Research Article

# COMPARATIVE DOCKING STUDIES OF ESTROGEN RECEPTOR INHIBITORS AND THEIR BINDING INTERACTION ANALYSIS

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**ABSTRACT**

Comparative docking studies have been performed on 10 drug molecules, which play a vital role in the treatment of breast cancer. These 10 drug molecules have the same target called Estrogen Receptor which acts as a DNA-binding transcription factor that regulates gene expression. The glide scores of these 10 drugs were compared to each other using the module Glide which is a part of the Schrödinger software, which provided a better understanding of the binding interactions of the ten drug molecules. Among the 10 drugs taken, toremifene had the lowest glide score, i.e. -9.82, which shows that it had better binding interaction with the protein.

**Keywords:** Breast Cancer, Estrogen Receptor, Molecular Docking, Glide, Binding Interactions, Schrödinger 2009.

## INTRODUCTION

Breast cancer is a cancer which originates in the tissues of the breast, mostly from the inner lining of the lobules or the milk ducts which supply milk. The cancer which is related to the ducts is called as ductal carcinoma[1], where as the cancer related to the lobules is called as lobular carcinoma[2].

Malignant breast neoplasm (breast cancer) may be invasive or non invasive. When the cancer has spread from the lobules to the other breast tissues, it is called as Invasive Breast Cancer.[3] Non Invasive Breast Cancer, is the cancer which has not spread to the other tissues in the breast, it is generally called as in situ.

Estrogens[4] are one among the five steroidal hormones which occur naturally as the female reproductive hormone. The mechanism of the estrogen receptor is given in figure 1.

Even before the hormone estrogen is used up by the body, it has to bind to the proteins called estrogen receptors. Breast cancers are tending to be sensitive to the hormone estrogen which will enhance the growth of the tumor. The cancerous cells which have the estrogen receptor on their surface are thus called as, estrogen receptor-positive cancer or ER-positive cancer. The mechanism, of the action of estrogen receptor is given in the figure 1. The proteomics of estrogen receptor signifies that it is of two different forms, which is usually referred to as α and β[5]. These are encoded by two different genes ESR1 and ESR2 which are present on the sixth and fourteenth chromosomes respectively. The ERα protein is expressed in the breast cancer cells, endometrial, and epithelium of efferent ducts. The expression of the ERβ is found in kidney, brain, bone, prostate, heart, lungs, and endothelial cells[6]. The binding and the functional selectivity of the ER helix with 12 domains play a vital role in the determination of the binding interactions with coactivators and corepressors along with the effect of the ligand on their respective agonists and antagonists[7]. E.g. 17-beta-estradiol binds equally well to both the receptors. In 70% of the cases the estrogen receptor is over expressed which are referred to as ER-positive.

In order to explain the mechanism of the tumorigenesis in the breast cancer two hypothesis have been proposed, they are:

1. Binding of the estrogen hormone to the estrogen receptor ER stimulates the proliferation of the mammary cells which results in the increased cell division and DNA replication, thus leading to mutations.
2. Genotoxic wastes are produced by the metabolism of the estrogen.[8]

In the study conducted includes the comparative docking of the 10 drug molecules, Chlorotrianisene, Clomifene, Estradiol, Estriol, Estrone, Ethinyl Estradiol, Fulvestrant, Quinestrol, Tamoxifen, and Tormifene.

1. Chlorotrianisene: Chlorotrianisene which is a non-steroidal synthetic estrogen is used to prevent the breast engorgement, treating the deficiencies in the ovary function, infertility and menopausal symptoms[9].
2. Clomifene: Clomifene is an ovulatory stimulant which is administered orally that acts as a selective estrogen receptor modulator (SERM). It is primarily used to induce ovulation [10].

