Synthesis, Characterization and Study of in vitro and in silico Anticancer Activity of (e)-2-Arylidene-1-Indanones

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Abstract Novel substituted (E)-2-benzylidene-1-indanones have been synthesized and evaluated for their cytotoxicity. 2-arylidene-4,7-diethylindan-1-one inhibited significantly the growth of MFC-7 cells in a dose dependent manner, without producing any alteration to the HBL-100 cells. Molecular docking studies were carried for all the synthesized compounds against estrogen receptor-alpha. Both in vitro and in silico studies suggest anticancer activity of (1-5) certain substituted (E)-2-benzylidene-1-indanones.

1. Introduction

2-Arylidene indan-1-ones are reported to be either biologically active or very useful synthons for the synthesis of various carbocyclic and heterocyclic molecules of biological importance [1-5]. Some substituted (E)-2-aryl methylene-6,7-dimethoxy-1-tetralones act as ceramide stimulants which in turn expressed inhibition of tumor cell growth [6,7]. A series of conformational analogues of (E)-2-benzylidene-1-tetralone (1) and (E)-2 benzylidene-1-indanone (2) [Fig.1] were synthesized to optimize the structure-activity relationship and to develop the more potent and selective antitumor agents. The compounds with 3'-lipophilic, 3'-5'- dilipophilic or 3'-5'-dilipophilic-4'-hydrophilic groups on (E)-2-benzylidene moiety were shown to possess high cytotoxic effects [8,9]. Indanone constitutes the nucleus of a series of compounds with a wide range of biomedical applications [9]. 5-Halo-1-indanones are probably the nearest to the molecule whose biomedical interest is better documented. Thus, the bromoderivative has been used in the synthesis of pesticides and antihelmintics. Chloroderivative, finds its use in the preparation of anticonvulsants, fungicides and dozapine analogues of high anticholiergic activity as well as metabolite of compounds of diaryl sulfonylurea types, which have great activity in many solid tumors [10]. The estrogen receptor-alpha (ERa) is a complex protein involved in breast cancer, thus an important target for the anti-cancer drug discovery. The breast cancers are estrogen receptor (ER) positive and depend on estrogen for growth and the only possible way for the treatment of this type of receptor is blocking estrogen mediated interaction with the receptor. Tamoxifen, a potent drug for breast cancer, inhibits estrogen receptor-alpha (ERa). In this study, we synthesized thirteen (E)-2-arylidene-1-indanones related novel compounds for targeting the estrogen receptor-alpha and act as potent anti breast cancer drug.
2. Materials and Methods

2.1. Synthesis and Characterization of 2-Arylidene Indan-1-Ones

The classical methods for preparation of biological compounds involve the synthesis of the indan-1-one moiety, followed by aldol condensation with suitable aldehydes [11, 12]. Basavaiah et al. [13] have reported a novel route for the synthesis of 2-arylidene indan-1-ones. Arylidene moiety was first prepared and then the indan-1-one framework was constructed. It is a convenient one-pot synthesis of (E)-2-arylidene indan-1-ones from tert-butyl-3-aryl-3-hydroxy-2-methylene propanoates, the Baylis-Hillman adducts, obtained from t-butylacrylate, [14,15] which essentially involved one inter-and one intra-molecular Friedel Crafts reaction in an one pot operation. Drawing inspiration from the above synthetic process, indanones with various structural modifications were synthesized using different substrates and Baylis Hillman adducts (Scheme-1). They were characterized by spectral and elemental analysis. The E-configuration, of these compounds was determined by $^1$HNMR according to the method of Bayer et al. [16, 17, and 18].

![Scheme 1](image)

**Scheme 1.** Reagents and Condition: i) Arenes (Toluene), H$_2$SO$_4$, ref., 1-2h.; ii) TFAA, CH$_2$Cl$_2$, 50 °C, 2h

(a) All reactions were carried out in 10 Mmole scale. The synthesis of the indan-1-one was achieved in two steps. In the first step, Baylis Hillman adducts of the corresponding aldehyde and t-butyl acrylate was prepared. This was carried out in solid phase using silica (200-400 mesh) in the presence of DABCO (1.5 Mmole) as catalyst.

