Identification of certain chemical disruptors of the estrogen receptor α: Structural and docking analyses

Due to the active role of estrogen receptor α (ERα) in certain breast cancer, finding possible ERα antagonists is important for lowering the growth rate of ERα-motivated breast cancer cells. Here, the current work was conducted to computationally find ERα inhibitors based on the use of certain docking methods. The three-dimensional (3D)-ERα structure was freely docked with the 3D structure of certain ligands, including milbemycin_A3_5-oxime (MA), Baloxavir marboxil (BM), tadafalil (TF), sesamol (SM), raspberry ketone (RK), guaiacol (GuC), proanthocyanidins (PAC), epicatechin (EC), and catechin (CC). The results of the protein-ligand based binding affinity values (kcal/mol) revealed the following: MA= -9.7, TF= -8.5, BM= -8.2, PAC= -8.1, CC= -7.5, EC= -7.2, RK= -6.1, SM= -5.7, and GuC= -4.8. According to the above-mentioned data, milbemycin_A3_5-oxime, tadafalil, Baloxavir marboxil, and proanthocyanidins show the highest binding affinity, which can further be studied in vitro and in vivo for their proposed activity. In addition, catechin, epicatechin, raspberry ketone, sesamol, and guaiacol can also further be investigated for their anti-ERα activity, in which some structural modification to the ligand molecules can be performed to increase their binding action, if they show low ERα-based blocking via in vitro and in vivo research.

Introduction

Estrogens are hormones that play key roles in a variety of physiological functions, including female reproduction, growth, and homeostasis. They can be triggered by attaching to certain transcription factors, such as the ERs. The N-terminal domain, the DNA-binding-domain, and the C-terminal ligand-binding domain are the three functional domains that make up the nuclear receptors (NRs) (1). The receptor comes in two different isoforms, ERα and ERβ. Considering that each subtype serves as a distinct function in estrogenic activity, both isoforms demonstrate significant levels of sequence similarity within their C-terminal ligand-binding domain and comparable affinity for the primary endogenous regulator, 17-estradiol (2).
Following their recognition, ER ligands have been found useful in a variety of therapeutic settings, most notably in the treatment of breast cancer. As a result, several small compounds were created with the intention of controlling ER behavior. Yet, some substances that fall within the classification of exogenous substances known as endocrine disrupting chemicals (EDC) can also interact with ER (3). EDCs can enter the body by ingestion, respiration, or contact with the skin. Because they can replicate the effect of endogenous hormones, they can affect the endocrine system in human and animals (4). The initial negative impacts of EDCs were linked to estrogens and included learning difficulties, endometriosis, breast cancer, and issues with fertility (5). EDCs are currently viewed as a hazard to public health due to the possibility that human exposure to these substances would raise the likelihood of biological processes including reproduction, cognition, and metabolism being impaired (6).

Empirical methods have always been employed in the advancement of ER drugs, and in more recent years, high throughput screening experiments have been crucial for identifying possible drug members. Via intensive molecular docking and free energy perturbation simulations, computer-aided drug design (CADD) methods, recent significant contributions to the cheminformatics and bioinformatics based drug design showed extraordinary success in the process. This has accelerated early drug discovery endeavors. According to the important of ERα in certain breast cancer, the present study was performed to find possible ERα antagonists for decreasing the multiplication rate of ERα-motivated breast cancer cells.

**Materials and methods**

In the present work, ligands were recruited to freely target the ERα (PDB ID: 3ERT; https://www.rcsb.org/) at the binding site of the protein, following the removal of any H2O, ligands, and unnecessary molecules from the 3D structure of the receptor using PyMOL 2.4. MA; a macrocyclic lactone, BM; an anti-viral substance, TF; phosphodiesterase type 5 inhibitor, SM; a natural phenolic component of the sesame seeds, RK; a phenol of the red raspberries, GuC; a natural compound with phenolic activity, PAC; a naturally present coloring compound of different fruits and flowers, EC; a flavan-3-ol compound from certain plants, and CC; a flavan-3-ol compound from plants. PyRx-Python Prescription (0.8) and Autodock vina plugin 12 were employed to evaluate the affinity values. In details, ligand files were downloaded from https://pubchem.ncbi.nlm.nih.gov/ and http://www.chemspider.com/.
PyMOL 2.4 also was recruited for the generation of docking images. The ligand-protein interactions and their images (2 and 3D models) were presented utilizing Discovery Studio Visualizer v20.1.0.19295 (BIOVIA).

Results

The results of the protein-ligand based binding affinity values (kcal/mol) revealed the following; MA= -9.7, TF= -8.5, BM= -8.2, PAC= -8.1, CC= -7.5, EC= -7.2, RK= -6.1, SM= -5.7, and GuC= -4.8 (Table 1).

