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Research Article

Exploring Brazilin as a Potential Breast Cancer Therapy via Molecular Dynamics Simulation Targeting ERα, 17β-HSD1, and NUDT5 Receptors

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Abstract

Breast cancer remains one of the leading causes of cancer-related mortality worldwide, suggesting the need for novel therapeutic agents with greater efficacy and fewer side effects. Brazilin, a natural flavonoid compound isolated from Caesalpinia sappan L., has shown promising anticancer activity, particularly against breast cancer cells. This study explores the therapeutic potential of brazilin by evaluating its interactions with three key molecular targets: estrogen receptor alpha (ERα), 17β-hydroxysteroid dehydrogenase 1 (17β-HSD1), and Nudix hydrolase 5 (NUDT5). Using molecular dynamics (MD) simulations, the study assesses the stability and binding interactions of complex systems. The results indicate that brazilin forms a stable complex with ERα, demonstrated by a low RMSD (2.6 Å) and strong hydrogen bonding with Glu353, occupancies of 97.8%, and minimal flexibility at the binding site (average RMSF < 2.5 Å). The binding free energy (ΔG Total) for the ERα–brazilin complex was -54.28 kcal/mol, indicating a stronger affinity than the reference ligand 4-hydroxytamoxifen. Brazilin also showed favorable binding with 17β-HSD1 and NUDT5, with binding energies of -39.71 kcal/mol and -23.23 kcal/mol, respectively. These findings suggest that brazilin may modulate critical targets involved in breast cancer progression, particularly in hormone receptor-positive subtypes. Further experimental validation is necessary to confirm its efficacy and optimize its therapeutic potential.

Keywords: Brazilin, breast cancer, ERα, molecular dynamics simulation, NUDT5, 17-β-HSD-1

1. INTRODUCTION

Breast cancer remains one of the most prevalent and life-threatening malignancies worldwide, particularly among women. According to the World Health Organization (WHO), breast cancer was reported for 23.8% of all new cancer cases in females in 2022, making it the most commonly diagnosed cancer globally [1,2]. Despite advances in treatment modalities, including chemotherapy, hormone therapy, and targeted therapies, the development of drug resistance and the adverse effects associated with current treatments underscore the need for novel therapeutic agents. Natural compounds have gained significant attention in recent years due to their potential efficacy, lower toxicity, and enhanced patient tolerance compared to synthetic drugs[3,4].

One such natural compound is brazilin, a bioactive flavonoid derived from *Caesalpinia sappan* L., a plant abundant in tropical and subtropical regions of Southeast Asia, including Indonesia, Thailand, Vietnam, and Malaysia. Traditionally, *C. sappan* L. has been used in medicine, dye production, and culinary applications [5,6]. Recent pharmacological studies have highlighted brazilin's potential therapeutic effects, including its ~~anti-cancer,~~ anticancer, antioxidant, anti-inflammatory, and antimicrobial properties[7]. Increasing evidence supports the anticancer potential of brazilin in various

tumor models, including breast cancer. For instance, brazilin has been shown to inhibit proliferation and migration of MCF-7 and MDA-MB-231 breast cancer cells[8], induce ferroptosis via the p53/SLC7A11/GPX4 pathway[9], and suppress lung metastasis in 4T1 murine models[10]. These findings collectively suggest that brazilin modulates multiple signaling pathways relevant to breast cancer progression and metastasis[11]. In line with this, recent virtual screening work of flavonoid derivatives reported favorable interactions with hormone and growth factor receptors (such as progesterone receptor, estrogen receptor and HER-2) in breast cancer models[12].

Breast cancer is a heterogeneous disease, and the dysregulation of key molecular pathways13 often drives its progression[13]. Among these, estrogen receptor alpha (ERα), 17-β-hydroxysteroid dehydrogenase 1 (17-β-HSD-1), and Nudix hydrolase 5 (NUDT5) play critical roles in hormone receptor-positive breast cancers. ERα is a well-established target in breast cancer therapy, as it mediates estrogen signaling, which promotes cancer cell proliferation[14]. Inhibiting ERα or modulating its activity can effectively suppress tumor growth. Similarly, 17-β-HSD-1 is an enzyme that converts weaker estrogens into more potent forms, thereby enhancing estrogenic activity within the tumor microenvironment. Inhibition of 17-β-HSD-1 has been shown to reduce estrogen levels and limit cancer progression[15]. Additionally, NUDT5, an enzyme involved in cellular energy metabolism and DNA repair, has emerged as a potential target in triple-negative breast cancer, where it contributes to cancer cell survival and treatment resistance[16].

Given the pharmacological potential of brazilin and the critical roles of ERα, 17-β-HSD-1, and NUDT5 in breast cancer, this study aims to investigate the molecular interactions of brazilin with these receptors using molecular dynamics (MD) simulations. MD simulations provide a powerful computational tool to model the stability and binding interactions of ligands with target proteins at an atomic level, offering insights into the potential therapeutic efficacy of novel compounds[17,18]. By evaluating brazilin's binding stability, structural fluctuations, and binding energy with ERα, 17-β-HSD-1, and NUDT5, this study seeks to elucidate its potential as a therapeutic agent for breast cancer.

The findings of this study could contribute to the growing body of knowledge on natural compounds as anti-cancer agents and provide a foundation for further experimental validation and optimization of brazilin-based therapies.

2. RESEARCH METHODS

Materials

Brazilin was used as the primary ligand. Reference ligands included 4-hydroxytamoxifen (OHT) for ERα, estradiol benzoate (E2B) for 17-β-HSD-1, and 958302 for NUDT5 were retrieved from Protein Data Bank (PDB). The three-dimensional structures of the target proteins were also obtained from the PDB with the following IDs, ERα (3ERT), 17-β-HSD-1 (3HB5), and NUDT5 (5NQR). The computational simulations were performed using the following hardware: processor Intel® Xeon(R) CPU E5-2670 v2 @ 2.50GHz (20 cores), graphics card NVIDIA GeForce RTX 3060, memory 64GB DDR4 ECC RAM, and storage 512GB SSD.

