.00 mmol), malononitrile (1.00 mmol) and BEC (10 wt%) was stirred at 80°C in PEG-400 for appropriate times for the different substrates. Scheme 1.1. After complete conversion as indicated by TLC, the catalyst was filtered out by simple filtration and the mother liquor poured onto ice-cold water, the solid separated out, neutralized, and filtered out the product. The resulting products were purified by column chromatography on silica gel (Merck, 60–120 mesh, chloroform–methanol) to afford the pure products.
1.3.2.1 8-(4-chlorobenzylidene)-4-(4-chlorophenyl)-2-((3-chlorophenyl)amino)-5,6,7,8-
tetrahydroquinoline-3-carbonitrile (4a)

M.P. 285–288 °C; Yield, 90%; IR (KBr, cm\(^{-1}\)): 3464 (N–H), 2917 (aliphatic, C-H), 2186
(C≡N), 1631 (C=N); \(^1\)H NMR (300 MHz, DMSO-d\(_6\), TMS, \(\delta\), ppm): 7.41 (s, 1H, -C=CH),
7.33–6.82 (m, 12H, Ar–H), 4.02 (s, 1H, NH), 2.59–1.53 (m, 6H, >CH\(_2\) protons of
tetrahydroquinoline ring); \(^1\)C NMR (100 MHz, DMSO-d\(_6\), TMS, \(\delta\), ppm): 158.05, 157.65,
135.54, 134.66, 130.40, 129.91, 123.75, 122.50, 122.46, 121.63, 121.08, 43.50, 27.14, 26.78,
22.15; EIMS: 515 [M⁺]; Elemental Analysis. Calculated (found) for C\(_{29}\)H\(_{20}\)Cl\(_3\)N\(_3\): % C, 67.39 (67.42); H, 3.90 (3.92); N, 8.13 (8.14).

1.3.2.2 8-(4-chlorobenzylidene)-4-(4-chlorophenyl)-2-((4-bromophenyl)amino)-5,6,7,8-
tetrahydroquinoline-3-carbonitrile (4b)

M.P. 276-279 °C; Yield, 95%; IR (KBr, cm\(^{-1}\)): 3484 (N-H), 2919 (aliphatic, C-H), 2188
(C≡N), 1630 (C=N); \(^1\)H NMR (300 MHz, DMSO-d\(_6\), TMS, \(\delta\), ppm): 7.27 (s, 1H, -C=CH),
7.24-6.70 (m, 12H, Ar–H), 4.75 (s, 1H, NH), 2.66-1.23 (m, 6H, >CH\(_2\) protons of
tetrahydroquinoline ring); \(^1\)C NMR (100 MHz, DMSO-d\(_6\), TMS, \(\delta\), ppm): 159.46, 158.20,
157.96, 140.44, 136.23, 129.19, 128.43, 127.58, 121.50, 120.70, 114.55, 113.84, 113.60,
56.29, 55.31, 54.78, 42.33, 26.52, 26.67, 21.45; EIMS: 561 [M⁺]; Elemental Analysis. Calculated (found) for C\(_{29}\)H\(_{20}\)BrCl\(_2\)N\(_3\): % C, 64.31 (64.34); H, 3.78 (3.79); N, 5.10 (5.14).

1.3.2.3 8-(4-chlorobenzylidene)-4-(4-chlorophenyl)-2-((3-nitrophenyl)amino)-5,6,7,8-
tetrahydroquinoline-3-carbonitrile (4c)

