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Original Article

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 IC_{50} 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.

INTRODUCTION

Chromones are an important class of oxygen-containing heterocyclic compounds. Synthetic and natural chromone derivatives possess important biological activities such as antitumor [1], antihepatotoxic [2], antioxidant [3], anti-inflammatory [4], antiallergic [5], estrogenic [6] and antibacterial [7] activities. The anticancer activity of chromones has also been related to its structural similarity with flavonoids.

Several compounds such as quercetin, genistein, daidzein, apigenin, biochanin A, kaempferol and naringenin that have chromone scaffold are known to bind to ER isoforms. Genistein, apigenin and kaempferol have a higher binding affinity 20 to 30-fold more for ERβ than ERα as indicated by a solid-phase binding assay [8-9].

The above literature precedence prompted us to investigate the docking and cytotoxicity of 2-vinylchromones on human breast cancer cell lines. Previous work in our own group had led to the synthesis of novel chromone derivatives such as 3,4-dihydro-2*H*-1-benzopyran-3,4 dione **1(a-c)** using $NaBH_4$ (unpublished results) and in another study *E*-3-aminochromen-4-one **2(a-b)** were synthesized (unpublished results). Herein, we report the *in silico* studies of chromone derivatives along with an assessment of cytotoxic activity of the compounds on MCF-7, MDA-MB-231 and MRC-5 cells.

MATERIALS AND METHODS

Determination of anticancer activity

MTT assay

$5x10^3$ cells/well were seeded in 96-multiwell flat bottom microtitre plate and cultured overnight in a humidified atmosphere of 5% CO_2 at 37 °C. The test compounds were serially diluted to the wells. Different concentrations ranging from 125µg/ml to 0.98µg/ml were analyzed. The cells were incubated for 24 h with test compounds with 5% CO_2 at 37 °C. After 24 h, 10 µl of MTT (5 mg/ml in 1X PBS) was added to each well. The cells were incubated for 3 h and the centrifuged at 1500 rpm for 10 min. The media were discarded and 150 µl of DMSO was added to each well. Optical density was measured using a microplate reader at 570 nm with reference optical density at 630 nm. IC_{50} values were calculated as the concentrations that show 50% inhibition of proliferation on the tested cell line.

Docking methodology

The compounds were modelled using the BUILD application of Maestro 8.0. The geometry was optimized by molecular mechanics using IMPACT in a dynamic environment using the standard TIP4P water model. The energy minimization was done using Optimized Potentials for Liquid Simulations 2005 (OPLS 2005) force field. Energy minimization was done using Polak-Ribier conjugate gradient and Truncated Newton conjugate gradient algorithms. The convergence threshold used was rms gradient of 0.01.

Conformational models of the ligands were generated. Docking of the ligands was carried out using an extra precision (XP) method called GLIDE (Grid-based Ligand Docking with Energetics) for flexible ligand docking. The ligands were prepared for docking using LIGPREP from Schrodinger's molecular modeling software [10]. The PDB entries chosen for the above studies were 2QTU, 2FSZ and 3ERT. The ligand interaction map was used to study the interactions of novel molecules with the residues of ER.

Statistical analysis

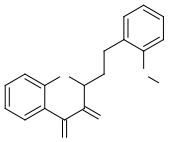
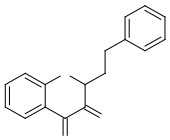
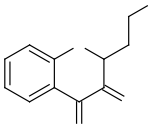
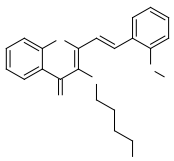
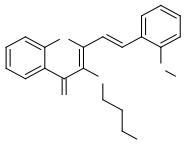
The experimental data obtained for each set of the experiment were expressed as the mean of change±SEM and analyzed by one-way and two-way ANOVA followed by a bonferroni post-test. Level of significance was set to $P \leq 0.05$. All the statistical calculations were performed using the evaluation version of Graph Pad Prism 5.1 statistical software.

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 hydroxy-tamoxifen (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 a complex of HT with ERβ. The compounds were modelled using the BUILD application of Maestro 9.6. The energy minimization was performed using optimized potentials for liquid simulations 2005 (OPLS 2005) force fields. In case of ERβ isoform, the docking studies were performed at the cognate site (2FSZ-101) as well as the allosteric binding site (2FSZ-103) of HT. The docking score of compounds **1(a-c)** and **2(a-b)** are indicated in table 1.

