DOCKING AND CYTOTOXICITY STUDIES OF 2-VINYLCHROMONE DERIVATIVES ON HUMAN BREAST CANCER CELL LINES

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ABSTRACT

Objective: Estrogen receptor (ER) is over-expressed in 70% of breast cancers. The ER has two isoforms, ERα and ERβ. The ER ligand binding domain (LBD) has been the target for hormone-responsive breast cancer. Due to tissue-specific effects currently available drugs for hormone positive breast cancer presents serious limitation. The dynamic and plastic nature of ER LBD plays a crucial role in ligand design that discriminates between the ER subtypes. Agents that selectively target ER isoform are a formidable challenge to researchers. The chromone scaffold is a privileged scaffold for exploration of anticancer agents. The objective of the present study was to evaluate the anticancer activity of a small library of 2-vinylchromones in human breast cancer cell lines MCF-7 and MDA-MB-231.

Methods: The compounds were synthesized by the reported procedures. Docking studies of the substituted 2-vinylchromone was performed using GLIDE tool in Maestro 8.0. The compounds were evaluated for anticancer activity against MCF-7 (ERα positive), MDA-MB-231 (ERβ positive) and MRC-5 (ERα, β negative) cell lines using MTT assay.

Results: The in silico studies indicated that substituted 2-vinylchromones, 1(a-c) and 2(a-b) exhibited comparable docking score at LBD of ERα and ERβ. However, the binding affinity of the compounds for the allosteric binding site in ERβ was negligible. The dose-dependent studies using MTT assay depicted that compounds 1(a-c) and 2(a-b) exhibited anticancer activity in ERα positive cell line MCF-7 as compared to ERβ positive cell line MDA MB 231. The most potent anticancer activity was observed for compound 2b against MCF-7 cells with IC₅₀ value of 15.625 µg/ml.

Conclusion: The present investigation indicated that 2-vinylchromone derivatives exhibited ER isoform selectivity and the presence of bulky group in 2-vinylchromones resulted in significantly higher cytotoxicity in ERα positive cell lines as compared to the ERβ positive cell line.

Keywords: Estrogen receptor, 2-vinylchromone, Docking, Anticancer, MCF-7, MDA-MB-231.
RESULTS

The synthesized compounds were evaluated for their binding affinity towards ERα and ERβ by docking studies. The PDB entry chosen for the docking studies were 2QTU [11], 2FSZ [12] and 3ERT [13]. The PDB entry 3ERT is a complex of ERα with hydroxytamofoxen (HT). The selection of 2QTU entry was based on structural similarity of the synthesized ligands with benzopyranones. Native ERβ pdb entry is not available therefore 2FSZ was selected which is similar to the synthesized ligands with benzopyranones. Native tamoxifen (HT).

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The dose-dependent studies using MTT assay depicted that compounds 1(a-c) and 2(a-b) exhibited anticancer activity in ERα positive cell line MCF-7 as compared to ERβ positive cell line MDA-MB-231. The compound 1b, 2a and 2b exhibited anticancer activity in ERα positive cell line. Amongst them compound 2b was most potent. Remarkable differences in the IC50 value were seen for 1b, 2a and 2b in MCF-7 and MDA-MB-231. The IC50 value for 1b, 2a and 2b were 62.5μg/ml, 31.25μg/ml and 15.625μg/ml, respectively in MCF-7 cell line. All the compounds exhibited an IC50 value of more than 125μg/ml in MDA-MB-231 cell line as indicated in table 2.

The glide energy of the compound 1b and 2b with ERα was found to be -27.40Kcal/mol and -55.59Kcal/mol with -7.01 and -8.27 docking score. Fig. 1 (A and B) illustrates the interaction of compound 1b and 2b with amino acid residues of ERα (PDB 1D 3ERT).