**Table 1**: Ligands and their binding affinity reads of the human estrogen receptor α.

<table>
<thead>
<tr>
<th>Ligand</th>
<th>Binding affinity (kcal/mol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Milbemycin_A3_5-oxime</td>
<td>-9.7</td>
</tr>
<tr>
<td>Tadalafil</td>
<td>-8.5</td>
</tr>
<tr>
<td>Baloxavir marboxil</td>
<td>-8.2</td>
</tr>
<tr>
<td>Proanthocyanidins</td>
<td>-8.1</td>
</tr>
<tr>
<td>Catechin</td>
<td>-7.5</td>
</tr>
<tr>
<td>Epicatechin</td>
<td>-7.2</td>
</tr>
<tr>
<td>Raspberry ketone</td>
<td>-6.1</td>
</tr>
<tr>
<td>Sesamol</td>
<td>-5.7</td>
</tr>
<tr>
<td>Guaiacol</td>
<td>-4.8</td>
</tr>
</tbody>
</table>

The binding activity of the ligands revealed unique attachment behaviors only to the active binding sites of the ERα, which can be explored via (Figure 1).
Figure 1: Binding activity of the ligands to the active binding sites of the estrogen receptor α; in: A. Milbemycin_A3_5-oxime, B. Tadalafil, C. Baloxavir marboxil, D. Proanthocyanidins, E. Catechin, F. Epicatechin, G. Raspberry ketone, H. Guaiacol.
The ligand-protein interactions (3D-based hydrogen bond based style) with the active binding sites of the ERα are presented in (Figure 2).

**Figure 2**: Ligand-protein interactions (3D-based hydrogen bond based style) with the active binding sites of the estrogen receptor α; in: A. Milbemycin_A3_5-oxime, B. Tadalafil, C. Baloxavir marboxil, D. Proanthocyanidins, E. Catechin, F. Epicatechin, G. Raspberry ketone, H. Sesamol, and I. Guaiacol.
The ligand-protein interactions (2D-based hydrogen bond based style) with the active binding sites of the ERα are presented in (Figure 3-11).

**Figure 3**: Milbemycin_A3_5-oxime-protein interactions (2D-based hydrogen bond based style) with the active binding sites of the estrogen receptor α.
Figure 4: Tadalafil-protein interactions (2D-based hydrogen bond based style) with the active binding sites of the estrogen receptor α.
Figure 5: Baloxavir marboxil-protein interactions (2D-based hydrogen bond based style) with the active binding sites of the estrogen receptor α.
Figure 6: Proanthocyanidin-protein interactions (2D-based hydrogen bond based style) with the active binding sites of the estrogen receptor α.
Figure 7: Catechin-protein interactions (2D-based hydrogen bond based style) with the active binding sites of the estrogen receptor α.
Figure 8: Epicatechin-protein interactions (2D-based hydrogen bond based style) with the active binding sites of the estrogen receptor α.
Figure 9: Raspberry ketone-protein interactions (2D-based hydrogen bond based style) with the active binding sites of the estrogen receptor α.
**Figure 10:** Sesamol-protein interactions (2D-based hydrogen bond based style) with the active binding sites of the estrogen receptor α.
Discussion

Computational-biology becomes the gold-standard step of the discovery of new drugs for the treatment of numerous difficult-to-treat health conditions, such as estrogen-dependent breast cancer. The present

**Figure 11:** Guaiacol-protein interactions (2D-based hydrogen bond based style) with the active binding sites of the estrogen receptor α.
study utilized this step to identify ligands with strong binding ability to the active site of the human ERα.

Based on the current results, MA was found to have the highest binding activity to the active site of the receptor. Milbemycins are subfamilies of the class of recognized anti-parasitics called as macrocyclic lactones. Milbemycins, such as milbemycin oxime is generated from compounds formed by Streptomyces hygroscopicus. A 16-member macrocyclic lactone ring serves as a universal pharmacophore for all groups (7). Some MLs, such as ivermectin have been utilized extensively in human medicine for the past 30 years in mass drug programs to manage and eradicate human onchocerciasis, lymphatic filariasis, and other human disorders. The US Food and Drug Administration (FDA) lately authorized moxidectin for the management of onchocerciasis. Some of these drugs are still being investigated for new purposes, including as an antiviral agent against a wide variety of RNA viruses, including HIV-1 and SARS-CoV-2 (8–10). A study by Li et al (11) found that milbemycin was effective in decreasing the activity of a cancer cell line.

Tadalafil recorded the second highest binding affinity with the active sites of the ERα. Aversa et al (12) detected a decrease in the expression of the ERα mRNA expression in the human osteoblast-like cells SAOS-2 after the use of tadalafil. Tadalafil alters the Cyp19α1 (ARO) behavior as well as the expression and feature of the androgen receptors in cell lines of bone, prostate, breast, and adipose tissues, indicating a potential direct interplay with steroid hormones, according to a series of preclinical and clinical findings (13–18).

The current docking analysis revealed that Baloxavir marboxil, proanthocyanidins, catechin, epicatechin, raspberry ketone, sesamol, and guaiacol were active in their binding to the active site of the ERα. These ligands have active phenolic groups that are favorable for the binding with the active binding site of the ERα, which mimics the binding activity of the 17β-Estradiol (19,20).

**Conclusion**

According to the above-mentioned data, milbemycin_A3_5-oxime, tadalafil, Baloxavir marboxil, and proanthocyanidins show the highest binding affinity, which can further be studied *in vitro* and *in vivo* for their proposed activity. In addition, catechin, epicatechin, raspberry ketone, sesamol, and guaiacol can also further be investigated for their anti-ERα activity, in which some structural modification to
the ligand molecules can be performed to increase their binding action, if they show low ERα-based blocking via *in vitro* and *in vivo* research.

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**Conflicts of interests**

The author states that there are no conflicts of interest about the publication or funding of this manuscript.

**References**


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