M.P. 273–276 °C; Yield, 93%; IR (KBr, cm\(^{-1}\)): 3364 (N-H), 3364 (N-H), 2948 (aliphatic, C-H), 2254
(C≡N), 1616 (C≡N), 1568 & 1327 (N-O, two strong band); \(^1\)H NMR (300 MHz, DMSO-d\(_6\),
TMS, \(\delta\), ppm): 8.10 (s, 1H, -C=CH), 7.44-8.08 (m, 12H, Ar–H), 4.33 (s, 1H, NH), 1.31-2.59
(m, 6H, >CH\(_2\) protons of tetrahydroquinoline ring), ppm; \(^1\)C NMR (100 MHz, DMSO-d\(_6\),
TMS, \(\delta\), ppm): 159.45, 158.09, 157.88, 140.61, 136.01, 129.20, 128.13, 127.60, 121.50,
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120.66, 114.71, 113.87, 56.21, 55.74, 54.89, 42.41, 26.22, 26.40, 21.84; EIMS: 527 [M+]; Elemental Analysis. Calculated (found) for C_{29}H_{20}Cl_{2}N_{4}O_{2}: % C, 66.04 (66.00); H, 3.82 (3.80); N, 10.62 (10.64); O, 6.07 (6.04).

1.3.2.4 \textit{8-(4-florobenzylidene)-4-(4-florophenyl)-2-((3-chlorophenyl)amino)-5,6,7,8-tetrahydroquinoline-3-carbonitrile} (4d)

M.P. 279-282°C; Yield, 92%; IR (KBr, cm^{-1}): 3375 (N-H), 3033 (>CH_{2}), 2255 (C≡N), 1608 (C=N); \textsuperscript{1}H NMR (300 MHz, DMSO-d_{6}, TMS, δ, ppm): 8.25 (s, 1H, -C=CH), 7.80-7.52 (m, 12H, Ar-H), 3.89 (s, 1H, NH), 2.6-1.3 (m, 6H, >CH_{2} protons of tetrahydroquinoline ring); \textsuperscript{13}C NMR (100 MHz, DMSO-d_{6}, TMS, δ, ppm): 158.66, 158.23, 158.24, 140.40, 136.06, 129.45, 128.65, 127.50, 121.59, 120.61, 114.69, 113.90, 112.74, 56.15, 55.10, 55.00, 42.37, 26.68, 26.43, 21.88; EIMS: 482 [M+]; Elemental Analysis. Calculated (found) for C_{29}H_{20}ClF_{2}N_{3}: % C, 71.97 (71.95); H, 4.15 (4.12); N, 8.68 (8.64).

1.3.2.5 \textit{8-(4-florobenzylidene)-4-(4-florophenyl)-2-((4-bromophenyl)amino)-5,6,7,8-tetrahydroquinoline-3-carbonitrile} (4e)

M.P. 281-284 °C; Yield, 94%; IR (KBr, cm^{-1}): 3364 (N-H), 2973 (aliphatic, C-H), 2258 (C≡N), 1614 (C=N); \textsuperscript{1}H NMR (300 MHz, DMSO-d_{6}, TMS, δ, ppm): 8.18 (s, 1H, -C=CH), 8.09-7.44 (m, 12H, Ar-H), 4.28 (s, 1H, NH), 2.52-1.68 (m, 6H, >CH_{2} protons of tetrahydroquinoline ring); \textsuperscript{13}C NMR (100 MHz, DMSO-d_{6}, TMS, δ, ppm): 159.45, 158.06, 157.34, 140.41, 136.23, 129.11, 128.63, 127.57, 121.63, 120.90, 114.33, 113.32, 113.21, 56.14, 55.10, 54.87, 42.20, 26.44, 26.34, 21.65; EIMS: 528 [M+]; Elemental Analysis. Calculated (found) for C_{29}H_{20}BrF_{2}N_{3}: % C, 65.92 (65.89); H, 3.82 (3.83); N, 15.12 (15.10).

1.3.2.6 \textit{8-(4-florobenzylidene)-4-(4-florophenyl)-2-((3-nitrophenyl)amino)-5,6,7,8-tetrahydroquinoline-3-carbonitrile} (4f)

M.P. 274–277°C; Yield, 91%; IR (KBr, cm^{-1}): 3366 (N-H), 2965 (aliphatic, C-H), 2249 (C≡N), 1630 (C≡N), 1548 & 1319 (N-O, two strong band); \textsuperscript{1}H NMR (300 MHz, DMSO-d_{6},...
8-(3-nitrobenzylidene)-4-(3-nitrophenyl)-2-((3-chlorophenyl)amino)-5,6,7,8-tetrahydroquinoline-3-carbonitrile (4g)

