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Microwave-Assisted Synthesis, Characterization and Cytotoxic Studies of Novel Estrogen Receptor α Ligands towards Human Breast Cancer Cells

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Abstract: A new, simple, and microwave-assisted, solution-phase T3P\textsuperscript{®}-DMSO mediated method for the preparation of a novel class of estrogen receptor alpha (ER\textalpha) ligands based on the 2-phenylquinoline scaffold was developed. Furthermore, the novel ER\textalpha ligands were tested for their bioactivity against ER\textalpha-positive and ER\textalpha-negative cell lines. The ligand (entry 4), with amine and nitro group substitution at C4 position, displayed significant cytotoxicity against MCF-7 and HepG2 cells with an IC\textsubscript{50} value of 6 and 11 \textmu M, respectively. On the other hand, ER\textalpha-negative cells displayed resistance to quinolines induced cytotoxicity with an IC\textsubscript{50} value >100 \textmu M and they does not induce cytotoxicity in normal breast epithelial cells. Molecular docking analyses suggest a consistent binding mode for these ER\textalpha ligands in the ligand binding domain of the human ER\textalpha and predict the ligands to occupy the hydrophobic cavity in a similar fashion as estradiol or GW2368.
INTRODUCTION

Breast cancer is a leading cancer in women worldwide and contributing to second cause of lethality after lung cancer.\(^1\),\(^2\) Most breast cancers are associated with interaction of estrogen receptors (ER) in the breast epithelial cells to estrogen. The physiological action of estrogen is induced via two types of estrogen receptors namely ER\(\alpha\) and ER\(\beta\).\(^3\) Research in the previous decade revealed that more than 70% of breast cancers are due to ER\(\alpha\) dependent epithelial cell proliferation. The role of ER\(\beta\) is not clear in initiation and progression of breast cancer.\(^4\) ER\(\alpha\) belongs to nuclear receptor superfamily which regulates the transcription of genes involved in proliferation, anti-apoptosis, metastasis and immunosurveillance.\(^5\)-\(^7\) The binding of 17\(\beta\)-estradiol to ER\(\alpha\) induces the receptor dimerization and facilitates binding of the ligand-receptor complex to the promoter of target genes.\(^8\) Also, ER-dependent pathways regulate the synthesis and distribution of glycosaminoglycans in cancer cells.\(^9\) Several small molecule ER\(\alpha\) antagonists including Tamoxifen, Raloxifene and Fulvestrant have been implemented in the treatment of breast cancer.\(^10\),\(^11\) Benzisoxazole tethered azoles have known to be the better ER ligands.\(^12\)-\(^14\) Therefore, probing small molecules against ER\(\alpha\) is considered to be the most attractive therapeutic target to treat breast cancer.\(^15\)

Quinoline derivatives are the pharmacologically important heterocycles which have been studied extensively for their anticancer properties. Multiple reports have demonstrated the preparation of quinolines using strong base like tertiary butoxide.\(^16\),\(^17\) We previous reported the anti-cancer effect of various heterocyclic compounds \(^18\)-\(^20\) and recently reported the solution phase synthesis of 2-amino-chromene-3-carbonitriles from alcohols, malanonitrile and phenols.\(^21\) Using a similar strategy, herein, we report a simple and efficient method for the preparation of T3P\(^9\)-DMSO mediated 2-phenylquinoline derivatives using amino alcohols and acetophenones under microwave irradiation and evaluated for their cytotoxicity. The newly developed method was generalized using variety of aromatic 2-amino alcohols
and different substituted acetophenones to obtain 1-10 molecules (Scheme 2; Table 1). Our docking analysis validated the interaction of quinoline derivatives with the estrogen receptor in the similar fashion as estradiol to induce its anticancer effect.

**Table 1. The physical characterization of new quinoline-based ERα ligands, whose core scaffold has different substitutions at R⁴ to R⁶ positions.**

<table>
<thead>
<tr>
<th>Entry</th>
<th>R¹</th>
<th>R²</th>
<th>R³</th>
<th>R⁴</th>
<th>R⁵</th>
<th>R⁶</th>
<th>Y</th>
<th>mp (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>H</td>
<td>H</td>
<td>OH</td>
<td>H</td>
<td>H</td>
<td>H</td>
<td>H</td>
<td>85</td>
</tr>
<tr>
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<td>CH₃</td>
<td>H</td>
<td>Br</td>
<td>H</td>
<td>H</td>
<td>H</td>
<td>H</td>
<td>89</td>
</tr>
<tr>
<td>3</td>
<td>H</td>
<td>H</td>
<td>Cl</td>
<td>H</td>
<td>Cl</td>
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<td>H</td>
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<td>H</td>
<td>95</td>
</tr>
<tr>
<td>5</td>
<td>CH₃</td>
<td>H</td>
<td>Cl</td>
<td>H</td>
<td>Cl</td>
<td>H</td>
<td>H</td>
<td>90</td>
</tr>
<tr>
<td>6</td>
<td>H</td>
<td>H</td>
<td>H</td>
<td>H</td>
<td>H</td>
<td>Br</td>
<td>H</td>
<td>88</td>
</tr>
<tr>
<td>7</td>
<td>CH₃</td>
<td>H</td>
<td>H</td>
<td>H</td>
<td>H</td>
<td>H</td>
<td>H</td>
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<tr>
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<td>H</td>
<td>NH₂</td>
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<td>H</td>
<td>91</td>
</tr>
<tr>
<td>9</td>
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<td>89</td>
</tr>
<tr>
<td>10</td>
<td>CH₃</td>
<td>H</td>
<td>H</td>
<td>NO₂</td>
<td>H</td>
<td>H</td>
<td>H</td>
<td>93</td>
</tr>
</tbody>
</table>

Additionally, all the new molecules were characterized completely using IR, ¹H NMR, ¹³C NMR, and LC-MS spectral analysis (please refer supplementary data).

