

Genistein as an Estrogen Receptor-Beta Selective Phytoestrogen: Molecular Docking Insights for Advanced Wound Healing Therapies

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Abstract. Wound healing is highly hormonal based and is stepwise process involving inflammation, proliferation, and remodeling. Estrogen is a key regulator having two receptor subtypes having various functions. ER α (Estrogen receptor alpha) promotes inflammatory signaling and ER β (Estrogen receptor beta) helps extracellular matrix (ECM) deposition, epithelial regeneration, and also angiogenesis. Excessive ER α activity will prolong inflammation and hinder repair, whereas ER β activation action is generally restorative. This difference makes ER β -selective ligands suitable for therapeutic treatments, mainly in estrogen-deficient chronic wounds. SERMs have attained importance as they mimic estrogen but carry minimum systemic risks than other hormone therapy. Genistein, an isoflavone abundant in soy, is best candidate because of its receptor-biased activity. Twelve phytoestrogenic ligands were docked with ER α and ER β using SwissDock to assess receptor selectivity. Genistein shows maximum affinity for ER β (-8.09 kcal/mol), which forms hydrogen bonds with residues ARG346, GLU305, HSE475. ER α shows a weaker interaction (-7.80 kcal/mol), enhancing ER β preference. Liquiritigenin and glycitein shows ER β preference but less strongly. These findings concludes that genistein accelerates repair through angiogenesis and ECM remodeling by ER beta signaling over ER alpha. However, the direct use is limited due to its poor solubility, making clinical application of nanocarrier or hydrogel delivery systems preferable.

Keywords: Genistein, Wound healing, Estrogen receptor alpha, Estrogen receptor beta, Angiogenesis

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1 Introduction

Wound healing is highly overlapping sequence of haemostatic, inflammatory, proliferative, and remodeling phases that integratably restore tissue integrity. Estrogen regulates these stages by inflammation, angiogenesis, and extracellular matrix formation. While limiting excessive inflammatory responses, estrogen promotes fibroblast and keratinocyte proliferation, re-epithelialization enhancement, and collagen synthesis. Estrogen deficiency disrupts immune cell signaling, impairs matrix deposition, leading to prolonged inflammation and delayed tissue repair effects seen in diabetic conditions. Within fibroblasts and endothelial cells, estrogen contributes to dermal density, balanced collagen turnover, angiogenic maintenance, and extracellular matrix remodeling through estrogen-receptor signaling that protects skin architecture [1]. Reduced estrogen availability clinically relates with delayed closure, impaired re-epithelialization, higher infection susceptibility, and decreases tensile strength. Experimental and clinical studies confirm that estrogen supplementation increases tensile integrity, angiogenesis and ECM quality. Topical estrogen has been shown to facilitate neovascularization and collagen deposition by reactivating endothelial progenitor cells and also mesenchymal stem cells in diabetic models, there is a high need for alternative approaches as conventional systemic hormone replacement therapy (HRT) has tissue-specific side effects and thromboembolic risk [2].

Estrogen receptor alpha (ER α) and estrogen receptor beta (ER β) is the core for estrogen functioning, which also has complementary roles in skin biology. ER α is connected to macrophage polarization and inflammatory clarification, whereas ER β contributes immensely to angiogenesis, epithelial repair and ECM production. Disruption of this balance in diabetic wounds often with ER α dominance can make inflammation sustenance, fibroblast survival reduction and also inhibit keratinocyte migration. Receptor activity imbalances influence systemic metabolism by regulating GLUT4 and glucose homeostasis. Current evidence labels ER β as a restorative factor for cutaneous wound repair, whereas excessive ER α activation seems to impede healing [3].

Plant-derived phytoestrogens and other Selective estrogen receptor modulators (SERMs) can give estrogen-like benefits without any HRT risks. Genistein has attained special interest as an ER β -biased ligand. Studies in both rodents and cell cultures show that genistein outperforms estradiol and raloxifene by enhancing wound closure, improving tensile strength, and remodeling ECM. Genistein decreases NF- κ B-driven inflammation, antioxidant responses via Nrf2 is restored, and key growth factors including TGF- β 1, TG2, and VEGF is promoted. Non-ER pathways such as IGF-1R and MAPK/ERK, promotes keratinocyte migration and dampens immune infiltration [4].

Genistein has shown to restore oxidative-nitrosative balance, increase nitric oxide bioavailability, and stimulate SIRT1/FoxO1 signaling, hence supporting angiogenesis in diabetic models [5]. Estradiol promotes closure through ER α -mediated macrophage activity, while genistein achieves same results while deleting all ER α -related risks [6]. Polyphenol-loaded nanocarriers, incorporating quercetin or genistein, have been shown to enhance solubility, reduce antibiotic dependence, and increase tissue repair and sustainable nanotechnology platforms have worked on these merits [7]. Genistein-loaded formulations have shown great perfusion after an ischemic injury, restoring redox balance, and facilitating endothelial migration, hence highlighting their translational potential. Genistein exerts systemic benefits by increasing insulin sensitivity, glycaemic control, lipid regulation, and blood pressure [8]. The suitability of Genistein in diabetic wound is due to this dual local and systemic effects.

Computational docking has become a main approach for identifying ligands with therapeutic potential. Platforms such as Auto Dock Vina, Galaxy workflows, and SwissDock 2024 used to predict receptor–ligand interactions with reproducibility by applying refined algorithms [9]. Clinical application of genistein remains limited by its poor solubility, rapid metabolism, and low bioavailability. Recent progress in nanotechnology-based carriers has increased its bioavailability, and tissue penetration [10]. These tools can enhance ligand specialization and track experimental validation within biomaterial systems. *In-silico* with *in-vivo* findings showing that genistein and related isoflavones preferentially binds with $Er\beta$. Icaritin and small-molecule conjugates (e.g., JW-127, JW-191, VB-165), shows even stronger $ER\beta$ selectivity and regenerative capacity. This data confirms $ER\beta$ as the prime captain of tissue repair, while $ER\alpha$ has more context-dependent roles [11]. Genistein with $ER\beta$ -mediated activity and multiple wound-healing mechanisms represent strong candidate for localized therapies. Nanocarriers or hydrogels incorporation enhances a way to avoid solubility and delivery problems, causing a more targeted yet sustained release. *In-silico* docking with biomaterial-assisted delivery system can be integrated as core of $ER\beta$ -selective ligands for chronic wounds, for clinically, safe, and effective therapies [12].