M.P. 286-289°C; Yield, 92%; IR (KBr, cm⁻¹): 3327 (N-H), 2929 (aliphatic, C-H), 2191 (C≡N), 1603 (C=N), 1519 & 1352 (N-O, two strong band); ¹H NMR (300 MHz, DMSO-d₆, TMS, δ, ppm): 8.17 (s, 1H, -C=CH), 8.16-6.97 (m, 12H, Ar-H), 4.31 (s, 1H, NH), 2.72-1.57 (m, 6H, >CH₂ protons of tetrahydroquinoline ring); ¹³C NMR (100 MHz, DMSO-d₆, TMS, δ, ppm): 159.66, 158.18, 158.01, 140.50, 136.00, 129.15, 128.55, 127.60, 121.56, 120.62, 114.79, 113.93, 113.77, 56.19, 55.04, 54.99, 42.34, 26.66, 26.47, 21.84; EIMS: 538[M⁺] (100), 537[M⁺2] (50); Elemental Analysis. Calculated (found) for C₂₉H₂₀ClN₅O₄: % C, 64.75 (64.77); H, 3.75 (3.79); N, 13.02 (13.04).

8-(3-nitrobenzylidene)-4-(4-nitrophenyl)-2-((4-bromophenyl)amino)-5,6,7,8-tetrahydroquinoline-3-carbonitrile (4h)

M.P. 288-291°C; Yield, 90%; IR (KBr, cm⁻¹): 3334 (N-H), 3029 (aliphatic, C-H), 2253 (C≡N), 1610 (C=N), 1541 & 1332 (N-O, two strong band); ¹H NMR (300 MHz, DMSO-d₆, TMS, δ, ppm): 8.17 (s, 1H, -C=CH), 7.51-8.02 (m, 12H, Ar-H), 4.29 (s, 1H, NH), 1.56-2.40 (m, 6H, >CH₂ protons of tetrahydroquinoline ring); ¹³C NMR (100 MHz, DMSO-d₆, TMS, δ, ppm): 159.15, 158.31, 157.84, 140.32, 136.07, 129.23, 128.50, 127.52, 121.18, 120.58, 114.42, 113.57, 113.49, 56.33, 55.20, 54.37, 42.40, 26.82, 26.16, 21.74; EIMS: 582 [M⁺];
Elemental Analysis. Calculated (found) for C_{29}H_{20}BrN_{3}O_{4}: % C, 59.81 (59.78); H, 3.46 (3.43); N, 12.02 (12.00); O, 10.99 (10.96).

**1.3.2.9** 8-(3-nitrobenzylidene)-4-(3-nitrophenyl)-2-((3-nitrophenyl)amino)-5,6,7,8-tetrahydroquinoline-3-carbonitrile (4i)

M.P. 292–295°C; Yield, 95%; IR (KBr, cm\(^{-1}\)): 3345 (N-H), 3031 (>CH\(_2\)), 2263 (C≡N), 1616 (C=N), 1575 & 1346 (N-O, two strong band); \(^1\)H NMR (300 MHz, DMSO-d\(_6\), TMS, \(\delta\), ppm): 8.10 (s, 1H, -C=CH), 8.08-7.41 (m, 12H, Ar-H), 4.41 (s, 1H, NH), 2.50-1.33 (m, 6H, >CH\(_2\) protons of tetrahydroquinoline ring); \(^1^\)C NMR (100 MHz, DMSO-d\(_6\), TMS, \(\delta\), ppm): 159.51, 158.23, 157.44, 140.12, 136.51, 129.34, 128.22, 127.19, 121.65, 120.33, 114.25, 113.87, 113.32, 56.25, 55.36, 54.54, 42.21, 26.37, 26.42, 21.56; EIMS: 548 [M+]; Elemental Analysis. Calculated (found) for C\(_{29}\)H\(_{20}\)N\(_6\)O\(_6\): % C, 63.50 (63.48); H, 3.68 (3.65); N, 15.32 (15.30); O, 17.50 (17.47).

