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Computational Screening of Natural Compounds for the Discovery of Potential Aromatase Inhibitors: A Promising Therapy for Estrogen-Dependent Breast Cancer

Misbahuddin M. Rafeeq[1], Ziaullah M. Sain[2], Norah A. Alturki[3], Ahmad Alzamami[4], Saeed A. Asiri[5], Mutaib M. Mashraqi[5], Amany I. Alqosaibi[6], Mashael M. Alnamshan[6], Abdulrahman Almutairi[7], Abdulkhaliq Munawir Alanazi[8] and Qamre Alam[9*]

[1]Department of Pharmacology. Faculty of Medicine, Rabigh. King Abdulaziz University. Jeddah, 21589, KSA.
[2]Department of Microbiology Faculty of Medicine, Rabigh. King Abdulaziz University, Jeddah. 21589. KSA.
[3]College of Applied Medical Science, Clinical Laboratory Science Department, King Saud University, Riyadh, Saudi Arabia.
[4]College of Applied Medical Science, Clinical Laboratory Science Department, Shaqra University, Saudi Arabia.
[5]Department of Clinical Laboratory Sciences, College of Applied Medical sciences, Najran University, Saudi Arabia.
[6]Department of Biology, College of Science, Imam Abdulrahman bin Faisal University, P.O. Box 1982, Dammam 31441, Saudi Arabia.
[7]Department of Pathology and Laboratory Medicine, King Abdulaziz Medical City, Ministry of National Guard Health Affairs (MNGHA), Riyadh, Saudi Arabia.
[8]Department of Respiratory Services, Ministry of National Guard Hospital and Health Affairs (MNGHA), P.O.Box 22490, Kingdom of Saudi Arabia.
[9]Medical Genomics Research Department, King Abdullah International Medical Research Center King Saud Bin Abdulaziz University for Health Sciences, King Abdulaziz Medical City Riyadh 11426, Saudi Arabia.

Authors' contributions

This work was carried out in collaboration among all authors. Authors MMR and QA designed the study, wrote the protocol, and wrote the first draft of the manuscript. Authors ZMS, NAA, AA, and SAA managed the analyses of the study. Authors MMM, AIA, MMA, AA, and AMA managed the literature searches. All authors read and approved the final manuscript.

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**Corresponding author: E-mail: alamqa@ngha.med.sa;*

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ABSTRACT

Aromatase plays a significant role in the progression of estrogen receptor-positive (ER-positive) breast cancer. The adverse side effects of currently used aromatase inhibitors (AIs) necessitate the development of new AIs that are more active, selective, and less toxic. This study used a computational approach to screen 503 natural compounds ZINC database against the aromatase active site. The best scoring hits ZINC69482055, ZINC69482510, and ZINC406719 exhibited strong binding with aromatase, with binding energy values of -8.45, -10.35, and -8.75 kcal/mol, respectively, which is comparatively higher than that of the control compound Anastrozole (-6.43 kcal/mol). Docking analysis showed that the selected hits interacted with the crucial residues of the aromatase active site. This study suggested that these compounds could be used as possible AIs in the cure of breast cancer. Hands-on bench work validation is needed to optimize these compounds as AIs.

Keywords: Aromatase; breast cancer; natural compounds; estrogen receptor.

1. INTRODUCTION

In every country on the planet, cancer is the foremost reason for death and a major impediment to rising life expectancy. In terms of morbidity and death, breast cancer (BC) is the most prevalent illness in women universally [1]. According to statistics published by the International Agency for Research on Cancer (IARC) in December 2020, BC has now surpassed lung cancer as the most frequently diagnosed cancer worldwide [2]. The various BC forms are determined by which cells become malignant. According to the American Cancer Society, ER-positive BC accounts for about two out of every three cases (almost 80%). The majority of these cases are ER-positive, indicating that ER on the cell surface bind to estrogen. By binding and activating the ER, estrogen promotes natural and cancerous breast epithelial cell growth and longevity [3]. The triggered receptor then binds to gene promoters in the nucleus, activating several other genes involved in cell division, cell death suppression, new blood vessel development, and protease action [3,4]. For women with ER-positive BC, hormonal therapy is the hallmark of adjuvant systemic care.