Mechanistic insight into how phytoestrogens differentially engage $ER\alpha$ and $Er\beta$ is limited even though estrogen signaling has been widely used. This gap is critical because conventional hormone replacement therapy, while clinically effective, has risks [13]. Selective estrogen receptor modulators (SERMs) role in skin regeneration has not been systematically even though it represent as a potential alternative. No receptor-focused docking study has identified a candidate suitable for simultaneously helping these processes although wound healing needs regulation of inflammation, oxidative stress, angiogenesis, and extracellular matrix remodelling. The structural determinants of $ER\beta$ -selective binding remain insufficiently defined while both receptors shows contrasting roles, with $ER\beta$ strongly linked to pro-regenerative outcomes such as keratinocyte migration and matrix organization. To fill these gaps, this work uses molecular docking in order to assess phytoestrogenic ligands for differential binding with both the receptors. We hypothesize that genistein is the best promising candidate, given its reported pleiotropic activity, and also propose that docking analysis will confirm its stronger affinity for $Er\beta$. There is scope for prioritizing genistein in a molecular fashion for safer, targeted therapies for wound healing.

Objective of this study was to systematically examine a panel of phytoestrogenic ligands using molecular docking and to compare their binding affinities toward $ER\alpha$ and $ER\beta$, with the aim of identifying candidates that preferentially target $ER\beta$ and hence has wound-healing applications. We hypothesized based on all mentioned evidence, that genistein would demonstrate the strongest $ER\beta$ selectivity, making it the most promising alternative to conventional HRT since it simultaneously engages multiple repair-associated pathways and enhances regenerative processes in the best manner possible.

2 Materials and Methods

2.1 Data Retrieval

2.1.1 Protein Data Retrieval

The amino acid sequences, structural details of estrogen receptor alpha ($ER\alpha$, UniProt ID: P03372) and estrogen receptor Beta ($Er\beta$, UniProt ID: Q92731) was downloaded from the UniProt database (<https://www.uniprot.org/>). For structure-based molecular docking, crystal structures of the ligand-binding domains were retrieved from the Protein Data Bank (PDB; <https://www.rcsb.org/>). The structures with PDB ID 1A52 ($ER\alpha$) and 4Z11 ($ER\beta$) were selected. The basis of selection was that the resolution is better than 3.0 Å, to define a perfect the binding pocket presence of a co-crystallized ligand, and zero missing residues due to the

structural completeness of ligand-binding domain . These PDB files(curated) were then used for Molecular docking as receptor models.

2.1.2 Ligand Structure Retrieval

Docking final panel consists of 12 ligands, identified through database-based screening and literature survey . Compounds were selected based on three criteria's : (i) previous reports of phytoestrogenic and SERM-like activity, (ii) documented and predicted role in wound-healing mechanisms such as fibroblast proliferation and migration, angiogenesis, collagen deposition, and anti-inflammatory responses (iii) the availability of correct three-dimensional structures using the PubChem database. The panel also consists of well-studied isoflavones like genistein and daidzein, combined with structurally diverse flavonoids and glycosides, thus maintaining both chemical diversity and clinical relevance. A set of twelve ligands was deemed sufficient to represent the major phytoestrogen subclasses, while also remaining computationally practical for systematic docking comparisons. For receptor models, ER α and ER β were deliberately chosen, as they are the two key estrogen receptor subtypes in skin biology ER α being more closely linked with inflammatory pathways and ER β with regenerative responses.

The 2D chemical structures of twelve selected phytoestrogenic and polyphenolic compounds were downloaded from the PubChem database (<https://pubchem.ncbi.nlm.nih.gov/>) in SMILES format (Table 1). The selection of these ligands was strictly based on their reported phytoestrogenic activity, selective estrogen receptor modulation (SERM), and potential wound-healing properties, as documented in existing literature. The PubChem Compound ID (CID) for each ligand was recorded for reproducibility.

Table 1. Ligands and their PubChem IDs

No	Ligands	Pubchem ID
1	Genistein	5280961
2	Daidzein	5281708
3	Biochanin A	5280373
4	Baicalein	5281605
5	Formononetin	5280378
6	Glycitein	5317750

7	Oroxylin A	5320315
8	Silymarin	5213
9	Puerarin	5281808
10	Icariin	5318997
11	Pratensein	5281803
12	Liquiritigenin	114829

2.1.3 Protein Preparation

The protein structures of ER α (PDB ID: 1A52) and ER β (PDB ID: 4ZI1) were prepared for molecular docking using PyMOL and the Protein Preparation Wizard within the Maestro Schrödinger academic version. The following steps were carried out to optimize the receptor structures. Except for the ligand-binding domain (LBD), which was retained to increase computational efficiency, all non-essential molecules, including crystallographic water molecules, ions, and other non-relevant heteroatoms, were removed. To ensure proper hydrogen bond formation during docking, polar hydrogen atoms were also added. Finally, the receptor structures were checked for any missing atoms and steric clashes, and all needed optimizations were performed to ensure a high-quality model for the subsequent docking. Receptor optimization and hydrogen-bond correction were performed using the Maestro Protein Preparation Wizard [14].

