The Structural Bioinformatics Analysis of Biophenolic Lignan-Estrogen Receptor Interaction

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Abstract: Background: Plant lignans have proven efficacious in blocking estrogen receptors of breast cancer cells. However, available studies have mostly dealt with anti-cancer effects of groups of lignans in certain foods or plants and the effects of specific lignans, especially from a molecular interaction viewpoint, has been rarely addressed in the literature.

Objective: We aimed to computationally predict the binding ability and binding strength of pinoresinol, matairesinol, lariciresinol and secoisolariciresinol as potent ligands of estrogen receptor alpha (ER-α), in order to study these lignans as drugs.

Methods: Blind Docking method was utilized to predict the binding orientation of lignans to their targets by AutoDock 4.2 software. Docking results of lignan-receptor complexes were compared to tamoxifen-receptor complex separately. Hydrophobic interactions and hydrogen bonds between lignans and ER were perused and the binding energy was calculated.

Results: The best binding affinity of tamoxifen, matairesinol, pinoresinol, lariciresinol and secoisolariciresinol were -8.7, -7.5, -6.7, -6.7, -5.8 kcal/mol, respectively, and matairesinol showed the minimum binding energy than other studied lignans. Matairesinol showed the most similar interactions with tamoxifen with small molecule-receptor complex in the following residues: Leu: 391, Ala:350, Ile:424 and Phe:404.

Conclusion: Among the studied lignans, matairesinol showed the least binding energy as well as the most similar hydrophobic interactions to tamoxifen suggesting that matairesinol can display more efficacious biological activity to inhibit ER in comparison with pinoresinol, lariciresinol and secoisolariciresinol. Thus, our results introduce matairesinol as a potentially effective anti-ER drug.

Keywords: Biophenolic lignan, structural bioinformatics, matairesinol, docking, breast cancer, estrogen receptor.

1. INTRODUCTIONS

Breast cancer is considered to be the leading cancer among women in industrialized countries as well as being the second biggest killer after lung cancer [1]. Three major subtypes of breast cancers have been identified through gene expression studies: HER2-positive, Endocrine receptor-positive (estrogen or progesterone receptors) and triple positive, i.e. positive for estrogen receptors (ER), progesterone receptors and HER2 [2]. “ER-positive” breast cancers comprise approximately 80% of all breast cancers occurring, which means cancer cells grow in response to the hormone estrogen. By blocking hormone receptors and preventing hormones from binding to them, hormone therapy drugs inhibit cancer recurring again [3]. Having a chemical structure similar to 17-estradiol, phytoestrogens may compete with estrogens for binding to ERs and in doing so they may act as estrogen agonists or antagonists. Thus, it is believed that phytoestrogens would behave like selective estrogen receptor modulators (SERM) being effective in chemoprevention of breast cancer [4]. Considering the growing interest towards nutraceuticals, plant lignans, which are biophenolic compounds widely distributed in the plant kingdom, are becoming promising therapeutically active compounds thanks to their presumed favorable health benefits including antitumor, antioxidant, both estrogenic and antiestrogenic activity and protection against coronary heart disease [5].

Due to their estrogen-like potency in various in vitro and in vivo assays, some phenolic compounds are categorized as phytoestrogens. Therefore, phytoestrogens have been posited to affect the etiology of estrogen-related diseases such as hormone responsive breast cancer [6].

Secoisolariciresinol diglucoside (SDG) and matairesinol are major lignans containing traces of pinoresinol, lariciresinol and isolariciresinol which are found in roots, stems, cereals, oilseeds, nuts, legumes and fruits [7]. Intestinal bacteria
can convert these plant lignans into mammalian lignans like enterolignans, enterodiol and enterolactone.

Secoisolariciresinol diglucoside (SDG) is a plant lignan which is mainly found in dietary food and different plants. SDG and its metabolites (mammalian enterolignan) have different pharmacological activities, viz. antioxidant, partial agonist to estrogen receptor and inhibitor of tyrosine kinase and topoisomerase. In spite of the fact that human studies have their own limitations, its applicability in breast, colon and prostate cancers and in cardiovascular diseases can be explained by its pharmacological actions [8-9].