3. Estradiol: Estradiol is the principal intracellular human estrogen, which is used for treating the urogenital symptoms associated with post –menopausal atrophy of the vagina or the lower urinary tract [11].
4. Ethinyl Estradiol: Ethinyl estradiol is synthetically derived from the natural estrogen estradiol. It is primarily used to treat severe menopausal symptoms, female hypogonadism and is used in the contraceptives[12, 13].
5. Quinestrol: Quinestrol is used in treating hot flashes seen during menopause, also used to treat breast and prostate cancer. It is the active metabolite of ethinyl estradiol with the name 3-cyclopentyl ether[14].
6. Estriol**:** Estriol is the major and naturally occurring estrogen of the three estrogens produced during pregnancy in the human fetus. It is used as a test to determine the general health of an unborn fetus. It is considered to be less carcinogenic [15].
7. Estrone: Estrone is prepared synthetically or occurs naturally and is produced primarily from the androstenedione from the gonads or adrenal cortex. Used for the management of premenopausal and postmenopausal symptoms [16].
8. Fulvestrant: Fulvestrant for intramuscular administration is an estrogen receptor antagonist without known agonist effects. For the treatment of hormone receptor positive metastatic breast cancer in postmenopausal women with disease progression following anti-estrogen therapy[17].
9. Tamoxifen**:** Tamoxifen is an anti-estrogen, belonging to a class of drugs called selective estrogen receptor modulators (SERM's), used in treating and preventing breast cancer[18].
10. Toremifene: Toremifene is an antineoplastic hormonal agent which is primarily administered in the treatment of metastatic breast cancer usually after attaining menopause in women with estrogen receptor-positive or receptor-unknown tumors [19].

**Mechanism of Estrogen Receptor**

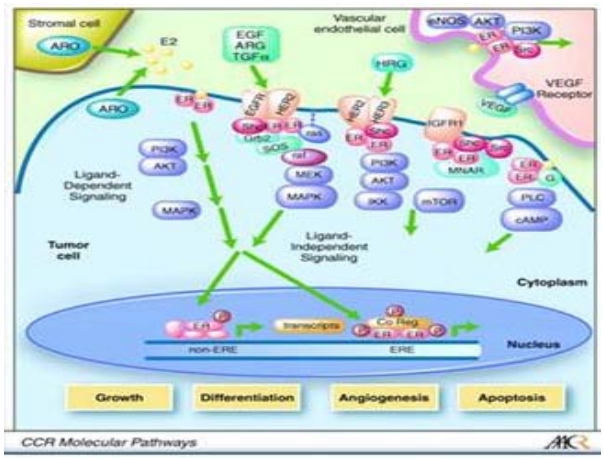



**Figure 1:** Mechanism of Estrogen Receptor.

## MATERIALS AND METHODS

### Docking Studies

The docking studies of the above 10 drug molecules with the protein have been carried out by the application LIGAND DOCKING in the module GLIDE of SCHRODINGER software. By the help of this tool we can predict the best binding interaction between the ligand and the protein. Creating an output directory for every work is a must while working in Schrodinger. Before initiating the ligand preparation, the structures have to be converted into maestro format (.**mae** format) from **.mol** format. The structures of the 10drug molecules have been drawn with the help of the, CHEMBIODRAW ULTRA 11.0 tool. Each structure has been imported into the MAESTRO 9.0.211: from the project table and are then exported from the mol format to the .mae format.

### Ligand Preparation

LigPrep or ligand preparation is an application where we generate a simple 2D structure to 3D structure which includes the generation of the tautomeric, stereochemical and ionization variations. LigPrep helps in generating an accurate 3D molecular model. The important characteristic of LigPrep application is the energy minimization with optimized potentials for liquid simulations-2005 (OPLS_2005) as the applied force field which filters customized ligands which can thus be used for further computational analysis. All the 10 ligands have been minimized using Schrödinger suite, whose corresponding results are given in the table 1.

The output folder for LigPrep will be displayed as out.maegz [20].

### Impact Minimization

The output folder of LigPrep is used as the input folder for impact minimization. The impact minimization is performed with the help of IMPACT module in which we have a tool called minimization. Among many conformers obtained in the LigPrep, the conformer with least potential energy will be minimized with the help of impact minimization under the applied OPLS_2005 force field. The significance of impact minimization is to observe the Lennard Jones Energy, which should be in negative [21].