The docking studies indicated that **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 [12] was negligible. Amongst the analogues synthesized, best docking score for ERα was exhibited by compound 3-(butylamino)-2-[(*E*)-2-(2-methoxyphenyl) ethenyl]-4*H*-chromen-4-one **(2b)** containing aminoalkyl group and 2-[2-(2-methoxy phenyl)ethyl]-3,4-dihydro-2*H*-1-benzopyran-3,4 dione **(1b)** having a keto group at C-3 position. A comparison of the ligand interaction map represented in fig. 1 indicated that majority of the poses obtained for **2b** share the following common residues: Glu 353, Arg 394, Trp 383, Ala 350, Met 343, Leu 391, Ile 424, Leu 525, Leu 346, Leu 387 as compared with HT. Compound **2b** also depicted other significant interactions with the following residues: Asp 351, Leu 349, Leu 354, Leu 536, Met 528, Thr 347, His 524, Leu 428, Met 388, Leu 384, Phe 404, Ala 350.

Table 1: Docking score and glide energy of the compounds 1(a-c) and 2(a-b) against ERα and ERβ

S. No.	Compound	Structure	3ERT		2QTU		2FSZ (101)		2FSZ (103)	
			Docking Score	Glide energy of the model (Kcal/mol)	Docking Score	Glide energy of the model (Kcal/mol)	Docking Score	Glide energy of the model (Kcal/mol)	Docking Score	Glide energy of the model (Kcal/mol)
1	1a		-5.19	-36.75	-7.8	-40.29	-6.57	-34.87	-3.42	-23.76
2	1b		-7.1	-27.4	-7.78	-38.64	-6.7	-36.56	-4.25	-29.98
3	1c		-6.14	-26.81	-6.03	-30.17	-5.23	-27.11	-3.42	-20.63
4	2a		-6.4	-34.35	-3.09	-21.86	-6.83	-43.35	-4.61	-30.49
5	2b		-8.27	-55.59	-3.87	-24.97	-5.84	-39.08	-3.39	-26.98

3ERT is a complex of ERα with HT. 2QTU pdb entry is a complex of benzopyranones with ERβ. 2FSZ is a complex of HT with ERβ where docking studies performed at the cognate site of HT was labeled as 2FSZ-101 and the allosteric binding site was labeled as 2FSZ-103.

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 ID 3ERT).

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 IC_{50} value were seen for **1b, 2a** and **2b** in MCF-7 and MDA-MB-231. The IC_{50} value for **1b, 2a, 2b** were 62.5µg/ml, 31.25µg/ml and 15.625µg/ml, respectively in MCF-7 cell line. All the compounds exhibited an IC_{50} value of more than 125µg/ml in MDA-MB-231 cell line as indicated in table 2.

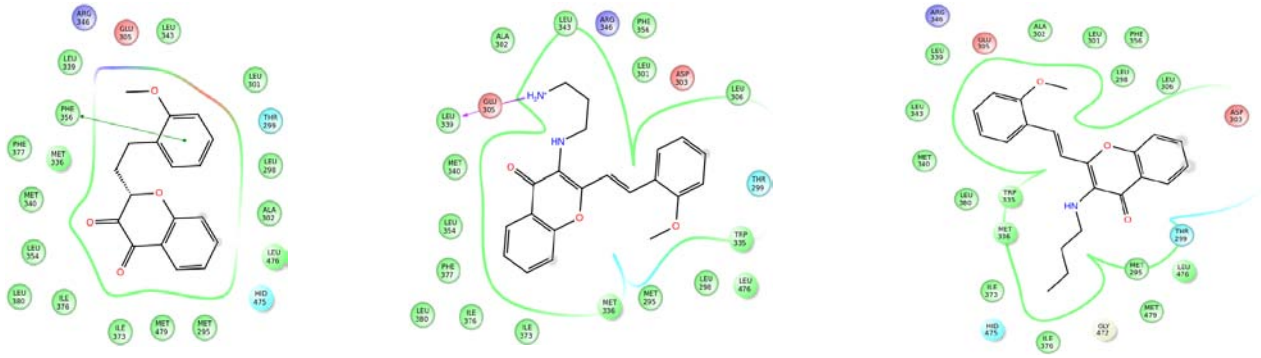
Table 2: Comparative IC_{50} value of compounds in MCF-7 and MDA-MB-231 cell lines

Compound	IC_{50}-MCF-7 (µg/ml)	IC_{50}-MDA-MB-231 (µg/ml)
1a	125	>125
1b	62.5	>125
1c	>125	>125
2a	31.25	>125
2b	15.62	>125

