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Molecular docking study of chalcone derivative with Human estrogen receptor as target protein and its anti-cancer activity against HepG2 Cells

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Abstract: The compound, 3-[(2-Aminobenzyl)amino]-1-phenylbut-2-en-1-one was synthesized by the reaction of aminobenzylamine and benzylacetone (1:1) in warm ethanol. Induced fit docking study was carried out for the compound and compared with the co-crystal ligand. The oxygen atom of the co-crystal ligand interacts with the nitrogen atom of the residue ARG 394 at a distance of 2.9Å with the glide score of −10.16 and glide energy of −62.46kcal/mol and the nitrogen atom attached with the phenyl ring interacts with the residue (HIS 524) at a distance of 2.9 Å with the glide score of −8.19 and glide energy of 43.25 kcal/mol. The anticancer activity of the compound against HepG2 cells was assessed by MTT assay. The cancer cell line-HepG2 cells showed exponential responses towards the increasing concentration of compound. The compound showed IC_{50} values of 19µg/mL against the HepG2 cells.

Keywords: Synthesis of Chalcone; Anticancer activity; Docking study; IC_{50} value.

Introduction

Chalcones are a major class of natural products with widespread distribution in fruits, vegetables, spices, tea and soy based foodstuff and have recently been the subject of great interest for their interesting pharmacological activities[1]. Chalcones and flavonoids have been reported to be active anti-tuberculosis agents[2]. Breast Cancer, is the second most wide spread type of cancer after the lung cancer with the rate of 10.4% and also in 2008, 458,503 deaths caused due to breast cancer (World health organization international agency for research on cancer. Breast cancer is more than 100 times more common in men[3,4]. Both normal and breast cancer cells have receptors to bind estrogen and progesterone circulating in the blood[5].

Women who started having periods earlier or entered menopause later than usual have a high risk of developing breast cancer. Because their bodies have been exposed to estrogen for longer. Estrogen exposure begins when period starts and drops during the menopause. Obesity is one of the causes for cancer. Post-menopause obese and overweight women may have a high risk of developing breast cancer. Different lifestyle and eating habits of female in rich and poor countries are also playing the role of developing the cancer.

Estrogen interacts with the estrogen receptors ERα and ERβ, and it controls multi functions in mammalian tissues. It also plays an important role in female reproduction, bone formation, and cardio vascular and CNS health[6]. Since the introduction of selective estrogen receptor modulators (SERMs), much interest has been focused on this class of compounds as an alternative approach for hormone replacement therapy (HRT)[7].

The breast consists of billions of microscopic cells. These cells multiply in an orderly fashion- new cells are made to replace the once they died. But in cancer, the cells multiply uncontrollably and there are too many cells progressively more and more than there should be. The breast cells are positive and more likely to respond hormonal therapies with Tamoxifen, Raloxifene,Toremifene. These cells also have a better prognosis than cancers that are hormone receptor negative[5]. Tamoxifen (Nolvadex R) is a drug, taken orally as a tablet, which interferes with the activity of estrogen. Some of the common side effects of Tamoxifen are serious side effects of Tamoxifen include blood clots, stroke, uterine cancer, and cataracts. Side effects of Raloxifene are serious blood clots in the legs, lungs, or eyes. Other reactions including leg swelling/pain, trouble breathing, chest pain, vision changes. Therefore these side effects make these drugs are resistance for use and require studies on a better alternate.

Materials and methods:

Synthesis of the compound 3-[(2-Aminobenzyl)amino]-1-phenylbut-2-en-1-one.

To a warm ethanolic solution (25 ml) of 2-aminobenzylamine(0.25 g, 0.2 mmol), an ethanolic solution of benzylacetone (0.3 g, 0.2 mmol) was added dropwise and the resulting solution was refluxed for 3 h. The solution was then filtered hot and allowed to stand at room temperature. After slow evaporation of the solvent at 298 K, block-like colourless crystals of the title compound were obtained. They were filtered off, washed with cold methanol and dried [Yield 0.45 g, 83%].