(b) In the second step, the hydroxy ester formed was dissolved in different alkyl benzenes (5 ml) and treated with a catalytic amount of conc. H$_2$SO$_4$ (0.4ml) under reflux for 1-2 hr. The solvent was removed under reduced pressure and the residue was treated with TFAA (2 ml) in refluxing CH$_2$Cl$_2$ for 2 hr. The residue was purified by column chromatography using silica gel (5% EtOAc-hexane) which afforded crystals. All the products gave satisfactory IR, $^1$HNMR (400MHz), $^{13}$CNMR (100MHz) spectral data and elemental analysis.

(c) The yields mentioned in Table 1 are the yields of pure indanones obtained after silica gel column chromatography (5% EtOAc-hexane) or by crystallization from CHCl$_3$-hexane (2:3) mixture. d) Structure was also confirmed by mass spectral analysis. Selected data for 4a:

- M.P:118 C, IRmax 1693, 1627 and 1581cm$^{-1}$, HNMR (400MHz CDCl$_3$): 7.65-7.70 (m,4H), 7.42-7.45 (m,5H), 3.98 (s,2H), 2.42 (s,3H), 13CNMR (100MHz CDCl$_3$): 18.12, 21.29, 31.31, 32.21, 76.88, 77.13, C17H14O: C,87.17; H,6.02 and found C,87.25; H,6.08.

2.2. Anticancer Activity

Human breast cancer cell lines (MCF-7) are obtained from the American Tissue Culture Collection (ATCC) (Rockville, MD) and were cultured in 100 µl of Roswell Park Memorial Institute medium (RPMI) 1640 media supplemented with 10% fetal bovine serum (FBS), 100 µg/ml penicillin and 100 µg/ml streptomycin. MCF-7 and MDA-MB-231 cells were incubated overnight at 37°C for cell attachment. Cell viability and Cytotoxicity of compounds in cells was determined by MTT assay based on mitochondrial reduction of yellow MTT tetrazolium dye to a highly colored blue formazan product. The cell suspension of $10^5$ cells/ml was prepared in complete growth medium. Stock solutions of compounds were prepared and serially diluted with complete growth medium containing 100 µg/ml penicillin and 100 µg/ml streptomycin. MCF-7 and MDA-MB-231 cells were incubated overnight at 37°C in 5% CO for cell attachment. Cell viability and Cytotoxicity of compounds in cells was determined by MTT assay based on mitochondrial reduction of yellow MTT tetrazolium dye to a highly colored blue formazan product. The cell suspension of $10^5$ cells/ml was prepared in complete growth medium. Stock solutions of compounds were prepared and serially diluted with complete growth medium containing 100 µg/ml penicillin and 100 µg/ml streptomycin. The 100 µL of cell suspension was added to each well of the 96-well tissue culture plates. Cells in 96-well plates were incubated with various concentrations of test compounds for 48 hr at 37°C. The above media was replaced with 90 µL of fresh serum free media and 10 µL of MTT reagent (5 mg/ml) and plates were incubated at 37°C for 10 min. The plates were measured for absorbance at 570
nm using ELISA reader and cell viability was determined.

2.3. Docking Studies Protein Preparation and Glide Docking

Ligands were drawn on Chem Draw tool, cleaned and minimized with Merck Molecular Force Field (MMFF) using LigPrep in Schrodinger suite. The structure of the estrogen receptoralpha downloaded from protein database bank (pdb id: 3ERT) and protein was prepared for molecular docking by using the protein preparation wizard of the Schrodinger suite. The water molecules in the crystal structure were removed and energy minimized with force field of OPLS2005 and RMSD of 0.30 Å using the protein preparation wizard. Grid was generated using the centroid of workspace ligand in the crystal structure of the receptor. The prepared 12, 11 receptor grids were used for Glide XP docking using the Glide module of the Schrodinger suite. The docking was performed using default parameter option in the panel.

