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Computational binding analysis and toxicity evaluation of estrogen receptor with estradiol and the approved SERMs raloxifene, tamoxifen, and toremifene

Esma Eryilmaz Dogan

Department of Biomedical Engineering, Faculty of Technology, Selcuk University, Konya, Turkey

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Abstract

Estrogen receptor is a key feature of many complex disorders. Natural estrogen ligand, estradiol has been investigated in the pharmaceutical aspect of breast cancer, Parkinson's, Alzheimer's, risk of stroke in postmenopausal women, and dementia. From the similar manner, synthetic selective estrogen receptor modulators (SERMs) have been investigated, and their pharmaceutical effects have been evaluated in compared to the natural ligand, estradiol, in literature. To design better alternatives to the approved SERMs and to improve the clinical observations, it is crucial to understand the molecular basis of drug-target interactions of estrogen receptor with the natural and synthetic ligands in a comparative manner. We used molecular modeling softwares PyRX, Avogadro, and Arguslab for in silico calculations. The results were analyzed using PyMol. We, in this study, provided a computational binding analysis of the estrogen receptor with the endogenous ligand estradiol and the FDA approved SERMs raloxifene, tamoxifen, and toremifene. We investigated the toxicity profile of the SERMs and estradiol and interpreted the results according to the reported clinical observations. We found that designing new molecules based on the estradiol structure instead of the approved tamoxifen analogs could result in better clinical observations for future estrogen targeting therapeutics.

Keywords: Estrogen receptor; estradiol; in silico; tamoxifen; raloxifene; molecular docking; toxicity

Introduction

Breast cancer, described by uncontrolled growth of epithelial cells in breast is the second most common type of cancer in women. It is also seen rare cases in men. Over time, breast cancer has been classified based on the mechanism and the pathways of carcinoma, and these classifications have resulted in the fundamental targets for prognosis and treatment of the disease. Hormonal targets such as progesterone, androgen, and especially estrogen receptors (ER) constitute an important group of molecular targets as the hormone-receptor positive breast cancer constitutes two cases out of three diagnosis according to the American Cancer Society [1]. Estrogen receptor is actively used in today's diagnosis of breast cancer and the treatment has been based on the level of estrogen receptor found in the body.

***Coresponding Author:** Esma Eryilmaz Dogan, Department of Biomedical Engineering, Faculty of Technology, Selcuk University, Konya, Turkey
E-mail: eeryilmaz@selcuk.edu.tr

Estradiol, the natural and endogenous ligand, also known as E2 is a hormone binding the estrogen receptor, circulating within the human body, and acting as a main female sex hormone. Estradiol plays important roles in many biological process such as regulating ERα levels in hypothalamic neurons [2], signaling for estrogen dependent behavior [3], and protecting hippocampal neurons [4]. Its neuroprotective effect has also been clearly established from animal studies [5, 6]. Binding of estradiol with ER gives insight regarding binding energy, binding pocket, and other binding related parameters. Therefore, we used estradiol for investigating the binding dynamics of ER and comparing the results with the FDA approved synthetic ligands, raloxifene, tamoxifen, and toremifene, called selective estrogen receptor modulators (SERMs). Binding of estrogen receptor with a bioactive small molecule has been manipulated to control the functionality of estrogen with synthetic ligands. For that, tamoxifen has been approved by FDA in 1977 to treat ER+ breast cancer upon with the proven benefits of the molecule in clinic. However, later studies have shown that patients treated with tamoxifen have been found developing an

increased risk of liver tumor and endometrial cancers [7-9]. Those results have led to the search for better alternatives to tamoxifen. Therefore, its structural analogs toremifene and raloxifene have been approved later, and their clinical results of toxicity are not quite convincing yet.

Binding parameters of ER with a ligand could be experimentally determined by controlling equilibrium binding assays performed mostly with radiolabeled compounds. Computational investigation of binding details, however, is less time-consuming and relatively easy to perform. Therefore, we, in this study, investigated the binding parameters including the types of interaction, association constant, and the binding energy of the complex structure of estrogen with bounded ligands. We tried to understand the reported clinical results with the resulting molecular based computational analysis. Receptor protein estrogen is bound with the natural ligand estradiol, and synthetic SERMs, tamoxifen, toremifene and raloxifene. We, in this study, investigated the binding affinity and the types of binding between the natural and the synthetic ligands listed above in a comparative manner using computational technique, molecular modeling, and molecular docking procedure.