Aromatase is a cytochrome P450 (CYP450) monooxygenase that is involved in estrogen synthesis that catalyzes the transformation of androgen (testosterone) to estrogen (estradiol) [5]. Aromatase inhibitors (AIs) have been shown in clinical trials to decrease the risk of BC in high-risk postmenopausal women (by more than half) [6]. AIs block the ability of the body to generate estrogen from androgens by inhibiting the action of the aromatase enzyme. Three well-known AIs are anastrozole, exemestane, and letrozole. For postmenopausal patients, AIs (letrozole, anastrozole, and exemestane) are the preferred hormone treatment [7]. Since AIs can cause a reduction in bone density, women who take medication must be screened for osteoporosis. Muscle pain, knee pain, and menopausal symptoms are also possible side effects of AIs (such as hot flashes). AIs are also linked to a decrease in bone density. Women that take an aromatase inhibitor have their bone density measured at a baseline such that changes in bone density can be tracked.

The stage of the disease at the time of diagnosis is a significant predictor of treatment expenses. Treatment for advanced disease is frequently more rigorous than treatment for early stages [8]. Consequently, the advancing stage is related to increased resource utilization as well as lower health consequences [9]. BC treatment costs frequently increased as the disease stage at diagnosis advanced [10].

Natural compounds are generally safer, simpler, cheaper, and less hazardous in the treatment of a variety of diseases. Natural compounds are gaining popularity as anticancer medications due to their widespread

use and low side effects [11,12]. It has been documented that more than 60% of new anticancer medicines are extracted from natural products [13]. Herbal extracts were found to inhibit protective signaling and suppression of host cell gene expression, as well as acting against viral oncogenes. Natural compounds target tumor cells by regulating apoptosis and autophagy pathways, conquering NF-κB, and inhibiting angiogenesis in prostate cancer. In addition, several natural compounds have been tested against the HPV E6 oncoprotein [14,15].

Computational methods are widely used in nearly all current drug discovery efforts, and computer-aided lead generation and optimization have achieved significant success. These methods are generally more reliable, simpler, and less expensive [16]. In this study, we used computational screening of a publicly accessible natural compound library to identify novel leads or hits as potent AIs to cut costs and time.

2. METHODOLOGY

2.1 Protein Preparation

3D structure of aromatase (PDB ID: 3EQM) was obtained from the protein data bank, water molecules and co-crystallized ligands were separated and saved separately. The clean 3D structure has proceeded for structure minimization using UCSF Chimera [17].

2.2 Natural Compound Library Retrieval and Preparation

UEFS Natural Products (database of the UEFS, The State University of Feriera De Santana, Brazil) library was accessed from the ZINC database (https://zinc.docking.org/catalogs/uefsnp/).

The database includes 503 natural compounds that were extracted in .sdf format and subjected to ligand preparation using the MMFF94 force field through energy minimization.

2.3 Receptor Based Virtual Screening and Molecular Docking

The prepared natural compound library as well as the 3D structure of the target protein (aromatase) were imported into PyRx, a virtual screening tool. Vina wizard was run to screen the compound library against the catalytic site of the aromatase. The top-scoring compounds were chosen for in-depth docking analysis.

The automatic molecular docking of lead hits with aromatase was conducted using the docking software Autodock 4.2 [18]. In order to deal with inhibitor–enzyme interactions, a Lamarckian genetic algorithm was used. The grid center points X, Y, and Z were set as 85.534, 53.554, and 45.296, respectively. The Autogrid software was used to produce a grid map of 40 x 40 x 40 points spaced evenly at 0.375 to determine the binding energies (BEs) between the compounds and the protein. The binding pose with the highest negative BE value was deemed to be the most promising.