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<table>
<thead>
<tr>
<th>S. No.</th>
<th>Compound</th>
<th>Structure</th>
<th>Docking Score</th>
<th>Glide energy of the model (Kcal/mol)</th>
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<th>Docking Score</th>
<th>Glide energy of the model (Kcal/mol)</th>
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<td>-36.75</td>
<td>-7.8</td>
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<td>-3.42</td>
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<tr>
<td>2</td>
<td>1b</td>
<td><img src="image2" alt="Structure" /></td>
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<td>3</td>
<td>1c</td>
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<td>-39.08</td>
<td>-3.39</td>
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</table>
Table 2: Comparative IC₅₀ value of compounds in MCF-7 and MDA-MB-231 cell lines

<table>
<thead>
<tr>
<th>Compound</th>
<th>IC₅₀-MCF-7 (μg/ml)</th>
<th>IC₅₀-MDA-MB-231 (μg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1a</td>
<td>125</td>
<td>&gt;125</td>
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<tr>
<td>1b</td>
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<td>&gt;125</td>
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<tr>
<td>1c</td>
<td>&gt;125</td>
<td>&gt;125</td>
</tr>
<tr>
<td>2a</td>
<td>31.25</td>
<td>&gt;125</td>
</tr>
<tr>
<td>2b</td>
<td>15.62</td>
<td>&gt;125</td>
</tr>
</tbody>
</table>

The docking results of compound 1b, 2a and 2b on ERβ (PDB ID 2FSZ) on the other hand did not exhibit interactions with the significant residues in the ERβ LBD. The results are presented in fig. 2.

The three-dimensional structure of ERα docked with compound 1b and 2b are represented in fig. 3 which also displays the superimposition of compound 1b and 2b with HT in the ligand binding pocket of ERα.
In an attempt to corroborate the results of docking studies for compounds 1(a-c) and 2(a-b) MTT assay were performed in ERα and ERβ positive cell lines. The cell lines chosen for the studies were MCF-7 (ERα positive), MDA-MB-231 (ERβ positive) and MRC-5 (ERα,β negative). The results of these studies are depicted in fig 4.

**DISCUSSION**

The LBD of ERα and ERβ has 60% homology [14]. The dynamic and plastic nature of ER LBD plays a crucial role in ligand design that discriminates between the ER subtypes.

The interior of the ligand binding pocket of ERα and ERβ have been reported to have 22-24 residues which are identical and involved in interactions with ligands [15]. The two pockets are however different in size and flexibility and this aspect is crucial for the development of selective subtype agents [16].

Apart from this the discovery of allosteric binding pocket in the coactivator groove of ERβ further provides avenues for identification of agents known as coactivator binding inhibitors [12, 17]. In the present exploratory work we have attempted to identify ER isoform-selective compounds from a small library of 2-vinylchromones. For this purpose docking studies have been carried at both ERα and ERβ LBD and allosteric binding pocket of ERβ. The findings of docking studies have been correlated with MTT assay on ERα, ERβ and ER α, β negative cell lines.

The in silico studies revealed that the synthesized compounds preferentially binds to the ligand binding domain of ERα and ERβ with a comparable docking score except for 2b which exhibits preferential docking to ERα. It was also found that these compounds exhibited poor binding to the allosteric binding site of ERβ. Highest docking score amongst the series was also observed for compound 2b. Prominent interacting residues including Glu 353, Arg 394 and Asp351 as compared to HT clearly indicate high fold selectivity for ERβ [19]. Similarly, electron density maps of HT complexed to ERβ indicate one of the HT molecules is located in the cognate ligand binding pocket with a confirmation indistinguishable from ERα/HT structure [20]. However, a comparison of IC_{50} values of HT in ERα and ERβ indicates ERα selectivity [21]. These observations suggest the involvement of different residues of ERα and ERβ LBD that may contribute towards isoform selectivity.

In our studies, it was observed that in all cases the chromones with methoxy substitution 1b, 2a and 2b exhibited the highest cytotoxicity. The presence of a bulky aminoalkyl group at C-3 of the chromone ring as compared to the keto group as in the case of 2a and 2b respectively led to significantly higher cytotoxicity. Further; structure-activity relationship studies will help to correlate contribution of long chain aminoalkyl substitution to cytotoxicity.

**CONCLUSION**

2-vinylchromone derivatives exhibited higher cytotoxicity in ERα positive cell lines as compared to the ERβ positive cell line. Our results also indicated that these derivatives exhibited ER isoform selectivity and presence of bulky group as in the case of 2a and 2b resulted in a significant increase in cytotoxic activity.

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**CONFLICT OF INTERESTS**

Declared None

**REFERENCES**