Materials and Methods

Ligand Structures Preparation

SMILES codes of the small molecule ligands estradiol, raloxifene, tamoxifen and toremifene were obtained from the website of PubChem, the National Institutes of Health (NIH), USA. Four ligand molecules approved by FDA, their DrugBank identification numbers, the first year of approval, and their SMILES codes were given in Table 1. The 2D and 3D structures of the ligands were provided in Figure 1. The sdf files of the ligands were downloaded from the DrugBank website, https://www.drugbank.ca/ [10], and the molecules were minimized to their stable 3D configuration using the geometry optimization tool of Avogadro software to be used in molecular docking procedure. The minimized 3D structures obtained from Avogadro were verified with the 3D structure provided in DrugBank. Geometry optimization was repeated many times till no change is seen in the molecular geometry meaning that a stable minimized structure was reached. ArgusLab [11] and PyRX [12] softwares were also used for energy minimization of the ligand structures, but they could not reach a stable configuration of their molecular geometry.

Table 1. The natural and the FDA approved synthetic ligands interacting with ER. The chemical names of the molecules, their ID codes, the year of approval, and the SMILES codes

Chemical name	Drug Bank ID	Year of approval	SMILES
Estradiol	DB00783	1954	[H][C@@]12CC[C@H](O)[C@@]1(C)CC[C@]1([H])C3=C(CC[C@@]21[H])C=C(O)C=C3
Raloxifene	DB00481	1997	OC1=CC=C(C=C1)C1=C(C(=O)C2=CC=C(OCCN3CCCCC3)C=C2)C2=C(S1)C=C(O)C=C2
Tamoxifen	DB00675	1977	CC\C(=C(/C1=CC=CC=C1)C1=CC=C(OCCN(C)C)C=C1)C1=CC=CC=C1
Toremifene	DB00539	1997	CN(C)CCOC1=CC=C(C=C1)C(=C(\CCCl)C1=CC=CC=C1)\C1=CC=CC=C1

Target Molecule Preparation

The crystal structure of ER was downloaded from the Protein Data Bank, http://www.rcsb.org/ [13] in a complex form of the protein molecule itself with different ligands and water molecules. For searching ER structure in Protein Data Bank, we restricted the database search to `Homo sapiens only', and the crystal structure of 6SQ0 at the resolution of 1.77 Å was downloaded [14]. The ER structure was cleaned by removing miscellaneous two groups using ArgusLab, a molecular modeling software package (http://www.arguslab.com/ arguslab.com/ArgusLab.html). We kept water molecules to able to observe possible hydrogen bindiureg with the ligands. The cleaned pdb file of the target molecule ER was saved to be used in docking procedure.

Molecular Docking

Molecular docking of the ligand molecules into the ER was performed by using PyRX, an open source virtual screening software, with an exhaustiveness value of 8 [12]. The minimized 3D structures of the ligands were opened in PyRX and converted to pdbqt file. Only chain A of all ligands was incorporated into the blind docking. Vina search space was selected as a cube of 25x25x25 Å3. The best possible interaction poses and their interaction energies were calculated by AutoDock Vina. Visualization and analysis of the docking poses of the complex structures were performed by PyMol, a molecular graphics system [15].

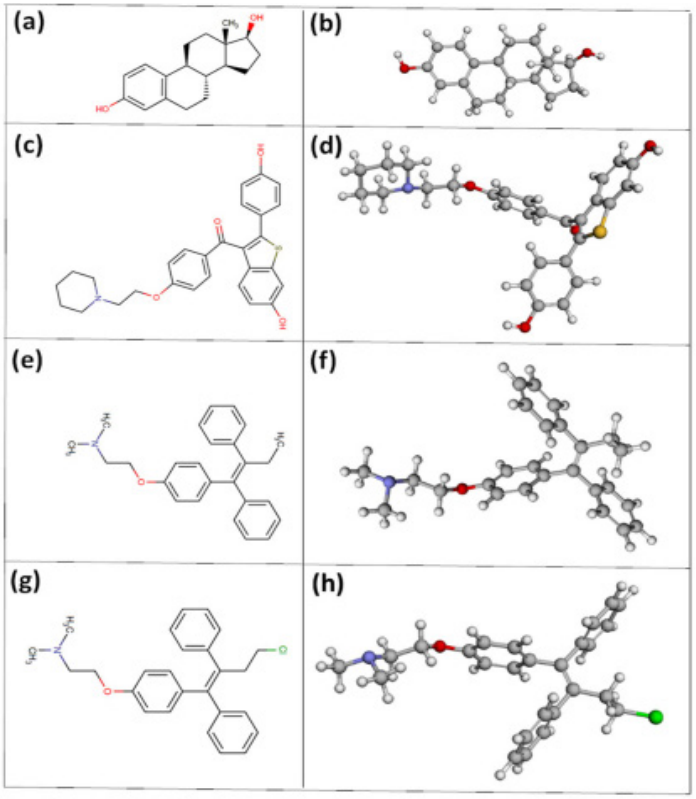

Figure 1. The 2D and 3D minimized structures of estradiol (a,b), raloxifene (c,d), tamoxifen (e,f), and toremifene (g,h)

