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Phytoestrogens: Next generation ERα modulators in osteoporosis treatment – in silico approach and ADMET evaluation

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Abstract

Objective: Osteoporosis is a common metabolic bone disease caused by an imbalance between bone formation and resorption. Estrogen deficiency plays a critical role, especially in postmenopausal osteoporosis. The side effects of current treatments (bisphosphonates, SERMs) necessitate the investigation of safe phytoestrogen-based alternatives that exert a bone-protective effect via the estrogen receptor alpha (ERα). The aim of this study is to investigate the potential agonistic effects of nine different phytoestrogens on ERα using in silico methods.

Materials and methods: In this study, molecular docking simulations were performed with phytoestrogens and the reference modulator tamoxifen using the ERα crystal structure (PDB: 3ERT). Binding affinities (kcal/mol) and ligand-protein interactions were determined with these simulations. In addition, ADMET (Absorption, Distribution, Metabolism, Excretion, Toxicity) analyses were performed to evaluate drug similarity and safety profiles.

Results: In docking analyses, coumestrol and genistein (-8.9 kcal/mol) stood out as the compounds with the highest binding affinity and achieved better scores than tamoxifen (-8.7 kcal/mol). These high-affinity compounds exhibited an agonistic binding mode similar to the native agonist and tamoxifen by forming hydrogen bonds with Glu353 and Arg394, key residues in the ERα ligand binding site. ADMET analyses showed that all phytoestrogens studied conformed to Lipinski rules and had generally acceptable pharmacokinetic and toxicity profiles.

Conclusion: The obtained in silico data support the idea that phytoestrogens, particularly coumestrol, genistein, and daidzein, have the potential for tamoxifen-like agonistic activity by targeting ERα and could be developed as new and safer therapeutic agents in the treatment of osteoporosis.

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Introduction

Osteoporosis, the most common metabolic bone disease, is a condition that affects the balance between bone formation and breakdown, leading to deterioration in bone mineralization and microarchitecture (1). It has been reported that the prevalence of osteoporosis approximately doubles with every five-year increase in age; the rate, which was found to be 3.3% in the 45–49 age range, reaches 50.3% in individuals aged 85 and over (1).

There are two different types of osteoporosis, depending on the factors affecting the disease: primary osteoporosis and secondary osteoporosis (2). Primary osteoporosis is caused by postmenopausal (due to estrogen deficiency) and senile (age-related) factors, while secondary osteoporosis can be caused by factors such as endocrine diseases, chronic inflammatory conditions, chronic kidney disease, neuromuscular diseases, gastrointestinal diseases, glucocorticoids, and unfavorable lifestyle factors (3). The fundamental mechanism of osteoporosis is when bone resorption (destruction) exceeds bone formation, which is a negative homeostasis in bone metabolism (4). The bone remodeling process physiologically occurs through the resorption of old and damaged bone by osteoclasts, followed by the formation of a new bone matrix by osteoblasts. Disruption of this balance leads to bone loss, deterioration of bone microstructure, and ultimately osteoporosis (5).

Advancing age and female gender are the most important risk factors. The sharp drop in estrogen levels during menopause leads to a rapid loss of bone mineral density and an increased risk of fractures. This condition is called postmenopausal osteoporosis (1,6). The most significant complication in osteoporosis patients is bone fractures. Bone fractures occurring in various areas have serious effects that reduce the quality of life. The physical problems caused by fractures carry a high risk of morbidity and mortality (7).

The pathophysiology of osteoporosis is explained by cellular and molecular mechanisms that differ in primary and secondary forms. The fundamental underlying mechanisms of the disease lead to bone resorption exceeding bone formation due to increased osteoclast activity and/or decreased osteoblast activity (2,5).

The most important of these mechanisms is the system consisting of receptor activator of nuclear factor κB (RANK), receptor activator of nuclear factor κB ligand (RANKL), and osteoprotegerin (OPG) proteins, which play a central regulatory role in bone metabolism and whose dysfunction leads to disease. The RANK/RANKL/OPG system supports bone homeostasis by causing the activation or inhibition of osteoclastogenesis (8). In osteoporosis, an increase in the RANKL/OPG ratio is observed, which leads to bone destruction (9). In addition to this system, various signaling pathways influence bone formation. Disruptions in these signaling pathways cause osteoporosis.

