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Molecular docking analysis of bioactive compounds from *Bauhinia thonningii* against estrogen receptor alpha as potential breast cancer therapeutic agents

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Abstract: Breast cancer remains a leading cause of cancer-related mortality among women globally, with approximately 70% of cases exhibiting estrogen receptor positivity. The limitations of current therapies necessitate the development of novel therapeutic agents with improved efficacy and reduced side effects. This study aimed to investigate the binding interactions of three bioactive compounds from *Bauhinia thonningii* (6,8-di-C-methyl kaempferol 3,7-dimethyl ether (compound 1), 6-C-methylquercetin-3,4′-dimethyl ether (compound 2), and quercetin3OαLrhamnopyranoside) (compound 3) with estrogen receptor alpha (ER-α) using molecular docking. The docking protocol was performed using AutoDock 4.2 and validated with an RMSD value of 0.92 Å. The native ligand 4-hydroxytamoxifen and tamoxifen showed binding energies of -11.32 and -10.33 kcal/mol, respectively. Among the tested compounds, quercetin3OαLrhamnopyranoside demonstrated the strongest binding affinity (-8.66 kcal/mol) and formed six hydrogen bonds with key amino acid residues in the receptor binding site. Detailed interaction analysis revealed that quercetin3OαLrhamnopyranoside's (compound 3) binding profile most closely resembled that of the native ligand, suggesting its potential as an ER-α modulator. These findings indicate that quercetin3OαLrhamnopyranoside represents a promising scaffold for further development of novel anticancer agents targeting ER-α positive breast cancer.

Keywords: molecular docking, breast cancer, estrogen receptor, *Bauhinia thonningii*, quercetin derivatives

Introduction

Breast cancer is a malignancy that originates in breast tissue, specifically from epithelial cells in the ducts and lobules [1]. This disease ranks among the most commonly diagnosed cancers in women worldwide, accounting for approximately 1 in 10 new cancer diagnoses annually. Breast cancer remains the second leading cause of cancer-related mortality among women globally [1]. Significantly, approximately 70% of breast cancers exhibit overexpression of estrogen receptors; this condition is known as estrogen receptor positive (ER+) breast cancer, which estrogen receptor α (ER-α) plays a critical role in cancer development and progression [2].

ER-α represents a primary molecular target in breast cancer treatment, with its inhibition offering substantial therapeutic potential. Tamoxifen, a drug from the selective estrogen receptor modulators (SERM) group, functions as an antagonist by binding to estrogen receptors and is widely prescribed for ER+ breast cancer treatment. However, clinical evidence has associated tamoxifen with serious adverse effects, including increased risk of endometrial cancer, venous thromboembolism, and cataracts [2]. These limitations underscore the urgent need for novel anti-cancer agents with enhanced selectivity for cancer cells and minimized side effects.

The development of conventional anti-cancer drugs typically requires extended timeframes and substantial financial investment. To address these challenges, computer-aided drug design (CADD) has emerged as an efficient alternative approach. CADD, which includes molecular docking techniques, utilizes advanced technologies to complement experimental analysis by predicting ligand-receptor interactions and binding affinities [4]. In breast cancer research specifically, molecular docking enables the identification of compounds that can effectively target ER-α with high specificity, accelerating the discovery of potential therapeutic candidates.

Bauhinia thonningii (Schumach.) Milne-Redh. (Caesalpiniaceae) is a medicinal plant with extensive traditional applications across various therapeutic domains. Researchers recommend screening plants traditionally used to treat immune disorders, skin conditions, inflammation, and various infections for potential cancer drug discovery, as many of these conditions share underlying pathological mechanisms with cancer [3]. *B. thonningii* contains several bioactive compounds including 6,8-di-C-methyl kaempferol 3,7-dimethyl ether (compound 1), 6-C-methylquercetin-3,4'-dimethyl ether (compound 2), and quercetin-3-O-α-L-rhamnopyranoside (compound 3) that have demonstrated significant antioxidant activity [3]. Their antioxidant properties correlate with chemopreventive potential, and their structural resemblance to known ER-α inhibitors, such as tamoxifen analogs, further supports their therapeutic potential [3].

Previous studies on related plant-derived flavonoids have shown promising anti-proliferative effects against various cancer cell lines, including breast cancer [4]. However, despite these encouraging findings and the traditional medicinal uses of *B. thonningii*, the specific mechanisms by which these compounds interact with molecular targets like ER-α remain inadequately characterized. This study aims to investigate the binding interactions between three bioactive compounds from *B. thonningii* and ER-α using molecular docking, to evaluate their potential as novel therapeutic agents for ER+ breast cancer treatment.