Results

Molecular Docking

In silico molecular docking is often used to study the binding affinity of a drug candidate with a receptor molecule, mostly proteins. We, in this study, investigated the theoretical interaction profile of the estrogen receptor bounded with the natural ligand estradiol and the three FDA approved drugs raloxifene, tamoxifen, and toremifene to compare the binding parameters of the natural ligand with the synthetic ones and to understand their clinical observations better. The complex structure of the ER upon molecular docking of four ligand molecules was shown in Figure 2. The lowest energy binding poses of the three synthetic ligands were computationally observed as bounded at the same binding pocket of ER with estradiol as shown in Figure 2.

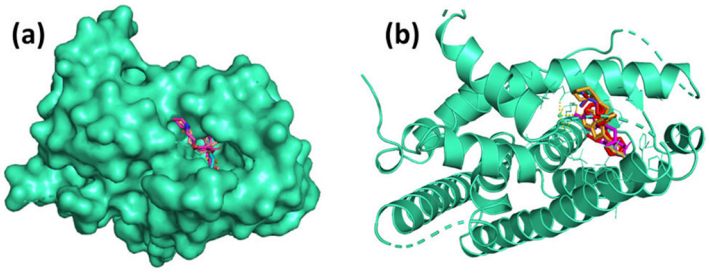

Figure 2. a) The overall topology of ER and b) the binding site of four ligands, estradiol, raloxifene, tamoxifen, and toremifene docked into the same binding pocket of ER (6SQ0).

The binding energies of the ER - ligand complex structures calculated by AutoDock Vina and shown in Figure 3 were provided in Table 2. Estradiol, as expected, was resulted in the highest number of polar bonds in the bond lengths of 3.2 Å, 2.4 Å, and 2.3 Å. Estradiol bound ER was resulted in one of the highest energy of -9.7 kcal/mol. Raloxifene which was approved in 1997 followed the natural ligand estradiol with the same binding energy and only one polar bond in a length of 3.2 Å. Tamoxifen and toremifene approved in 1977 and 1997, respectively, followed them with the interaction energy of -8.8 kcal/mol and with only one polar bond of 2.6 Å. An animal study performed on rats showed that raloxifene resulted in more stable down-regulation of ERβ and ERβ2 after 72 h of treatment compared to tamoxifen and toremifene [16]. The results of this animal study support our computational results leading to the more stable binding complex of raloxifene bound with estrogen than the other two ligands, tamoxifen and toremifene. Time dependent regulation of ODC (ornithine decarboxylase), an essential enzyme for cell growth was also studied with estradiol and the SERMs mentioned [16]. The enzyme activity study on the comparative efficacy of estradiol and the SERMS showed that tamoxifen and toremifene resulted in a similar time–dependent regulation of ODC, but the marked effect was less than that of estradiol. And yet, Raloxifene which was resulted in the same binding energy with natural estradiol showed even weaker ODC activity. When we compared the effect of estradiol and the synthetic SERMs on the induction of ODC genes, estradiol showed a quite high activity than those of SERMs. Moreover, another animal study showed that even though raloxifene increased the estrogenic effect in brain, that effect of raloxifene did not resulted in positive influence of estrogen in cognitive performance of brain [17]. That might be related to the less number of interactions with a specific binding site of estrogen performed by estradiol, but not by raloxifene. Neuroprotective effect of estradiol and some SERMs have also been studied on animals, and different mechanisms of neuroprotection have been found in SERMs and in estradiol [18]. Based on these animal tests, clinical reports, and our in silico results, estradiol structure could be used for designing new drug molecules and for searching better alternatives than that of tamoxifen analogs.