2.1.4 Molecular Docking and Analysis

Molecular docking was carried out using the SwissDock web server (<http://www.swissdock.ch/>) with the prepared receptor structures of ER α (PDB ID: 1A52) and ER β (PDB ID: 4ZI1) and the optimized ligand structures. Docking parameters were defined as follows: sampling exhaustiveness of 90, cavity prioritization score of 70, and grid box size of 40 Å. The docking grid centres were set at (96.0, 13.0, 95.0) for ER α and (39.0, 26.0, -1.0) for ER β . Docking outputs were evaluated using Chimera tool and Maestro,2025. The top-ranked binding poses for each ligand–receptor complex was examined for orientation within the binding pocket. Main non-covalent interactions, including hydrogen bonding, hydrophobic contacts, and π – π stacking, were identified and interaction strength was also determined. The amino acid residues interacting were annotated for both the receptors. To illustrate the binding networks two-dimensional ligand–protein interaction diagrams were also generated.

3 Results

3.1 Molecular Docking of Estrogen Receptor Alpha

To evaluate the binding interactions of estrogen receptor alpha (ER α) with a panel of phytoestrogenic and polyphenolic ligands, molecular docking studies were carried out using the SwissDock web-based platform. In Figure 1, the docking generated binding affinity scores are summarized. These values show a comparative assessment of receptor interaction potential and ligand binding strength. Baicalein displayed the most suitable binding energy (−8.1189 kcal/mol), suggesting a strong affinity toward ER α as seen in Figure 1. Pratensein (−8.017 kcal/mol) and biochanin A (−8.0028 kcal/mol) was followed. Formononetin, icariin, and glycitein, also showed notable binding affinities, with docking scores ranging from −7.95 to −7.93 kcal/mol.

To gain further insight into the molecular determinants of binding, the interaction profiles of

the top three ligands baicalein, pratensein, and biochanin A were analyzed in detail. The interactions of these molecules are summarized in Figure 2. Their specific interactions with amino acid residues within the ER α binding pocket are presented in Table 2.

Table 2. Interaction profiles of the ligands(top three)docked by ER α

Drug Name	Interacted Ligand Atom	Interacted Residue	Interacted Residue Atom	Bond Length (Å)	Binding Energy (kcal/mol)
Baicalein	H	GLU 353	H	1.75	-8.1189
	H	GLU 353	H	1.73	
Pratensein	H	GLU 353	O	1.72	-8.017
	O	ARG 394	H	2.08	
	O	HSD 524	H	2.26	
Biochanin A	O	HSD 524	H	2.30	-8.0028
	H	GLU 353	O	1.71	
	O	ARG 394	H	2.10	

Key hydrogen bond interactions for Baicalein were observed between its hydrogen atoms and GLU353 of ER α , with bond lengths of 1.75 Å and 1.73 Å (Figure 3A–B). Multiple stabilizing hydrogen bonds shown by Pratensein - one between a ligand hydrogen and the oxygen atom of GLU353 (1.72 Å), a second between a ligand oxygen and the hydrogen atom of ARG394 (2.08 Å), and a third with HSD524 (2.26 Å). Its high binding affinity is contributed by these combined interactions (Figure 4A–B). A strong interaction network, engaging HSD524, GLU353, and ARG394 through hydrogen bonds, with bond lengths ranging from 1.71 to 2.30 Å (Figure 5A–B) is formed for Biochanin A. This result highlights the importance of GLU353, ARG394, and HSD524 in stabilizing ligand binding within the ER α active site. The presence of these residues across the top-ranked ligands suggests that they play a crucial role in mediating receptor modulation and phytoestrogen binding.

3.2 Molecular Docking of Estrogen Receptor Beta

To investigate the binding interactions of estrogen receptor beta (ER β) with the selected phytoestrogenic and polyphenolic ligands molecular docking studies were carried out using the SwissDock. Binding affinity scores were generated by docking, which are shown in Figure 6. These values provide a Comparative assessment of the ligands ability to interact with Er β is shown by these values.

Genistein showed the most stable binding affinity toward ER β , with a docking score of -8.0905 kcal/mol is shown in Figure 3. This was followed closely by, liquiritigenin (-8.028 kcal/mol) and glycitein (-7.9808 kcal/mol) follows the trend of strong receptor interactions. Daidzein, baicalein, and silymarin, exhibited moderately high affinities, with docking scores ranging from -7.90 to -7.81 kcal/mol. Molecular basis of these interactions were elucidated, complete docking profiles of the top three ligands genistein, liquiritigenin, and glycitein were analyzed in the diagram below. In Figure 7, these molecular interactions are summarized. In Table 3, the detailed interactions of these compounds with key residues in the ER β active pocket are shown.

Table 3. Interaction profiles of the ligands(Top Three) docked with ER β

Drug Name	Interacted Ligand Atom	Interacted Residue	Interacted Residue Atom	Bond Length (Å)	Binding Energy (kcal/mol)
Genistein	O	ARG 346	H	1.93	-8.0905
	H	GLU 305	O	1.81	
	H	HSE 475	N	1.79	
Liquiritigenin	H	GLU 305	O	1.75	-8.028
	O	ARG 346	H	2.07	
Glycitein	O	ARG 346	H	1.85	-7.9808
	H	GLU 305	O	1.78	
	H	HSE 475	N	1.89	

Strong hydrogen bond interactions were observed with multiple residues in ER β for Genistein. A ligand oxygen atom formed a specific bond with ARG346 (1.93 Å), while hydrogen atoms engaged GLU305 (1.81 Å) and HSE475 (1.79 Å) (Figure 8A–B). Superior binding affinity of Genistein is due to this multiple stabilizing contacts. Liquiritigenin exhibited stable binding through two hydrogen bonds - one between a ligand hydrogen and the other with oxygen of GLU305 (1.75 Å), and another between a ligand oxygen atom and ARG346 (2.07 Å) (Figure 9A–B). Glycitein also showed suitable interactions, forming three hydrogen bonds with ARG346 (1.85 Å), GLU305 (1.78 Å), and a histidine residue (HSE) (1.89 Å) (Figure 10A–B). These findings emphasize the role of ARG346, GLU305, and HSE475 in stabilizing ligand binding within the ER β pocket. These interactions are repeated across the top-ranked ligands shows their main role in facilitating the selective ER β modulation and phytoestrogen binding.