2.4 LIGPLOT+ Analysis

The LIGPLOT^{+} Version v.2.1 program was used to evaluate the hydrogen bond and hydrophobic interaction of 'hits–aromatase' complexes. The LIGPLOT algorithm was used to convert the generated 3-D structures of the 'hits–aromatase' interaction into 2-D figures.

3. RESULTS AND DISCUSSION

Natural products obtained from various sources may have the ability to activate several metabolic processes, which may be useful in the treatment of stubborn disorders such as cancer [19,20]. Aromatase plays a key role in the progression of ER-positive BC [21], making it a potential curative target for its treatment. The existence of significant side effects associated with the long-term therapeutic use of AIs necessitates the development of new AIs that are more active, selective, and less toxic. With this purpose, in the present study 503 natural compounds from the ZINC database (UEFS natural products database) have been screened against the aromatase using the state of art insilico approach. Amon them, selected hits (ZINC69482055, ZINC69482510, and ZINC406719) exhibit strong binding with the aromatase. ZINC69482055 bind with aromatase through 17 amino acid residues namely, Arg115, Ile133, Phe134, Phe221, Trp224, Ile305, Ala306, Asp309, Thr310, Val370, Leu372, Val373, Met374, Phe430, Cys437, Leu447, and Ser478 (Fig. 1a); while Arg115, Ile132, Ile133, Phe148, Leu152, Ala306, Ala307, Thr310, Met311, Met364, Val370, Pro429, Phe430, Gly436, Cys437, Ala438, Gly439, Ala443, and Met446

residues of aromatase was found to interact with ZINC69482510 (Fig. 1b). Further, ZINC406719 was observed to interact with Arg115, Ile133, Phe134, Trp224, Thr 310, Val370, Leu372, Val373, Met374, Phe430, Cys437, Leu477, and Ser478 residues of the aromatase (Fig. 1c).

The active site residues of aromatase have been described as Met374, Arg115, Ile133, Ala306, Thr310, Asp309, Val370, and Ser478 [22]. Consistent with this, in this study, the hits (ZINC69482055, ZINC69482510, and ZINC406719) have been found to bind with these residues of the aromatase (Fig. 1a, b, and c).

The BE of ZINC69482055, ZINC69482510, and ZINC406719 with aromatase were found to be -8.45, -10.35, and -8.75 Kcal/mol, respectively, while the inhibition constants were 25.9, 12.63, and 23.56 µm, respectively (Table 1).

Anastrozole is the third generation aromatase inhibitor [23] and has been the control ligand in this study. Anastrozole has been report to bind with Arg115, Ile133, Phe134, Phe221, Trp224, Ile305, Ala306, Asp309, Thr310, Val369, Val370, Arg435, Leu477, Ser478, Arg115, Ile133, Phe134, Trp224, Leu228, Ile305, Ala306, Asp309, Thr310, Val370, Leu372, Val373, Met374, Arg435, and Leu477 residues of the aromatase [24]. Interestingly, in this study ZINC69482055, ZINC69482510, and ZINC406719 have also been found to interact with these aromatase residues (Fig. 1a, b, and c). BE of Anastrozole with aromatase was found to be -6.43 Kcal/mol (Table 1).