**1.3.2.10** 8-(4-nitrobenzylidene)-4-(4-nitrophenyl)-2-((3-chlorophenyl)amino)-5,6,7,8-tetrahydroquinoline-3-carbonitrile (4j)

M.P. 286–289°C; Yield, 92%; IR (KBr, cm\(^{-1}\)): 3353 (N-H), 3034 (aliphatic, C-H), 2242 (C=N), 1615 (C=N), 1536 & 1330 (N-O, two strong band); \(^1\)H NMR (300 MHz, DMSO-d\(_6\), TMS, \(\delta\), ppm): 8.21 (s, 1H, -C=CH), 8.07-7.43 (m, 12H, Ar-H), 4.21 (s, 1H, NH), 2.56-1.03 (m, 6H, >CH\(_2\) protons of tetrahydroquinoline ring); \(^1^\)C NMR (100 MHz, DMSO-d\(_6\), TMS, \(\delta\), ppm): 159.16, 158.27, 157.73, 140.55, 136.12, 129.45, 128.43, 127.19, 121.13, 120.12, 114.63, 113.82, 113.54, 56.16, 55.13, 54.65, 42.71, 26.37, 26.65, 21.44; EIMS: 537 [M+]; Elemental Analysis. Calculated (found) for C\(_{29}\)H\(_{20}\)ClN\(_5\)O\(_4\): % C, 64.75 (64.70); H, 3.75 (3.72); N, 13.02 (13.00); O, 11.90 (11.89).

**1.3.2.11** 8-(4-nitrobenzylidene)-4-(4-nitrophenyl)-2-((4-bromophenyl)amino)-5,6,7,8-tetrahydroquinoline-3-carbonitrile (4k)
M.P. 287–290°C; Yield, 91%; IR (KBr, cm\(^{-1}\)): 3367 (N-H), 3016 (aliphatic, C-H), 2254 (C≡N), 1619 (C≡N), 1525 & 1325 (N-O, two strong band); \(^1\)H NMR (300 MHz, DMSO-d\(_6\), TMS, δ, ppm): 8.05 (s, 1H, -C=CH), 7.94-5.56 (m, 12H, Ar-H), 4.22 (s, 1H, NH), 2.71-1.66 (m, 6H, >CH\(_2\) protons of tetrahydroquinoline ring); \(^1\)C NMR (100 MHz, DMSO-d\(_6\), TMS, δ, ppm): 159.36, 158.12, 157.58, 140.19, 136.12, 129.26, 128.43, 127.32, 121.21, 120.57, 114.70, 113.43, 56.22, 55.14, 54.55, 42.71, 26.33, 26.14, 21.36; EIMS: 582 [M+];

Elemental Analysis. Calculated (found) for C\(_{29}\)H\(_{20}\)BrN\(_5\)O\(_3\): % C, 59.81 (59.79); H, 3.46 (3.42); N, 12.02 (12.00); O, 10.99 (10.97).

\subsection*{8-(4-nitrobenzylidene)-4-(4-nitrophenyl)-2-((3-nitrophenyl)amino)-5,6,7,8-tetrahydroquinoline-3-carbonitrile (4l)}

M.P. 292–295°C; Yield, 93%; IR (KBr, cm\(^{-1}\)): 3360 (N-H), 2992 (aliphatic, C-H), 2251 (C≡N), 1613 (C≡N), 1555 & 1335 (N-O, two strong band); \(^1\)H NMR (300 MHz, DMSO-d\(_6\), TMS, δ, ppm): 8.19 (s, 1H, -C=CH), 8.05-7.20 (m, 12H, Ar-H), 4.31 (s, 1H, NH), 2.64-1.77 (m, 6H, >CH\(_2\) protons of tetrahydroquinoline ring); \(^1\)C NMR (100 MHz, DMSO-d\(_6\), TMS, δ, ppm): 159.16, 158.11, 157.65, 140.32, 136.23, 129.34, 128.33, 127.43, 121.11, 120.16, 114.70, 113.46, 56.28, 55.32, 54.14, 42.42, 26.75, 26.55, 21.86; EIMS: 548 [M+];

Elemental Analysis. Calculated (found) for C\(_{29}\)H\(_{20}\)BrN\(_5\)O\(_3\): % C, 63.50 (63.46); H, 3.68 (3.62); N, 15.32 (15.30); O, 17.50 (17.46).

