Docking simulations of steroidal oximes toward Estrogen Receptor alpha. Analysis of their potential anticancer activity

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ABSTRACT

Breast cancer is the most common cause of cancer deaths in women. Normal breast cells and most breast cancer cells have receptors that attach to circulating estrogen and progesterone. Estrogen and progesterone bind to the receptors and may cause cancer cells to proliferate. Drugs, currently being used to treat this disease are not so effective and cause adverse effects. For this reason, the quest of new compounds that bind to the estrogen receptor (ER), activating or inhibiting ERs selectively, is a very active field of research. In this task, molecular docking plays a vital role in drug design, either as a tool in drug discovery and analysis of protein-ligand interactions. In this work, 26 steroidal oximes, which are derivatives of diosgenin, were analyzed using docking studies, and compared against the estrogen receptor (ERα). The 3D structures of ERα were downloaded from Protein Data Bank (3ERT) and optimized using pdb2pqr.py. Modifications in rings A and B were made, and both oxime configurations (E or Z) were considered. Results for hydroximino steroids were compared with those obtained for compounds exhibiting biological activity against this type of cancer, namely, 4-hydroxytamoxifen, diosgenin and 6,23-dihydroximino diosgenin derivative. Molecular docking studies on ERα revealed that three mono oximes were found to interact with the protein more efficiently and have the best docking score. Thus, modification of diosgenin with oxime groups could lead to antiproliferative steroidal compounds, and therefore they could be considered for further research and in vivo evaluations for breast cancer treatment and management strategies.

Keywords: Breast cancer, estrogen receptor, Docking, Hydroximino steroid.

1. INTRODUCTION

Breast carcinoma is the most common malignant neoplasm in women worldwide, and the hormone-dependent breast cancer accounts for 70% of the cases [1]. This type of breast cancer depends on estrogen for continued growth [2]. Estrogen plays a critical role in the growth, development, and maintenance of a diverse range of tissues. They exert their physiological effects via the estrogen receptor (ER), which functions as a ligand-activated transcriptional regulator. There are two types, the estrogen receptor alpha (ERα) and the estrogen receptor beta (ERβ). ERα is generally expressed in the reproductive organs (uterus, breast, ovaries), the liver, and the central nervous system. Furthermore, ERβ is predominantly expressed in bone, endothelium, lungs, urogenital tract, ovary, central nervous system, and prostate [3, 4]. Estrogen receptors (ERs) are members of the nuclear receptor superfamily of ligand inducible transcription factors that mediates the biological effects of the estrogen steroid hormone.

On the other hand, estradiol is synthesized by aromatase and binds to ER to provoke receptor dimerization. This ER-estradiol complex is translocated into the nucleus where binds to DNA at specific binding sites. As a result of this process, several co-regulators are activated inducing estrogenic effects. Any failure or deregulation leads to uncontrolled cellular proliferation [4].

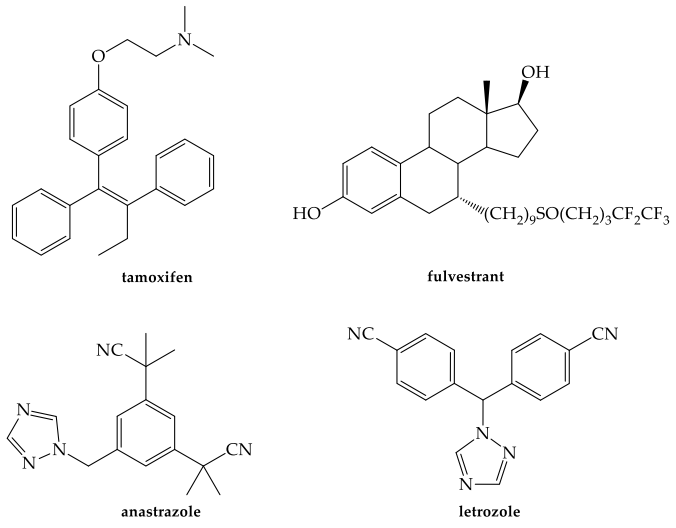
Thus, one of the most effective therapeutic approaches to treat hormone-dependent breast cancer is to deprive cancer cells of estrogens by using drugs acting on the estrogen receptor (ER) or by inhibiting the aromatase enzyme [2, 5-7].