3.3 Comparative Docking Interaction of Genistein between ER α and ER β

In Figure 11, the heat map shows Genistein was the ER β -preferential ligand in the panel with higher binding energy than its ER α interaction. It is due to this that we chose to specially compare the two receptor subtypes with genistein. The binding interactions of Genistein with both ER α and ER β were compared to further explore selectivity of ligands.

Table 4. Comparative docking interaction profile of Genistein with ER α and ER β

Target protein	Drug Name	Interacted Ligand Atom	Interacted Residue	Interacted Residue Atom	Bond Length (Å)	Binding Energy (kcal/mol)
ER α	Genistein	O	HSD 524	H	2.20	-7.8049
		H	GLY 521	O	1.91	
		O	ARG 394	H	2.17	
		H	GLU 353	O	1.68	
ER β	Genistein	O	ARG 346	H	1.93	-8.0905
		H	GLU 305	O	1.81	
		H	HSE 475	N	1.79	

Genistein exhibited stable interactions with both receptors, but the nature of the interacting residues and overall binding affinities differed as detailed in Table 4. An overview of the specific interaction pathways from the receptor to the drug atoms is visualized in Figure 12. For ER α , Genistein established hydrogen bonds with HSD 524 (2.20 Å), GLY 521 (1.91 Å), ARG 394 (2.17 Å), and GLU 353 (1.68 Å), resulting in a binding energy of -7.8049 kcal/mol (Figure 13A–B). These interactions suggest that Genistein primarily anchors within the canonical ligand-binding pocket of ER α through a network of polar contacts with critical residues.

For ER β , Genistein showed stronger binding affinity (-8.0905 kcal/mol), supported by shorter and more stabilizing hydrogen bonds with ARG 346 (1.93 Å), GLU 305 (1.81 Å), and HSE 475 (1.79 Å) (Figure 8A–B). Engagement of ARG 346 and GLU 305 and reduced bond lengths are responsible for the increased stability of Genistein in the ER β binding pocket compared to ER α pocket. In the bar chart in Figure 14, the comparative bond lengths for these key interactions are visualized.

Genistein exhibits preferential binding toward ER β over ER α , consistent with its power to act as a selective estrogen receptor modulator (SERM). Arginine and glutamate residues has a shared involvement across both receptors projects a special binding mechanism, while the special residues causes the receptor subtype specificity. The shorter bond lengths and engagement of ARG 346, GLU 305, and HSE 475 in ER β provides stronger stabilization of the ligand within the binding pocket compared to ER α . Enhanced hydrogen-bonding network shown here explains the more favorable binding energy shown for ER β , suggesting that Genistein might exert its biological effects more efficiently through ER β than ER α . All ligand–receptor complexes were visualized and analyzed using UCSF Chimera, a widely used molecular graphics system [15].

For clearer visualization, we generated comparative figures that highlight ligand performance across ER α and ER β . In Figure 11, the heat map provides an overview of docking scores for all 12 ligands, showing receptor-specific trends in binding affinity. Genistein showed the strongest binding to ER β , while baicalein and pratensein prefers ER α . Sankey diagram was added to clarify residue-level interactions (Figure 12) mapping genistein’s hydrogen bonds with critical amino acids in both receptors. A comparative bar chart (Figure 14) shows hydrogen bond lengths. These images allows direct comparison across ligands and receptors, interpretation of results easier. It also reinforces genistein’s preferential stabilization within ER β .

To strengthen data visualization and highlight comparative trends, The figures were redesigned to provide more analytical value in order to focus in strengthening data visualization and to focus the comparative trends. Figures 1 and 6 bar charts summarize docking scores of all 12 ligands with ER α and ER β , which allows a direct comparison of binding affinities across all the receptor subtypes. Sankey diagrams (Figures 2 and 7) further shows the hydrogen bond interactions of the top ligands, tracing from each compound to the specific residues involved. These identifies the most suitable ligands for each receptor but also reveal key residues such as ARG394, HSD524, and GLU353 in ER α and ARG346, GLU305, and HSE475 in ER β as critical for stabilization. These redesigned figures provide clear proof into ligand–receptor selectivity patterns and Genistein’s preferential binding to ER β is reinforces the mechanical basis for this .

Figure Legends

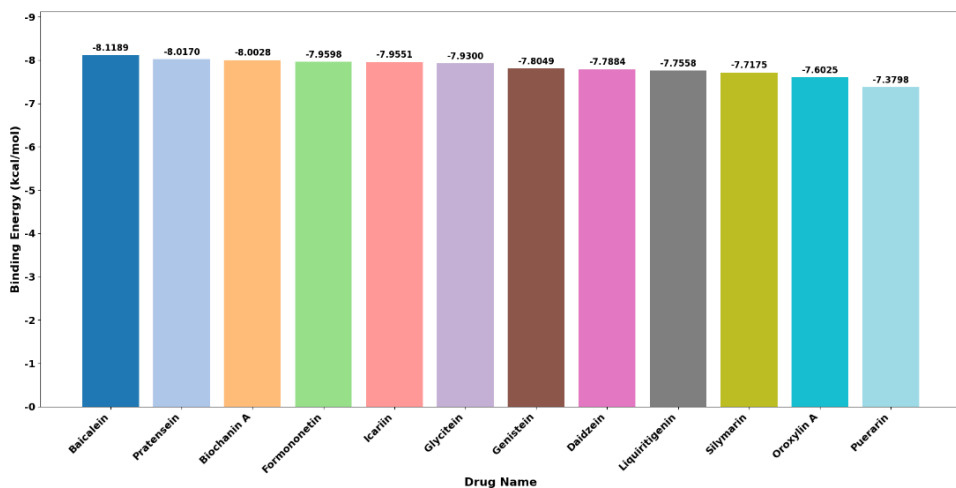


Fig.1. Binding energy values of selected ligands with ER α predicted using SwissDock. Bar chart shows the docking scores (kcal/mol) of 12 phytoestrogenic ligands against the ER α receptor. Each bar shows the binding affinity of 1 ligand, with more negative values is showing stronger binding. Baicalein, pratensein, and biochanin A exhibited the strongest interactions with ER α , due to their relatively low binding energy values.