System Preparation

Missing residues in the protein structure of ERα were modeled and refined using the SWISS-MODEL server https://swissmodel.expasy.org/ using 3ERT as a template. The ligands (brazilin, OHT, E2B, and 958302) were parameterized using the General Amber Force Field (GAFF) and the ff14SB force field for protein parameterization. The cysteine and histidine residue types were modified using the APBS-PDB2PQR server (https://server.poissonboltzmann.org/pdb2pqr). The system was solvated using the TIP3P water model in a cubic box with a minimum distance of 10 Å between protein and the edge of the box, and sodium ions were added to neutralize the system. Initial coordinates for all complexes were retrieved from our previous docking study by[19].

Molecular Dynamics Simulation

AMBER22 was used to minimize energy and perform the MD simulation. The process of MD simulation was adapted from our previous study[20]. It began with energy minimization, which was performed using 2000 steps of the steepest descent algorithm followed by 2000 steps of the conjugate gradient algorithm to remove steric clashes and optimize the protein structure. The system was then heated from 0 K to 310 K over 60 ps under NVT (constant number of particles, volume, and temperature) conditions. The harmonic restraint of 5 kcal/molÅ^2 on the backbone atoms was applied. Furthermore, the system was equilibrated under NPT (constant number of particles, pressure, and temperature) conditions for 1000 ps to stabilize the temperature and pressure. The harmonic restraint on the backbone protein was slowly decreased by 1 kcal/molÅ^2 until it reached zero. Finally, MD simulations were run for 100 ns with a 2 fs time step. The NPT ensemble was performed in the production run with the SHAKE algorithm applied to all hydrogen

atoms. The temperature was regulated using a Langevin thermostat with a collision frequency of 1 ps^{-1}. At the same time, the pressure was maintained with a Berendsen barostat, applying a coupling constant of 1 ps and a target pressure of 1 bar. A nonbonded cutoff value of 9 Å was applied, and long-range electrostatics were handled using the particle mesh Ewald method. Trajectories were saved every 1000 steps for analysis.

Trajectory Analysis

Root Mean Square Deviation (RMSD) was calculated to assess the stability of the protein-ligand complex over time. Root Mean Square Fluctuation (RMSF) was used to measure the flexibility of individual residues in the protein. Hydrogen bonds between the ligand and protein were analyzed to determine interaction stability and occupancy rates. The binding free energy (ΔG) was calculated using the Molecular Mechanics Generalized Born Surface Area (MMGBSA) method. The energy terms included: Van der Waals energy (E Van der Waals), Electrostatic energy (E Electrostatic), Polar solvation energy (E GB), Non-polar solvation energy (E Surf), Total binding energy (ΔG Total = ΔG Gas + ΔG Solv).
All simulations were analyzed using AmberTools22 and Biovia Discovery Studio Visualizer. The results were visualized and interpreted to evaluate the binding stability and interactions of brazilin with ERα, 17-β-HSD-1, and NUDT5, and compare it with the reference.

3. RESULTS AND DISCUSSION

Brazilin's Interaction with ERα

Conformational Stability

The conformational stability of the ERα-brazilin complex was evaluated using RMSD analysis over a 100 ns MD simulation. The RMSD measures the deviation of atomic positions in the protein-ligand complex from its initial structure, providing insights into the stability of the binding interaction. The average RMSD for the ERα receptor when bound to brazilin was 2.6 Å, indicating a stable binding conformation. In comparison, the reference ligand OHT showed an average RMSD of 2.9 Å, suggesting that brazilin exhibits comparable or slightly better stability (**Figure 1**). RMSD values below 3 Å are generally considered indicative of stable protein–ligand complexes in MD simulations, reflecting the absence of major conformational rearrangements and consistent intermolecular interactions over time[21,22]

The RMSD of the brazilin ligand remained consistently low (0.2 Å), indicating minimal deviation from its initial binding pose throughout the simulation. In contrast, the reference ligand 4-hydroxytamoxifen (OHT) exhibited slightly higher fluctuations (1.2 Å). These observations suggest that brazilin maintains a stable binding orientation within the ERα pocket and forms a stable complex, a property desirable in drug candidates due to its potential to enhance binding affinity and reduce off-target interactions[17]. This stability is further supported by the strong hydrogen bonding interactions observed with Glu353.

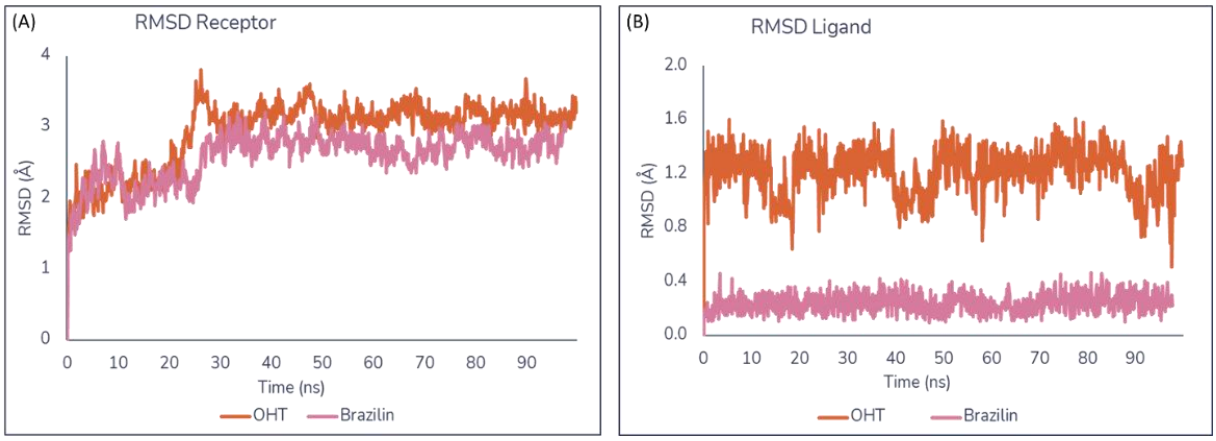

Figure 1. RMSD receptor and ligand throughout MD simulation with ERα. (A) RMSD receptor and (B) RMSD ligand