The differentiation of osteoblasts, which play a role in bone formation, begins in precursor mesenchymal stem cells and is primarily regulated by the Wnt/β-catenin and BMP–Smad signaling pathways. These pathways induce the expression of Runx2 and Osterix, key transcription factors of osteogenesis, respectively, through β-catenin stabilization and Smad activation (10). Inactivation of these pathways leads to bone formation loss (11,12).

In age-related osteoporosis, both resorption and formation decrease, but formation decreases more significantly; therefore, the risk of developing osteoporosis increases with age (13). Furthermore, reactive oxygen species (ROS), which increase with aging, trigger oxidative stress, contributing to a decrease in bone mass and strength. ROS activation of FOXO transcription factors causes β-catenin to move away from the Wnt signaling pathway, thus leading to a decrease in osteoblastogenesis (14).

Hormonal interactions play a role in the pathophysiology of osteoporosis. In glucocorticoid-induced osteoporosis, high levels of glucocorticoid exposure accelerate the death of osteoblasts and osteocytes, weakens Wnt signaling which supports bone formation, and increases the formation of fat cells instead of bone cells. It also disrupts the RANKL/OPG balance, leading to increased bone resorption (1,3). Estrogen plays a crucial role in maintaining bone homeostasis by regulating the balance between bone-forming osteoblasts and bone-resorbing osteoclasts. The biological effects of estrogen primarily occur through binding to specific intracellular receptors, namely Estrogen Receptor Alpha (ERα) and Beta (ERβ). Of these, ERα is the predominantly expressed subtype in bone tissue and is essential for estrogen's protective

effects against bone loss (15). In the postmenopausal period, estrogen deficiency leads to impaired bone remodeling, accelerating bone resorption and causing the development of osteoporosis (16). Therefore, targeting ERα is the primary strategy in the treatment and prevention of postmenopausal osteoporosis. The goal of osteoporosis treatment is to reduce the risk of fractures and increase bone mineral density; in this context, drugs are generally classified as antiresorptive or anabolic agents.

Antiresorptive therapies (e.g., bisphosphonates, Denosumab, Raloxifene) slow bone resorption, while anabolic agents (Teriparatide, Abaloparatide) stimulate new bone formation. These treatments have various side effects such as gastrointestinal problems, jaw osteonecrosis, atypical femur fracture, hypercalcemia, or venous thromboembolism (17).

Phytoestrogens are plant-derived compounds that have functions similar to estrogen. Phytoestrogens play a role in cellular growth by binding to ERα and Erβ (18). Especially when it comes to bone tissue, phytoestrogens generally function as agonists, providing a beneficial effect on osteoporosis. Phytoestrogens act similarly to Selective Estrogen Receptor Modulators (SERMs). The main mechanism is to inhibit osteoclastogenesis (bone breakdown) by reducing the RANKL/OPG ratio. Furthermore, thanks to their strong antioxidant properties, they protect osteoblast function and limit bone breakdown by reducing oxidative stress. Increasing osteoblast activity and reducing pro-inflammatory cytokines are also among the main mechanisms by which phytoestrogens support bone health (19-21).

In drug discovery processes today, "Computer-Aided Drug Design" (CADD) methods are widely used to save time and cost. Molecular docking, one of the leading methods, is a powerful tool for predicting how small molecule ligands (polyphenols) bind to the active site of the target protein (ERα) and their binding affinity (22).

The aim of this study is to investigate the binding of several different polyphenols, known in the literature for their various biological activities, to ERα, a potential target in osteoporosis treatment, using in silico methods. Using molecular docking simulations, the interactions and binding energies of these compounds with the protein's active site were analyzed and compared with a reference modulator.

Materials and methods

Protein structure preparation

The three-dimensional crystal structure of ERα was obtained from the Protein Data Bank (PDB) (https://www.rcsb.org), and the structure coded 3ERT PDB was used in this study. Protein preparation steps were performed using AutoDock Tools 1.5.7 software. Crystallographic water molecules, the original inhibitor, and other cofactors/metabolites on the protein structure were removed. To ensure structural integrity, missing atoms were completed, polar hydrogen atoms were added, and Kollman coupled charges were assigned to the protein structure. The prepared protein structure was saved in PDBQT format for use in molecular docking analyses.

Preparation of ligand structures

In total, the chemical structures of eight different ligands and the reference modulator tamoxifen were obtained from the PubChem database in SDF format (Table 1). The creation of the three-dimensional conformations of the ligands, format transformations, and determination of protonation states suitable for physiological pH were performed using Open Babel GUI and AutoDock Tools 1.5.7 software. Subsequently, geometry optimization for each ligand was performed using UCSF Chimera 1.17.3. The optimized ligand structures were saved in PDBQT format, suitable for docking studies, and made ready for analysis.

