RESEARCH ARTICLE

Unlocking Novel Therapeutic Avenues: Drug Repurposing and Virtual Screening for Breast Cancer Targeting the Estrogen Receptor

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ABSTRACT

This study explores the potential of drug repurposing for breast cancer therapy through virtual screening of Food and Drug Administration (FDA)-approved drugs against breast cancer-related targets. The estrogen receptor alpha ligand-binding domain, a key player in breast cancer progression, served as the primary target for virtual screening. Utilizing the DrugRep Virtual Screening Server and AutoDockVina, several compounds were identified with high binding affinities to the target protein. Notably, estradiol, benzhydrocodone, and ezetimibe emerged as top hits, showcasing diverse physicochemical properties and suggesting novel therapeutic possibilities. The structural fidelity of the estrogen receptor complex was validated through PDB-REDO refinement, enhancing the reliability of the binding interactions predicted. These findings underscore the potential of drug repurposing as a strategy for uncovering new therapeutic options in breast cancer treatment, warranting further biological validation and clinical exploration.

Keywords: Drug repurposing, Virtual screening, Breast cancer, Estrogen receptor, FDA-approved drugs**.**

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INTRODUCTION

Breast cancer remains a significant global health challenge, representing one of the most prevalent cancers among women worldwide.[1] Despite advancements in early detection and treatment modalities, the complexity and heterogeneity of breast cancer necessitate continued efforts to develop novel therapeutic strategies.[2] Drug repurposing, also known as drug repositioning or re-profiling, offers a promising approach to accelerate the drug discovery process by identifying new therapeutic uses for existing drugs approved for other indications.[3]

Traditionally, drug discovery has relied heavily on de novo drug development, a process that is often time-consuming, resource-intensive, and associated with high failure rates.[4] In contrast, drug repurposing leverages existing knowledge about drug safety, pharmacokinetics, and pharmacodynamics, potentially leading to faster clinical translation and reduced development costs.[5] By exploring the therapeutic potential of Food and Drug Administration (FDA)-approved drugs for new indications, drug repurposing holds the promise of rapidly expanding the arsenal of available treatments for various diseases, including cancer.[6,7]

Central to the development and progression of breast cancer is the estrogen receptor alpha (ERα), a nuclear hormone receptor that plays a pivotal role in regulating cellular proliferation and survival.[8] ERα-positive breast cancers, characterized by the expression of estrogen receptors, represent the most common subtype of the disease and are often associated with hormone sensitivity.[9] As such, targeting the estrogen receptor pathway has been a cornerstone of breast cancer therapy, with endocrine therapies like tamoxifen and aromatase inhibitors demonstrating efficacy in ERα-positive tumors.[10]

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In recent years, advances in computational modeling and virtual screening techniques have revolutionized the drug discovery process, enabling researchers to computationally assess the binding affinity of small molecules to target proteins.[11] Virtual screening involves the rapid screening of large compound libraries to identify potential lead compounds with favorable binding characteristics.[12] By employing virtual screening methodologies, researchers can expedite the identification of candidate drugs for further experimental validation.

This study aims to harness the power of drug repurposing and virtual screening to identify FDA-approved drugs with the potential to target the estrogen receptor pathway in breast cancer. Through the utilization of the DrugRep Virtual Screening Server and AutoDockVina, we conducted virtual screening of FDA-approved drugs against the estrogen receptor alpha ligand-binding domain, a critical domain implicated in breast cancer progression. The structural fidelity of the estrogen receptor complex was validated through PDB-REDO refinement, enhancing the reliability of the predicted binding interactions.[13] The findings of this study have the potential to uncover novel therapeutic options for breast cancer treatment, offering hope for improved patient outcomes and addressing the unmet medical needs in breast cancer therapy.