Much effort has been dedicated to the quest of compounds that bind to ER activating or inhibiting ERs selectively [6, 8, 9]. This group of compounds, called Selective Estrogen Receptor Modulators (SERMs), effectively block the activation of ERα by endogenous ligands and prevent the transcription of genes mediated by estrogen response elements. This class of compounds exhibit tissue-specific effects on breast tissues, resulting in the antagonist activity toward ERα [8, 9].

Tamoxifen (1) was the first SERM to be approved for clinical use. Although tamoxifen has been used for many years to treat and prevent breast cancer, it has also been shown that its agonist effect on other tissues like endometrium could produce other type of cancer [9]. On the other hand, fulvestrant [2] is a Selective Estrogen Receptor Degrader (SERD) that binds to ERs accelerating their degradation.

Finally, anastrazole and letrozole are drugs that inhibit aromatase enzyme and have been approved for postmenopausal breast cancer.

Figure 1. Most common drugs in use for hormonal-treatment of breast cancer.



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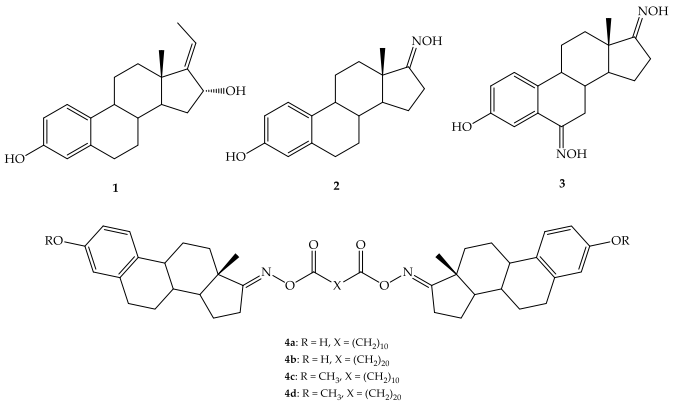
However, drug resistance and side effects, such as blood clots and endometrial cancer, are main concerns arising from endocrine treatment of breast cancer, which should be considered [1]. Therefore, the quest for new compounds to be used in hormone therapy is a matter of current interest, and different scaffolds structures have been explored to synthesize an increasing number of SERM and SERD [10,11]. In this effort, Computational Biology and Bioinformatics have the potential not only of speeding up the drug discovery process, with an associated cost reduction, but also of changing the way drugs are currently designed. One of such methods is the molecular docking of a family of structurally related molecules toward a receptor active site [12].

For example, using estrone as scaffold several steroidal analogs with different substitution at the 6, 16 and 17 positions have been synthesized [13-17] (Figure 2). From these, compound 1 (Figure 2) has demonstrated strong binding to ER, good anticancer potential in ER responsive cells (IC50 = 5.49 µM) and exhibited a very similar binding mode to estradiol [16]. On the other hand, a series of steroid oximes have been reported as anticancer agents [17], and therefore hydroximino steroids based on different scaffolds have been synthesized [14,17-20]. These compounds represent a distinct class of modulator and varying the hydroximino group location on the parental steroid skeleton results in remarkable changes in the anticancer activity [17]. On the other hand, 3-, 6- and 17-hydroxiimino steroids have proved that could be used in hormone therapy, although they have only been evaluated as aromatase inhibitors [18]. Bivalent estrogens have been synthesized using oxime esters of estrone and spacers of varying length [21] (Figure 2, compound 4).