Further, the library of ERα ligands was tested for its cytotoxicity against ERα-positive, ERα-negative cancer cells and their counterpart non-diseased breast epithelial cells as described previously and detailed methodology is provided in supplementary information.²², ²³ The results are summarized in Table 2.
Table 2. Cytotoxicity data for the new ERα ligands IC_{50} against human cancer cells.

<table>
<thead>
<tr>
<th>Entry</th>
<th>ERα-positive cells</th>
<th>ERα-negative cells</th>
<th>Breast epithelial cells</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MCF-7 (µM)</td>
<td>HepG2 (µM)</td>
<td>MDA-MB-231 (µM)</td>
</tr>
<tr>
<td>1</td>
<td>&gt;50</td>
<td>&gt;50</td>
<td>NT</td>
</tr>
<tr>
<td>2</td>
<td>&gt;50</td>
<td>&gt;50</td>
<td>NT</td>
</tr>
<tr>
<td>3</td>
<td>25</td>
<td>31.6</td>
<td>&gt; 100</td>
</tr>
<tr>
<td>4</td>
<td>6</td>
<td>11</td>
<td>&gt; 100</td>
</tr>
<tr>
<td>5</td>
<td>&gt;50</td>
<td>&gt;50</td>
<td>NT</td>
</tr>
<tr>
<td>6</td>
<td>12</td>
<td>9</td>
<td>&gt; 100</td>
</tr>
<tr>
<td>7</td>
<td>&gt;50</td>
<td>&gt;50</td>
<td>NT</td>
</tr>
<tr>
<td>8</td>
<td>34</td>
<td>41</td>
<td>NT</td>
</tr>
<tr>
<td>9</td>
<td>28</td>
<td>19</td>
<td>NT</td>
</tr>
<tr>
<td>10</td>
<td>23</td>
<td>37</td>
<td>&gt; 100</td>
</tr>
</tbody>
</table>

*NT – Not tested

Among the tested compounds 4, 6, and 10 significantly inhibited the proliferation of ERα-positive cells. Further, compounds (4, 6, and 10) with high cytotoxicity were tested against ERα-negative cells. All the ERα-negative cells were resistance to the lead compounds with IC_{50} values more than 100 µM. However, the shortlisted three molecules did not induce cytotoxicity on MCF-10A cells up to 72 h at 100 µM. These results indicate that the newly synthesized ERα ligands are selectively cytotoxic against ERα expressing cancer cells and does not interfere with viability of their counterpart.

Additionally, the structural models for molecular interactions between the newer ERα ligands and the human estrogen receptor were generated using in silico docking analysis as described previously and detailed methodology is provided in supplementary information.24-26 Docking
was based on the co-crystal structure of the naphthalene derivative GW2368 with the estrogen receptor (PDB: 3DT3). Molecular docking suggests a consistent binding mode for the series of quinoline-based ERα ligands in the ligand binding domain of the human estrogen receptor. Thereby, the compounds occupy the hydrophobic cavity in a similar fashion as estradiol or GW2368 (Figure 1). The docking scores of the ERα ligands were comparable with estradiol or GW2368 (Supplementary Table 1). The quinoline scaffold of the ERα ligands occupies the position of rings A and B in the steroid and show major overlap with the naphthalene ring system of GW2368. Furthermore, presence of benzene substituents allows for interactions with His-524 in agreement with other estrogen receptor ligands. Presence of a hydrogen bond donor function is predicted to facilitate a second binding mode, where hydrogen bonds to Arg-394 and Glu-353 are formed. The structure-activity-relationship studies for the compound 4, which bearing chlorine atom at R² renders significant anti-proliferative activity towards ER positive cancer cells, whereas the presence of methyl group at R¹ decreases the activity. This observation was evidenced with strong binding of compound 4 to ER alpha LBD, when compared to compound 8.

In conclusion, we have identified a novel quinoline-based ERα ligands as biologically active compounds against ERα expressing human cancer cells. This study also introduces a novel microwave-assisted synthesis pathway to the compound series. Therefore, method will be useful to develop libraries of quinoline-based ERα ligands to treat breast cancer.

Acknowledgement

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University, Kingdom of Saudi Arabia is also acknowledged. Basappa & CDM thank Karnataka University, India & DST for DC PAVATE & INSPIRE Fellowships.

References and Notes


**Figure Legends**

**Figure 1:** ERα Scaffold Evolution

**Figure 2:** Schematic representation of the synthesis of quinoline-based ERα ligands.

**Figure 3:** Predicted interactions of quinoline-based ERα molecules towards the ligand binding domain of the human estrogen receptor. The protein is shown as green cartoon with main polar interaction centers Arg-394, Glu-353, and His-524 highlighted as sticks in atomic coloring. Interactions of estradiol with estrogen receptor (A) are resembled by the naphthalene-derived compound GW2368 (B) and predicted to be similar for the quinoline series (C).
Scheme 2

1a-c + 1d-h → 1-10

T3P®-DMSO
Ethanol, mwi
60 °C, 10 min
NaOH, 5 min
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Lead ERα ligand

MCF-7 cells IC₅₀ = 6 μM

Newer ligands occupy the ligand binding domain of ERα similar to estradiol or GW2368