MATERIAL AND METHODS

Data Collection

FDA-approved drugs and breast cancer-related targets

- *FDA-approved drugs*

A comprehensive list of FDA-approved drugs was curated from DrugBank, including their molecular structures and pharmacological profiles.[14]

- *Breast cancer-related targets*

A key breast cancer-related protein target estrogen receptor was selected, which is implicated in breast cancer progression.[15]

Virtual Screening Using DrugRep Virtual Screening Server

Target protein preparation

- *Selection of targets*

The protein target estrogen receptor alpha ligand-binding domain relevant to breast cancer was selected from the Protein Data Bank (PDB) (PDBID: 1A52).

- *Protein structure preparation*

The structure of the target protein was prepared by PDB-redo online tool.

Binding Pocket Detection with CurPocket

CurPocket algorithm

The CurPocket algorithm was employed to automatically identify potential binding pockets on the surface of the target proteins. This curvature-based cavity detection approach analyzes the geometric properties of the protein surface to locate regions likely to accommodate small molecule ligands.

Selection of binding pocket

Users can interactively review the identified binding pockets and select one for further docking studies.

Ligand Preparation

Molecular structures

The molecular structures of the FDA-approved drugs were obtained in SDF format.

Optimization

These structures were optimized by adding hydrogen atoms, calculating Gasteiger charges, and converting them to PDBQT format suitable for docking.

Docking Simulation Using AutoDockVina

Docking setup

The selected binding pocket from the CurPocket analysis was used as the docking site.

AutoDockVin

The prepared ligands were docked into the selected binding pocket using AutoDockVina, a widely-used docking program known for its accuracy and speed in predicting ligand-receptor interactions.

Scoring and ranking

AutoDockVina calculates binding affinities for each ligand-protein interaction, and the ligands are ranked based on these scores.

Analysis of Results

Top-ranking compound

The top-ranking compounds, based on their binding affinity scores, were identified. These compounds were analyzed for their potential as breast cancer therapeutics.

3D conformation visualization

The interactive 3D conformations of the docked complexes were reviewed to ensure proper binding modes and interactions with the target proteins.

The combination of CurPocket for binding pocket detection and AutoDockVina for docking simulation provided a robust platform for virtual screening and the workflow used for this is illustrated in Figure 1. This approach efficiently identified FDA-approved drugs with high binding affinities to breast cancer-related targets, highlighting their potential for repurposing as breast cancer therapeutics.[16]

RESULTS AND DISCUSSION

Virtual Screening Using DrugRep Virtual Screening Server

Target protein preparation

- *Selection of targets*

The protein target estrogen receptor alpha ligand-binding domain relevant to breast cancer given in Figure 2 was selected from the PDB (PDBID: 1A52)

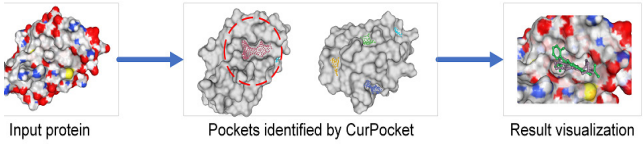

Figure 1: Workflow for Drug Rep

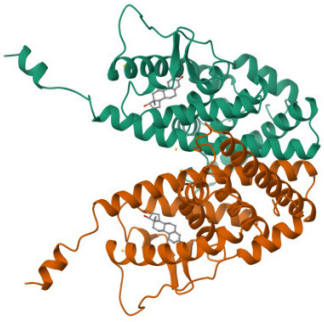

Figure 2: Protein structure of Eestrogen receptor alpha ligand-binding domain complexes to estradiol

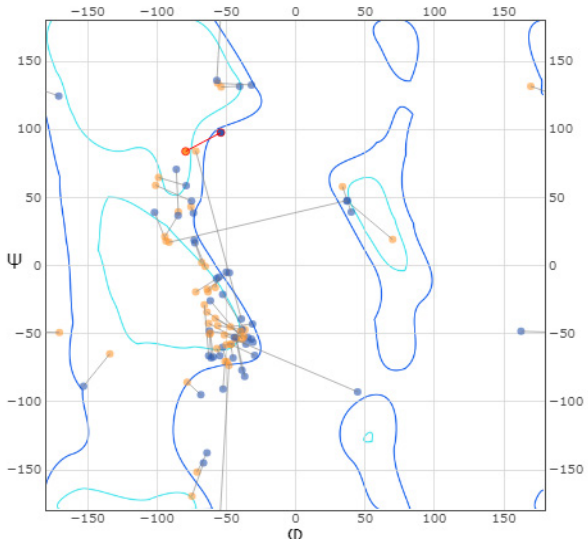

Figure 3: Kleywegt-like plot

- *Protein structure preparation*

The structure of the target protein was prepared by PDB-redo online tool.