Table 2. The binding parameters of the structure of ligand-ER upon docking

Ligand molecule	Energy (kcal/mol)	Number of polar interaction	Polar bond lengths (Å)
Estradiol	-9.7	3	3.2, 2.4, 2.3
Raloxifene	-9.7	1	3.2
Tamoxifen	-8.8	1	2.6
Toremifene	-8.8	1	2.6

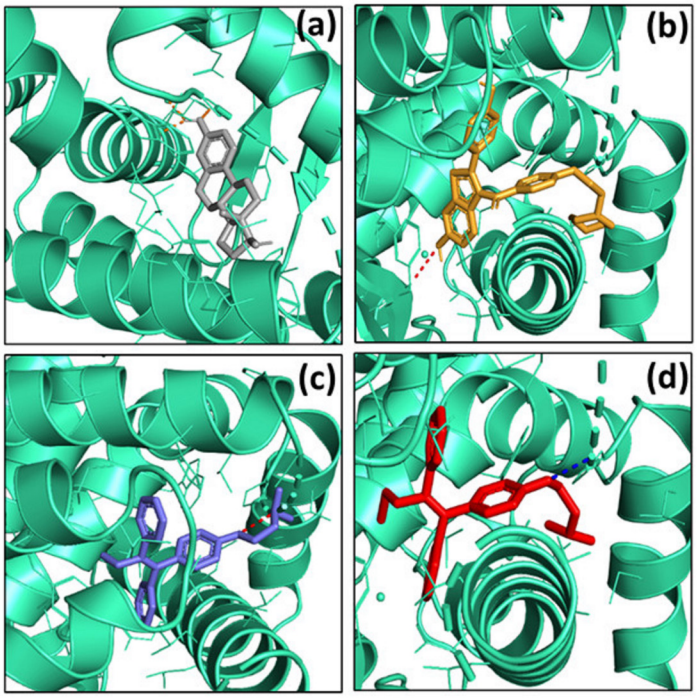

Figure 3. The binding site and the interactions of a) estradiol, b) raloxifene, c) tamoxifen, and d) toremifene with estrogen receptor (6SQ0).

Physico-chemical Properties

Calculating physico-chemical properties and evaluating its drug-likeness profile is a common way of investigating how a ligand is molecularly and pharmaceutically appropriate to be a bioavailable and effective drug. Based on this fact, we calculated the molecular structural properties of the ligands estradiol and SERMs listed above in order to help with understanding the clinical results of the natural and the synthetic ligands in a comparative manner. The results of the calculations were provided in Table 3. While the natural ligand estradiol gave appropriate drug-likeness properties explained in our previous study [19], the approved drug molecules, the synthetic ligands raloxifene, tamoxifen, and toremifene resulted in not a very convenient picture with a miLog P value above 6. A Log P value higher than five is considered as a violation of the 'rule of five' reported by Lipinski as a drug-likeness criteria [20]. A previous study performed on the patented molecules in between the year of 2000 and 2010, Log P value was ranged from 3.5 to 4.5 [21].

A higher Log P value meaning a higher molecular hydrophobicity could cause negative effects on the pharmacological properties of the molecule such as solubility and even toxicity [22]. Although docking results of raloxifene was promising, its molecular weight is also quite higher than the threshold of 500 g/mol in terms of the 'rule of five', the common drug-likeness criteria. So, the effort of lowering Log P value and lowering the molecular weight could be a good approach to designing new SERMs. Because of the higher Log P value, all synthetic ligands raloxifene, tamoxifen, and toremifene were resulted in one violation of drug-likeness criteria, although estradiol obeyed all of them.

Table 3. Physico-chemical properties of the natural and the synthetic ligand molecules

Ligand molecule	miLog P	TPSA	MW	NOH	NOHNH	Nvio
Estradiol	3.43	40.46	272.4	2	2	0
Raloxifene	6.22	70.00	473.5	5	2	1
Tamoxifen	6.06	12.47	371.5	2	0	1
Toremifene	6.06	12.47	405.9	2	0	1

MiLog P: Logarithm of partition coefficient, MW: molecular weight in the unit of gr/mol, TPSA: topological polar surface area in the unit of Å, NOH: the number of hydrogen bond donor, and NOHNH: the number of hydrogen bond acceptor