### Protein Preparation

The protein was retrieved from PDB (http://www.pdb.org) with the PDB id 1UOM**.** The ProPrep was processed with the help of the protein preparation wizard from the workflows option of the Schrodinger suite. The force field applied for the preparation of the protein is optimized potentials for liquid simulations-2005 (OPLS_2005). The water molecules, hetero atoms, residues were deleted while one of the chains is retained along with H-bond. The active site of the protein is identified by the help of PDB-CASTp and Q-site finder [22].

**Table 1:** Details of the ligands or drugs with the minimized energies

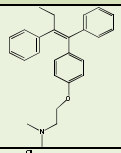
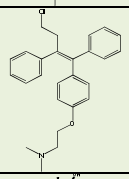
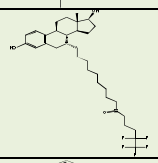
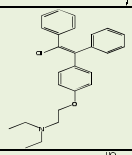
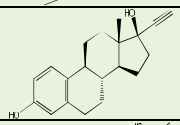
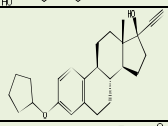
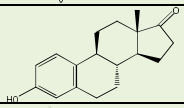
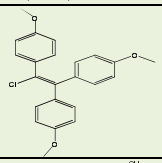
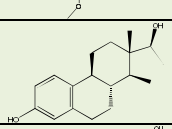
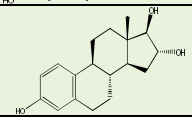
| S. No | Name | Structure | Molecular formula | Molecular weight | Minimized energy |
|---|---|---|---|---|---|
| 1. | Toremifene |  | $C_{26}H_{28}ClNO$ | 405.96 | 48.616593 |
| 2. | Tamoxifen |  | $C_{26}H_{29}NO$ | 371.5146 | 50.506078 |
| 3. | Fulvestrant |  | $C_{32}H_{47}F_5O_3S$ | 606.771 | 37.475968 |
| 4. | Clomifene |  | $C_{26}H_{28}ClNO$ | 405.96 | 45.340806 |
| 5. | Ethinyl-estradiol |  | $C_{20}H_{24}O_2$ | 296.4034 | 27.452440 |
| 6. | Quinestrol |  | $C_{25}H_{32}O_2$ | 364.5204 | 51.464681 |
| 7. | Estrone |  | $C_{18}H_{22}O_2$ | 270.3661 | 30.887580 |
| 8. | Chloro-trianisene |  | $C_{23}H_{21}ClO_3$ | 380.864 | 32.932608 |
| 9. | Estradiol |  | $C_{18}H_{24}O_2$ | 272.382 | 30.337806 |
| 10. | Estriol |  | $C_{18}H_{24}O_3$ | 288.3814 | 34.450553 |

**Table 2:** Schrödinger Docking Results.

| S.No | DRUG NAMES | G-SCORE | NO.OF H-BONDS | RESIDUES | H-BOND DISTANCE in Å |
|---|---|---|---|---|---|
| 1. | Estriol | -1.68 | 2 | ASP 351<br>PRO 355 | 1.823<br>2.011 |
| 2. | Estradiol | -1.91 | 1 | ASP 351 | 1.800 |
| 3. | Fulvestrant | -8.65 | 1 | GLU 353 | 1.746 |
| 4. | Ethinyl Estradiol | -2.72 | 1 | ASP 351 | 1.552 |
| 5. | Estrone | -2.34 | 1 | ASP 351 | 1.855 |
| 6. | Clomifene | -3.83 | 0 | - | 0 |
| 7. | Chlorotrianisene | -2.01 | 1 | PRO 535 | 1.952 |
| 8. | Quinestrol | -2.70 | 0 | - | 0 |
| 9. | Toremifene | -9.82 | 1 | ARG 394 | 2.210 |
| 10. | Tamoxifen | -9.55 | 1 | ARG 394 | 2.427 |

### Grid Generation

The receptor-grid is generated by the help of the module glide. Grid generation represents the physical properties like volume of the receptor (specifically the active site) that is needed for carrying out the ligand-docking process. Import the output file of the protein preparation; also define the receptor, active site, the positional constraints, and then monitor the generation of the grid calculation. The generated receptor grid is used for the comparative docking studies conducted in ligand docking procedure[23].