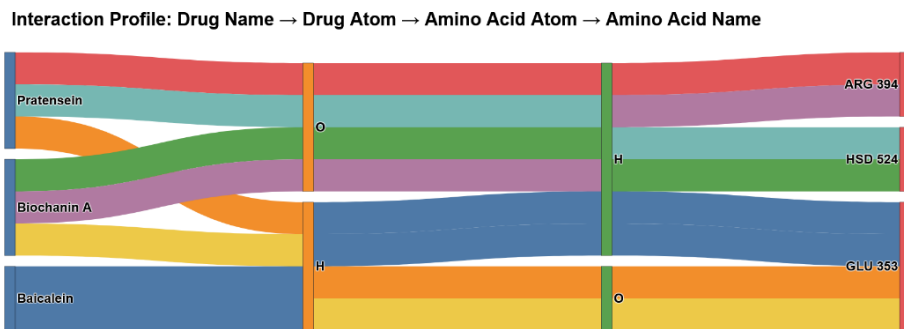
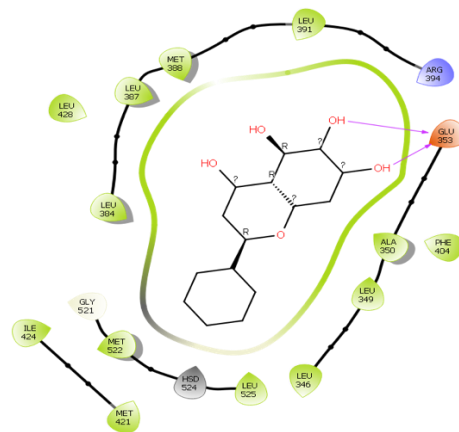
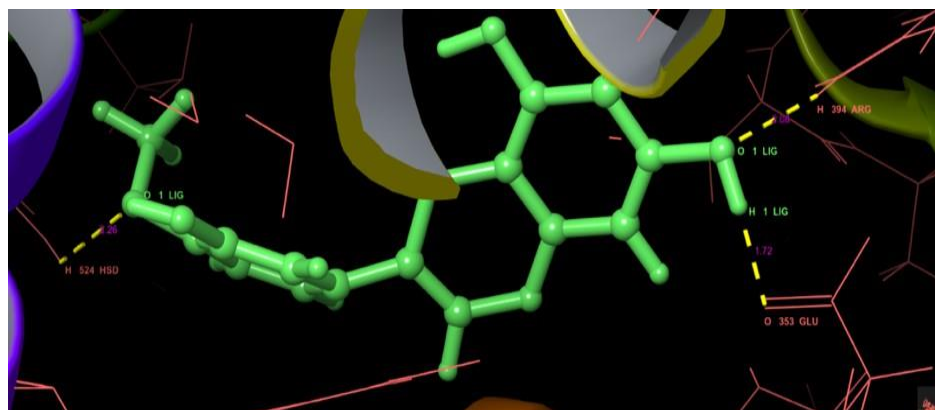


Fig. 2. Interaction profile of the top ligands with ER α .

Sankey diagram showing the hydrogen bond interactions between the top-performing ligands (pratensein, biochanin A, baicalein) and key amino acid residues of ER α . The flows trace the connections from the drug name (left), through the specific ligand atom involved in bonding, to the corresponding amino acid atom and finally to the amino acid residue (right). Notably, ARG394, HSD524, and GLU353 were the principal residues forming stable hydrogen bonds, confirming their importance in ligand recognition and stabilization within the ER α binding pocket.



(A)

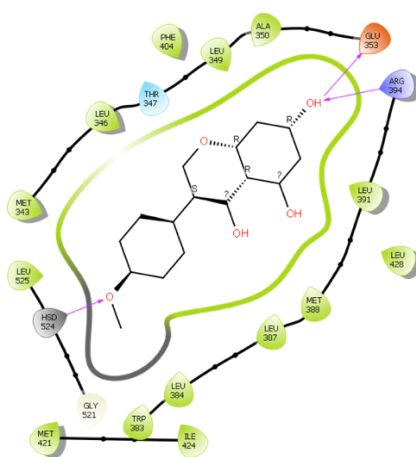


(B)

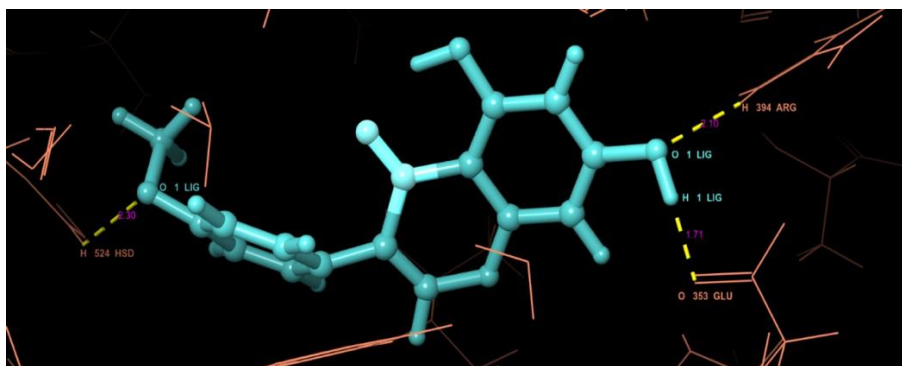
Fig. 4. Binding interactions of pratensein with estrogen receptor alpha (ER α).

(A) **2D interaction diagram** shows pratensein (green) docked within the ER α binding pocket. Hydrogen bonds are seen as purple lines connecting the ligand to GLU353 (orange), ARG394 (blue), and HSD524 (grey). Residues around are color-coded, hydrophobic residues in green (e.g., LEU, MET, ILE, PHE), polar residues in grey, acidic residues in orange, and basic residues in blue.

(B) **3D docking pose of pratensein within ER α :** The ligand is shown in green stick, while receptor residues are shown as cyan sticks and helices as orange structures. Hydrogen bonds with GLU353, ARG394, and HSD524 are shown as yellow dashed lines, with bond distances of 1.72 Å and 2.26 Å, focusing the stabilizing role of these contacts in anchoring pratensein within the cavity.