\subsection*{8-(4-methybenzylidene)-4-(4-methylphenyl)-2-((3-chlorophenyl)amino)-5,6,7,8-tetrahydroquinoline-3-carbonitrile (4m)}

M.P. 273–276°C; Yield, 90%; IR (KBr, cm\(^{-1}\)): 3352 (N-H), 2980 (aliphatic, C-H), 2261 (C≡N), 1625 (C≡N); \(^1\)H NMR (300 MHz, DMSO-d\(_6\), TMS, δ, ppm): 8.16 (s, 1H, -C=CH), 8.07-7.38 (m, 12H, Ar-H), 4.38 (s, 1H, NH), 2.52-1.05 (m, 6H, >CH\(_2\) protons of tetrahydroquinoline ring), 2.34 (s, 6H, -CH\(_3\)); \(^1\)C NMR (100 MHz, DMSO-d\(_6\), TMS, δ, ppm): 159.32, 158.12, 157.88, 140.14, 136.23, 129.10, 128.23, 127.12, 121.26, 120.70,
114.45, 113.30, 113.12, 56.22, 55.84, 54.90, 42.38, 26.26, 26.40, 21.86, 21.00, 21.00; EIMS: 476 [M+]; Elemental Analysis. Calculated (found) for C_{31}H_{26}ClN_{3}: % C, 78.22 (78.19); H, 5.51 (5.49); N, 8.83 (8.80).

1.3.2.14 8-(4-methoxybenzylidene)-4-(4-methoxyphenyl)-2-((3-chlorophenyl)amino)-5,6,7,8-tetrahydroquinoline-3-carbonitrile (4n)

M.P. 281–284°C; Yield, 92%; IR (KBr, cm^{-1}): 3366 (N-H), 3030 (aliphatic, C-H), 2257 (C≡N), 1632 (C=N); ¹H NMR (300 MHz, DMSO-d$_6$, TMS, δ, ppm): 8.09 (s, 1H, -C=CH), 7.91-7.30 (m, 12H, Ar-H), 4.31 (s, 1H, NH), 2.58-1.42 (m, 6H, >CH₂ protons of tetrahydroquinoline ring); ¹³C NMR (100 MHz, DMSO-d$_6$, TMS, δ, ppm): 159.52, 158.24, 157.78, 140.65, 136.45, 129.17, 128.81, 127.43, 121.40, 120.45, 114.95, 113.40, 113.65, 56.53, 55.24, 55.02, 54.63, 42.71, 26.63, 26.32, 21.74; EIMS: 508 [M+] (100); Elemental Analysis. Calculated (found) for C$_{31}$H$_{26}$ClN$_{3}$O$_2$: % C, 73.29 (73.25); H, 5.16 (5.13); N, 8.27 (8.24); O, 6.30 (6.26).

1.3.2.15 8-(4-chlorobenzylidene)-4-(4-chlorophenyl)-2-((4-(4-chlorophenyl)thiazol-2-yl)amino)-5,6,7,8-tetrahydroquinoline-3-carbonitrile (4o)

M.P. 291–294°C; Yield, 90%; IR (KBr, cm^{-1}): 3369 (N-H), 3000 (aliphatic, C-H), 2253 (C≡N), 1609 (C=N); ¹H NMR (300 MHz, DMSO-d$_6$, TMS, δ, ppm): 8.16 (s, 1H, -C=CH), 8.04-7.54 (m, 13H, Ar-H), 4.32 (s, 1H, NH), 2.44-1.28 (m, 6H, >CH₂ protons of tetrahydroquinoline ring); ¹³C NMR (100 MHz, DMSO-d$_6$, TMS, δ, ppm): 162.05, 161.71, 153.47, 150.38, 149.73, 156.49, 156.40, 146.75, 145.31, 144.76, 142.23, 136.33, 133.49, 132.00, 131.66, 130.94, 130.90, 129.35, 129.31, 127.18, 124.62, 124.06, 123.96, 123.09, 122.39, 122.30, 120.46, 114.33, 109.37, 89.05, 45.10, 35.64, 27.00; EIMS: 600 [M+]; Elemental Analysis. Calculated (found) for C$_{32}$H$_{21}$Cl$_3$N$_4$S: % C, 64.06 (64.02); H, 3.53 (3.52); N, 9.34 (9.31).
1.3.2.16 8-(4-florobenzylidene)-4-(4-florophenyl)-2-((4-(4-chlorophenyl)thiazol-2-yl) amino)-5,6,7,8-tetrahydroquinoline-3-carbonitrile (4p)