Figure 2. Family of molecules synthesized and tested for activity against breast cancer cells



Furthermore, diosgenin, a sapogenin steroid, is a major bioactive constituent of various edible pulses and roots. Over the past decade, a series of preclinical and mechanistic studies have shown that diosgenin could inhibit proliferation and induce apoptosis in a wide variety of tumor cells, including osteosarcoma, colon carcinoma, leukemia, hepatoma, and breast carcinoma [22, 23]. Several diosgenin derivatives have been synthesized and their anti-proliferative activity has been assessed [24-26]. Interestingly, diosgenin-derived oximes have shown enhanced anti-proliferative activity as compared to diosgenin [20, 24]. Therefore, use of diosgenin as scaffold for attachment of hydroximino groups to the steroidal rings A and B should lead to derivatives with increased anticancer activity. However, no studies of ERα inhibition by diosgenin-derived oximes have been found in the literature. In this work a docking study of a library of mono- and bis-hydroximino diosgenin derivatives toward ERα was carried out.

2. EXPERIMENTAL

1. Ligan/Protein preparation

ERα 3D structure resolved by X-ray diffraction [PDB code: 3ERT, resolution (R= 1,90 Å)] [8] respectively; was downloaded from Protein Data Bank (http://www.rcsb.org). The missing sequences from receptors were built using structure homology with the SWISS-MODEL server [27]. The structure was optimized using pdb2pqr.py (Version 2.1.0) online server [28] with AMBER force field [12] and the protonation states of ionizable groups at pH = 7.0 were assigned by using PROPKA [12].

The 3D structure of 4-hydroxytamoxifen (OHT) was extracted from 3ERT and used as a control. The 2D structures of 26 designed compounds were drawn with ChemBioDraw Ultra 16.0. Their 3D coordinates were generated with Avogadro 1.1.1. [29] using default parametrization. Next, a preliminary optimization through Steepest Descent algorithms using MMFF94 [30] force field was performed in Avogadro 1.1.1. [36]. Then a semi-empirical Hamiltonian PM6-DH2 optimization was made with MOPAC 2016 [31].

Receptors and ligands PDB files were converted to PDBQT format using AutoDockTools [32]. Partial charges were calculated using the Gasteiger model. Non-polar hydrogen atoms were merged with the heavy atoms. In the case of ligands, rotatable bonds were set to default using the TORSDOF utility in AutoDockTools. All protein residues were kept rigid. A simulation box of size 24 × 16 × 20 Å3 (ERα) was constructed so that it could include the ligands flexible residues. The center of the simulation box was placed at the center of the active site.

2.2. Molecular docking simulation and analysis of receptor-ligand complexes
Multiple rigid molecular docking simulations were performed using AutoDock Vina 1.1.2 program (Vina) [33]. The docking parameters were set to default except the following: exhaustiveness = 32 and num_modes = 2, then 10 independent runs were carried out. The Vina predicted enzyme-ligand complexes (20 docked poses per ligand) were clustered using an RMSD < 2.5 Å. The mean binding energy (kcal/mol) was determined for each cluster. Then a contact-based analysis of the best-scoring pose in each group was carried out. Non-covalent interactions within ligands and ERα were determined using the Python-implemented computer algorithm BINANA [34]. The most promising ligands were selected based on their binding energy and the number of common interactions with the receptor. The binding modes of promising ligands were represented using PyMOL 2.4.1. The program AuPosSOM (Automatic analysis of Poses using SOM) [35] was employed to compare the contact fingerprint similarity between OHT and designed compounds. This approach is complementary to the scoring function and previous investigations have revealed that it is as efficient as conventional energy-based scoring functions or gives better results.