(A)



(B)

Fig. 5. Binding interactions of Biochanin A with estrogen receptor alpha (ER α).

(A) **2D interaction diagram** shows biochanin A (cyan) docked within the ER α binding pocket. Hydrogen bonds are shown by purple lines with GLU353 (orange), ARG394 (blue), and HSD524 (grey). Hydrophobic residues surrounded by LEU, MET, and ALA are shown in green, polar residues in grey.

(B) **3D docking pose** of Biochanin A inside the ER α cavity. The ligand is shown as cyan sticks, receptor residues as orange sticks, and secondary structure in orange helices. Hydrogen bonds are shown by yellow dashed lines with bond distances of 1.71 Å (GLU353), 2.10 Å (ARG394), and 3.00 Å (HSD524), proves strong stabilization of biochanin A in its active site. These short -directional interactions shows the value of GLU353 and ARG394 in ligand anchoring.

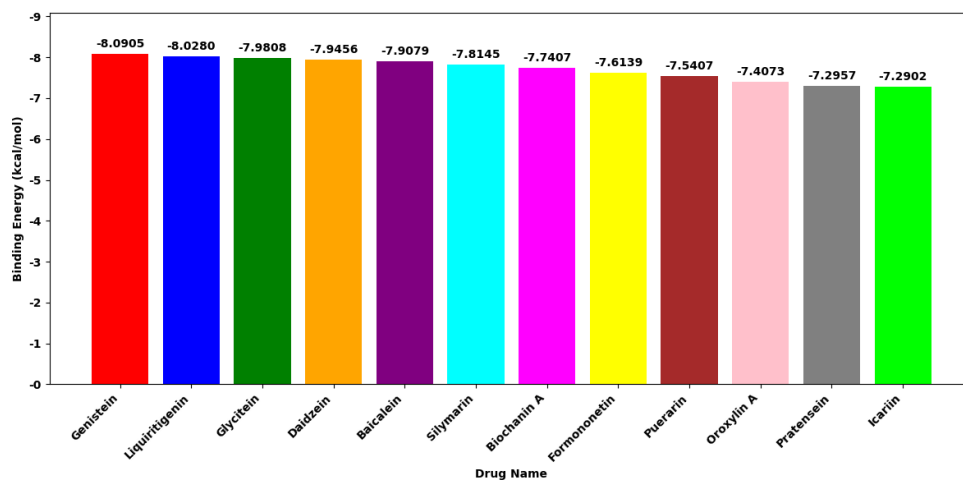


Fig. 6. Binding energy values of selected ligands with ER β predicted using SwissDock. Bar chart showing the docking scores (kcal/mol) of twelve phytoestrogenic ligands against the ER β receptor. More negative binding energy values represent stronger predicted binding affinities. Genistein, ligniritigenin, and glycitein shows the most favorable interactions with ER β , in accordance with their proposed pro-regenerative activity.

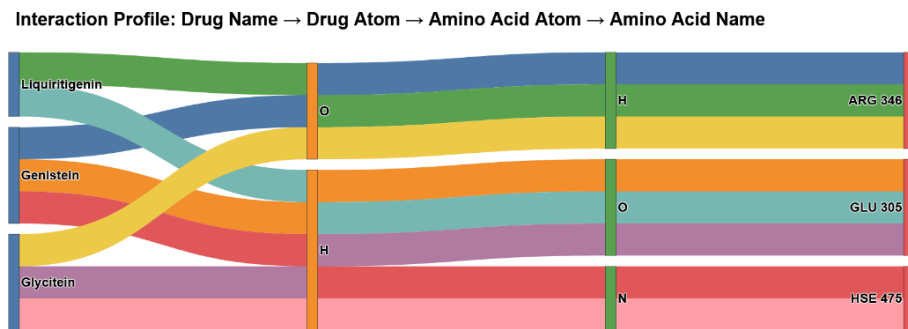
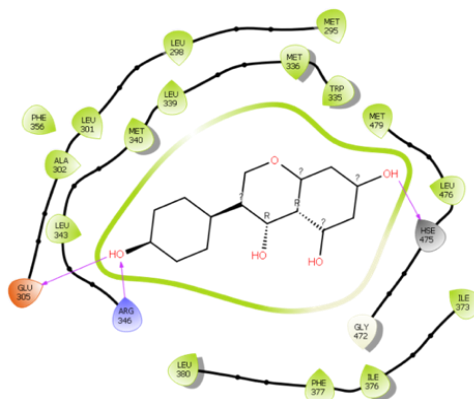


Fig. 7. Interaction profile of the top ligands with ERβ.

Sankey diagram shows hydrogen bond interactions between the highest-affinity ligands (genistein, liquiritigenin, glycitein) and main amino acid residues of ERβ. The diagram shows the progression from the ligand (left), followed by the specific drug atom forming the bond, then to interacting amino acid atom and last to the amino acid residue (right). ARG346, GLU305, and HSE475 were seen as the main residues forming stabilizing interactions, implying their importance in mediating ligand selectivity and anchoring within the ERβ binding site.



(A)



(B)

Fig. 8. Binding interaction of Genistein with estrogen receptor Beta (ER β).

(A) **2D interaction diagram** showing Genistein (pink) docked within the ER β binding site. Hydrogen bonds are shown by purple lines connecting the ligand to GLU305 (orange), ARG346 (blue), and HSE475 (grey). Hydrophobic residues includes LEU, MET, ILE, and PHE are shown in green, while polar residues are in grey.

(B) **3D docking pose** of Genistein in the ER β cavity. The ligand is displayed as magenta stick model, receptor residues as orange sticks, and helices as orange cartoons. Stabilizing hydrogen bonds are seen as yellow dashed lines with distances of 1.81 Å (GLU305), 1.93 Å (ARG346), and 2.18 Å (HSE475). The short hydrogen bonds shows genistein's strong and selective anchoring within the ER β active site, showing its preference for ER β over ER α .