Structural Fluctuations

The flexibility of individual residues in the ERα receptor during the MD simulation was assessed using RMSF analysis. RMSF measures the extent to which each residue fluctuates from its average position over time, providing insights into the dynamic behavior of the protein-ligand complex. The average RMSF for ERα when bound to brazilin was 3.7 Å, compared to 1.3 Å for the ERα-OHT complex (**Figure 2**). Although the overall RMSF of the ERα-brazilin complex was higher than that of the ERα-OHT complex, this increase primarily reflects enhanced flexibility in several loops and regions. A closer inspection of the binding site residues revealed that most residues exhibited RMSF values above 2 Å, indicating local fluctuations during the simulation. However, the helix region containing GLU353 showed RMSF values below 2 Å, suggesting that this site remained

structurally stable (**Figure 3**). This observation is consistent with the firm and persistent hydrogen bond between brazilin and Glu353, stabilizing the ligand within the binding pocket. These findings align with previous studies showing that natural flavonoids like brazilin can induce conformational changes in target proteins, leading to enhanced therapeutic effects[7].

The combination of a low ligand RMSD and a stable key interaction, despite local receptor flexibility, suggests that the ERα–brazilin complex maintains dynamic yet functionally stable binding characteristics. Such behavior is commonly observed in systems where ligand binding induces localized conformational adaptation, induced fit, rather than global destabilization [23–25]. The snapshot throughout the MD simulation showed that both brazilin and OHT can maintain their structure.

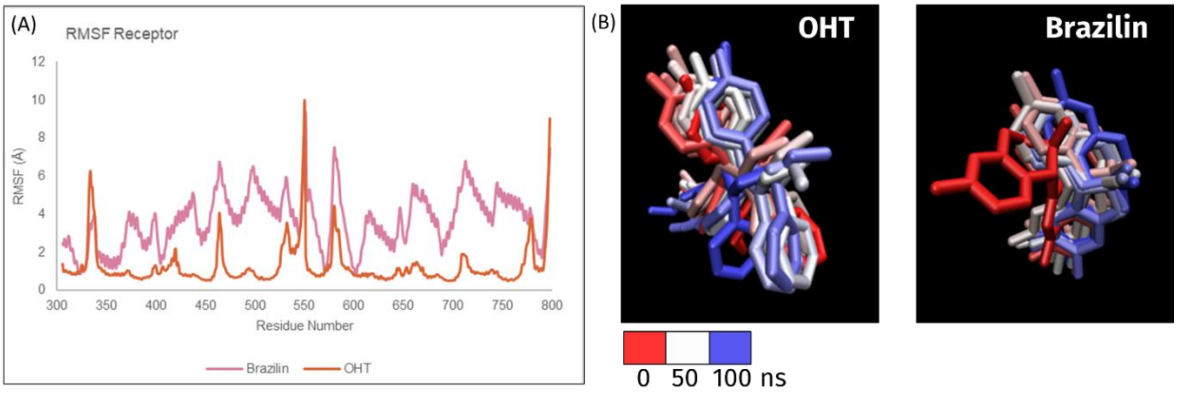

Figure 2. (A) RMSF receptor of ERα and (B) snapshot of ligands through MD simulation.

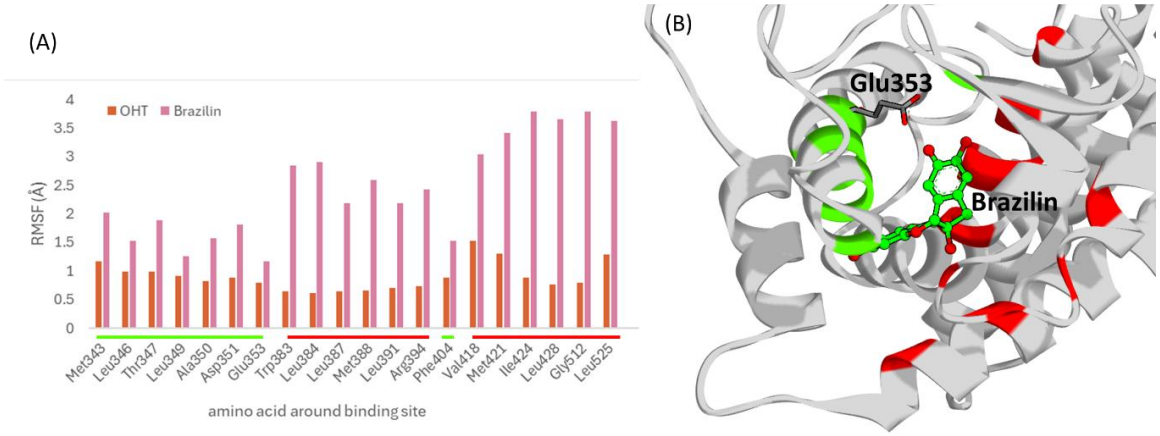

Figure 3. RMSF values of residues around binding site (A), and the structural representation of ERα showing regions with high (red) and low (green) RMSF (B).

Hydrogen Bond Interactions

The hydrogen bond plays a crucial role in stabilizing ligand-receptor interactions [26]. The hydrogen bond occupancy rates between brazilin and key residues in ERα were analyzed to evaluate the strength and persistence of these interactions. Brazilin formed strong hydrogen bonds with Glu353, with occupancy rates of 97.8% and 96.7% for two key interactions (**Table 1**). These high occupancy rates indicate stable and persistent hydrogen bonding, which is critical for maintaining ligand-receptor stability (**Figure 4**). Importantly, Glu353 is a highly conserved residue within the ligand-binding domain of ERα and plays a well-established functional role in ligand recognition and stabilization. In the canonical binding mode of 17β-estradiol and selective estrogen receptor modulators (SERMs) such as 4-hydroxytamoxifen, the phenolic hydroxyl group of the ligand forms a direct hydrogen bond with Glu353, often complemented by interactions with Arg394 and His524 [27,28]. This conserved interaction network anchors the ligand in the binding pocket, contributing to receptor activation or inhibition. Therefore, the stable and persistent hydrogen bonding between brazilin and Glu353 observed in this study is functionally relevant and consistent with the canonical ERα–ligand binding mechanism.

