RESEARCH ARTICLE

Repurposing FDA-Approved Anastrozole-based Drugs for Breast Cancer through Drug-Drug Transcriptomic Similarity and Cavity Detection Guided Blind Docking

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ABSTRACT

Breast cancer remains a significant global health concern, necessitating innovative strategies for drug discovery and development. Repurposing existing drugs offers a promising avenue, leveraging the wealth of information available on food and drug administration (FDA) approved compounds. This study investigates the repurposing potential of anastrozole-based drugs, already established for their efficacy in certain conditions, for breast cancer treatment.

This research presents a multifaceted investigation into the identification, structural refinement, and virtual screening of potential therapeutics targeting the aromatase CYP19A enzyme, a crucial player in hormone-related conditions such as breast cancer. The study commences with the identification of anastrozole-based drugs exhibiting transcriptomic profiles closely resembling known breast cancer therapeutics. Through a comprehensive analysis, a subset of compounds demonstrates high transcriptomic similarity, suggesting shared molecular pathways and target interactions. Notably, anastrozole, a well-known aromatase inhibitor, emerges as a top candidate, highlighting its potential in breast cancer treatment. The crystallographic structure of aromatase CYP19A is subjected to meticulous preprocessing using the PDB-REDO server, resulting in significant improvements in various validation metrics. Structural changes, including alterations in rotamers, removal of water molecules, and peptide flips, indicate the success of the refinement process in enhancing the accuracy of the protein model. The refined structure serves as a reliable foundation for subsequent studies. Further, structure-based cavity detection unveils potential binding sites on the aromatase enzyme. Docking studies employing the cb-dock server elucidate the interaction patterns and binding affinities of selected compounds within these cavities. Anastrozole, along with other candidates like dolasetron and stiripentol, exhibits promising binding scores and interacts with specific residues crucial for enzyme activity.

This integrative approach, combining transcriptomic similarity analysis, structural refinement, and virtual screening, provides valuable insights into potential lead compounds for the inhibition of aromatase in breast cancer therapy. The identified compounds offer a starting point for further experimental validation and drug development. The most promising compound that can be repurposed for as an aromatase inhibitor is dolasetron. Overall, this research contributes to the ongoing efforts to leverage computational methodologies for the rational design of targeted therapeutics against hormone-related disorders.

Keywords: Breast cancer, FDA-approved, Transcriptomic similarity, Repurposing and Drug discovery

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INTRODUCTION

Breast cancer, a complex and heterogeneous disease, remains a formidable challenge in the realm of oncology.[1] Central to the pathogenesis of a significant subset of breast cancers is the dysregulation of hormone signaling, particularly the aberrant activity of aromatase, encoded by the CYP19A gene. Aromatase is responsible for the conversion of androgens to estrogens, thereby contributing to the growth and progression of hormone-sensitive breast tumors. As such, targeted inhibition of aromatase has become a cornerstone in the therapeutic arsenal against hormone receptor-positive breast cancers.[2,3]

This research embarks on a comprehensive exploration aimed at advancing our understanding and therapeutic strategies for breast cancer, with a focal point on aromatase as a pivotal molecular target. The study integrates advanced computational methodologies, including transcriptomic similarity analysis, structural refinement, and virtual screening, to identify and refine potential therapeutics targeting aromatase. The overarching goal is to unveil novel compounds with enhanced efficacy, reduced side effects, and improved selectivity, ultimately contributing to the refinement of breast cancer treatment paradigms.[4]

The initial phase of our investigation involves the identification of anastrozole-based drugs exhibiting transcriptomic profiles closely resembling known breast cancer therapeutics. Leveraging transcriptomic similarity analysis, we aim to elucidate shared molecular pathways and potential target interactions, providing a foundation for the subsequent stages of our research. The crystallographic structure of aromatase is then subjected to rigorous preprocessing using the PDB-REDO server, ensuring the accuracy and reliability of the structural model. This refined structure becomes instrumental in our virtual screening endeavors.[5]