3. Results and Discussion

3.1. In vitro Anticancer Activity

The in vitro anticancer activity of all the synthesized compounds was evaluated against MCF7 cancer cell line using MTT assay method and Tamoxifen was used as the reference compound. All the synthetic compounds produced a dose dependant inhibition of growth of the cells. Out of 13 compounds, 6 compounds inhibited the growth of cytotoxicity against MCF-7 cancer cells. Compounds 12 and 9 were the most active (IC50 = 7.3_M) and compound 10, 13 and 5 also showed moderate activity with IC50 values 70.9_M, 61.9_M and 54.3_M, respectively.

3.2. Docking Studies

Extra precision docking was performed for compounds with estrogen receptor alpha (pdb id: 3ERT) and results were tabulated (Table 2). Maximum docking score was found for tamoxifen (Fig. 2), with -11.34 and glide energy of -30.83. All the thirteen compounds docked estrogen receptor hydrophobically, no hydrogen bonds were found for these compounds. Maximum docking score was found for compounds 10 and 9 with -7.46 and glide energy of -35.72 and -34.34 respectively (Table 2). The hydrophobic residues of phe 435, asp 273, leu 276, trp 305, cys 269, ala 431, his 434, leu 256, leu 440, glu 275, tyr 326, leu 309, val 313, ile 310, met 306, ile 438, leu 345 were docked with estrogen receptor alpha (Fig. 3). Compounds were analysed by their absorption, distribution, metabolism, excretion and toxicity (ADMET), using QikProp. For all compounds, the partition coefficient (QPlogPo/w), water solubility (QPlogS) values, percent human oral absorption and total surface area were from 300 to 700. These parameters showed fitness within the acceptable range defined for human use, thereby indicating their potential as drug-like molecules (Table 3).

![Image 1](https://example.com/image1.png)

**Figure 1.** (E)-2-benzylidene-1-teralone (1) and (E)-2 benzylidene-1-indanone (2).

![Image 2](https://example.com/image2.png)

A : Active site amino acids intercations of Tamoxifen with receptor,
B: hydrophobic interactions of Tamoxifen with receptor,
C: Docking interactions of Tamoxifen with receptor.

**Figure 2.** A and B Shows the hydrophobic interactions of Tamoxifen with estrogen receptor alpha, C: Docking of Tamoxifen with estrogen receptor alpha.
Figure 3. A and B Shows the hydrophobic interactions of Compound 9 with estrogen receptor alpha.

A: Active site amino acids interacions of Compound 9 with receptor,
B: hydrophobic interactions of Compound 9 with receptor,

Figure 4. A and B Shows the hydrophobic interactions of Compound 10 with estrogen receptor alpha.

A: Active site amino acids interacions of Tamoxifen with receptor,
B: hydrophobic interactions of Tamoxifen with receptor,
C: Docking interactions of Tamoxifen with receptor.

Table 1. Structural modifications using different substrates.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Arenes</th>
<th>Aryl (Yield %)</th>
<th>Phenyl</th>
<th>4-Methyl Phenyl</th>
<th>4-Isopropyl phenyl</th>
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<tr>
<td>4 a-c</td>
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<td>67</td>
<td>61</td>
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Table 2. Docking studies of all synthesized 2-arylidene indan-1-ones against estrogen receptor alpha.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Glide gscore</th>
<th>Glide energy</th>
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4. Conclusions

This study reports thirteen novel (E) 2-Arylidene indan-1-ones compounds which have in vitro activity against breast cancer and also displayed good binding interactions against estrogen receptor-alpha through docking studies. The anticancer activity suggests that among the synthesized compounds, 12 and 9 showed good activity against MCF-7 breast cancer cell line. Compounds 10, 9, 12 showed good docking score compared with known drug Tamoxifen against estrogen receptor-alpha. All thirteen compounds satisfied the ADME properties within the allowed ranges and showed drug-likeness with less toxicity. In vitro and in silico studies suggest that these compounds would be the potent drug against breast cancer.

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References