In contrast, OHT showed lower hydrogen bond occupancy with Glu353 (46.7% and 19.3%), suggesting that brazilin forms more stable interactions with this key residue, which likely contributes to its low RMSD and favorable binding free energy in the ERα–brazilin complex.

Table 1. H-Bond analysis of brazilin (BRZ) and ERα throughout MD simulation

Acceptor	Donor H	Donor	Occupancy (%)
Glu353@OE1	OHT@H13	OHT@O4	46.7
Glu353@OE2	OHT@H13	OHT@O4	19.3
Glu353@OE2	BRZ@H3	BRZ@O4	97.8
Glu353@OE2	BRZ@H2	BRZ@O3	96.7

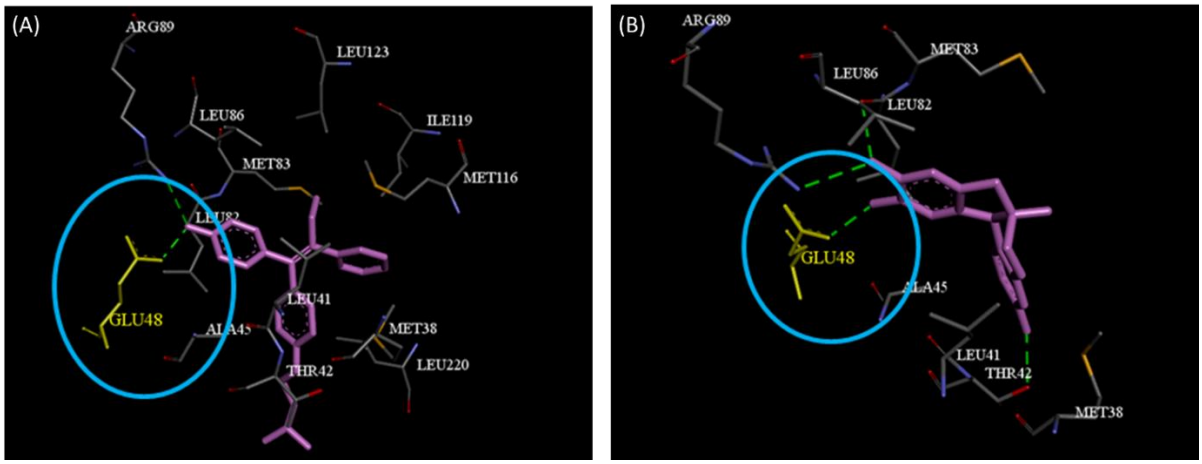

Figure 4. H-Bond interaction around OHT (A) and brazilin (B). The hydrogen bond is presented in a green dashed line. Residue numbering follows the MD trajectory; Glu48 corresponds to Glu353 in the original ERα sequence (PDB ID: 3ERT).

Binding Energy Calculations

The binding free energy (ΔG) of the ERα-brazilin complex was calculated using the MMGBSA method. This provides an estimate of the overall binding affinity between brazilin and ERα. The ΔG Total for the ERα-brazilin complex was -54.28 kcal/mol, which was more favorable than that of the ERα-OHT complex (-34.36 kcal/mol) (**Table 2**). This indicates that brazilin has a stronger binding affinity for ERα. The favorable binding energy of brazilin was primarily driven by van der Waals interactions (-51.89 kcal/mol) and electrostatic contributions (-16.98 kcal/mol), highlighting the importance of these forces in stabilizing the complex. These results suggest that brazilin has the potential to effectively inhibit ERα, making it a promising candidate for further development. For instance, Raptania, et al[7] reported that brazilin exhibits cytotoxic effects on breast cancer cells by modulating estrogen receptor signaling pathways. The ability of brazilin to form stable interactions with ERα makes it a promising candidate for hormone receptor-positive breast cancers, where ERα plays a central role in tumor growth and progression. These results demonstrated that natural compounds like brazilin can exhibit strong binding affinities for enzymes involved in cancer progression, making them promising candidates for therapeutic development[3,29].

The stable interaction between brazilin and ERα observed in this study, supported by a low RMSD and strong hydrogen bonding with Glu353, may provide a structural explanation for its previously reported anticancer activity. Experimental studies have shown that brazilin and its derivatives can reduce the viability and migration of MCF-7 (ERα-positive) and MDA-MB-231 (triple-negative) breast cancer cells at concentrations around 20–50 µM, accompanied by decreased focal adhesion kinase (FAK) activation[10,11]. These findings suggest that the stable binding of brazilin to ERα could contribute to its inhibitory effects on breast cancer cell growth. However, further experimental validation is needed to confirm this relationship.

Table 2. MMGBSA energy calculation of brazilin and ERα throughout MD simulation.

	ERα-Brazilin		ERα-OHT	
	Average (kcal/mol)	SD	Average (kcal/mol)	SD
Energy Van der Waals	-51.8912	4.4109	-31.3198	3.5393
Energy Electrostatic	-16.9781	4.9006	-39.4182	5.9154
Energy GB	22.0551	3.1495	41.7097	3.5927
Energy Surf	-7.4658	0.3385	-5.3271	0.3653
ΔG Gas	-68.8693	4.1422	-70.738	6.6373
ΔG Solv	14.5893	3.2006	36.3827	3.2499
ΔG Total	**-54.2799**	4.1946	**-34.3554**	3.8034

Brazilin's Interaction with 17-β-HSD-1

Conformational Stability

The conformational stability of the 17-β-HSD-1-brazilin complex was assessed over a 100 ns MD simulation. The average RMSD for the 17-β-HSD-1 receptor when bound to brazilin was 1.6 Å, compared to 2.0 Å for the reference ligand estradiol benzoate (E2B). This indicates that brazilin exhibits comparable or slightly better stability than E2B (**Figure 5**). The RMSD of the brazilin ligand itself was 0.4 Å, demonstrating minimal deviation from its initial binding pose, whereas E2B showed slightly higher fluctuations (0.5 Å). These results suggest that brazilin forms a stable complex with 17-β-HSD-1, which aligns with previous studies showing that natural compounds can exhibit stable binding to enzymes involved in steroid metabolism[30].