Our approach includes structure-based cavity detection to unveil potential binding sites on the aromatase enzyme. Subsequent docking studies employing the cb-dock server facilitate the characterization of the interaction patterns and binding affinities of selected compounds within these cavities. The integration of these diverse computational methodologies aims to streamline the identification of lead compounds with the potential to inhibit aromatase activity, thereby disrupting the hormone-driven pathways crucial for breast cancer progression.[6,7]

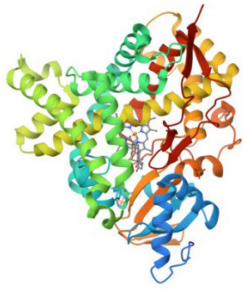


Figure 1: Crystal structure of human placental aromatase cytochrome P450 aromatase (CYP19A)

Through this research, we aspire to contribute to the burgeoning field of precision medicine for breast cancer by providing a rational framework for the identification and optimization of therapeutics targeting aromatase. The computational insights garnered herein are envisioned to guide subsequent experimental validations and, ultimately, pave the way for the development of innovative and more effective treatment strategies against hormone-related breast cancers.[8,9]

MATERIALS AND METHOD

Drug-drug Transcriptomic Similarity Analysis

Utilizing large-scale transcriptomic datasets, we assess the similarity between anastrozole-based drugs and established breast cancer therapeutics. Identification of common gene expression patterns provides insights into potential shared mechanisms of action.

The Connectivity Map Touchstone tool (https://clue.io/touchstone) was employed to systematically select FDA-approved anastrozole-based drugs for potential application in breast cancer through drug-drug transcriptomic similarity. The investigation commenced with access to the tool's website, followed by the exploration of the Touchstone dataset, rich in expression profiles from various perturbagens, including FDA-approved drugs. Running the tool initiated a connectivity mapping analysis, comparing the input gene expression signature against the extensive Touchstone dataset.[10]

Results were critically reviewed, and priority was given to FDA-approved anastrozole-based drugs exhibiting high transcriptomic similarity to the input signature. Subsequently, an in-depth exploration of the connections between the identified drugs and the input query was conducted, shedding light on shared molecular pathways and potential therapeutic targets. The insights gained from this analysis formed the basis for hypotheses regarding the efficacy of the selected drugs for breast cancer treatment.[11]

Protein Pre-preparation using PDB REDO and Molecular Docking

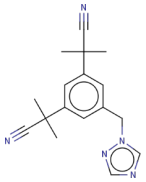
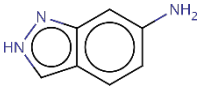
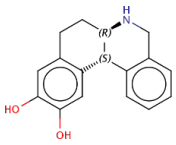
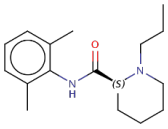
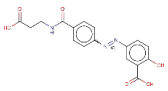
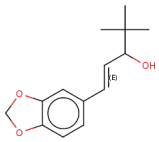
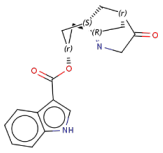
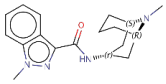
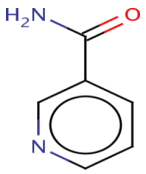
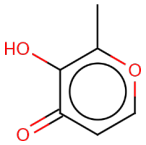
Pre-preparation of the protein of aromatase enzyme was done using PDB REDO server. Molecular docking simulations were executed to assess the binding affinity and interaction patterns of selected compounds with the human placental aromatase cytochrome P450 (CYP19A) enzyme (PDB ID: 3EQM) (Figure 1). The AutoDock tool from the cb-dock server was utilized for this purpose. Virtual screening results were meticulously analyzed based on docking scores, and compounds were ranked according to their predicted binding affinities. Compounds demonstrating high binding affinity, favorable interaction patterns, and structural compatibility with CYP19A were identified as potential lead compounds.[12,13]

RESULTS

Transcriptomic Similarity

Identification of anastrozole-based drugs with transcriptomic profiles closely resembling known breast cancer therapeutics.