Pinoresinol has displayed the strongest anti-inflammatory properties by affecting NF-xB signaling pathway, likely via its furofuran structure and/or its intestinal metabolism. Pinoresinol glucoside of prunes contained antioxidant and anti-inflammatory properties [10-11]. Matairesinol, a plant lignan present in a wide variety of foods such as seeds, vegetables and fruits, has diverse biological functions for instance antiangiogenic, anti-cancer, anti-fungal and anti-osteoporotic activities [12-13]. The chemical structure of phytoestrogens is similar to natural and synthetic estrogens. Thus, phytoestrogens may serve as weak estrogen analogs by binding to the ER exposed on cell membranes. In other situations, phytoestrogens may act like estrogen antagonists and prevent estrogens from binding to their receptors.

In the present study, we computationally evaluate the ligand-receptor interaction of four phytoestrogen lignans: secoisolariciresinol, matairesinol, pinoresinol and lariciresinol, as positively appraised lignans for ER-positive breast cancer targeted therapy. We then compared the inhibitory potential of lignans by molecular docking approach. Blind Docking method was utilized to predict the binding orientation of lignans to their targets. Docking of tamoxifen (a common cancer drug) on ER was also carried out and then lignan-receptor complexes were compared against tamoxifen-receptor ones.

2. METHODS

2.1. Software and Databases

In this study, docking method was utilized to predict the binding orientation of lignans including matairesinol, pinoresinol, lariciresinol and secoisolariciresinol to their protein target, estrogen receptor alpha (ER-α). Protein Data Bank (PDB), PubChem and Drug Bank databases were used to extract the corresponding files of attributed lignans and protein. In order to optimize the small molecules for docking, ChemSketch and HyperChem were employed as well.

AutoDock4.2 was used in order to predict the affinity and activity of the small molecules to ER-αforming a stable complex [14]. Afterwards, the lignan-receptor complexes were compared and effective bonds involved in complex stability were investigated.

The related results were analyzed using AutoDockTools (ADT) and Discovery Studio Visualizer.

2.2. Receptor and Ligand Preparation

A typical PDB structure file is composed of heavy atoms and may contain a co-crystallized ligand, water molecules, and metal ions and so on, which makes it unsuitable for immediate use in molecular modeling calculations. In order to refine such structures, firstly they should be fixed. Afterwards unwanted chains and waters are to be deleted. The fixed structures are then optimized after fixing and deleting het groups. For this aim, we retrieved 3D crystal structures of ER-α from PDB. Then the water molecules and ligands were removed from the corresponding proteins in AutoDock Tools (ADT) software.

The representation of receptors and ligands in AutoDock 4.2 is required in a file format called “pdbqt”, which is a modified PDB format containing atomic charges, atom type definitions and rotatable bonds. For all the drugs and drug-like compounds, the geometry optimization results were obtained using the aforementioned software.

2.3. Creation of Grid box and Configuration file

A grid center and grid dimensions needs to be determined for AutoDock calculation. For ER-alpha, a grid box surrounding the active site was chosen. The grid box size was set at 88, 88 and 126 while the grid box x-center, y-center and z-center were set on 0.167, -5.278 and 0.0, respectively. Before the program can be run, the grid parameter file (GPF) dictates AutoGrid 4 which receptor to compute the potentials around and the types of maps to compute. The location and extent of the maps were determined by the grid parameter file (GPF).

3. RESULTS

The 3D structure of ER-alpha can be seen in (Fig. 1), which is refined using WebLab software. ER is composed of 3 domains; however, we chose LBD (ligand binding domain). Figs. (2A-D) show the structures of lignans, which are listed as matairesinol, pinoresinol, secoisolariciresinol and lariciresinol. The results of molecular docking for all lignan and tamoxifen are presented in Table 1. AutoDockTools and Discovery Studio Visualizer were used in order to analyze the interactions between docked potent agents and macromolecule.

![Image](377x65 to 530x268)

**Fig. (1).** The 3D structure of Estrogen Receptor alpha.
Table 1. Docking results of small molecules against estrogen receptor.

<table>
<thead>
<tr>
<th>The Range of Binding Energy (Kcal/mol)</th>
<th>Hydrophobic Interactions</th>
<th>Hydrogen Bonds</th>
<th>The Best Binding Energy (Kcal/mol)</th>
<th>Compound Name</th>
</tr>
</thead>
<tbody>
<tr>
<td>-6.0 — -6.7</td>
<td>TYR:526</td>
<td>GLU:523</td>
<td>-6.7</td>
<td>Pinoresinol</td>
</tr>
<tr>
<td>-6.0 — -6.7</td>
<td>PRO:325, PRO:324 GLY:390</td>
<td>PRO:324</td>
<td>-6.7</td>
<td>Lariciresinol</td>
</tr>
<tr>
<td>-5.6 — -5.8</td>
<td>CYS:381, HIS:377 THR:460, TYR:459</td>
<td>LYS:520</td>
<td>-5.8</td>
<td>Secoisolariciresinol</td>
</tr>
</tbody>
</table>

Fig. (2). The structure of biophenolic lignans and tamoxifen: (A) Matairesinol, (B) Pinoresinol, (C) Lariciresinol, (D) Secoisolariciresinol and (E) Tamoxifen.