Molecular docking protocol

Molecular docking simulations were performed using AutoDock Vina software to evaluate the possible binding modes and binding affinities of ligands with ERα. The exhaustiveness parameter was set to 8 to ensure computational accuracy and efficiency. Ten (10) binding poses were generated for each ligand and these poses were ranked based on the obtained binding affinities (kcal/mol). A grid box was created for the docking studies; the center coordinates were determined as x = 27.432, y = –2.033 and z = 26.269 to encompass the active region using Discovery Studio Visualizer software. Grid spacing was set to 0.375 Å and a grid box with dimensions of 40 X 40 X 40 was used for the molecular docking process. The binding poses obtained from the simulations were analyzed with Discovery Studio Visualizer software for detailed visualization and analysis of the interactions

Table 1: Docking ccores and PubChem ID information for compounds

Compound	Ligand short name	PubChem ID	Docking score (kcal/mol)
Tamoxifen	L1	CID: 2733526	-8.7
Equol	L2	CID: 91469	-8.2
Ipriflavone	L3	CID: 3747	-7.4
Coumestrol	L4	CID: 5281707	-8.9
Formononetin	L5	CID: 5280378	-6.9
Biochanin A	L6	CID: 5280373	-6.7
Sophoraflavanone B	L7	CID: 480764	-8.2
Daidzein	L8	CID: 5281708	-8.8
Genistein	L9	CID: 5280961	-8.9

(23). The studies thoroughly evaluated hydrogen bonds, hydrophobic interactions, π–π stacking, π–cation interactions, and other complementary ligand–protein interaction types.

Absorption, distribution, metabolism, excretion, toxicity (ADMET) estimates

ADMETlab 3.0, an in silico prediction tool developed to characterize the pharmacokinetic and toxicological properties of a candidate molecule in drug discovery and development processes, was used in our study (24). SMILES (Simplified Molecular Input Line Entry System) codes, a text-based line encoding of compounds, were obtained from the PubChem database and used as the input format to the ADMETlab 3.0 web server in the analyses.

Drug similarity assessment was estimated based on a concept previously developed by Lipinski et al. (MW≤500; logP≤5; H-bond acceptor≤10; H-bond donor≤5) (25). Pharmacokinetic and toxicological profiles were predicted using ADMETlab 3.0. Through this platform, absorption parameters (HIA, Caco-2 permeability), distribution characteristics (blood-brain barrier crossing (BBB), plasma protein binding rate), potential metabolic interactions (interaction with CYP450 isoforms), excretion trends (LogS and LogP values) were evaluated. In toxicity analysis, AMES mutagenicity, hERG channel inhibition risk, and hepatotoxicity indicators were predicted. Thus, the drug similarity, pharmacokinetic behavior, and safety profile of the compounds were predicted holistically.

Results

Results of molecular docking analysis of ERα and ligands

In the molecular docking study, the calculated binding energies (kcal/mol) of the tested compounds against ERα were ranked as follows (Table 1): Coumestrol and genistein (-8.9), daidzein (-8.8), tamoxifen (-8.7), equol and sophoraflavanone B (-8.2), ipriflavone (-7.4), formononetin (-6.9) and biochanin A (-6.7).

The analyzed interactions revealed that the ligands formed different types of bonds with various amino acid residues in the ERα binding pocket (Table 2). Tamoxifen bonded with GLY420 via a conventional hydrogen bond and with ASP351 via a carbon-hydrogen bond; it formed alkyl interactions with LEU346 and MET388, and also performed multiple π-alkyl hydrophobic contacts with residues MET421, LEU525, and ALA350. Coumestrol formed conventional hydrogen bonds with GLY521 and LEU387; it exhibited intense π-alkyl hydrophobic interactions with residues LEU384, LEU346, ALA350, LEU525, LEU387, and LEU391. Genistein bonded with GLU353 via a conventional hydrogen bond. Figure 1 shows the binding interactions of coumestrol and genistein, which had the lowest docking scores with the reference inhibitor (Figure 1). Sophoraflavanone B exhibited π-σ bonding with ILE424 and π-sulfur interaction with MET421. In addition, it formed multiple π-alkyl hydrophobic contacts with residues LEU384, MET388, ALA350, LEU387, LEU391, and

Table 2: Details of the interaction between ERα and ligands.

