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Short Communication

In silico Virtual Screening on Estrogen Receptor Alpha Protein Ligand Binding Domain for the Development of New Selective Estrogen Receptor Modulators

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Abstract

While early detection technology for cancer has been peruse as one of the priorities to extend life expectancy, small molecule therapeutic drugs treatment remains as the first line of defend again cancer problems. The hormone dependency for Estrogen Receptor positive breast cancer has led to the development of therapeutic drugs known as selective estrogen receptor modulators. However, selective estrogen receptor modulators drugs have been limited to only few candidates were tamoxifen is considered the golden standard. Despite the short-term success of tamoxifen application, long-term treatment of this drug has been associated with: breast cancer resistance in hormone therapy and an increased incidence of endometrial cancer by 4 to 6.9-fold. New advances in the characterization, at atomic level, of protein-ligand complexes and the development of new algorithms and computational chemistry, the rational development of more selective and active drugs has been possible in medicinal chemistry. In this context, an in silico virtual screening on the Estrogen Receptor-Ligand Binding Domain has performed in order to identify new lead candidates as templates for new drugs in this study. A library of commercial available compounds (~650,000 drug-like small molecules) were docked into the Estrogen Receptor-Ligand Binding Domain using as template the protein-ligand complex crystal structure with 4-hydroxytamoxifen. Particularly attention has been made to the principal structural components of the best docking results for the library compounds entering the protein ligand binding domain and lateral side chain toward the alpha helix 12 of the Estrogen Receptor alpha that could result in the stabilization of protein antagonist form.

ABBREVIATIONS

ER+: Estrogen Receptor Positive; E2: Estradiol; ERα: Estrogen Receptor Alpha; SERMs: Selective Estrogen Receptor Modulators; H12: Helix 12; PDB: Protein Data Bank; OHT: 4-Hydroxytamoxifen; LBD: Ligand Binding Domain; ADT: AutoDockTools; VS: Virtual Screening

INTRODUCTION

Estrogen Receptor positive (ER+) breast cancer represents more than 50% of breast cancer. High expression of the protein estrogen receptor alpha (ERα) and its interaction with estrogens represent the main mechanism responsible for the high rate of cell proliferation in this cancerous tissue [1]. Among estrogens, estradiol (E2) is the most relevant and responsible endogenous estrogen that binds estrogen receptor. This interaction is considered to trigger events at molecular levels, that could result in receptor dimerization, recruitment, and interaction with coactivators and corepressors that involve both, transcriptional regulation (genomic action), and direct signaling cascades (non-genomic actions), which involve a cross coupling with grow factor pathways.

Selective Estrogen Receptor Modulator (SERMs) has been developed in the past decades as therapeutics drugs with the purpose to block the transcription factor which involve the complexation between ERα and Estradiol [2]. It is known that the anti-estrogenic power that some SERMs elicit, upon complexation to the estrogen receptor, is due to its ability to induce changes in the position of the receptor helix12 (H12), thus inhibiting the recruitment with coactivators that are responsible for the transcription factor once inside the cell nucleus. The gold standard SERM, in hormone dependent breast cancer, is tamoxifen [3]. However, despite the short-term success of tamoxifen application [4], long-term treatment of this drug has been associated with: breast cancer resistance in hormone therapy [5] and an increased incidence of endometrial cancer by 4 to 6.9-fold [6]. New advances in the characterization, at atomic level, of protein-ligand complexes by X-ray crystallography [7], and the development of new algorithms and computational chemistry to understand fundamental aspects such as bonding and energetic parameters, allows the rational design toward more selective and active drugs in medicinal chemistry. In this context, an *in silico* virtual screening (VS) on the estrogen receptor-ligand binding domain (ER-LBD) with library of commercial available

compounds has been performed in order to identify new lead candidates as templates for the new generation of SERM drugs.