Structural Fluctuations

The flexibility of individual residues in the 17-β-HSD-1 receptor was analyzed to understand the dynamic behavior of the protein-ligand complex. The average RMSF for 17-β-HSD-1 when bound to brazilin was 1.0 Å, compared to 1.1 Å for the 17-β-HSD-1-E2B complex (**Figure 6**). This indicates that brazilin induces comparable flexibility in the receptor, with no significant increase in residue fluctuations. The low RMSF values suggest that 17-β-HSD-1 remains structurally stable throughout the simulation, consistent with the behavior of well-folded enzymes in MD simulations[25]. These findings are supported by studies highlighting the role of residue flexibility in modulating enzyme-ligand interactions, particularly in steroidogenic enzymes like 17-β-HSD-1[29].

Hydrogen Bonding Interactions

The hydrogen bond occupancy rates between brazilin and key residues in 17-β-HSD-1 were analyzed to evaluate the strength and persistence of these interactions. Brazilin formed hydrogen bonds with Asn90, with an occupancy rate of 10.8% (Table 3). Although this occupancy rate is lower than that of E2B (55% with Ser142), the interaction is still significant and contributes to the overall stability of the complex (**Figure 7**). The lower hydrogen bond occupancy rates for brazilin suggest that other non-covalent interactions, such as van der Waals forces, may be more prominent in stabilizing the 17-β-HSD-1-brazilin complex. This observation aligns with studies showing that natural flavonoids often rely on a combination of hydrogen bonding and hydrophobic interactions to stabilize their binding to target proteins[3,31].

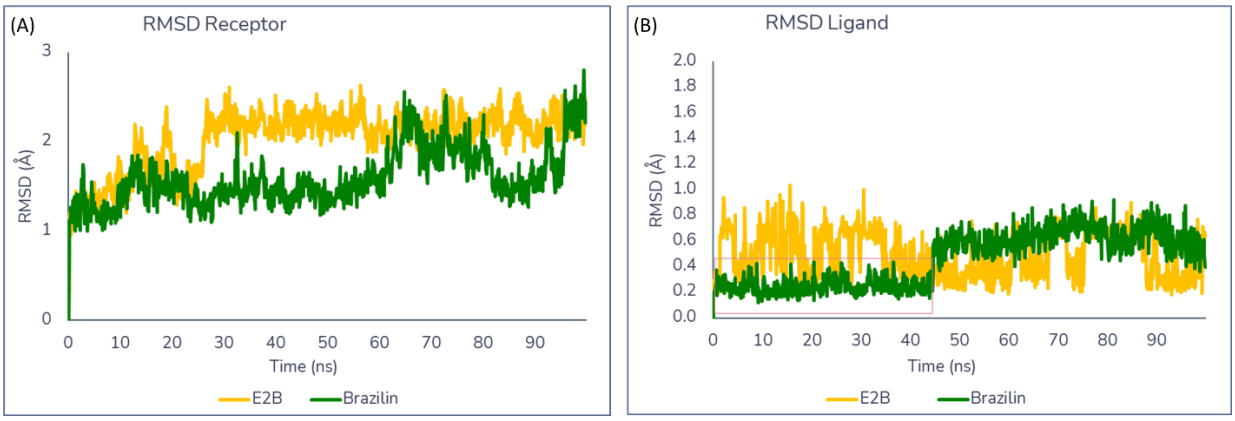

Figure 5. RMSD receptor and ligand throughout MD simulation with 17-β-HSD. (A) RMSD receptor and (B) RMSD ligand.

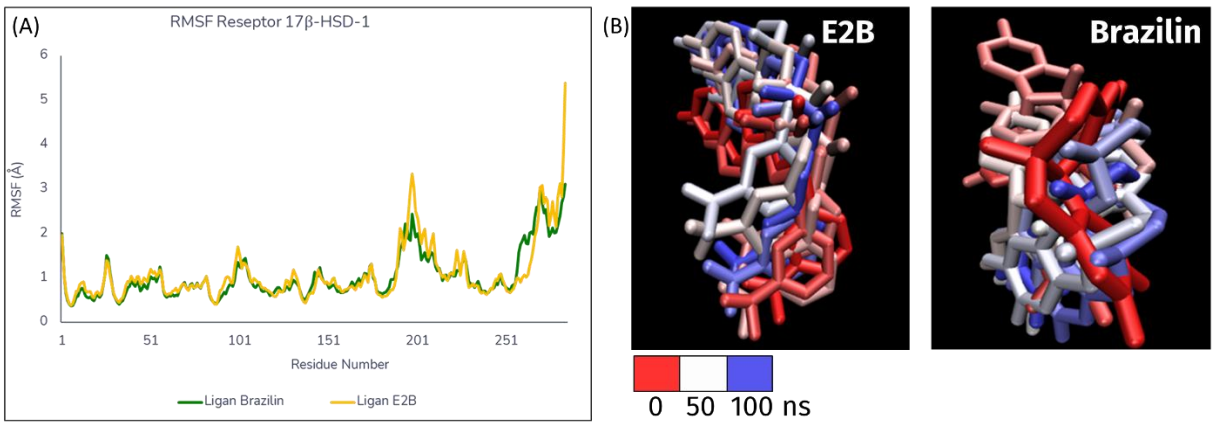

Figure 6. (A) RMSF receptor of 17-β-HSD and (B) snapshot of ligands through MD simulation.