Methods

Tools and materials

The hardware used was a Lenovo ideapad 3-114IML05 type 81WA laptop with 4 Gigabyte DDR4 2400 RAM, Intel® Core™ i3-10110U Processor with Intel UHD graphics. The software tools employed included BIOVIA Discovery Studio Visualizer 2023, AutoDock 4.2 with AutoDockTools (ADT), AutoDock Vina version 1.2.4, Avogadro for molecular optimization, and PubChem and Protein Data Bank (PDB) as molecular databases [5][6][7].

The independent variables were previously reported compounds: 6,8-di-C-methyl kaempferol 3,7-dimethyl ether (compound 1); 6-C-methylquercetin-3,4'-dimethyl ether (compound 2); and quercetin-3-O-α-L-rhamnopyranoside (compound 3). The dependent variable was the estrogen receptor alpha (ER-alpha) target protein obtained from RCSB PDB with PDB ID: 2BJ4 [8].

Protein preparation

ER Alpha (PDB ID: 2BJ4) was downloaded from the Protein Data Bank (http://www.rcsb.org/). The crystallographic resolution of the structure was 1.9 Å. Prior to docking experiments, the target protein was separated from its original ligand 4-hydroxytamoxifen using Discovery Studio Visualizer [9]. Hydrogen atoms were added to optimize hydrogen bonding networks, and all water molecules were removed from the structure to prevent interference with ligand binding. Proper protonation states were assigned to ionizable residues at physiological pH. The prepared protein structure was then saved in .pdb format for further processing [6][7].

Docking validation

Validation was performed using AutoDockTools (AutoDock 4.2 and Autogrid) to verify the accuracy of the docking protocol [10]it is not known exactly how the most ideal runs in the docking process with AutoDock 4. This study aims to determine the effect of the number of runs docking processes with AutoDock 4 on the validity of the docking results.\n \n \n Materials and methods\n . The method used is the redocking process with AutoDock 4.2.6. The receptor used is an estrogen receptor with ligand reference estradiol (PDB ID 1GWR. The original co-crystallized ligand 4-hydroxytamoxifen was redocked to the binding site to assess the docking parameters. The grid box center was defined with coordinates X = 19.091, Y = 37.385, and Z = 31.608, grid box dimensions of X = 22 Å, Y = 30 Å, Z = 29 Å, and grid point spacing of 0.375 Å. The docking validation was considered successful when the root-mean-square deviation (RMSD) value was less than 2 Å, which is the standard criterion for evaluating the accuracy of docking poses compared to experimental structures [10]it is not known exactly how the most ideal runs in the docking process with AutoDock 4. This study aims to determine the effect of the number of runs docking processes with AutoDock 4 on the validity of the docking results.\n \n \n Materials and methods\n. The method used is the redocking process with AutoDock 4.2.6. The receptor used is an estrogen receptor with ligand reference estradiol (PDB ID 1GWR.

Optimization of structure

Two-dimensional and three-dimensional structures of compound 1, compound 2, and compound 3 were downloaded from PubChem (https://pubchem.ncbi.nlm.nih.gov/) [5]. The structures were then geometrically optimized using Avogadro with the MMFF94 force field to achieve the lowest energy conformations. Energy minimization was performed until the convergence criterion of 10^{-7} kcal/mol was reached. This optimization process ensured that the ligand structures had proper bond lengths, angles, and torsions before docking [7][11].

Molecular docking of compounds with estrogen receptor alpha

The optimized compounds were docked to the target protein using AutoDock 4.2 with the Lamarckian Genetic Algorithm [6]. The grid box size and center coordinates were identical to those used during the validation process. For each compound, 100 independent docking runs were performed to generate diverse binding poses [10]. The genetic algorithm parameters included a population size of 150, maximum number of energy evaluations of 2.5 × 106, and maximum number of generations of 27,000. The docking results were saved in .dlg format, and only poses with RMSD values below 2 Å were considered for further analysis. The protein-ligand interactions were then visualized and analyzed in both 2D and 3D formats using BIOVIA Discovery Studio to identify key binding interactions, including hydrogen bonds, hydrophobic interactions, and π-stacking [7].

Data analysis

The docking results were analyzed based on binding free energy (ΔG) values and interaction patterns. The best docking poses were selected according to the lowest binding energy and optimal interaction profiles. Binding affinities were calculated using the AutoDock scoring function, which incorporates van der Waals forces, hydrogen bonding, electrostatic interactions, and desolvation energy [6]. Protein-ligand interactions were quantitatively assessed by measuring the number and strength of hydrogen bonds, hydrophobic contacts, and other non-covalent interactions. [12].