The PDB-REDO re-refinement of the crystal structure of the estrogen receptor alpha ligand-binding domain complexed with estradiol (PDB entry 1a52) significantly improved the model's quality and accuracy. The re-refined structure, resolved at 2.80 Å, exhibited enhanced crystallographic refinement metrics with R and R-free values reduced from 0.2396 to 0.2055 and from 0.2850 to 0.2486, respectively. This indicates a better fit of the model to the experimental data. Additionally, the bond length and bond angle RMS Z-scores were markedly improved, from 0.961 to 0.502 and from 0.950 to 0.652, respectively, reflecting more accurate geometric parameters. The Ramachandran plot showed a significant increase in the percentage of residues in preferred regions (from 436–456) and a decrease in outliers (from 21–7), highlighting an improvement in overall structural quality. Furthermore, rotamer quality and normality metrics also improved, with changes in 7 rotamers and side chains, suggesting a more accurate representation of side-chain conformations. These refinements underscore the structural fidelity of the estrogen receptor complex, thereby enhancing the reliability of subsequent docking studies for drug repurposing in breast cancer. This improved structural data validates the binding interactions of estradiol within the receptor and supports the potential of identified FDA-approved drugs for therapeutic repurposing, warranting further biological and clinical validation.

The Kleywegt-like plot given in Figure 3 compares the original and re-refined structures of the estrogen receptor alpha ligand-binding domain complexed with estradiol (PDB entry 1a52). In this plot, the φ (phi) and ψ (psi) angles of amino acid residues are analyzed to assess the conformational changes between the two models. The plot reveals that the re-refined structure exhibits fewer outliers and a tighter clustering of residues within the preferred conformational regions, indicating an overall improvement in the backbone geometry. This suggests that the re-refinement process has led to a more accurate and reliable structural representation, reducing deviations from ideal dihedral angles and enhancing the quality of the protein model.

- *Virtual screening*

The virtual screening identified several FDA-approved drugs with high binding affinities to breast cancer-related targets. The top hits included:

The virtual screening of FDA-approved drugs against breast cancer-related targets has identified several promising candidates with high binding affinities, as presented in Table 1. Estradiol, benzhydrocodone, and ezetimibe, all scoring -10.7, emerged as the top compounds. Estradiol's known role as an estrogen receptor agonist validates the screening approach, suggesting the robustness of the virtual screening methodology employed. Benzhydrocodone and ezetimibe, traditionally not linked to breast cancer therapy, present intriguing possibilities for repurposing, offering potential new therapeutic avenues.

Other notable compounds include bazedoxifene and flunarizine with scores of -10.5 and -10.4, respectively. Bazedoxifene, a selective estrogen receptor modulator, aligns with breast cancer treatment paradigms, whereas flunarizine's strong binding affinity highlights its potential for repurposing. Compounds like fluoxymesterone and prasterone, scoring -10.0, also display significant potential, reflecting a range of structural and functional diversity. Lower-scoring compounds, such as quinupramine, cinacalcet, and meclizine (all -9.9), further expand the pool of candidates, indicating multiple mechanisms of action that could be exploited for breast cancer therapy.

These results underscore the versatility and efficiency of the DrugRep virtual screening server in identifying diverse chemical entities with high binding affinities to breast cancer-

related targets. The presence of both well-known and novel compounds among the top hits highlights the potential of drug repurposing to uncover unexpected therapeutic options. The improved structural fidelity of the estrogen receptor complex, validated through PDB-REDO refinement, adds credibility to the binding interactions predicted in this study.