Protein, ligand preparation and Induced fit docking:

The protein human estrogen acceptor has been downloaded from the Protein data bank (PDB id: 2IOK). The water molecules are removed and side chains have been fixed using the module prime and energy minimized using OPLS force field using the module protein preparation wizard. All computational works were performed on CentOS EL-5 workstation using the molecular modeling software Maestro[8]. GLIDE-5.5 (Grid-based Ligand Docking with Energetics) performs flexible (IFD) docking between the ligand molecule with a macromolecule, usually a protein. PyMOL software was used for graphical visualization, analyzing hydrogen bond interactions and producing quality images. The crystal structure of the compound was drawn using the software Chemsketch and energy minimized using the impact minimization. The ligand preparation is to prepare the three dimensional structure of drug like molecules in maestro format. The impact module performs conversions, apply corrections to the structures, generate variations on the structures and optimize the structures. The structures were minimized using impact energy minimization with 1000 cycles of Steepest Descent and 5000 cycles of Conjugate gradient.

Induced fit docking study was carried out for the compound and compared with the co-crystal ligand. The results are following:

Results and discussion:

Molecular docking study has shown that the compound has bind well at the active site of human estrogen acceptor. The oxygen atom of the co-crystal ligand interacts with the nitrogen atom of the residue ARG 394 at a distance of 2.9Å with the glide score of -10.16 and glide energy of 62.46 kcal/mol. In the compound, the nitrogen atom attached with the phenyl ring interacts with the residue (HIS 524) at a distance of 2.9 Å with the glide score of -8.19 and glide energy of 43.25 kcal/mol.

Table: Induced fit docking studies of the compound with the Human estrogen receptor

Compound	H-bond interaction D-H…A	Distance (Å)	Glide score	Glide energy (kcal/mol)
IOK (cocrystal)	(ARG 394)N-H…O O-H…O(GLU 353)	2.9 2.5	-10.16	-62.46
Chalcone derivative	N-H…N(HIS 524)	2.9	-8.19	-43.25

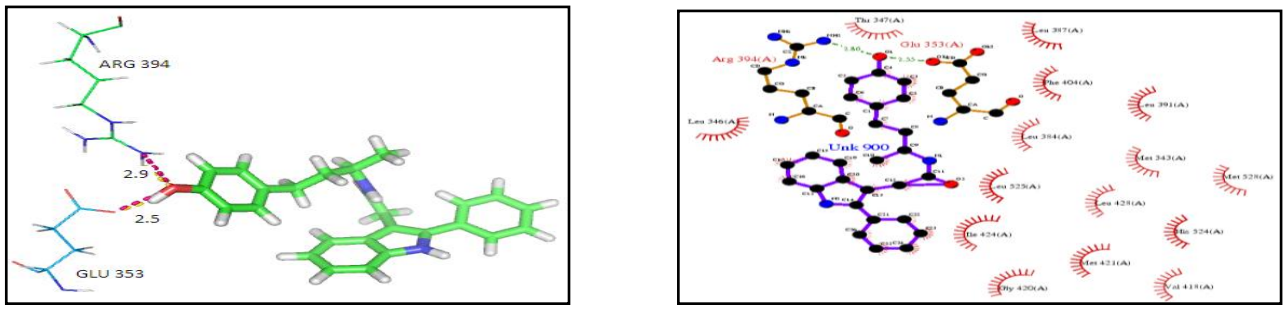

Figure:1 Interactions of Cocrystal ligand (IOK) at the active site residues: a) The pymol picture shows the interactions with the active site residues. b) ligplot shows the inteaction of the compoud at the active site and hydrophobic residues.

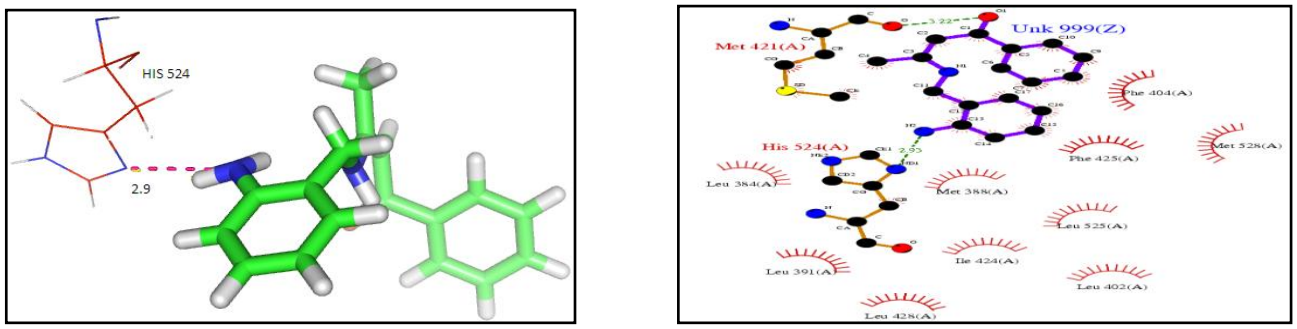

Figure:2 Interactions of chalcone at the active site residues: a) the pymol picture shows the interaction of the compound at the active site residue b) ligplot of the compound at the active site and hydrophobic residues.