Table 1. Molecular docking result of native ligand, *Bauhinia thonningii* bioactive compounds and tamoxifen against ER-α

Protein target	Ligand	Binding energy (kcal/mol)
ER-α (PDB ID: 2BJ4)	4-hydroxytamoxifen (native ligand)	-11.32
	Compound 1	-6.07
	Compound 2	-7.26
	Compound 3	-8.66
	Tamoxifen	-10.33

Results

Molecular docking

The molecular docking analysis demonstrated significant binding affinity differences among the tested compounds when docked against the estrogen receptor alpha (ER-α) protein (Table 1). Quercetin-3-O-α-L-rhamnopyranoside (compound 3) exhibited the strongest binding with an energy value of -8.66 kcal/mol, while 6,8-di-C-methyl kaempferol 3,7-dimethyl ether (compound 1) showed the weakest binding with -6.07 kcal/mol. The reference compound 4-hydroxytamoxifen (native ligand) demonstrated superior binding with -11.32 kcal/mol, while tamoxifen displayed a binding energy of -10.33 kcal/mol. These findings suggest that compound 3 possesses the highest potential among the three tested compounds for interaction with the ER-α target.

Protein-ligand interaction

Interaction analysis revealed that compound 3 and tamoxifen most closely resembled the binding pattern of the native ligand (Table 2). This correlation aligns with their respective binding energy values, which were the lowest among the tested compounds. The interaction profile showed that the native ligand interacted with 11 amino acid residues of ER-α, while compound 1 interacted with 5 residues, compound 2 with 6 residues, compound 3 with 8 residues, and tamoxifen with 8 residues (Figure 1).

Two-dimensional visualization of the ligand-receptor interactions using BIOVIA Discovery Studio 2019 revealed diverse interaction types across all compounds. Compound 1 (6,8-Di-C-methyl kaempferol 3,7-dimethyl ether) formed several interactions including conventional hydrogen bonds with Asp351, Pi-sigma