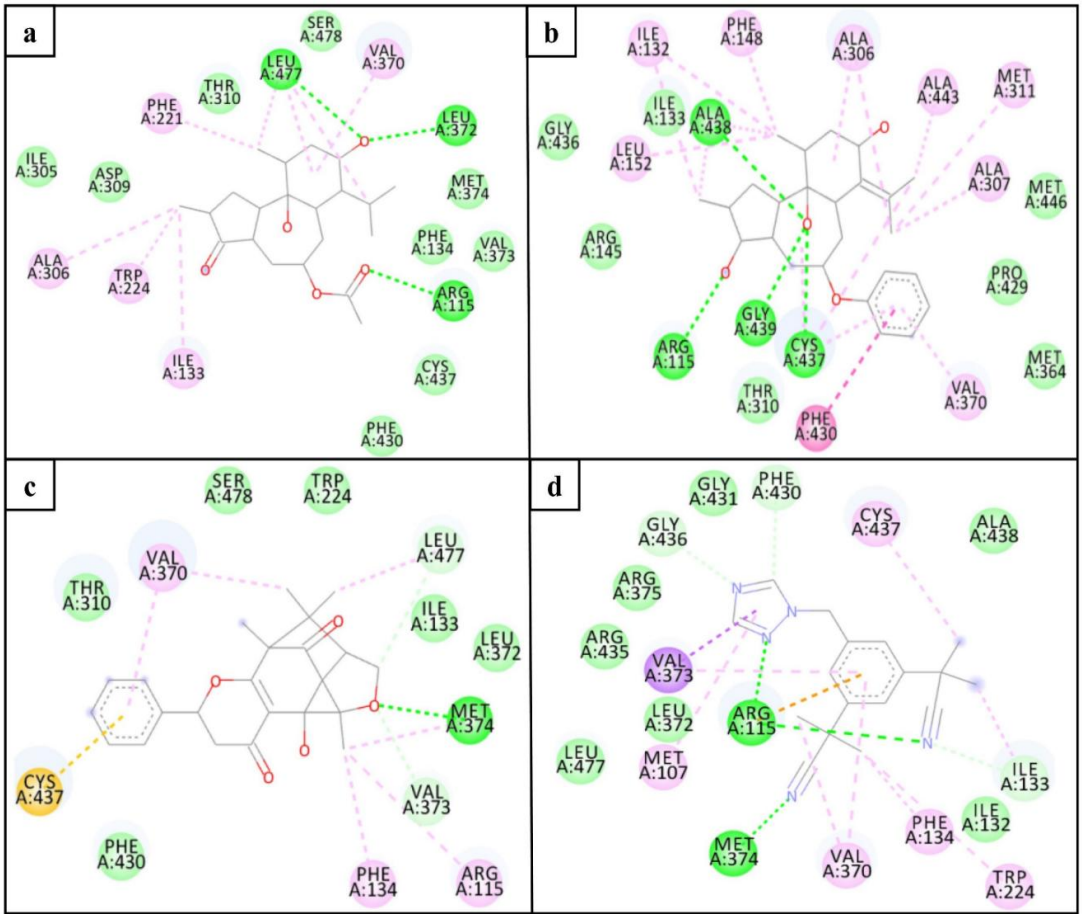
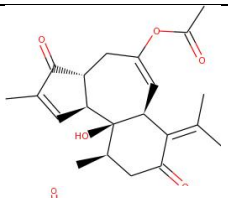
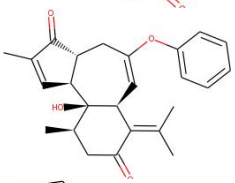
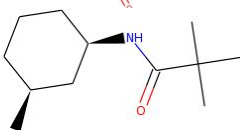


Fig. 1. Interacting residues of aromatase with ZINC69482055 (a), ZINC69482510 (b), ZINC406719 (c), and Anastrozole (d)

Table 1. BE of hit compounds with aromatase

S. No.	Compounds	2D structure	Binding energy (kcal/mol)	Inhibition constant (μM)
1.	ZINC69482055		-8.45	25.9
2.	ZINC69482510		-10.35	12.63
3.	ZINC406719		-8.75	23.56
4.	Anastrozole*	-	-6.43	92.27