M.P. 134–136°C; Yield, 89%; IR (KBr, cm⁻¹): 3366 (N-H), 2983 (aliphatic, C-H), 2247 (C≡N), 1610 (C≡N); ¹H NMR (300 MHz, DMSO-d₆, TMS, δ, ppm): 8.01 (s, 1H, -C=CH), 7.97-7.37 (m, 13H, Ar-H), 4.38 (s, 1H, NH), 2.50-1.30 (m, 6H, >CH₂ protons of tetrahydroquinoline ring); ¹³C NMR (100 MHz, DMSO-d₆, TMS, δ, ppm): 162.58, 161.11, 155.35, 155.20, 150.38, 149.53, 149.05, 146.72, 144.39, 143.22, 142.54, 135.35, 133.42, 132.38, 131.90, 130.41, 130.04, 129.27, 129.23, 128.60, 125.68, 125.09, 123.37, 123.30, 121.06, 121.00, 120.44, 115.37, 109.32, 89.15, 45.71, 34.64, 24.62; EIMS: 567 [M+]; Elemental Analysis. Calculated (found) for C₃₂H₂₁ClF₂N₄S: % C, 67.78 (67.71); H, 3.73 (3.67); N, 9.88 (9.82).

1.3.2.17 8-(3-nitrobenzylidene)-4-(3-nitrophenyl)-2-((4-(4-chlorophenyl)thiazol-2-yl) amino)-5,6,7,8-tetrahydroquinoline-3-carbonitrile (4q)

M.P. 293–296°C; Yield, 91%; IR (KBr, cm⁻¹): 3370 (N-H), 3024 (aliphatic, C-H), 2259 (C≡N), 1630 (C≡N), 1545 & 1330 (N-O, two strong band); ¹H NMR (300 MHz, DMSO-d₆, TMS, δ, ppm): 8.08 (s, 1H, -C=CH), 8.08-7.46 (m, 13H, Ar-H), 4.33 (s, 1H, NH), 2.70-1.93 (m, 6H, >CH₂ protons of tetrahydroquinoline ring); ¹³C NMR (100 MHz, DMSO-d₆, TMS, δ, ppm): 162.07, 161.55, 153.91, 151.26, 151.20, 150.04, 149.34, 146.28, 144.00, 143.49, 141.53, 134.36, 132.41, 131.96, 130.74, 129.42, 129.04, 128.93, 128.45, 128.40, 124.34, 124.28, 123.78, 123.71, 122.56, 122.44, 120.43, 114.42, 107.18, 89.56, 42.71, 33.38, 25.76; EIMS: 620 [M+]; Elemental Analysis. Calculated (found) for C₃₂H₂₁ClN₆O₄S: % C, 61.88 (61.85); H, 3.41 (3.42); N, 13.53 (13.50); O, 10.30 (10.28).