Ligand	Category	Type	Residues (Distance A°)
Tamoxifen	Hydrogen Bond	Conventional Hydrogen Bond	GLY420 (2.70)
	Hydrogen Bond	Carbon Hydrogen Bond	ASP351 (3.54)
	Hydrophobic	Alkyl	LEU346 (5.13), MET388 (5.36), LEU346 (4.81)
	Hydrophobic	Π-Alkyl	MET421 (5.49), LEU525 (4.65), ALA350 (3.83), LEU525 (4.81)
Coumestrol	Hydrogen Bond	Conventional Hydrogen Bond	GLY521 (2.74), LEU387 (2.55)
	Hydrophobic	Π-Alkyl	LEU384 (5.33), LEU346 (4.83), ALA350 (4.71), LEU525 (4.70), ALA350 (5.37), LEU387 (4.42), LEU391 (5.15)
Genistein	Hydrogen Bond	Conventional Hydrogen Bond	GLU353 (2.72)
	Hydrophobic	Π-Sigma	ILE424 (3.87)
	Other	Π-Sulfur	MET421 (5.15)
	Hydrophobic	Π-Alkyl	LEU384 (5.41), MET388 (4.99), ALA350 (5.16), LEU387 (4.41), LEU391 (5.19), LEU525 (5.18)
Sophoraflavanone B	Hydrogen Bond	Conventional Hydrogen Bond	VAL534 (1.94)
	Hydrophobic	Π-Sigma	VAL533 (3.99)
	Hydrophobic	Π-Π Stacked	TRP383 (5.02), TRP383 (3.77)
	Hydrophobic	Alkyl	VAL533 (5.35), LEU536 (5.33), LEU539 (5.22)
	Hydrophobic	Π-Alkyl	VAL533 (5.35), VAL533 (5.32), LEU536 (5.27), ALA350 (5.10), LEU354 (5.27), LEU536 (5.38)
Daidzein	Hydrogen Bond	Conventional Hydrogen Bond	ARG394 (3.22), HIS524 (2.91), LEU387 (2.18)
	Hydrophobic	Π-Sigma	ILE424 (3.97)
	Hydrophobic	Π-Alkyl	LEU384 (5.46), MET388 (5.09), ALA350 (5.02), LEU387 (4.26), LEU391 (5.34), MET421 (5.10), LEU525 (5.12)

Formononetin	Hydrogen Bond	Conventional Hydrogen Bond	ARG394 (3.00), HIS524 (3.20)
	Hydrogen Bond	Carbon Hydrogen Bond	GLY420 (2.62), LYS520 (3.63)
	Hydrophobic	Π-Sigma	LEU346 (3.97)
	Hydrophobic	Alkyl	ILE424 (4.62)
	Hydrophobic	Π-Alkyl	HIS524 (4.64), ALA350 (5.15), LEU346 (4.66), ALA350 (5.47), LEU391 (5.01), MET421 (5.31), LEU525 (4.88)
Biochanin A	Hydrogen Bond	Carbon Hydrogen Bond	LEU346 (3.36)
	Hydrophobic	Π-Sigma	LEU525 (3.81), LEU525 (3.85)
	Other	Π-Sulfur	MET343 (5.28)
	Hydrophobic	Amide-Π Stacked	LEU346;THR347 (5.05)
	Hydrophobic	Π-Alkyl	LEU346 (4.71), ALA350 (4.91), LEU346 (5.21), MET388 (5.42), MET421 (5.46), ALA350 (4.73)
Ipriflavone	Electrostatic	Π-Anion	ASP351 (3.90)
	Hydrophobic	Π-Π Stacked	TRP383 (5.12), TRP383 (3.86)
	Hydrophobic	Alkyl	PRO535 (4.45)
	Hydrophobic	Π-Alkyl	LEU536 (3.82), VAL533 (5.38), LEU536 (4.70), ALA350 (5.03), LEU354 (5.23), LEU536 (5.45)
Equol	Hydrogen Bond	Conventional Hydrogen Bond	LEU387 (2.42)
	Hydrophobic	Π-Sigma	LEU387 (3.85)
	Other	Π-Sulfur	MET343 (5.30)
	Hydrophobic	Alkyl	LEU346 (5.06)
	Hydrophobic	Π-Alkyl	LEU525 (4.65), ALA350 (5.27), LEU391 (5.01)