3. RESULTS

The binding mode of a series of 26 diosgenin-derived oximes has been analyzed by docking studies (Table S1, Supporting Material). Modifications in rings A, B, and both were made, and also both configurations (E or Z) were considered. All diosgenin derivatives were docked toward ERα cavity. The crystal structure of ERα complexed with 4-hydroxytamoxifen (OHT), the active metabolite of tamoxifen, were downloaded from Protein Data Bank. The results obtained in a preliminary docking simulation using OHT, revealed that the docked structure has few modifications on its configuration in the experimental complex [8]. The low RMSD value (2.99 Å) found in the redocking analysis of OHT suggested that the chosen parameters, such as location and size of the simulation box are suitable.

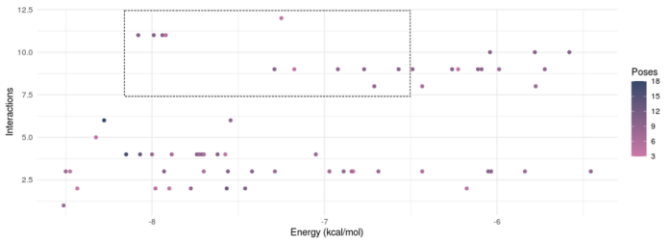
Most ligand binding modes showed low binding energy toward the tested breast cancer-related receptor (Supporting materials). All designed compounds satisfy Rosenfeld´s criteria for a binder, i.e. present low binding energy and few different binding modes in docking with ERα [36].

The contact-based analysis using BINANA was carried out to analyze the kind and strength of interactions that maintain receptor-ligand complexes. OHT maintains several contacts with ERα active site (MET343, LEU346, THR347, ALA350, ASP351, GLU358, TRP383, LEU387, ARG394, PHE404, GLU419, GLY420, MET421, LEU428, and LEU525).

Therefore, it can be assumed that a potential inhibitor will preserve similar interactions with these amino acids [37]. Minimum values of binding energy, $\Delta G \leq -6.5$ kcal/mol, and number of common interactions with OHT (over 50% of total interactions, *i.e.* 8), have been applied as filtering conditions. Results, indicate that 11 compounds could act as potential inhibitors of Erα (Figure 3).

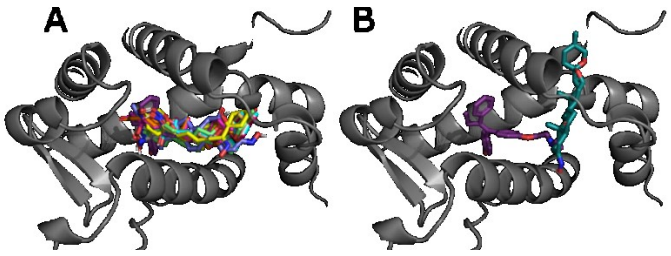
For the rest of ligands, even though their binding energy toward ERα is low, the amounts of different binding modes or a small percent of common interactions make them bad binders of ERα.

Figure 3. Distribution of Binding Energy predicted by the Vina scoring function and the number of common interactions with 4-hydroxytamoxifen.



Selected compounds can be separated in two major clusters by considering their binding mode. Most of ligands display a binding mode similar to OHT (Figure 4A, OHT in purple) whereas, one ligand adopts a 90 degrees' orientation as compared to OHT (Figure 4B).

Figure 4. Clustering of the selected compound according to their binding mode. A) Cluster 1: 5a, 6a, 6b, 7a, 8a, 8b, 9a, 10c, 11b and 12c. B) Cluster 2: 11a. In purple OHT.



The effect of free and entrapped EA extract on PC-3 cells and nuclear morphology was analyzed by phase contrast and fluorescence microscopies, respectively (Figure 3).

In Table 1 are listed the interactions of designed compounds with ERα active site that are in common with those shown by OHT. Most contacts correspond to van der Waals interactions, and the more frequent involves PHE (404), ALA (350), TRP (383), LEU (525), THR (347), and MET (343), which are key residues for ERα inhibition. In general, hydrogen bonds were absent, although compounds 6b and 9a maintain polar contacts with Leu525 and Glu419 at 3.32 and 3.30 Å, respectively (Figure 5A and Figure 5B). Short distance interactions were also found, *i.e.,* compound 9b has a strong hydrophobic contact that involves the methyl group at C-18 in the steroidal moiety (Figure 5B). Whereas the third binding mode of compound 10c displayed a polar interaction at 2.4 Å with Asp351. This compound also has a perpendicular orientation to OHT (Figure 5C).