Table 3. H-Bond analysis of brazilin (BZR) and E2B throughout MD simulation

Acceptor	Donor H	Donor	Occupancy (%)
E2B@O19	Ser142@HG	Ser142@OG	55.0
Asn152@OD1	E2B@H1	E2B@N30	16.9
His221@NE2	E2B@H29	E2B@O4	54.2
Asn90@O	BRZ@H1	BRZ@O1	10.8

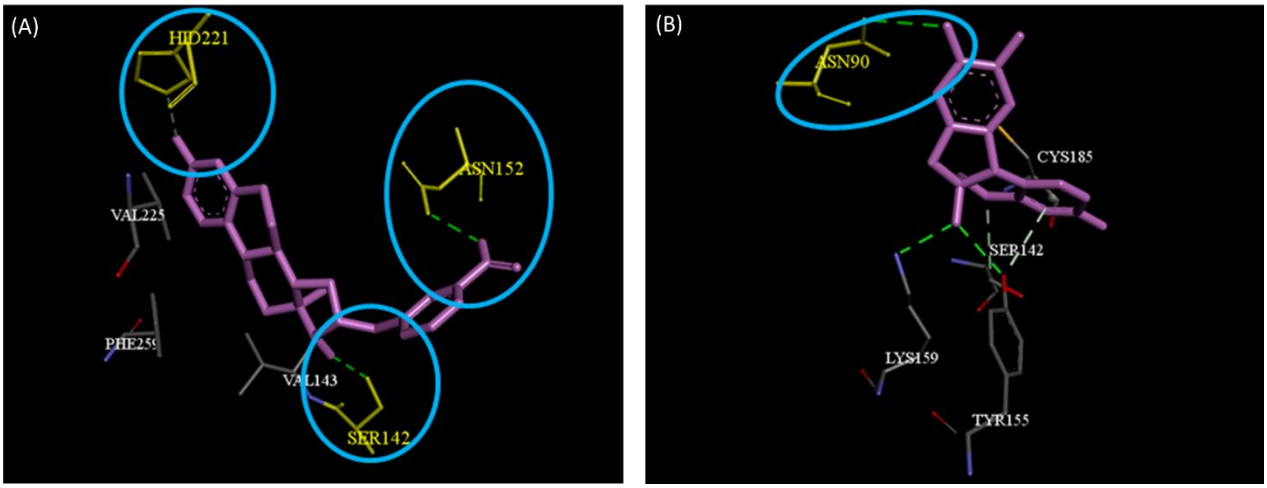

Figure 7. H-Bond interaction around E2B (A) and brazilin (B). Hydrogen bond is presented in a green dashed line

Table 4. MMGBSA energy calculation of brazilin and 17-β-HSD throughout MD simulation

	17-β-HSD-1-Brazilin		17-β-HSD-1-E2B	
	Average (kcal/mol)	**SD**	**Average (kcal/mol)**	**SD**
Energy Van der Waals	-41.5322	21.2751	-32.3546	2.626
Energy Electrostatic	-13.4079	8.508	-15.1879	11.1585
Energy GB	20.7016	10.8948	29.7501	8.3434
Energy Surf	-5.4716	2.7681	-4.2984	0.3916
ΔG Gas	-54.9401	28.6616	-47.5425	10.1695
ΔG Solv	15.23	8.3063	25.4517	8.1769
ΔG Total	**-39.7101**	21.3494	**-22.0908**	5.5231

Binding Energy Calculations

The binding free energy (ΔG) of the 17-β-HSD-1-brazilin complex was calculated to estimate the overall binding affinity. The total binding energy (ΔG Total) for the 17-β-HSD-1-brazilin complex was -39.71 kcal/mol, which was more favorable than that of the 17-β-HSD-1-E2B complex (-22.09 kcal/mol) (**Table 4**). This indicates that brazilin has a stronger binding affinity for 17-β-HSD-1. The favorable binding energy of brazilin was primarily driven by van der Waals interactions (-41.53 kcal/mol) and electrostatic contributions (-13.41 kcal/mol). These findings align with previous studies highlighting the anti-cancer potential of natural flavonoids[32].

The favorable binding free energy (ΔG Total) of brazilin compared to the reference ligand E2B suggests that brazilin could effectively inhibit 17-β-HSD-1, an enzyme that enhances estrogenic activity within the tumor microenvironment. This is particularly relevant for hormone-dependent breast cancers, where 17-β-HSD-1 has been implicated in cancer progression and resistance to therapy[29]. The ability of brazilin to modulate estrogen levels through 17-β-HSD-1 inhibition could provide a novel therapeutic strategy for managing hormone receptor-positive breast cancers.

Although the MM/GBSA analysis provided valuable insight into the binding affinity of brazilin and the reference ligand E2B toward 17β-HSD-1, several limitations of this method should be acknowledged. The MM/GBSA approach is a post-processing method that estimates binding free energy based on molecular mechanics and implicit solvation models. However, it does not fully account for entropic contributions, explicit solvent effects, or long-range electrostatics, which may influence the accuracy of absolute binding free energies[33,34]. Future studies could employ enhanced sampling techniques (e.g., metadynamics or accelerated MD) or free energy perturbation (FEP)/thermodynamic integration (TI) methods to obtain a more accurate estimation of binding free energy. Additionally, experimental validation, such as enzyme inhibition assays or surface plasmon resonance (SPR), would be essential to

confirm the predicted inhibitory potential of brazilin against 17β-HSD-1.

The interaction of brazilin with 17β-HSD1 observed in this study suggests a potential interference with the enzyme's catalytic function, which plays a crucial role in the conversion of estrone (E1) to the more active estradiol (E2). Previous structural and computational studies have demonstrated that the natural substrate and inhibitors of 17β-HSD1, such as E2 and E2B, occupy the steroid-binding pocket and stabilize key residues, including Tyr155, Ser142, and His221, which are involved in catalysis[15]. The comparable binding pattern and stable complex formation of brazilin in the present study indicate that it may exert an inhibitory effect similar to that of known ligands, E2B, potentially reducing local estrogen production. However, further biochemical and cell-based validation would be required to confirm whether this interaction translates into functional inhibition and contributes to the anticancer potential of brazilin in hormone-dependent breast cancer.