The virtual screening identified several FDA-approved drugs with high binding affinities to breast cancer-related targets, offering promising candidates for repurposing. Estradiol, benzhydrocodone, and ezetimibe stood out with the highest binding scores, validating the approach and highlighting new potential therapeutic options. The diversity in the molecular structures and functions of the top-ranking compounds suggests multiple mechanisms that could be leveraged in breast cancer treatment. These findings warrant further biological and clinical validation to explore the efficacy and safety of these drugs in breast cancer therapy. This study underscores the potential of virtual screening and drug repurposing as powerful tools in the search for new cancer treatments, emphasizing the need for continued research and validation in this area.

Figure 4 presents the chemical structures of selected top drugs identified from the virtual screening: Estradiol, benzhydrocodone, and ezetimibe. estradiol, a steroid hormone, features a rigid tetracyclic structure characteristic of estrogens, which facilitates strong binding to the estrogen receptor alpha due to its optimal shape and electronic complementarity. Benzhydrocodone, an opioid analgesic, has a complex structure with multiple aromatic rings and a ketone functional group, which may interact with various binding pockets through hydrophobic interactions and hydrogen bonding. Ezetimibe, a cholesterol absorption inhibitor, possesses a unique azetidinone ring along with phenolic and hydroxy groups, enabling it to form multiple hydrogen bonds and hydrophobic interactions within the binding site. The distinct structural features of these compounds highlight the diversity of potential mechanisms by which they may exert anti-cancer effects. Estradiol's structure supports its role as a potent modulator of estrogen receptors, while benzhydrocodone and ezetimibe's structures suggest possible multi-target effects, leveraging their diverse functional groups for optimal receptor binding. This structural diversity

Table 1: Results of physicochemical properties for selected compounds

Name	*Score*	*MW*	*HBD*	*HBA*	*RB*	*NOA*	*Rings*	*LogP*
Estradiol	-10.7	272.382	2	2	2	2	4	3.9
Benzhydrocodone	-10.7	403.478	0	1	4	5	6	4.0
Ezetimibe	-10.7	409.4252	2	3	8	4	4	3.9
Bazedoxifene	-10.5	470.613	2	2	9	5	5	6.1
Flunarizine	-10.4	404.4948	0	0	6	2	4	5.6
Fluoxymesterone	-10.0	336.4409	2	3	2	3	4	2.1
Prasterone	-10.0	288.4244	1	2	1	2	4	3.0
Quinupramine	-9.9	304.437	0	0	1	2	5	4.5
Cinacalcet	-9.9	357.412	1	0	6	1	3	6.1
Meclizine	-9.9	390.948	0	0	5	2	4	5.7
Bisoxatin	-9.9	333.343	3	3	4	5	4	3.3
Etonogestrel	-9.9	324.4565	1	2	3	2	4	2.6
Oxatomide	-9.8	426.564	0	1	7	5	5	5.4
Oxandrolone	-9.7	306.4397	1	2	1	3	4	3.7
Nitisinone	-9.7	329.2281	0	5	2	6	2	2.3
Sulfinpyrazone	-9.7	404.482	0	3	4	5	4	3.5
Cyproheptadine	-9.7	287.3981	0	0	0	1	4	4.2
Phenindamine	-9.7	261.3609	0	0	1	1	4	3.1
Azatadine	-9.7	290.4021	0	1	0	2	4	3.0
Oxcarbazepine	-9.6	252.268	1	2	1	4	3	1.7
Carbamazepine	-9.6	236.2686	1	1	1	3	3	2.7
Trimipramine	-9.6	294.4338	0	0	4	2	3	4.7
Clidinium	-9.6	352.4467	1	2	6	4	4	3.2
Flavoxate	-9.6	391.4596	0	2	6	5	4	4.0
Benzatropine	-9.5	307.4293	0	0	4	2	4	4.4