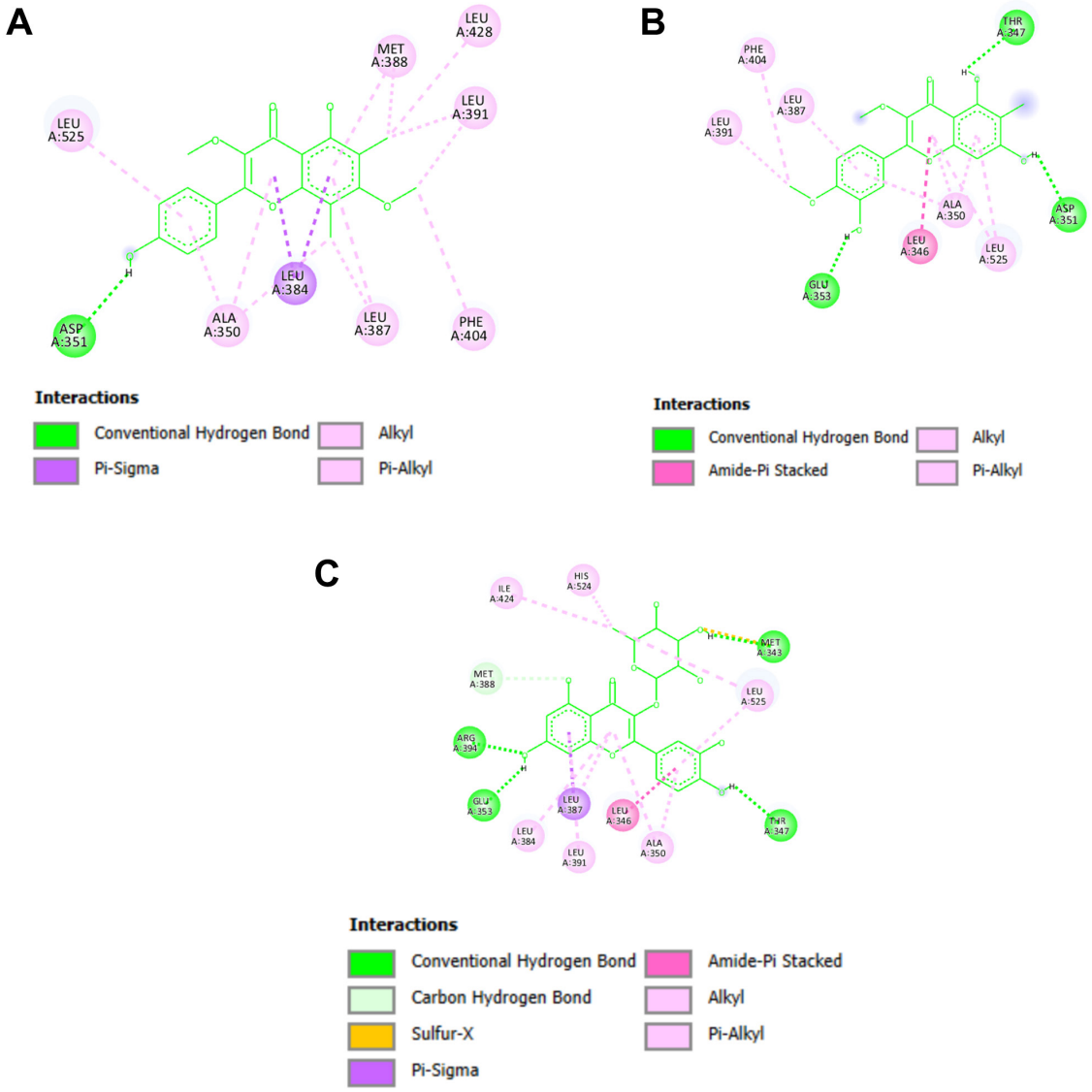

Figure 1. Two-dimensional visualization of ligand on amino acids of protein ER-alpha (PDB ID 2BJ4). A. 6,8-di-C-methyl kaempferol 3,7-dimethyl ether, (B) 6-C-methylquercetin-3,4'-dimethyl ether, (C) Quercetin-3-O-α-L-rhamnopyranoside

Table 2. Amino acid residues of native ligand, native ligand, *Bauhinia thonningii* bioactive compounds and tamoxifen against ER-α

Ligand	Met 421	Leu 525	Ile 424	Thr 347	Ala 350	Leu 391	Leu 387	Phe 404	Glu 353	Leu 428	Met 388
Native ligand	v	v	v	v	v	v	v	v	v	v	v
Compound 1		v			v		v	v		v	
Compound 2		v			v	v	v	v	v		
Compound 3		v	v	v	v	v	v		v		v
Tamoxifen	v	v	v	v	v	v	v				v

Table 3. Bond's type and distance of native ligand, *Bauhinia thonningii* bioactive compounds, and tamoxifen against ER-α

Ligand	Hydrogen bonds	Hydrophobic bonds	Other bonds	Distance (Å)
Native ligand	2	10	1	1.98677
Compound 1	2	14	0	2.17219
Compound 2	4	9	0	1.94132
Compound 3	6	11	1	1.62530
Tamoxifen	1	12	1	3.69694

bonds with Leu384, and alkyl/Pi-alkyl interactions with Ala350, Leu387, Phe404, Leu391, Leu428, Met388, and Leu525. Compound 2 (6-C-methylquercetin-3,4'-dimethyl ether) displayed conventional hydrogen bonds with Asp351, Thr347, and Glu353, Amide-pi stacked bonds with Leu346, and alkyl/Pi-alkyl bonds with Phe404, Leu391, Leu387, Ala350, and Leu525.

Compound 3 (quercetin-3-O-α-L-rhamnopyranoside) exhibited the most diverse interaction profile, with conventional hydrogen bonds to Arg394, Glu353, Thr347, and Met343, carbon hydrogen bonds with Met388, Pi-sigma bonds with Leu387, Amide-pi stacked bonds with Leu346, and alkyl/Pi-alkyl interactions with Ile424, His524, Leu525, Ala350, Leu391, and Leu384. Table 3 summarizes these interactions, with compound 3 forming the highest number of hydrogen bonds (6) and having the shortest bond distance (1.62530), suggesting the strongest and most stable interaction with the target protein.

Discussion

Molecular docking is an in silico approach for drug discovery that predicts interactions between potential therapeutic compounds and target proteins. In this study, we employed molecular docking to investigate the binding affinity of bioactive compounds from *Bauhinia thonningii* against the estrogen receptor alpha (ER-α) protein. The ER-α receptor (PDB ID: 2BJ4) was selected as the target protein due to its critical role in breast cancer development, where it mediates gene expression and cell proliferation through estrogen stimulation [8]. ER-α is particularly significant in breast cancer pathogenesis as it influences growth, defense mechanisms, and development of malignant cells.