Brazilin's Interaction with NUDT5

Conformational Stability

The conformational stability of the NUDT5-brazilin complex was evaluated over a 100 ns MD simulation. The average RMSD for the NUDT5 receptor when bound to brazilin was 4.5 Å, compared to 3.0 Å for the reference ligand 958302. This indicates that brazilin induces slightly higher fluctuations in the receptor compared to the reference ligand (**Figure 8**). The RMSD of the brazilin ligand itself was 0.3 Å, demonstrating minimal deviation from its initial binding pose, whereas 958302 showed higher fluctuations (1.6 Å). These results suggest that while brazilin induces slightly higher receptor fluctuations, it maintains a stable binding mode within the NUDT5 binding pocket. This aligns with studies showing that natural compounds can exhibit stable binding to enzymes involved in nucleotide metabolism[35,36].

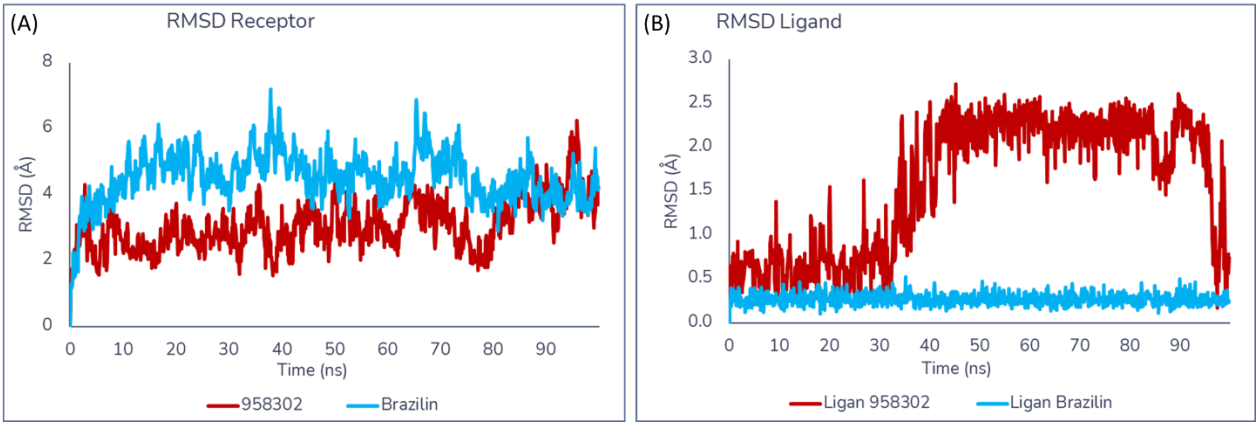

Figure 8. RMSD receptor and ligand throughout MD simulation with NUDT5. (A) RMSD receptor and (B) RMSD ligand.

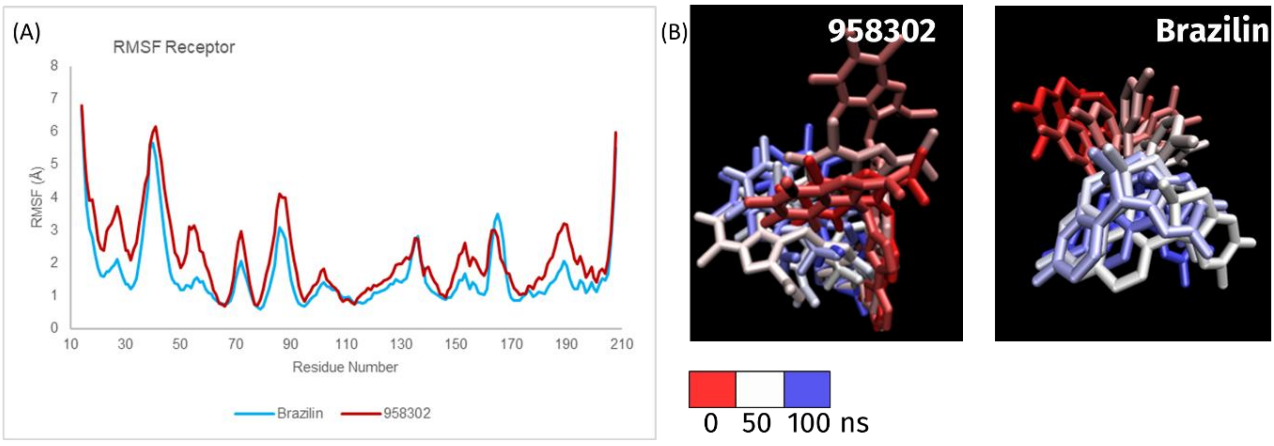

Figure 9. (A) RMSF receptor of NUDT5 and (B) snapshot of ligands through MD simulation.

Table 5. H-Bond analysis of brazilin (BZR) and NUDT5 throughout MD simulation

Acceptor	Donor H	Donor	Occupancy (%)
958302@O6	Arg52@HH12	Arg52@NH1	27.5
Gly98@O	BRZ@H1	BRZ@O1	2.8

Table 6. MMGBSA energy calculation of brazilin and NUDT5 throughout MD simulation

	NUDT5-Brazilin		NUDT5-958302	
	Average (kcal/mol)	**SD**	**Average (kcal/mol)**	**SD**
Energy Van der Waals	-32.3231	3.0104	-19.471	2.681
Energy Electrostatic	-8.7296	10.5491	-31.4445	17.2018
Energy GB	21.6636	8.9151	38.8191	12.7409
Energy Surf	-3.8431	0.4267	-3.2562	0.2185
ΔG Gas	-41.0526	12.4613	-50.9155	15.7296
ΔG Solv	17.8205	8.8234	35.5629	12.6599
ΔG Total	**-23.2321**	4.2111	**-15.3526**	4.2904