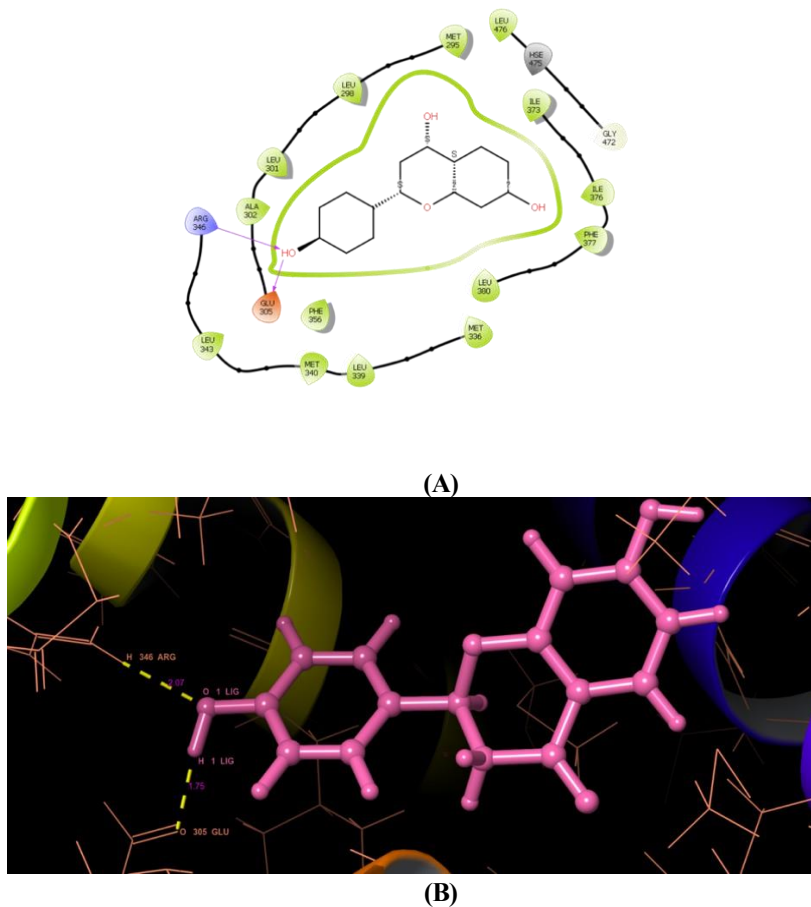


Fig. 9. Binding interactions of licirritigenin with estrogen receptor beta (ER β).

(A) **2D interaction diagram** showing licirritigenin (pink) docked in the ER β binding pocket. Hydrogen bonds are indicated as purple lines linking ligand hydroxyl groups with GLU305 (orange) and ARG346 (blue). Surrounding residues are represented according to type: hydrophobic (green, e.g., LEU, MET, PHE), polar (grey), and aromatic (green PHE)

(B) **3D docking pose** of licirritigenin within ER β . The ligand is shown in pink stick form, receptor residues as orange sticks, and helices as orange/yellow cartoons. Hydrogen bonds with GLU305 and ARG346 are visualized as yellow dashed lines, with bond distances of 1.75 Å and 2.07 Å, respectively. These short interactions demonstrate stable anchoring of licirritigenin, consistent with its moderate affinity for ER β .

selectivity patterns of different ligands also shown. The binding strength is shown by the color gradients, the darker shades shows stronger affinities. Most favorable interaction with Er β is shown by Genistein, while baicalein and pratensein showed stronger binding toward Er α .

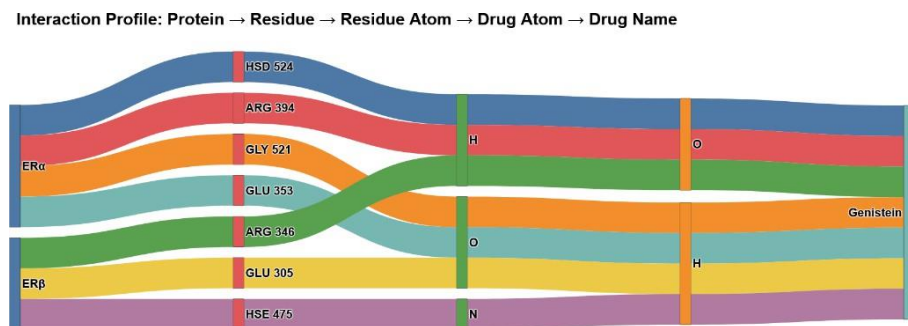


Fig. 12. Interaction profile of Genistein with ER α and ER β . Sankey diagram mapping the hydrogen bond interactions between genistein and the key residues of ER α and ER β which starts from the protein (ER α or ER β), then through the amino acid residues and their respective atoms, and proceeds to the corresponding atoms of the genistein. Main contacts were observed with GLU353, ARG394, HSD524, and GLY521 in ER α , and with ARG346, GLU305, and HSE475 in ER β . Genistein's preferential stabilization within ER β by maintaining stable interactions with residues needed for ER α binding is shown here.