Interestingly, three bis-oximes, 7a, 10c, and 12c, which are 3E,6E stereoisomers, exhibit similar binding mode to OHT. In other words, it seems that somehow this stereochemistry determines the way these compounds are accommodated in the active site. As can be seen in Figure 4D these three bis-oximes occupy the same region in the ERα cavity.

Table 1. Structure of the best docked designed compounds and in common interactions with 4-hydroxytamoxifen (OHT).

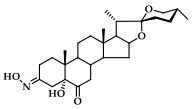
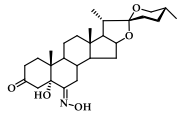
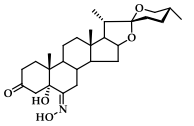
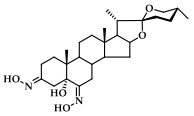
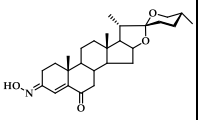
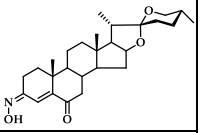
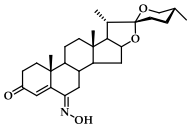
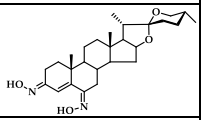
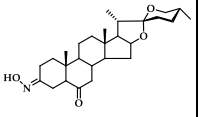
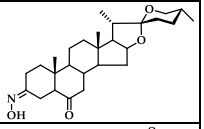
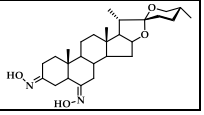
Selected Compounds	Common interactions with OHT	Structures
5a_1	PHE(404) ALA(350) TRP(383) LEU(387) ASP(351) LEU(525) THR(347) MET(343) LEU(346)	
6a_3	PHE(404) ALA(350) TRP(383) LEU(428) LEU(387) ASP(351) LEU(525) THR(347) GLU(419) MET(421) MET(343) LEU(346)	
6b_1	PHE(404) ALA(350) TRP(383) ASP(351) LEU(525) THR(347) MET(421) MET(343) LEU(346)	
7a_1	PHE(404) ALA(350) TRP(383) LEU(387) ASP(351) LEU(525) THR(347) MET(343) LEU(346)	
8a_2	PHE(404) ALA(350) TRP(383) LEU(387) ASP(351) LEU(525) THR(347) MET(343) LEU(346)	
8b_2	ALA(350) TRP(383) LEU(428) ASP(351) LEU(525) THR(347) MET(421) MET(343) LEU(346)	
9a_2	PHE(404) ALA(350) TRP(383) LEU(428) LEU(387) ASP(351) LEU(525) THR(347) GLU(419) MET(421) MET(343)	
10c_1	PHE(404) ALA(350) TRP(383) ASP(351) LEU(525) THR(347) MET(343) LEU(346	
11a_1	PHE(404) ALA(350) TRP(383) LEU(387) ASP(351) LEU(525) THR(347) MET(343) LEU(346)	
11b_1	PHE(404) ALA(350) TRP(383) LEU(387) ASP(351) LEU(525) THR(347) MET(343) LEU(346)	
12c_1	PHE(404) ALA(350) TRP(383) LEU(387) ASP(351) LEU(525) THR(347) MET(343) LEU(346)	