MATERIALS AND METHODS

Docking studies

Protein-drug docking studies were performed using AutoDock Vina [8]. Analysis of the binding site was performed employing Pocket Volume Measure (POVME 2.0) [9]. PDB file of the ERα-LBD protein was obtained from The Research Collaborator for Structural Bioinformatics Protein Data Bank (RCSB PDB) [10]. Molecular Docking calculations were run on the high-performance computing system Lone Star 5 at Texas Advance Computer Center. Commercial data bases were obtained from ZINC data base.

The monomeric structure (PDB: 3ERT) [11] of the ERα-LBD-OHT complex was selected for the study. The OHT inside the LBD, waters, metals, and any molecule that was not a fundamental part for the study was removed. The crystal structure was verified for missed atoms on some amino acids side chain of the protein crystal structure using modeller 9.18 [12]. Polar hydrogen and gasteiger charge were computed and added to the protein structure and convert it to pdbqt format using AutoDockTools (ADT) software [13]. Raloxifene and 4-OHT, from the structural point of view, both show a similarity of the distribution of its organic component within the LBD. Both shows, like the endogenous ligand E2, hydrogen bonds between their phenolic rings and the amino acid residues Arg394 and Glu353. However, we chose the ERα-4-OHT (PDB: 3ERT) crystal structure because it has better resolution.

Virtual screening

In silico virtual screening (VS) were performed on the ER-LBD to obtain new lead candidates as templates to develop new drugs. A library of commercial available compounds (~650,000 drug-like small molecules) [14] were docked into the ER-LBD-4-hydroxytamoxifen structure (PDB: 3ERT) with OHT removed. The top 5 molecules structure, name and affinity are in Table 1. Molecules numbering are according to its corresponding ranking in affinity.

RESULTS AND DISCUSSION

The antagonist OHT drug is positioned in the same binding pocket of the endogenous ERα ligand, E2 (PDB: 1A52) (Figure 1A) [15]. Both ligand share a phenolic group and shows the same hydrogen network between its hydroxy group and Arg394 and Glu353 residues. However, the structure of OHT represents a greater volume than E2 (359.1 Å^3 vs 248.2 Å^3). The ligand pocket that contains both ligands is about 338.3 Å^3. Although this comparison appears contradictory in relation to the size of the cavity and OHT, after inspection of the crystal structure (PDB: 3ERT), part of the structure of OHT points away of the ERα-LBD through an ethylene bridge. This leaves part of this bridge and the amino group of the OHT outside the LBD to stabilize the ERα H12antagonist conformation (Figure 1B). After this finding, the search box to perform the docking studies in the ERα-LBD was located at the center of the LBD and extended outside the binding site (including the residues where the ethylene and amino side chain of OHT reside) to obtain new lateral side chains capable to mimic the OHT activity. Figure 1C shows the result of the OHT docked in the ERα-LBD and compared with the 3ERT structure as validation of our docking parameters.

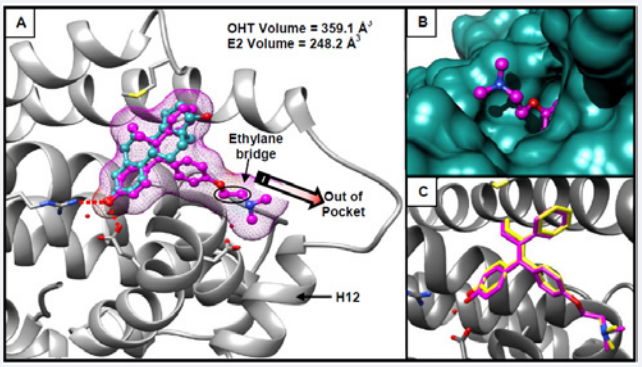


Figure 1 (A) E2 (**blue**) and OHT (**magenta**) relative superposition in ERα-LBD and important structural aspects of the protein-ligand complex of 3ERT.
(B) Top view of the amine group of HOT out the LBD.
(C) Superposition of the docking results of the OHT docked (**yellow**) on the crystallography position (**magenta**) of OHT into the ERα-LBD.