### Docking

The docking has been carried out by the application LIGAND DOCKING which is present in the module GLIDE5.0 version in the extra precision (XP) mode for clear and accurate details along with Epik state penalties to docking score. Select the selected entries for the ligand to be docked. Also load the output file of the grid generated. The output files of impact minimization of all the drugs are taken as the input files for the docking process. The docking of each drug along with the grid will be carried out which generates conformational changes with respect to the active site of the protein, estrogen receptor (PDB ID- 1UOM). The glide scores or docking scores will be displayed in a text document. Thus, the ligand which has the least glide score will be considered to have the best docked pose or best glide score.

## RESULTS

The glide scores of the above 10 drug molecules are tabulated in the table-2, which were docked using the module GLIDE 5.0 version of the Schrodinger software. The table-2 shows the G-score, number of hydrogen bonds, interacting residues, H-bond distance and glide or binding energy of all the drug molecules. The stability of the docking of the drugs with the protein depends on the above parameters, and thus the binding interactions describe how well the drug has interacted with the protein estrogen receptor retrieved from protein data bank with the PDB ID- 1UOM. After performing the docking for all the 10 drug molecules, the docking simulations resulted in a very closely related crystallographic structure which supports our study. The residue number ASP 351 plays an important role as it acts as the active site in the docking studies of the target estrogen receptor and all the 10 drug molecules.

## DISCUSSION

The drug molecules taken for the comparative computational docking studies viz., chlorotrianisene, clomifene, estradiol, estriol, estrone, ethinyl estradiol, fulvestrant, quinestrol, tamoxifen, toremifene, were docked against the protein and the best simulated results among all the compounds were evaluated based upon the glide score given in table 2. The glide scores of the ligands are estriol (-1.68), estradiol (-1.91), fulvestrant (-8.65), ethinyl estradiol (-2.72), estrone (-2.34), clomifene (-3.83), quinestrol (-2.70), chlorotrianisene (-2.01), toremifene (-9.82), tamoxifen (-9.55). Out of these ligands toremifene and tamoxifen were found be best docked to the estrogen receptor protein (PDB ID- 1UOM) with -9.82 and -9.55 glide scores respectively. Thus from the study conducted, depending upon the G-score, it is evident that these two drugs dock well with the protein (PDB ID-1UOM) which is shown in figure-2 and figure-3 respectively.

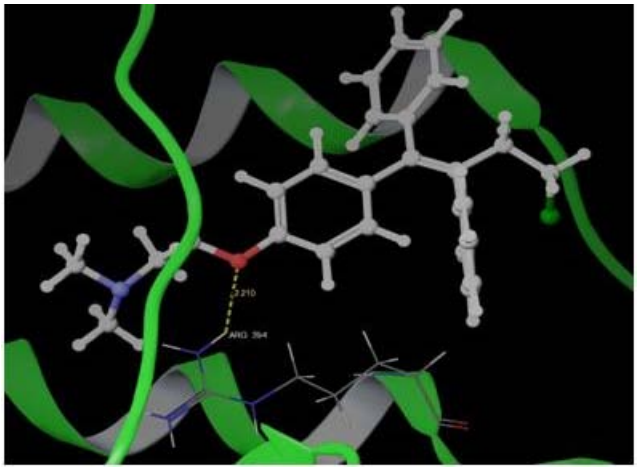


**Figure 2:** Interaction of Toremifene with ARG 394 protein in active site.

**Figure 3:** Interaction of Tamoxifen with ARG 394 protein in active site.

## CONCLUSION

The binding interaction of the ligand-protein has a significant role in the structural based drug designing. This approach has been successful in demonstrating that the two ligands toremifene and tamoxifen showed high binding affinity towards the protein, estrogen receptor. Among these two ligands, toremifene had high affinity against the target, estrogen receptor. According to the computational simulation docking studies, the drug toremifene showed highest binding affinity towards the protein estrogen receptor with -9.82 as the glide score, as given in table 2. Thus it is needed to be tested and evaluated in the laboratory for further analysis. We finally conclude that this drug acts as a potential drug in the treatment of breast cancer.

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