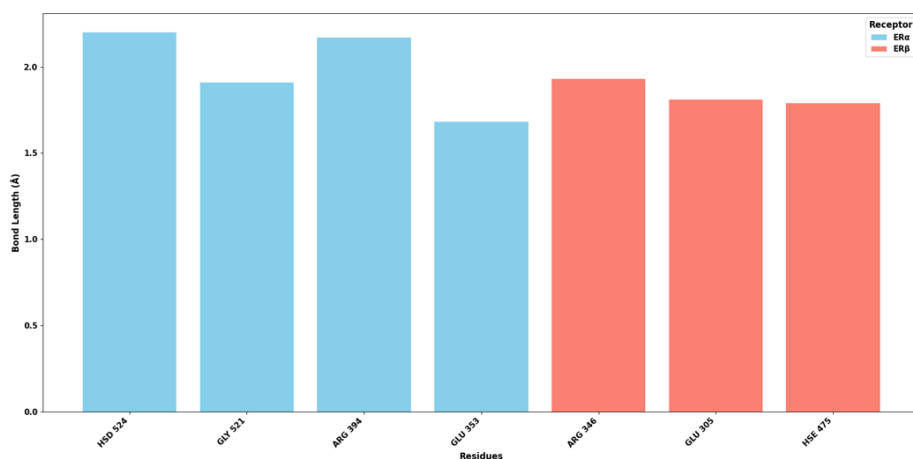


Fig. 14. ER α /ER β residues and Genistein Hydrogen bond comparison : Bar chart shows the hydrogen bond distances (Å) between Genistein and key amino acid residues of ER α (blue) and ER β (red). Shorter bond lengths shows stronger stabilizing interactions. In ER α , Genistein interacted more with HSD524, GLY521, ARG394, and GLU353, and ER β , strong contacts were with ARG346, GLU305, and HSE475. The shorter bond lengths with ER β residues supports genistein's preferential stabilization with the ER β binding pocket.

4 Discussion

The study provides molecular-level insight into different phytoestrogens interaction with the estrogen receptor isoforms ER α and ER β , and clarifying their contributions to wound-healing. Distinct receptor-specific patterns of selective estrogen receptor modulation were identified by systematically evaluating binding energies and mapping residue-level interactions. Most favorably binding for ER α , was found for baicalein (−8.1189 kcal/mol), pratensein (−8.017 kcal/mol), and biochanin A (−8.0028 kcal/mol). These ligands forms stabilizing interactions with GLU353, ARG394, and HSD524, residues which causes ER α –ligand stabilization. ER α has primary role in modulating inflammatory responses, which are relevant for the early inflammatory phase of wound healing, where fast, regulated resolution is important to avoid chronic progression. ER β docking revealed genistein as the most stable binder (−8.0905 kcal/mol), followed by liquiritigenin and glycitein. ER β is more strongly associated with regenerative signaling pathways, keratinocyte migration, extracellular matrix deposition, and angiogenesis. High-affinity ER β ligands has ARG346, GLU305, and HSE475, residues interaction which function as ER β selectivity determinants. Genistein emerged as the most balanced candidate while comparing receptor subtypes although it demonstrated stable affinity for ER α (−7.8049 kcal/mol). The interaction with ER β was characterized by shorter hydrogen-bond distances and stronger denser bonding forces. ER β activation predominates in promoting cutaneous tissue repair for experimental observations provides a mechanistic explanation. Computational findings are matching with earlier biological data and strengthen the view of genistein as an ER β -biased selective estrogen receptor modulator (SERM).

The results matches with experimental literature describing the wound-healing activity of genistein. *In - vivo* studies have shown that genistein accelerates wound closure, improves tensile strength, and enhances extracellular matrix remodelling, with more results compared to of estradiol or raloxifene. *In- vitro* work supports these effects, demonstrating that genistein promotes fibroblast migration, stimulates keratinocyte proliferation, and downregulates pro-inflammatory cytokine release. Flavonoids can form stable complexes with protein targets, with genistein displaying particularly strong interactions through hydrogen bonding and hydrophobic contacts that alter protein conformation. These properties

shows its structural adaptability and capability for stable ligand binding, which proves again its selective affinity for ER β and regulation of wound-healing pathways. This molecular evidence strengthens the concept for genistein's translational use in chronic wound management.

Although this study offers valuable results, limitations should be considered. The analyses are based on molecular docking, which cannot fully capture receptor flexibility, downstream signalling cascades, or the complexity of the microcellular microenvironment. Cell culture and animal wound-healing models will therefore be essential for validation. The ligand set was restricted to twelve phytoestrogens selected for their reported importance to tissue repair, but other compounds like synthetic derivatives can also be looked upon. Receptor-wide conformational changes or co-regulator interactions that can influence selectivity were not considered as the focus here was in to the ligand-binding domain. The work to our knowledge is one of the pioneer systematic docking-based comparisons of phytoestrogens across ER α and ER β in the context of wound healing. Genistein's preferential stabilization within ER β and also the key residues responsible for this selectivity was confirmed, which provides more clarity. Integration of docking predictions with biological pathways core to skin repair including fibroblast migration, angiogenesis, and extracellular matrix deposition linking computational outcomes to functional relevance is a highlight in this study. In short, all these aspects highlight the novelty of the study and also establishes genistein as a strong candidate for ER β -targeted therapeutic strategies.

5 Conclusion

In the process of wound healing, critical role is played by estrogen signalling, but two subtype receptors act in opposite ways. ER β supports epithelial repair, extracellular matrix deposition, and angiogenesis while ER α is primarily involved in inflammatory responses. In chronic estrogen-deficient chronic wounds this functional divergence highlights the therapeutic value of ligands that selectively activate ER β . Distinct residue-level preferences were observed during the molecular docking of twelve phytoestrogenic ligands with ER α and ER β . GLU353, ARG394, and HSD524 stabilizes the ER α interactions whereas ARG346, GLU305, and HSE475 governs ER β selectivity. ER α (-7.80 kcal/mol) showed a weaker binding, compared with the highest affinity for ER β (-8.09 kcal/mol), among the tested compounds. The greater stability in the ER β binding pocket was due to multiple hydrogen bonds. There is prior biological evidence that Genistein promotes angiogenesis, extracellular matrix remodeling, wound closure and our computational findings are consistent with that. Ligands such as liquiritigenin and glycitein demonstrated ER β interactions with low binding. The collective results strongly establish genistein as the lead ER β -preferential modulator in regenerative medicine. The utility of receptor-focused computational methods to identify and optimize ligands with selective pharmacological activity was also found in this docking experiment. Advanced biomaterial-based systems such as nanocarriers and hydrogels are recommended to ensure effective delivery, since poor solubility and low bioavailability limits genistein's therapeutic use. To translate these docking insights into clinically viable ER β -targeted therapies for wound healing, combining animal models, *in-vitro* studies and other structure-activity refinement is crucial.

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