In order to obtain a good sample of binding modes for conformational cluster analysis, it is advised to repeat a given docking 50 to 100 times [15]. As such, for each lignan-ER the whole process was repeated 100 times to gain more trustable results. According to the clustering results shown in Table 1, for 100 obtained conformers, the binding energy for matairesinol is between -5.3 and -7.5, while this value is in the range of -6.0 to -6.7 for pinoresinol and between -6.1 to -8.7 for tamoxifen. These results have been observed between -6.0 to -6.7 for lariciresinol, whereas the range of binding energy is between -5.6 and -5.8 for secoisolariciresinol with 100 iterations, both of which are demonstrated in Table 1.

The best binding energy considering hydrophobic interactions and hydrogen bonds numbers are -7.5, -6.7, -6.7, -5.8 and -8.7 respectively for matairesinol, pinoresinol, lariciresinol, secoisolariciresinol and tamoxifen.

Hydrogen bonds and hydrophobic interactions between ER-alpha and matairesinol are presented in Fig. (3A). Thr:347 and Leu:387 form hydrogen bonds with this lignan, whereas Leu:391, Leu:384, Leu:346, Ala:350, Ile:424, Met:382 and Phe:404 have formed hydrophobic bonds with it. The 3D structure of the ER surface around matairesinol is shown in Fig. (3B).

Investigation of the interactions between pinoresinol and ER-α shows the formation of hydrogen bond with Glu:523 as well as hydrophobic interaction with Tyr:526. These results are demonstrated in Fig. (4A). The structure of the ER surface and pinoresinol is shown in Fig. (4B).

Lariciresinol has formed hydrogen bonds with Pro:325, Pro:324 and Gly:390. In contrast, secoisolariciresinol has formed the same bonds with Cys:381, His:377, Thr:460 and Tyr:459 in ER, which is shown in Figs. (5A and 6A).
4. DISCUSSION

Numerous natural products have biological activity that can be therapeutically exploited in treating a variety of diseases. Thanks to the advent of modern molecular biology and combinatorial chemistry, natural products are increasingly taking a secondary role in drug development. Recently there seems to be a renewed interest for natural compounds and their role as the main base for drug development [16-17]. In recent years, a great number of medicinal herbs have been used to treat various cancers, introducing these herbs or their analogs as anti-cancer sources [18-20]. Different medicinal
plants have been used for the treatment of breast cancer, which is the most common cancer found in women [21-22].

Estrogens have long been identified with major roles in stimulating the growth of several types of breast cancer. Now it is widely accepted that two receptors mediate estrogen actions and that the presence of ER is associated with better prognosis and a higher chance of response to hormonal therapy. More than half of all breast cancers over express ER and approximately 70% of them respond to anti-estrogen (for instance tamoxifen) therapy [1]. Drug resistance issues have brought about the search for and the development of various hormonal therapies designed to inhibit ER action, while research on the mechanisms underlying resistance has cast light on the cellular mechanisms besides ligand binding, which control ER function [23-24].

Normally, E2 (Estradiol) binds to ER and then the activated complex then binds to DNA sequences upstream of estrogen responsive genes, which in turn induces the recruitment of ER co-activators. This would lead to an increase in the transcription of the estrogen responsive genes PS2, PGR and CD1 and subsequent proliferation of cancer cells [25]. It has been revealed that the most noticeable effect of

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**Fig. (5).** Lariciresinol-associated ER: (A) The 2D diagram of hydrogen bonding and hydrophobic interactions between Lariciresinol and ER, (B) The receptor surface around Lariciresinol.

**Fig. (6).** Secoisolariciresinol-associated ER: (A) The 2D diagram of hydrogen bonding and hydrophobic interactions between Secoisolariciresinol and ER, (B) The receptor surface around Secoisolariciresinol.
SDG was to significantly reduce PS2 mRNA expression [26]. It is shown in two different in vivo models that lariciresinol, as one of the main dietary lignans, can inhibit mammary cancer growth in hormone responsive breast cancers [6].