Table 1: Results of transcriptomic similarity-based repurposing

Rank	*Score*	*ID*	*Name*		*Description*
1	99.98	BRD-K52172416	Anastrozole		Aromatase inhibitor
2	99.93	BRD-K51066026	Aminoindazole		Ionophore
3	99.93	BRD-K31471398	Dihydrexidine		Dopamine receptor agonist
4	99.89	BRD-K50938786	Ropivacaine		Sodium channel blocker
5	99.89	BRD-K41410256	Balsalazide		Cyclooxygenase inhibitor
6	99.89	BRD-A72441487	Stiripentol		GABA uptake inhibitor
7	99.86	BRD-K28029915	Dolasetron		Serotonin receptor antagonist
8	99.86	BRD-K10860596	Granisetron		Serotonin receptor antagonist
9	99.81	BRD-K28912512	Nicotinamide		Protein synthesis stimulant
10	99.79	BRD-K40619305	Larixinic-acid		Compound that interacts with metal centers

The results of the transcriptomic similarity-based repurposing analysis have unveiled a promising list of compounds with potential implications for aromatase inhibition in the context of breast cancer therapy. Anastrozole, identified as the top-ranked candidate with a remarkable score of 99.98, reaffirms its status as a potent aromatase inhibitor, aligning with its established role in breast cancer treatment. Additionally, other compounds such as aminoindazole, dihydrexidine, ropivacaine, balsalazide, stiripentol, dolasetron, granisetron, nicotinamide, and larixinic-acid exhibit high transcriptomic similarity all are listed in Table 1, suggesting their potential relevance to breast cancer therapeutics. The diverse nature of these compounds, ranging from ionophores and dopamine receptor agonists to cyclooxygenase inhibitors and GABA uptake inhibitors,

Figure 2: Introspect showing cell line specific response to perturbagens

underscores the multifaceted approach in identifying novel agents for aromatase modulation. This comprehensive analysis not only reaffirms the candidacy of established drugs like anastrozole but also proposes new avenues for investigation, opening doors for further experimental validation and potential drug repurposing strategies in the pursuit of more effective breast cancer treatments.

In Figure 2, Thick black bars signify transcriptional activity scores greater than or equal to 0.5; thinner black bars denote scores less than 0.5. The absence of a bar means no data is available. PC3 A375 and A549 cell lines show transcriptional activity scores greater than or equal to 0.5.

Molecular Docking: Protein Pre-preparation using PDB REDO

Significant structural changes were observed in the aromatase CYP19A enzyme shown in Table 2 following the preprocessing and refinement performed by the PDB-REDO server.

Significant structural changes were observed in the aromatase CYP19A enzyme following the preprocessing and refinement performed by the PDB-REDO server. The analysis revealed alterations in six rotamers, indicating adjustments in the conformations of specific amino acid side chains. Notably, no side chains were flipped during the refinement process. Additionally, five water molecules were removed from the structure, potentially impacting the enzyme's local hydration environment. Three peptides were flipped, suggesting changes in the orientation of peptide bonds within the protein. Chirality remained unchanged, with no alterations in the handedness of amino acid configurations. Impressively, 147 residues exhibited improved fitting to the electron density map, reflecting enhanced alignment with experimental data. Conversely, only one residue demonstrated a decrease in fitting to density, highlighting the overall success of the preprocessing steps in refining and optimizing the aromatase CYP19A enzyme's crystal structure. These significant structural changes underscore the utility of PDB-REDO in enhancing the accuracy and reliability of the protein model for subsequent analyses and investigations.

Results of Structure-based Cavity Detection

Five cavities were detected in the protein structure of aromatase all are illustrated in Figure 3.