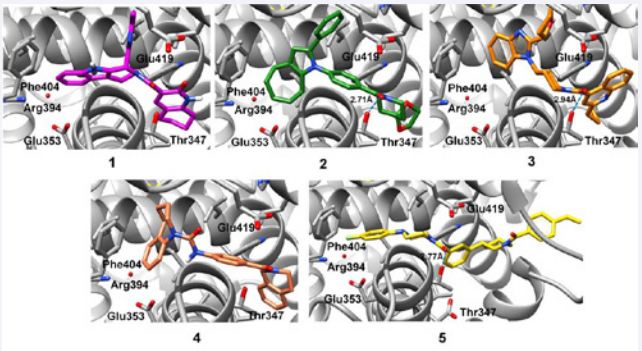


Figure 2 Top 5 molecules pose of the Drug like database library into the ERα-LBD. Each image is numbered according to the same molecule number and corresponding ranking in affinity.

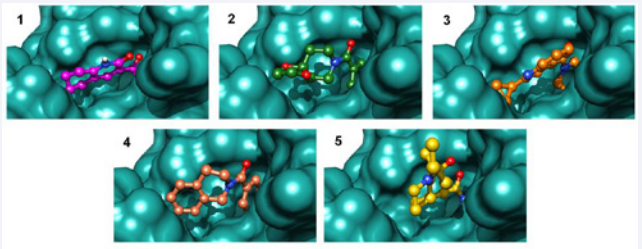
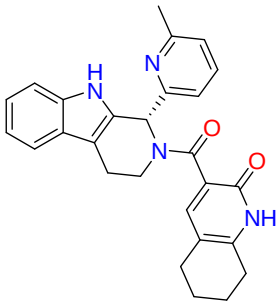
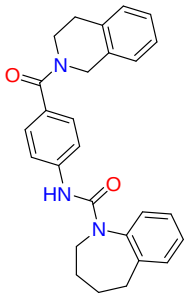
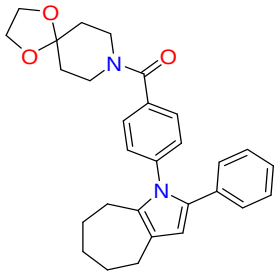
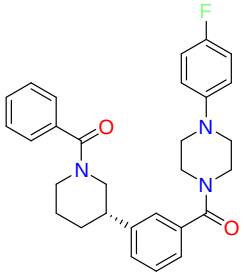
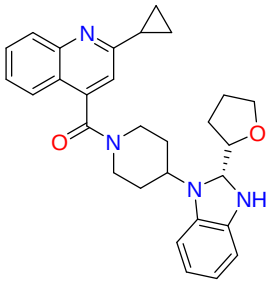
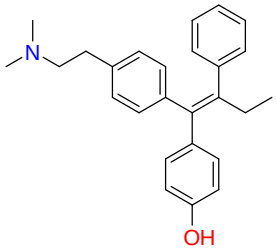


Figure 3 Top view of each compound component out of the protein pocket. Each image is numbered according to the same molecule number and corresponding ranking in affinity.

Drug like molecule library

The screened compounds from the drug-like molecule database shows important structural components of the families

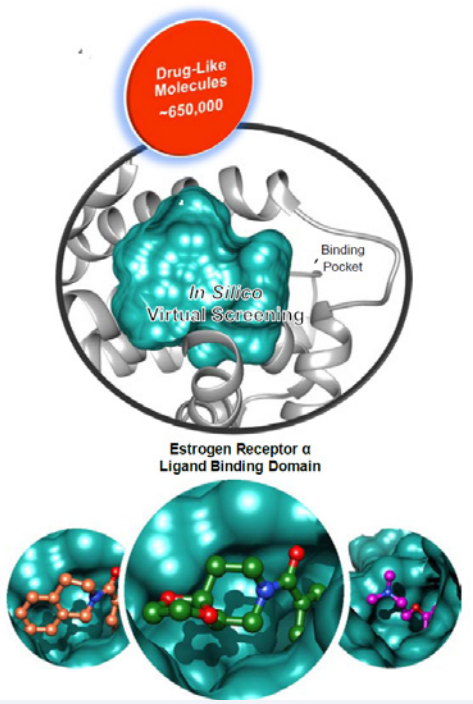
Table 1: Compounds names, numbering and affinity for each one of commercial library used in the docking studies on ERα-LBD.