LEU525. Sophoraflavanone B bonded to VAL534 via conventional hydrogen bonding; it displayed a significant π-π stacking interaction with TRP383, and showed hydrophobic interactions in the alkyl and π-alkyl categories with VAL533, LEU536, ALA350, and LEU354. Formononetin formed conventional hydrogen bonds with ARG394 and HIS524; and carbon-hydrogen bonds with GLY420 and LYS520. A π-σ bond was recorded with LEU346, an alkyl interaction with ILE424, and multiple hydrophobic π-alkyl interactions with residues HIS524, ALA350, LEU346, LEU391, and LEU525. Daidzein formed three conventional hydrogen bonds with ARG394, HIS524, and LEU387; showed a π-σ hydrophobic contact with ILE424; and exhibited strong π-alkyl interactions with LEU384, MET388, ALA350, LEU387, LEU391, MET421, and LEU525. Equol formed a conventional hydrogen bond with LEU387; exhibited a π-σ interaction with the

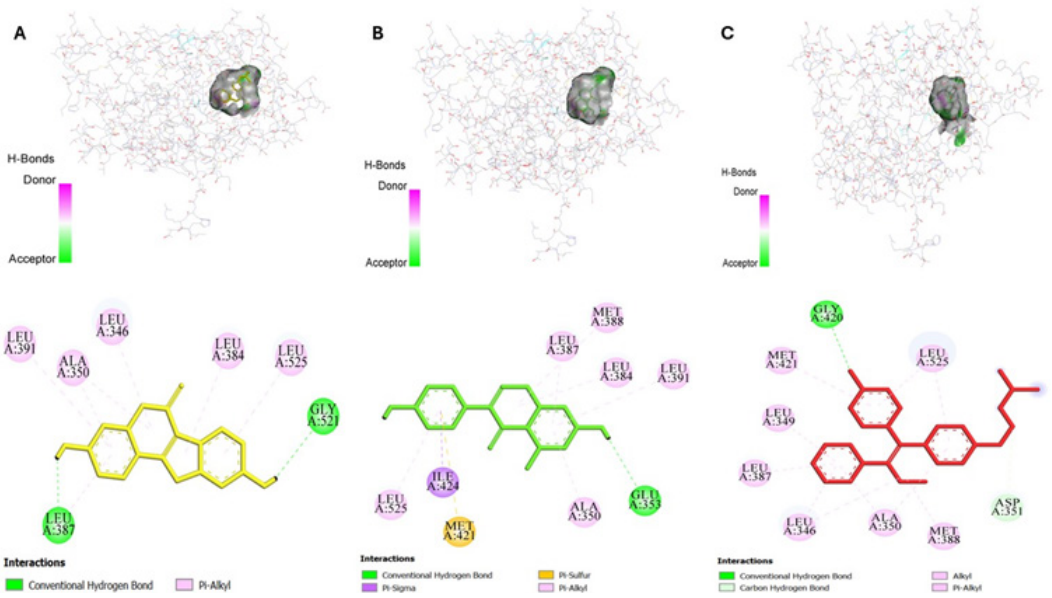

Figure 1: A: Interactions between coumestrol and the amino acids of ERα; B: Interactions between genistein and the amino acids of ERα; C: Interactions between the tamoxifen and the amino acids of ERα

same residue; and established a significant π-sulfide contact with MET343. Furthermore, hydrophobic bonds of alkyl and π-alkyl character were observed with residues LEU346, LEU525, ALA350, and LEU391. Biochanin A formed a carbon-hydrogen bond with LEU346; two π-σ hydrophobic interactions with LEU525; and a π-sulfur bond with MET343. In addition, amide-π stacking and π-alkyl contacts were observed with LEU346, and multiple π-alkyl interactions were observed with ALA350, MET388, and MET421. Ipriflavone, on the other hand, bound to ASP351 exhibiting a strong π-anion interaction; It established two different π-π stacking interactions with TRP383 and showed significant π-alkyl hydrophobic interactions with PRO535 (alkyl), LEU536, VAL533, ALA350, and LEU354.

ADMET results

Pharmacokinetic and toxicological properties of the compounds evaluated in the study were obtained by ADMET analyses (Table 3). According to the ADMET analysis results, all of the examined compounds, tamoxifen, equol, ipriflavone, coumestrol, formononetin, biochanin A, sophoraflavanone B, daidzein, and genistein, have a value of 0.0 in terms of violation of Lipinski's five rules.

When the physicochemical properties were examined, the water solubility (LogS) values ranged from -6.344 (tamoxifen) to -3.441 (equol). The LogS values of the other compounds were calculated as -4.594 for ipriflavone, -3.521 for coumestrol, -4.356 for formononetin, -3.725 for biochanin A, -4.651 for sophoraflavanone B, -3.79 for daidzein, and -3.471 for genistein, respectively. Lipophilicity (LogP) values were highest in tamoxifen at 6.151 and lowest in genistein at 2.075.