Due to the advances in structural biology, computational biologists have been able to design small-molecule drugs, which in turn has induced higher biological activity and minimal side effects for disease-specific targets [27]. Thus, plant lignans can be a good candidate to achieve this purpose. This study focuses on structural bioinformatics analysis at the level of ligand-binding interaction [28]. Molecular docking was employed to evaluate in silico potential of matairesinol, pinoresinol, lariciresinol and secoisolariciresinol to inhibit ER receptor. In order to investigate inhibitory of these lignans tamoxifen, a well-known drug in ER-positive breast cancer was docked on ER and then hydrophobic interactions, hydrogen bonds and binding energy for all lignan-ER complexes were compared to tamoxifen-ER complex.

The best binding energy was observed to be at -8.7, -7.5, -6.7, -6.7 and -5.8 kcal/mol for tamoxifen, matairesinol, pinoresinol, lariciresinol and secoisolariciresinol, respectively. Among the four lignans studied, calculated values for matairesinol are the closest to those of tamoxifen recalling that better ligand-receptor complex stability would be expected for tamoxifen. The stabilization of ligands at the interface of protein structures is affected by weak interactions between molecules, which has been proved previously [29-30]. Matairesinol has formed hydrogen bonds with Thr:347 and Leu:387 in ER, whereas Leu:391, Leu:384, Leu:346, Ala:350, Ile:424, Met:382 and Phe:404 have formed hydrophobic bonds with it. It is noteworthy that hydrophobic interactions in Leu:391, Alas:350, Ile:424 and Phe:404 are the same in tamoxifen.

Hydrogen bonds between pinoresinol and Glu:523 can be observed, while the ligand has formed hydrophobic interactions with Tyr:526. There is no common interaction in pinoresinol-ER and tamoxifen-ER complexes.

Lariciresinol has formed three hydrogen bonds with Pro:325, Pro:324 and Gly:390 in ER, comparable to the only one bond formed between tamoxifen and ER. Hydrophobic interaction between lariciresinol and ER-α can be seen in Pro:324. However, these bonds have less effects on the binding affinity of complex compared to the eight hydrophobic interactions, tamoxifen has formed in Leu:387, Ala:350, Leu:325, Phe:404, Ile:424, Met:388, Leu:391 and Leu:428.

There are four hydrogen bonds between Cys:381, His:377, Thr:460 and Tyr:459 in ER and secoisolariciresinol, as well as a hydrophobic interaction with Lys:520. Both lariciresinol and secoisolariciresinol makes more hydrogen bonds than tamoxifen; however, the binding energy of tamoxifen is the lowest. Thus, the greater number of hydrophobic bonds may make the complex more stable, highlighting the important role of hydrophobic interactions in drug design [27]. In conclusion, among lignans, matairesinol showed the lowest binding energy as well as the most similar hydrophobic interactions to tamoxifen, which confirms that in comparison with pinoresinol, lariciresinol and secoisolariciresinol, matairesinol can display more similar biological activity to inhibit ER.

CONCLUSION

In our study, four lignans have been specifically investigated as small molecules preventing estrogen from binding to its specific receptor. We evaluated the affinity of each molecule, as a potent drug, to bind to ER separately. In order to figure out the most effective and most potent lignan regarding its ER inhibitory effects, tamoxifen was used as the

Fig. (7). Tamoxifen-associated ER: (A) The 2D diagram of hydrogen bonding and hydrophobic interactions between Tamoxifen and ER, (B) The receptor surface around Tamoxifen.
reference and the receptor binding values of lignans were compared against it. At the end, we introduced matairesinol with the best binding energy and the most similar interactions compared to the reference drug.

**AUTHORS’ CONTRIBUTION**


Mahshid Sadat Kashef-Saberi: draft preparation and revised preparation.


**ETHICS APPROVAL AND CONSENT TO PARTICIPATE**

Not applicable.

**HUMAN AND ANIMAL RIGHTS**

No Animals/Humans were used for studies that are base of this research.

**CONSENT FOR PUBLICATION**

Not applicable.

**CONFLICT OF INTEREST**

The authors declare no conflict of interest, financial or otherwise.

**ACKNOWLEDGEMENTS**

This work was supported financially by Stem Cell Technology Research Center.

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