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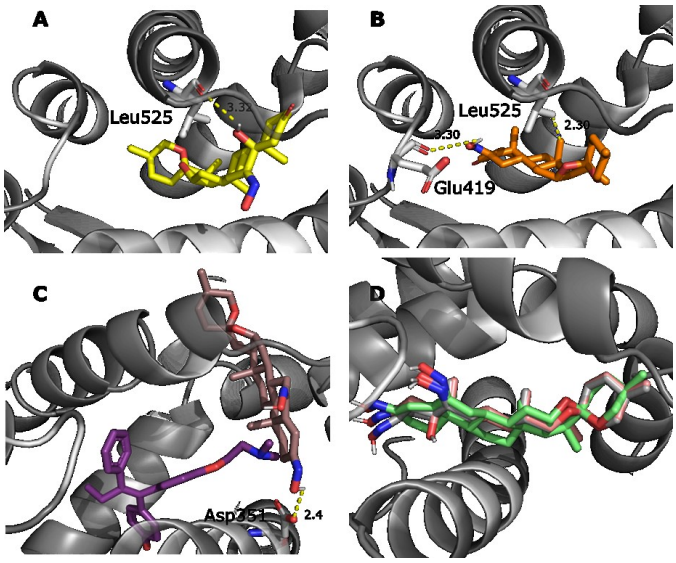
Figure 5. Representation of interactions of compound 6b_1 (A), 9a_2 (B) and 10c_3(C). D) Similar binding mode of the selected bis-oximes 7a_1 (pink), 10c_1 (green) and 12c_1 (gray). All distances are in Å.

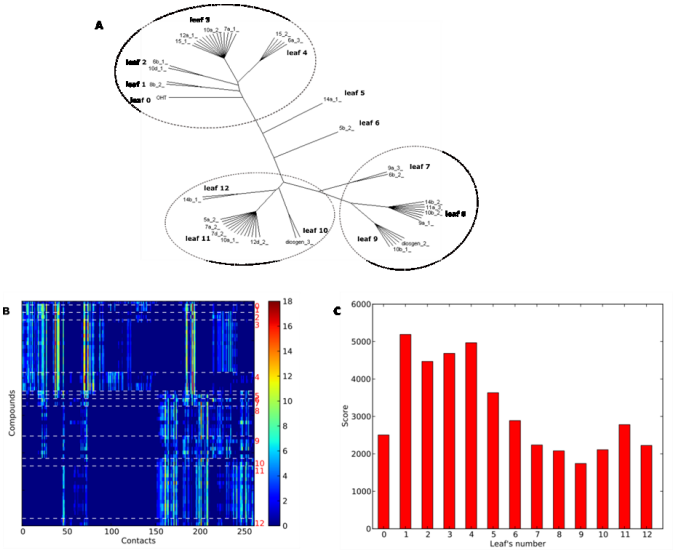


On the other hand, results obtained for mono-oximes suggest that both configurations originate good binding modes. Even though binding energies are the same, only mono-oximes have cluster populations of 50% (10 independent runs with the same docked pose), which means that binding modes found for these compounds are more probable than those obtained for bis-oximes.

Sometimes, application of Rosenfeld´s criteria and analysis of interatomic interactions are not enough to choose a proper group of potential inhibitors. In these cases, comparison of three-dimensional contact fingerprints of ligands with those from a known inhibitor provides an alternative way to discriminate between active or inactive compounds [37]. Structure interaction fingerprints have been used to score docking poses according to the number of common interactions, this method allowed not to limit the analysis to the use of a reference inhibitor. Poses with high numbers of common interactions the most likely these ligands would be "good inhibitors" [38]. Currently, the analysis of poses is automatically carried out using self-organizing maps (AuPosSOM). In this method, docked ligands are clustered in accordance with the similarity of their binding modes. The results are presented as a hierarchic tree where leaves contain compounds with similar binding modes.