The docking methodology was validated through redocking of the native ligand 4-hydroxytamoxifen with ER-α, yielding an RMSD value of 0.92 Å, which falls well within the acceptable range of <2 Å. This confirms the reliability and accuracy of our docking protocol [12]. The grid box parameters were carefully optimized to encompass the binding site, ensuring efficient and accurate docking simulations.

Analysis of binding energies revealed that quercetin-3-O-α-L-rhamnopyranoside (compound 3) exhibited the most promising interaction with ER-α (-8.66 kcal/mol) among the tested compounds. This enhanced binding can be attributed to its unique structural features that facilitate optimal interactions with the receptor's binding pocket. While compound 3's binding energy was higher (less negative) than that of the native ligand (-11.32 kcal/mol) and tamoxifen (-10.33 kcal/mol), it still demonstrated significant potential as an ER-α modulator.

The superior binding affinity of compound 3 correlates with its extensive interaction profile, forming six hydrogen bonds and eleven hydrophobic bonds with key amino acid residues in the receptor binding site. These multiple interactions contribute to its stability within the binding pocket, as evidenced by the shortest bond distance (1.62530) among all tested compounds. Binding energy values directly reflect the strength of ligand-receptor interactions, with lower values indicating higher affinity [12].

Quercetin derivatives, including compound 3, have demonstrated significant anticancer properties in previous studies. Research has shown that quercetin can modulate ER activity through type II estrogen-binding sites [13][14]. Wang et al. [15] reported that quercetin derivatives could reverse tamoxifen resistance in breast cancer models by inhibiting cellular proliferation and inducing apoptosis in resistant cell lines. This suggests that compound 3 may offer therapeutic benefits in cases where conventional treatments fail.

Recent research on *Bauhinia thonningii* compounds has revealed their cytotoxic potential against various cancer cell lines [3]. Flavonoids isolated

from *B. thonningii*, including quercetin-3-O-α-L-rhamnopyranoside, have shown promising antiproliferative activity against drug-resistant cancer phenotypes [3][16]. These compounds induce apoptotic cell death through multiple mechanisms, including caspase activation, mitochondrial membrane potential alteration, and increased reactive oxygen species (ROS) production.

Rhamnopyranoside compounds, particularly those containing quercetin moieties, have demonstrated potential anticancer properties in previous studies. For instance, 3,5,7,3',5'-pentahydroxyflavanonol-3-O-α-L-rhamnopyranoside isolated from *Bauhinia strychnifolia* exhibited potent cytotoxic activity against various cancer cell lines, including MCF-7 breast cancer cells [16]. This further supports our findings regarding the potential of quercetin-3-O-α-L-rhamnopyranoside as an anticancer agent targeting ER-α.

The molecular mechanisms underlying the anticancer effects of quercetin derivatives involve modulation of multiple signaling pathways. Studies suggest that quercetin can induce apoptosis through ROS-regulated p53 signaling pathways and can inhibit cancer cell proliferation by regulating genes involved in cell cycle progression [17]. Additionally, quercetin affects glucose uptake and metabolism in breast cancer cells through an estrogen receptor-independent mechanism, suggesting multiple modes of action against breast cancer [18].

Conclusion

Our molecular docking study effectively utilized an in silico approach to identify potential anticancer compounds from *Bauhinia thonningii* that target ER-α. The docking protocol was validated with an RMSD value of 0.92 Å, confirming its reliability for predicting ligand-receptor interactions. Among the three tested compounds, quercetin-3-O-α-L-rhamnopyranoside (compound 3) demonstrated the strongest binding affinity with an energy value of -8.66 kcal/mol, suggesting its potential as an ER-α modulator.

We propose quercetin-3-O-α-L-rhamnopyranoside represents a promising candidate for further development as an anticancer agent targeting ER-α. Future research should focus on experimental validation through in vitro and in vivo studies to confirm its efficacy and elucidate its precise mechanisms of action. Additionally, structural optimization strategies could enhance its binding affinity and biological activity, potentially leading to more effective therapeutic agents for breast cancer treatment.

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Declaration of interest

The authors declare that none of them has any conflict of interest with any private, public or academic party related to the information contained in this manuscript.

Author contributions

Conceptualization, AHS, LZ, WNA; Methodology, SZ, A, CFR, MK, SND, WPA, ZAA, WNA; Investigation, WNA, AHS; Writing – Original Draft, LZ, SZ, A, CFR, MK, SND, WPA, ZAA, AHS; Writing – Review & Editing, AHS.

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