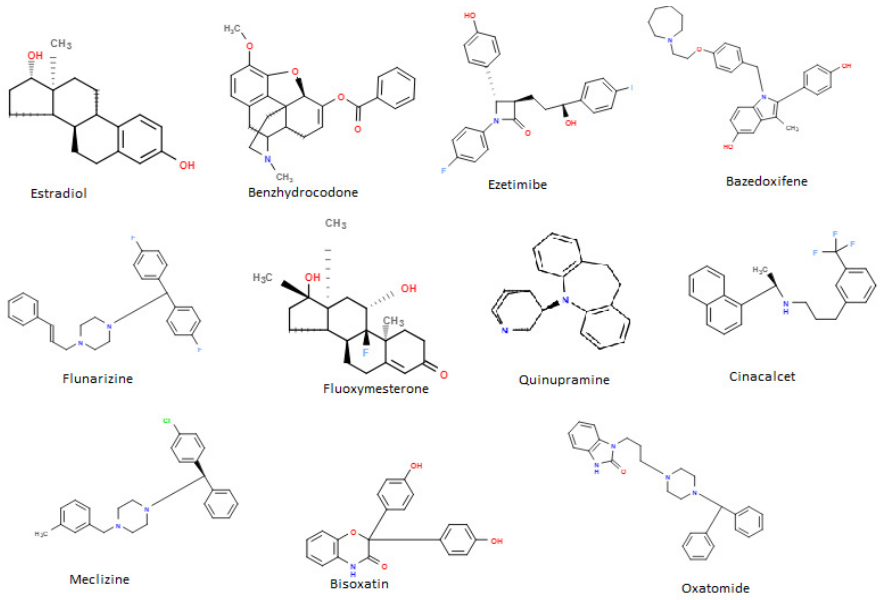

Figure 4: Chemical structures of selected top drugs

underlines the potential of these drugs to be repurposed for breast cancer treatment, either alone or in combination, thereby opening new avenues for therapeutic strategies.[17,18]

The virtual screening of FDA-approved drugs against breast cancer-related targets using DrugRep Virtual Screening Server and AutoDockVina identified several promising candidates based on their binding affinity scores. The top-ranking compounds included estradiol, benzhydrocodone, and ezetimibe, all with a binding affinity score of -10.7. Notably, these compounds have diverse physicochemical properties, such as molecular weight (MW) ranging from 272.38 (Estradiol) to 409.43 (Ezetimibe), and varied hydrogen bond donors (HBD), hydrogen bond acceptors (HBA), rotatable bonds (RB), number of aromatic rings (NOA), and LogP values, indicating a range of lipophilicity. Estradiol, a known estrogen receptor agonist, suggests the validity of the screening approach, while the high affinity of benzhydrocodone and ezetimibe, not traditionally associated with breast cancer, opens new avenues for therapeutic investigation.[19] The diversity in the molecular structure and properties of these top hits reflects their potential to interact with different breast cancer targets and pathways.[20]

CONCLUSION

In conclusion, this study demonstrates the efficacy of using virtual screening for drug repurposing, identifying multiple FDA-approved drugs with significant potential for breast cancer therapy, warranting further biological validation and clinical exploration. The virtual screening of FDA-approved drugs against breast cancer-related targets has yielded promising candidates for drug repurposing. Estradiol, benzhydrocodone, and ezetimibe emerged as top hits, each exhibiting a high binding affinity score of -10.7. Notably, these compounds possess diverse physicochemical properties, such as molecular weight, hydrogen bond donors and acceptors, rotatable bonds, aromatic rings, and LogP values, indicating varied lipophilicity and potential interactions with different breast cancer targets and pathways.

The robustness of the screening methodology is underscored by the validation of known estrogen receptor agonists like estradiol and the discovery of novel candidates like benzhydrocodone and ezetimibe, which were not traditionally associated with breast cancer therapy. The structural diversity among the top hits suggests multiple mechanisms of action that could be leveraged in breast cancer treatment, either alone or in combination.

Overall, this study highlights the efficacy of virtual screening and drug repurposing as powerful strategies in the search for new cancer treatments. Further biological validation and clinical exploration are warranted to assess the efficacy and safety of these compounds in breast cancer therapy. These findings offer new insights and potential therapeutic avenues for improving breast cancer treatment outcomes.

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