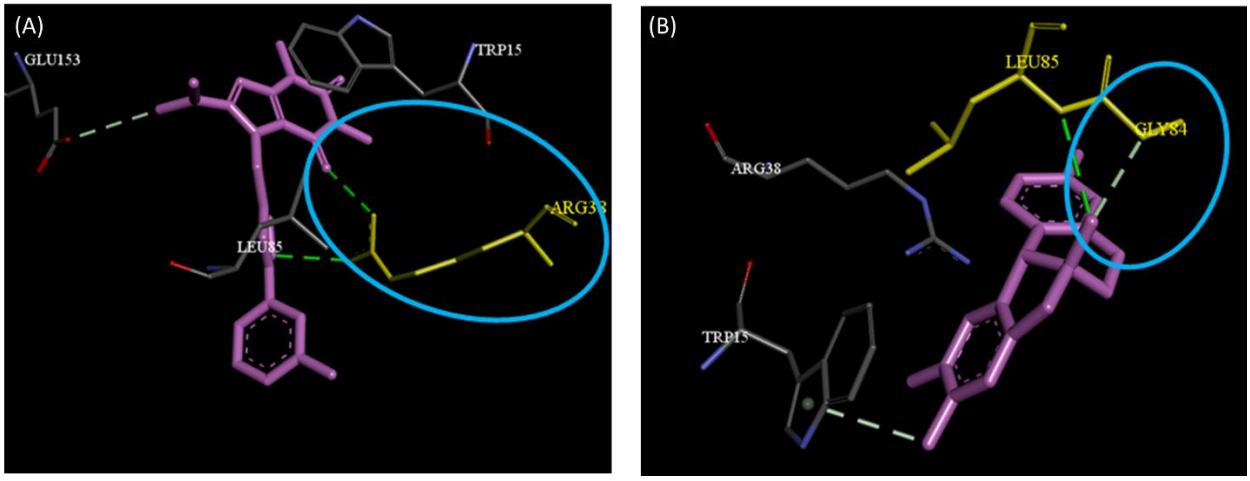

Figure 10. H-Bond interaction around 958302 (A) and brazilin (B). Hydrogen bond is presented in a green dashed line. Residue numbering follows the MD trajectory; Arg38 corresponds to Arg52; Gly84 corresponds to Gly98 in the original NUDT5 sequence (PDB ID: 5NQR).

Structural Fluctuations

The flexibility of individual residues in the NUDT5 receptor was analyzed to understand the dynamic behavior of the protein-ligand complex. The average RMSF for NUDT5 when bound to brazilin was 1.6 Å, compared to 2.1 Å for the NUDT5-958302 complex (**Figure 9**). This indicates that brazilin induces lower residue fluctuations in the receptor compared to the reference ligand. The lower RMSF values suggest that brazilin stabilizes certain regions of NUDT5, potentially enhancing its binding interactions. These findings are supported by studies highlighting the role of residue flexibility in modulating enzyme-ligand interactions, particularly in enzymes involved in DNA repair and nucleotide metabolism[37,38].

Hydrogen Bonding Interactions

The hydrogen bond occupancy rates between brazilin and key residues in NUDT5 were analyzed to evaluate the strength and persistence of these interactions. Brazilin formed a hydrogen bond with Gly98, with an occupancy rate of 2.8% (Table 5). Although this occupancy rate is lower than that of 958302 (27.5% with Arg52), the interaction is still significant and contributes to the overall stability of the complex (Figure 10). The lower hydrogen bond occupancy rates for brazilin suggest that other non-covalent interactions, such as van der Waals forces, play a more prominent role in stabilizing the NUDT5-brazilin complex. This observation aligns with studies showing that natural flavonoids often rely on a combination of hydrogen bonding and hydrophobic interactions to stabilize their binding to target proteins[3,39].

Binding Energy Calculations

The binding free energy (ΔG) of the NUDT5-brazilin complex was calculated to estimate the overall binding affinity. The total binding energy (ΔG Total) for the NUDT5-brazilin complex was -23.23 kcal/mol, which was more favorable than that of the NUDT5-958302 complex (-15.35 kcal/mol) (**Table 6**). This indicates that brazilin has a stronger binding affinity for NUDT5. The favorable binding energy of brazilin was primarily driven by van der Waals interactions (-32.32 kcal/mol) and electrostatic contributions (-8.73 kcal/mol). These results are consistent with previous studies demonstrating that natural compounds such as brazilin can exhibit strong binding affinities for enzymes involved in cancer progression, making them promising candidates for therapeutic development [37].

The favorable binding free energy (ΔG Total) of brazilin compared to the reference ligand 958302 suggests that brazilin could effectively inhibit NUDT5, an enzyme involved in cellular energy metabolism and DNA repair. This is particularly relevant for triple-negative breast cancers, where NUDT5 has been implicated in cancer cell survival and treatment resistance[37]. The ability of brazilin to target NUDT5 could provide a novel therapeutic strategy for managing triple-negative breast cancers, which currently have limited treatment options.

The lower binding energy of brazilin compared to the reference ligand in the NUDT5 (PDB ID: 5NQR) receptor suggests a stronger or more stable interaction within the active site. NUDT5 has been implicated in estrogen-regulated gene transcription and breast cancer cell proliferation through its role in ATP generation within the nucleus[40]. Therefore, the stronger binding affinity observed for brazilin may indicate its potential to interfere with NUDT5-mediated signaling, which could contribute to the modulation of breast cancer cell growth. Nevertheless, this interpretation remains hypothetical and requires further experimental confirmation to determine whether the interaction translates into a measurable biological effect.

4. CONCLUSION

This study explored the potential of brazilin as a therapeutic agent for breast cancer by evaluating its interactions with key receptors (estrogen alpha, 17-β-HSD-1, and NUDT5) through MD simulations. The results of this study underscore the multi-target potential of brazilin as a natural anti-cancer agent. By targeting those receptors, brazilin could modulate multiple pathways involved in breast cancer progression, making it a versatile candidate for further development. The results showed that brazilin demonstrated favorable binding stability with estrogen alpha, particularly through hydrogen bonding at Glu353, with high occupancy rates indicating strong receptor interactions. While brazilin's binding affinity was lower compared to native ligands, its stability and interactions with critical residues suggest potential therapeutic relevance, especially for hormone receptor-positive breast cancers. Further optimization and experimental studies are warranted to enhance its binding affinity and validate its efficacy as an anti-cancer agent.

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