Drug-Like Molecules					
Compound		**Affinity (kJ/mol)**	**Compound**		**Affinity (kJ/mol)**
1	 3-[(1R)-1-(6-methyl-2-pyridyl)1,3,4,9-tetrahydropyrido[3,4-b]indole-2-carbonyl]-5,6,7,8-tetrahydro-1	**-12.6**	**4**	 N-[4-(3,4-dihydro-1H-isoquinoline-2-carbonyl) phenyl]-2,3,4,5-tetrahydro-1-benzazepine-1-carboxamide	**-12.1**
2	 1,4-dioxa-8-azaspiro[4.5]decan-8-yl-[4-(2-phenyl-5,6,7,8-tetrahydro-4H-cyclohepta[b]pyrrol-1-yl)phen	**-12.2**	**5**	 (4-ethylphenyl)-[(3S)-3-[3-[4-(4-fluorophenyl) piperazine-1-carbonyl]phenyl]-1-piperidyl] methanone	**-12.1**
3	 (2-cyclopropyl-4-quinolyl)-[4-[2-[(2S)-tetrahydrofuran-2-yl]benzimidazol-1-yl]-1-piperidyl]methanone	**-12.1**	**OHT**	 4-hydroxytamoxifen	**-12.4**
Abbreviations: OHT: 4-Hydroxytamoxifen					

with 5, 6, and 7 membered heterocyclic compounds. Each compound is a combination of up to three different heterocyclic moieties, in most cases, bridged by an amide group. With the exception of 2, the remaining adopt the same orientation of their fused aromatic rings as the phenolic group of OHT towards amino acid residues Arg394 and Glu353 (Figure 2). On the other hand, although compound 3 shows its fused aromatic ring in the direction towards the amino acids residues Arg394 and Glu353, this ring is further away from the relative position to the other fused rings of the other compounds and OHT. Compound 3 shows the greatest difference in the displacement of its structural component contained within the LBD compared to the rest of the compounds. The slightly higher affinity for 1 and 2 to ERα-LBD could be justified due to the higher content of hydrophobic interactions of the compounds engulfed into the protein pocket. Each compound shows a structural component, lateral side chain, out of the protein pocket as OHT does (Figure 3). Compounds 2, 3, and 5 show hydrogen bonds within the oxygen of the amide group lateral side chain and Thr347 residue.

CONCLUSION

Virtual screening via docking studies is a fast, economic and effective way to study protein-drug interactions and provides a tool to identify possible target specific drugs candidates. This type of study provides the opportunity to significantly advance the development of drugs for a protein that has been directly implicated to a health problem; in this case, ER+. Being able to identify commercially available compounds that show drug-



Graphical Abstract 1

like properties and key interaction with the protein of interest is an advantage over the traditional synthetic chemistry. The excessive expenses of resources and time for the synthesis and characterization of organic products that could results in only few lead compounds with high specificity makes the *in silico* studies more suitable for drug design.

The compounds presented in this study shows the main structural features of the family of important 5 and 6 membered heterocyclic compounds with biological activity such as indole, pyrrole, benzoimidazole, oxazolines, and quinolines, tetrahydroisoquinlines, respectively. In addition, a 7-membered heterocyclic compound was found to dock into the LBD. This last heterocyclic family has been less explored, compared to the previous mentioned, as a template to develop new lead candidates and drugs. This opens a new frontier, from the synthetic and structural point of view, of developing and synthetizing new lead compounds with high affinity and selectivity for the estrogen receptor protein.

On the other hand, particular attention must be given to the side chain of each of the molecules found in the study. This part is, perhaps, the most important at the moment to develop new drugs to exhibit an antagonistic effect on ERα. This calls attention to the medicinal chemist, where the combination of a one pharmacophore could be combined with different side chains, resulting in a new family of SERMs. Finally, it would also result in the possibility of improving some physical features of the drug, such as stability, solubility and partition coefficient, while at the same time improving the selectivity and affinity for the target protein

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