*Control compound

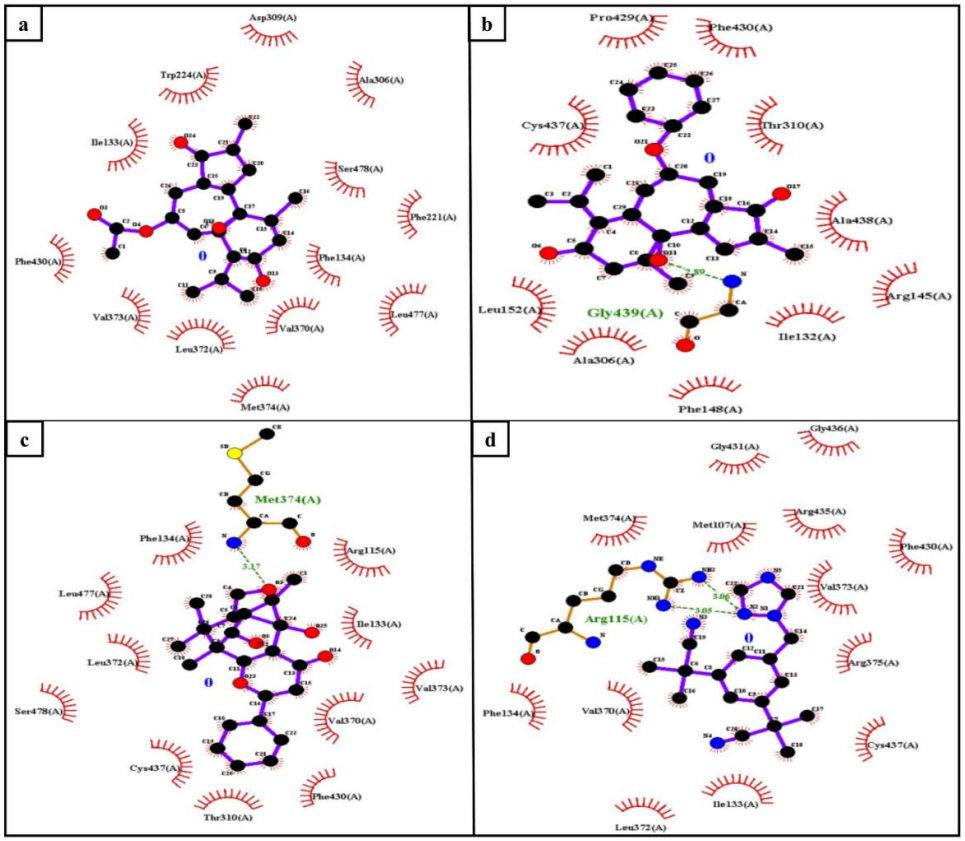


Fig. 2. H-bond (green dashed line) and hydrophobic interacting residues (red arcs) of aromatase with ZINC69482055 (a), ZINC69482510 (b), ZINC406719 (c), and Anastrozole (d)

The H-bond and hydrophobic association aid in determining the effectiveness of inhibitors against the target protein and play a vital role in the stabilization of the 'inhibitor–protein' complex [25,26]. ZINC69482510 was found to form an H-bond with Gly439 residue of aromatase, whereas Met374 residue of aromatase was observed to make an H-bond with ZINC406719. Further, Phe430 was the common hydrophobic interacting residue of aromatase with hits compounds and the Anastrozole (Fig. 2a, b, c, and d).

Molecular docking is an increasingly valuable method for drug discovery that investigates the binding modes of ligand molecules to their target protein [27,28]. The strength of the interaction between the ligand-protein complex is measured in terms of BE, with a lower BE value indicating a stronger ligand-target protein interaction [29]. Accordingly, in this study the hits (ZINC69482055, ZINC69482510, and ZINC406719) show strong interaction (lower BE) with aromatase than the Anastrozole (control compound), indicating that these compounds can be used as the AIs to cure the BC.

4. CONCLUSION

This study described the binding interactions of the aromatase enzyme with the hits compounds picked from a high throughput virtual screen. The top hits (ZINC69482055, ZINC69482510, and ZINC406719) exhibited strong binding with the aromatase. These findings pave the way for further research in the quest for new AIs to cure BC.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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