Table 2: Results of crystallographic structure of the aromatase (CYP19A) after PDB-REDO refinement

Validation metrics	*Original*	*PDB-REDO*
Crystallographic Refinement		
R	0.2164	0.1747
R-free	0.2449	0.2131
Bond length RMS Z-score	0.416	0.581
Bond angle RMS Z-score	0.642	0.845
Model quality raw scores		
Percentiles		
Ramachandran plot normality	-3.331	-2.616
Rotamer normality	-6.080	-3.475
Coarse packing	-0.583	-0.370
Fine packing	-1.136	-0.484
Bump severity	0.021	0.014
Hydrogen bond satisfaction	0.832	0.850

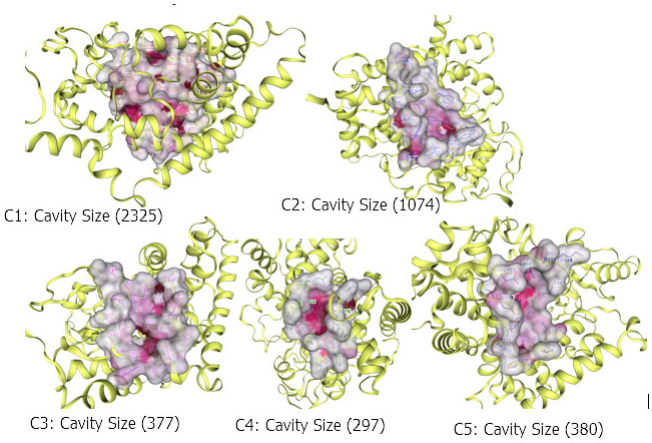


Figure 3: Cavities detected in CYP19A by structure-based cavity detection

Results of AutoDockVina-based Molecular Docking

The results of the docking studies (Results shown in Table 3) conducted by the cb-dock server have provided valuable insights into the potential binding affinities and interacting residues of selected compounds with the aromatase enzyme. anastrozole, the reference aromatase inhibitor, demonstrated a favorable score of -7.9 in pocket C1, interacting with key residues such as LYS354, TYR361, and MET444. Notably, aminoindazole, dihydrexidine, ropivacaine, balsalazide, stiripentol, dolasetron, granisetron, nicotinamide, and larixinic-acid exhibited diverse binding profiles in distinct pockets. Compounds like dolasetron (Figure 4) and granisetron displayed notably high scores in pocket C1, forming interactions with residues like ARG115 and THR310. These findings suggest the potential of these compounds to bind effectively to the aromatase enzyme, possibly influencing its activity. The variation in binding pockets and interacting residues underscores the importance of exploring multiple compounds to identify lead candidates with diverse modes of action. These results provide a foundation for further experimental validation and highlight the potential

Table 3: Results of docking studies by CB-dock server

S. No.	*Name*	*Score*	*Pocket and Interacting Residues*
1	Anastrozole	-7.9	Pocket C1 Chain A: LYS354 GLU357 ASN358 TYR361 PHE418 LYS420 ASN421 VAL422 PRO423 TYR424 PHE427 GLN428 PRO429 PHE430 GLY431 LYS440 MET444
2	Aminoindazole	-6.1	Pocket C3 Chain A: HIS62 ILE89 SER90 GLU92 PHE116 GLY117 SER118 LYS119 LEU228 ILE229 LYS230 PRO231 ASP232 LYS376 ASN393
3	Dihydrexidine	-8.9	Pocket C1 Chain A: ARG115 ILE133 PHE134 PHE221 TRP224 ALA306 ASP309 THR310 VAL370 LEU372 VAL373 MET374 LEU477 SER478
4	Ropivacaine	-7.2	Pocket C2 Chain A: GLU357 TYR361 PHE418 ASN421 VAL422 PRO423 TYR424 PHE427 GLN428 PRO429 PHE430 GLY431 PHE432 LYS440 TYR441 MET444
5	Balsalazide	-8.1	Pocket C2 Chain A: ARG115 ILE132 ILE133 PHE134 ARG145 TRP224 ALA306 THR310 VAL370 LEU372 VAL373 MET374 ARG375 ILE398 PHE430 ARG435 GLY436 CYS437 ALA438 LEU477 SER478
6	Stiripentol	-7.3	Pocket C1 Chain A: ILE132 ILE133 ARG145 PHE148 LEU152 SER199 GLU302 MET303 ALA306 ALA307 THR310 MET311 GLY436 CYS437 ALA438 GLY439 LYS440 ILE442 ALA443 MET446
7	Dolasetron	-9.2	Pocket C1 Chain A: ARG115 ILE133 PHE134 PHE221 TRP224 THR310 VAL370 LEU372 VAL373 MET374 ILE398 GLN428 PRO429 PHE430 GLY431 ARG435 CYS437 LEU477 SER478
8	Granisetron	-8.3	Pocket C1 Chain A: ARG115 ILE133 PHE134 PHE221 TRP224 ASP309 THR310 VAL370 LEU372 VAL373 MET374 PHE430 GLY431 ARG435 GLY436 CYS437 ALA438 LEU477 SER478
9	Nicotinamide	-5.4	Pocket C1 Chain A: ARG192 GLN218 PHE221 ASP222 PRO308 ASP309 VAL313 SER478 HIS480 PRO481 GLU483
10	Larixinic-acid	-4.8	Pocket C1 Chain A: ARG115 ILE133 PHE134 TRP224 VAL370 LEU372 VAL373 MET374 LEU477