Sample: Compound

Cell viability	Concentration in µg
92	5
81	10
77	15
52	20
41	25
33	30
11	35
8	40
6	45
5	50

IC_{50} value for compound is 19µg/mL

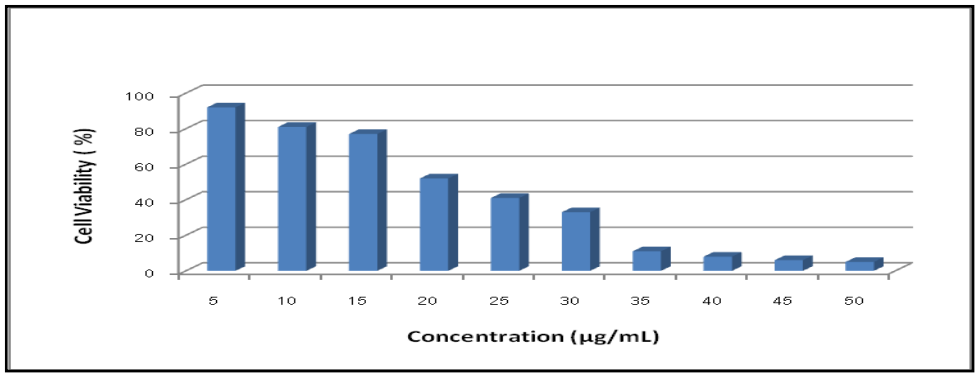

Figure: 3 Cytotoxic effects of compound against the HepG2 cell lines by MTT cell viability assay

MTT Assay:

The anticancer activity of the compound was checked by MTT assay. The stock solution of MTT dye (3-[4, 5-dimethylthiazol-2-yl]-2, 5-diphenyl tetrazolium bromide) 5 mg per ml,was made in sterile phosphate buffered saline. The lysis solution for the assay was prepared by mixing 0.6 ml of acetic acid and 99.4 ml of Di-methyl sulphoxide. The HepG2 cell line cells were incubated with the appropriate concentrations of test sample for 24 and 48 hours. Then after the incubation the consumed medium was carefully removed from all the wells of the assay plate and was replaced with freshly prepared sterile DMEM. The MTT dye (100µl per ml of stock solution) was added to each well and the culture plates were incubated for 3 hours in Carbon dioxide incubation chamber. The supernatant was aspirated carefully, taking care not to remove formed Formazan crystals with in the cells. The lysis solution in amounts equal to that of the DMEM added before incubation was added then the cells were lysated over 5 minutes and mixed well. Then 200 µl of the lysate from each well of 24 wells culture plate was transferred to the 96 well plate and then the optical density (OD) of the lysate was measured at 595nm using ELISA reader.

Results:

The anticancer activity of the compound against HepG2 cells was assessed by MTT assay. The cancer cell line - HepG2 cells showed exponential responses towards the increasing concentration of compound tested. The cells experienced a significant increase in viability at very low concentrations of compound with an eventual decline at the high concentrations of compound tested (Fig. 1). The compound1 showed IC_{50} values of 19µg/mL against the HepG2 cells.Thus the tested compound possesses very good anticancer activity against the HepG2 cells. Further studies on these samples may lead to isolation and designing of potential anticancer compound.

Conclusion

Docking study was carried out for the compound and compared with the co-crystal ligand. The oxygen atom of the co-crystal ligand interacts with the nitrogen atom of the residue ARG 394 at a distance of 2.9Å with the glide score of -10.16 and glide energy of -62.46kcal/mol and the nitrogen atom attached with the phenyl ring interacts with the residue (HIS 524) at a distance of 2.9 Å with the glide score of -8.19 and glide energy of 43.25 kcal/mol. Cancer cell line - HepG2 cells showed exponential responses towards the increasing concentration of compound and the compound showed IC_{50} values of 19µg/mL against the HepG2 cells.

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