AuPosSOM results are displayed in Figure 6 and a detailed leaf composition is given in Table 1. Three major clusters can be observed in Figure 6A, which correspond to different interaction pattern with ERα. In Figure 6B each line represents a binding mode footprint, and each column represents the contact with atoms from the protein. As can be seen, compounds from leaves 1-4 are in the same cluster as OHT and they have a similar contact pattern to the known inhibitor. AuPosSOM also uses a score function that corresponds to a combination between contact specificity and contact intensity for the different leaves. According to these results binding modes belonging to leaves 1-6 and leaf 7 have a higher score than OHT, this means that their interaction with ERα is stronger (Figure 6C). Thus, these results suggest that binding modes in leaves 1 (8b_2 and 7d_3) and 4 (6a_3, 9a_2, 10c_2, 15_2, 10d_2) are the best.

Figure 6. Representation of interactions of compound 6b_1 (A), 9a_2 (B) and 10c_3(C). D) Similar binding mode of the selected bis-oximes 7a_1 (pink), 10c_1 (green) and 12c_1 (gray). All distances are in Å. AuPosSOM results. A) Hierarchal tree map where clusters between the leaves are represented in circles. B) Contact map where leaf number is represented in red. The color scale indicates the contact intensity average where the dark blue color corresponds to a low-intensity contact (close to zero) and in this method leaves are separated by white dotted lines. C) Scoring plot.



4. DISCUSSION

It is widely known that some breast cancer tumor cells are sensitive to female steroidal hormones, where estrogen and estradiol play a crucial role. Inhibitors have shown their antagonistic effects in breast tissue; however, they exhibit side effects in other ones, mainly in the endometrium [35]. Consequently, the quest for new compounds with these activities is a very active field of research. Two control compounds were included in this study, namely diosgenin, a known steroid with antiproliferative activity in breast cancer tissues [39], and compound 15, a 6,23-dihydroximinium diosgenin derivative, [20, 40].

On one side, diosgenin showed significant differences in cell viability of MDA-MB-231 cell line at 20 µM whereas compound 15 had a promising GI50 of 19 and 12 µM in two cell lines related to breast cancer, namely HBL-100 and T-47D, respectively. Docking study of these compounds was carried out to analyze the observed experimental results in antiproliferative activity. The obtained poses for both compounds are represented in Figure 1.

Two populated binding modes were obtained for compound 15; with binding energies of –5.78 and –5.77 kcal/mol and several in common interactions with OHT (10 and 8 respectively) were identified. On the other hand, three different binding modes with energies ranging between –8.51 and –8.48 kcal/mol were found for diosgenin. Despite these low energy values, just a few common interactions of diosgenin with ERα were found in comparison with OHT. The steroidal fragment of diosgenin is structurally very similar to estrogen and/or estradiol, which are natural binders of this receptor, and therefore shows a high affinity by the ER cavity.

Thus, these results suggest that chemical modification of diosgenin skeleton could be used to enhance antagonist activity against Erα [8]. Previous structure-activity relationship studies have shown that methyl groups can improve antagonist activity and selectivity toward ERα [10]. In this context, it is expected that activity of compound 9a could be increased by the strong hydrophobic interaction originated by the methyl group at C-18 (Figure 5B).

In addition, it has also been described that hydrogen bonds with Leu346, Thr347, Asp351, Arg394, or Leu525 play a fundamental role to stabilize the binding conformation [41]. As mentioned above, only polar interactions with Asp351 and Leu525 were found for compounds 10c and 6b. The most outstanding result is the hydrogen bond interaction between the hydroximino group of 9b and Glu419. This polar contact has not been described, and only a hydrophobic interaction of Glu419 with OHT has been reported [8].

In summary, our results indicate that modifications of diosgenin with oxime groups could lead to potential inhibitors to treat breast cancer. Experimental and theoretical results suggest that introduction of hydroximino group can establish important polar interactions with key residues that are vital to inhibit the activity of ERα. The results of docking simulations on 26 diosgenin-derived oximes, application of Rosenfeld et al. criteria, and analysis of the interaction pattern, indicate that three mono-oximes (compounds 6a, 8b, and 9a) are the most promising compounds to become ER inhibitors.

5. ACKNOWLEDGEMENTS

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