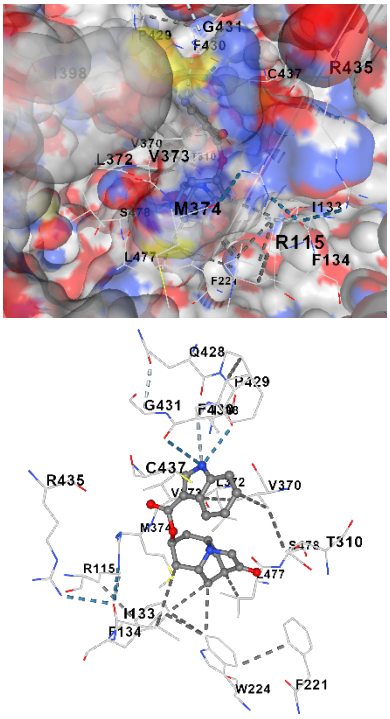


Figure 4: Interaction between dolasetron and aromatase enzyme in structure-based blind docking

of the identified compounds for the development of novel therapeutics targeting aromatase in breast cancer treatment.

CONCLUSION

In conclusion, our comprehensive investigation utilizing transcriptomic similarity analysis, structural refinement, and docking studies presents a systematic approach to identifying potential therapeutics for aromatase inhibition in the context of breast cancer. The analysis of transcriptomic similarity revealed a list of compounds with notable scores, indicating their potential to modulate aromatase activity. Anastrozole, a well-known aromatase inhibitor, emerged as the top candidate with an impressive score of 99.98, reaffirming its efficacy in breast cancer therapy. The PDB-REDO refinement process significantly enhanced the quality of the aromatase crystal structure, as evidenced by improved validation metrics such as a reduced R-value (0.2164–0.1747) and enhanced fine packing score (-1.136–-0.484).

Docking studies using the cb-dock server further elucidated the binding affinities and interacting residues of selected compounds with the aromatase enzyme. Notably, dolasetron and granisetron exhibited high scores of -9.2 and -8.3, respectively, in pocket C1, forming crucial interactions with residues like ARG115 and THR310. The diverse nature of these compounds, ranging from ionophores to serotonin

receptor antagonists, highlights the multifaceted approach employed for aromatase modulation. Furthermore, the analysis of significant structural changes post-refinement, including alterations in rotamers and water removal, underscores the success of the structural preprocessing in enhancing the accuracy of the aromatase model.

The integrative approach provides a robust foundation for the identification of lead compounds targeting aromatase, offering potential avenues for therapeutic intervention in breast cancer. The identified compounds, especially anastrozole, dolasetron, and granisetron, merit further experimental validation and hold promise for the development of innovative and more effective strategies in hormone-related breast cancer treatment. This research contributes to the ongoing efforts in precision medicine, emphasizing the importance of computational methodologies in drug discovery and repurposing.

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