1.3.2.18 8-(4-nitrobenzylidene)-4-(4-nitrophenyl)-2-((4-(4-chlorophenyl)thiazol-2-yl) amino)-5,6,7,8-tetrahydroquinoline-3-carbonitrile (4r)
M.P. 293–296°C; Yield, 90%; IR (KBr, cm⁻¹): 3356 (N-H), 2978 (aliphatic, C-H), 2257 (C≡N), 1622 (C≡N), 1551 & 1334 (N-O, two strong band); ¹H NMR (300 MHz, DMSO-d₆, TMS, δ, ppm): 8.18 (s, 1H, -C=CH), 7.95-7.29 (m, 13H, Ar-H), 4.23 (s, 1H, NH), 2.76-1.53 (m, 6H, >CH₂ protons of tetrahydroquinoline ring); ¹³C NMR (100 MHz, DMSO-d₆, TMS, δ, ppm): 162.33, 161.46, 152.49, 151.15, 151.03, 150.66, 149.87, 145.52, 143.01, 142.48, 140.39, 134.36, 132.77, 131.43, 130.24, 129.56, 129.50, 128.74, 128.08, 128.00, 125.84, 125.80, 123.46, 123.41, 122.28, 122.23, 121.44, 113.90, 107.21, 89.02, 43.75, 33.91, 26.42; EIMS: 610 [M+]; Elemental Analysis. Calculated (found) for C₃₂H₂₁Cl₂N₅O₂S: % C, 62.95 (62.92); H, 3.47 (3.44); N, 11.47 (11.45); O, 5.24 (5.22).

1.3.3 Determination of Anticancer Activity

1.3.3.1 SRB Assay:

The cell lines were grown in RPMI 1640 medium containing 10% fetal bovine serum and 2 mM L-glutamine. For present screening experiment, cells were inoculated into 96 well microtiter plates in 90 µL at 5000 cells per well. After cell inoculation, the microtiter plates were incubated at 37°C, 5% CO₂, 95% air and 100% relative humidity for 24 hrs prior to addition of experimental drugs. Experimental drugs were solubilized in appropriate solvent to prepare stock of 10⁻² concentration. At the time of experiment four 10-fold serial dilutions were made using complete medium. Aliquots of 10 µL of these different drug dilutions were added to the appropriate micro-titer wells already containing 90 µL of medium, resulting in the required final drug concentrations.

After addition compounds, plates were incubated at standard conditions for 48 hrs and assay was terminated by the addition of cold TCA. Cells were fixed in situ by the gentle addition of 50 µL of cold 30% (w/v) TCA (final concentration, 10% TCA) and incubated for 60 minutes at 4°C. The supernatant was discarded; the plates were washed five times with tap water and air dried. Sulforhodamine B (SRB) solution (50 µl) at 0.4% (w/v) in 1% acetic acid
was added to each of the wells, and plates were incubated for 20 minutes at room temperature. After staining, unbound dye was recovered and the residual dye was removed by washing five times with 1% acetic acid. The plates were air dried. Bound stain was subsequently eluted with 10 mM trizma base, and the absorbance was read on an Elisa plate reader at a wavelength of 540 nm with 690 nm reference wavelength. Percent Growth was expressed as the ratio of average absorbance of the test well to the average absorbance of the control wells X 100.

1.4 Conclusions

In summary, we described a simple, efficient, practical and green method for one pot synthesis of 8-(3/4-substituted benzylidene)-4-(4-substituted phenyl)-2-((3/4-substituted phenyl)amino)-5,6,7,8-tetrahydroquinoline-3-carbonitrile derivatives in the presence of catalytic amount of BEC (pH 12.5) and PEG-400, and screened for their in vitro anticancer activity against Human Breast Cancer Cell Line (MDA-MB-468). The present procedure has the following advantages, eco-friendly in nature, less reaction time, convenient work-up, improved yield of products and characteristic recyclability of catalyst, thus making it a useful and important addition to the existing methods. Additionally the study may exalt the scope of developing these results also revealed the suitability of synthesized tetrahydroquinoline pharmacophore as potential anticancer agents.
Fig 1.4 IR of compound 4a

Fig 1.5 $^1$H NMR of compound 4a
Fig 1.6 $^{13}$C NMR of compound 4a

Fig 1.7 Mass of compound 4a
Fig 1.8 IR of compound 4b

Fig 1.9 $^1$H NMR of compound 4b
Fig 1.10 Mass of compound 4b

Fig 1.11 IR of compound 4g
Fig 1.12 $^1$HNMR of compound 4g

Fig 1.13 $^{13}$C NMR of compound 4g
Fig 1.14 Mass of compound 4g
References