Toxicity Evaluation

We explored the potential toxicity of the ligand molecules in a color-coded manner using the Osiris Property Explorer program (www.organicchemistry.org/prog/peo/). Toxicity risk categories were classified in the program as mutagenic, tumorigenic, irritant effects, and reproductive effects. High risk of undesired effects was represented in red, whereas drug-like behavior is colored in green. The results showed that synthetic drugs did not really exhibit conform as of the natural ligand estradiol. Raloxifene and tamoxifen showed a safer profile in toxicity table (Table 4) except a high risk on reproduce effects, whereas toremifene showed a high risk of tumorigenicity and reproductive effect and a moderate risk on mutagenicity. Toremifene has been actually approved upon the undesirable effects of tamoxifen observed in the clinical trials. It was developed to produce a similar efficacy with tamoxifen but an improved safety profile. Several investigations showed that tamoxifen increased the risk of hepatocarcinoma in rats [23] and gastrointestinal and endometrial cancers in human [7, 24]. According to our toxicity analysis, toremifene had an even higher toxicity risk profile than that of tamoxifen. Clinical trials of toremifene and tamoxifen performed in 2014 have shown some common adverse effects such as hot flashes, vaginal dryness, discharge, bleeding, and vomiting [25]. More than that, phase III trials of toremifene have shown that efficacy and safety is comparable to that of tamoxifen in postmenopausal women with ER+ or ER- [26]. Moreover, the risk of endometrial cancer was evaluated for toremifene and tamoxifen, and almost the same number of incidence have been found in both group of patients [27]. The results showed that the molecular structure of the synthetic SERMS raloxifene, tamoxifen, and toremifene were not close enough to the best alternative the natural ligand estradiol. Instead of modifying the structure of tamoxifen approved in 1977 and not much been preferred for treatment any more (https://go.drugbank.com/drugs/DB00675), creating model drug structure on the lead of the molecular structure of estradiol would result in more effective clinical observations according to our in silico molecular drug-target interaction study.

Table 4. Physico-chemical properties of the natural and the synthetic ligand molecules

Ligand molecule	Mutagenic	Tumorigenic	Irritant	Reproductive
Estradiol	Green	Green	Green	Green
Raloxifene	Green	Green	Green	Red
Tamoxifen	Green	Green	Green	Red
Toremifene	Orange	Red	Green	Red

Discussion

Our computational results were analyzed in terms of three different aspect of molecular docking, physico-chemical properties, and toxicity evaluation. Molecular docking analysis provided that all of the ligands were bound with the same binding pocket of the ER molecule, however, more important point than that is the binding details with the number of polar bonds and polar bond lengths. Those details exhibited that estradiol has the best structure to bind with ER resulting with the highest binding energy and with three polar bonds. Raloxifene followed estradiol giving the same binding energy, but only one polar bond. Although these results were significant, ligands should be investigated in terms of drug-likeness profile and toxicity prediction.

Drug-likeness parameters with related physico-chemical properties were investigated for all of the ligand structure, and the results were meaningful. Tamoxifen analogs failed to obey one of the most important drug-likeness parameter, Log P value, although estradiol showed the best value of 3.43 leading to the reported range of Log P [21]. On the other hand, molecular weight of tamoxifen analogs is also close to the reported limit of 500 gr/mol [20]. For the ER targeted therapeutics, our results showed that tamoxifen analogs are not really best structure to continue with the investigation. Instead, based on the estradiol structure, its bioisosteric analogs with the new promising elements studied in recent medicinal chemistry such as silicon and/or boron are worth to investigate and could give a better pharmacological and toxicology profile [28].

Another important aspect of bioactive compounds to be a god oral bioavailable drug is toxicity evaluation. Although literature has a good number of ER targeted studies, they are lack of toxicity investigation [29]. Our results additionally provided a potential toxicity risk prediction for all ligands, and tamoxifen analogs exhibited one or more toxic effects on different fields. Tamoxifen and Raloxifene showed a relatively better toxic profile resulting in high risk of reproductive effect. Toremifene, on the other hand, exhibited even worse toxicity profile including mutagenic and tumorigenic effect in addition to the reproductive effect. These potential risk predictions are also consistent with the clinical cancer reports provided by patients taking the tamoxifen analogs.

Conclusion

We used the molecular docking procedure to investigate the binding interactions of estrogen receptor with a natural ligand estradiol and the synthetic ligands raloxifene, tamoxifen, and toremifene in a comparative manner. We computationally evaluated their relative toxicity profile and compared them with the reported clinical

observations. The most favorable binding pose was obtained with the details of the interaction types, and the coordinates of all ligands and target estrogen. The results showed that estradiol has the most appropriate structure to interact with estrogen receptor with the highest binding energy and three stable interactions among other ligands. Based on those in vitro studies and our computational toxicity and pharmacological results, we suggest that new design drug molecules using the estradiol structure as a lead molecule would be resulting in better pharmaceutical consequences both in vitro and in clinic. Our theoretical binding analysis of an hormone receptor, estrogen with experimentally studied natural and synthetic ligands is considered to give insight to the future estrogen-ligand interaction studies before performing costly experimental testing.

Conflict of interests
The authors declare that they have no competing interests.

Financial Disclosure
All authors declare no financial support.

Ethical approval
Manuscript does not include any human or animal materials.

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