[A] **[B]**

Fig. 1: [A] Interactions of compound 1b; [B] Interactions of compound 2b

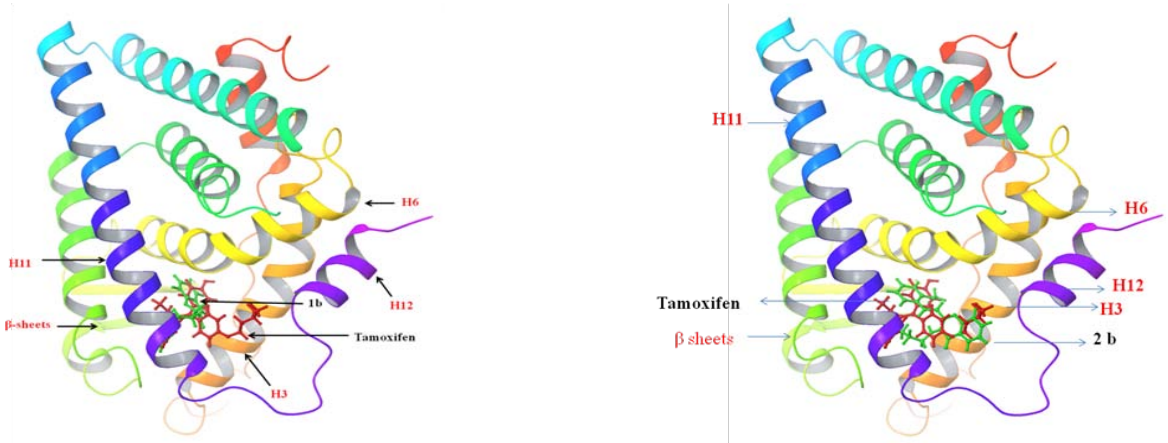
The docking results of compound **1b, 2a** and **2b** on ERβ (PDB ID 2FSZ) on the other hand did not exhibit interactions with the significant residues in the ERβ LBD. The results are presented in fig. 2.



[A] **[B]** **[C]**

Fig. 2: [A] Interactions of compound 1b; [B] Interactions of compound 2a; [C] Interactions of compound 2b

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.



[A] **[B]**

Fig. 3: [A] Three-dimensional structure of ERα docked with compound 1b; [B] Three-dimensional structure of ERα docked with compound 2b; where HT is represented in red and compound 1b and 2b in green

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.

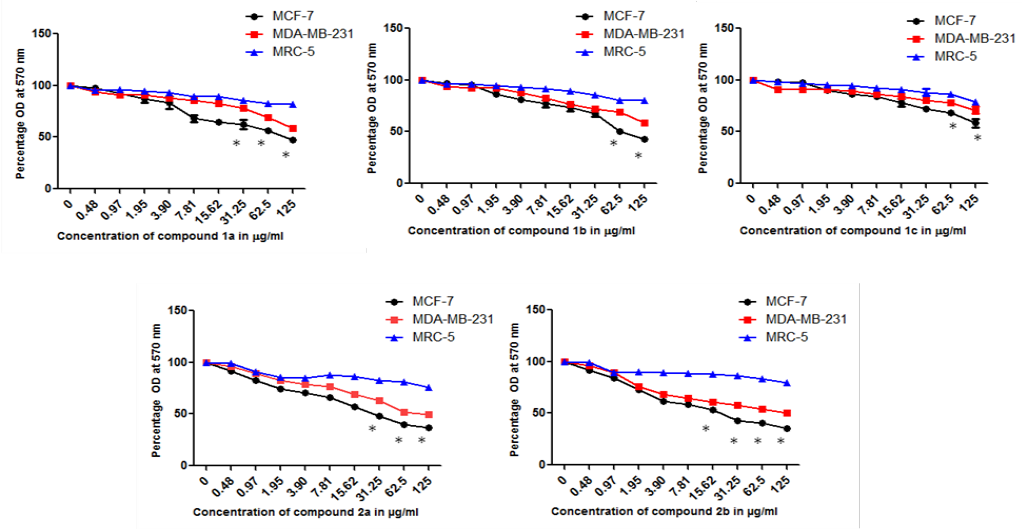


Fig. 4: Comparative graph for 1(a-c) and 2(a-b) compounds with % change in optical density in respective cell lines plotted against different concentrations of compounds. The data are mean±SEM from 2 samples for each group and analyzed by two-way ANOVA followed by Bonferroni Post-test where * P<0.01 significant from control

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-affinity binding which is further corroborated with a high docking score of **2b**. A comparison of docking residues of ERβ, on the other hand, did not reveal an interaction with these residues.

Further, the evaluation of the cytotoxic activity indicated that these novel compounds have exhibited significant cytotoxicity in ERα positive cell line instead of ERβ positive cell line. Studies on ligands such as genistein have clearly established that although it exhibits similar binding mode when complexed to ERα and ERβ [18] it exhibits 40-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

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