When absorption and distribution parameters were evaluated, Human Intestinal Absorption (HIA) probability values were determined as 0.0 for tamoxifen and equol, 0.18 for formononetin, and 0.085 for biochanin A. Caco-2 permeability (Log cm/s) values ranged from -4.549 to -5.051. Blood-Brain Barrier (BBB) permeability probability was 1.0 for tamoxifen, 0.224 for equol, while this value was below 0.031 for other compounds. Plasma Protein Binding (PPB) rates were above 90 percent for all compounds; The highest value was recorded in ipriflavone with 98.482%, and the lowest value in coumestrol with 91.026%. In the cytochrome P450 metabolism profile, the probability of being a CYP3A4 inhibitor is high for genistein (0.992), biochanin A (0.99), coumestrol (0.954), and tamoxifen (0.871). The probability of being a CYP3A4 substrate is 1.0 for tamoxifen, while it is below 0.053 for the other compounds. In terms of CYP2D6 inhibition, tamoxifen (0.992), formononetin (0.998), biochanin A (0.998), and genistein (0.997) have high probability values. The probability of being a CYP1A2 inhibitor was determined as 1.0 for coumestrol, 0.999 for genistein, and 0.998 for biochanin A. The probability of CYP2C8 inhibition is 1.0 or 0.999 for all compounds except equol (0.954). Furthermore, CYP2B6 inhibition was 1.0 for tamoxifen, ipriflavone, formononetin, sophoraflavanone B, and daidzein.

Table 3: The results of the ADMET test with AdmetLab3.0 (I: Inhibitor, S: Substrate)

ADMET Parameter	L1	L2	L3	L4	L5	L6	L7	L8	L9
Lipinski's rule	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
HIA	0.0	0.0	0.016	0.002	0.18	0.085	0.002	0.017	0.008
Caco-2 (Log cm/s)	-4.549	-4.844	-4.626	-5.048	-4.637	-5.009	-4.945	-4.692	-5.051
BBB	1.0	0.224	0.005	0.004	0.01	0.015	0.0	0.025	0.031
PPB (%)	98.336	91.42	98.482	91.026	93.82	97.164	94.733	91.802	95.865
AMES	0.145	0.65	0.266	0.679	0.642	0.661	0.537	0.537	0.556
hERG	0.884	0.297	0.302	0.076	0.269	0.188	0.073	0.194	0.128
Hepatotoxicity	0.996	0.884	0.57	0.492	0.478	0.465	0.474	0.474	0.461
LogS	-6.344	-3.441	-4.594	-3.521	-4.356	-3.725	-4.651	-3.79	-3.471
LogP	6.151	2.851	3.648	2.539	2.484	2.411	3.887	2.221	2.075
CYP1A2-I	0.001	0.982	0.791	1.0	0.98	0.998	0.002	0.994	0.999
CYP1A2-S	1.0	0.039	0.017	0.091	1.0	1.0	0.005	0.998	0.995
CYP2C19-I	0.05	0.706	1.0	0.967	1.0	0.947	1.0	0.999	0.86
CYP2C19-S	1.0	0.177	0.012	0.0	0.004	0.0	0.28	0.002	0.0
CYP2C9-I	0.038	0.845	0.985	0.548	0.995	0.295	1.0	0.996	0.309
CYP2C9-S	1.0	0.234	0.001	0.822	0.999	0.987	0.004	0.985	0.867
CYP2D6-I	0.992	0.691	0.032	0.175	0.998	0.998	0.0	1.0	0.997
CYP2D6-S	1.0	1.0	0.0	0.952	1.0	1.0	0.548	1.0	1.0
CYP3A4-I	0.871	0.843	0.002	0.954	0.455	0.99	0.461	0.857	0.992
CYP3A4-S	1.0	0.016	0.053	0.033	0.0	0.0	0.0	0.0	0.0
CYP2B6-I	1.0	0.131	1.0	0.049	1.0	0.965	1.0	1.0	0.968
CYP2B6-S	0.999	0.0	0.024	0.0	0.949	0.013	0.0	0.005	0.0

When toxicity parameters were examined, the probability of AMES mutagenicity was higher for coumestrol (0.679), biochanin A (0.661), and equol (0.65) compared to others; for tamoxifen, this value was 0.145. The probability of hERG inhibition was highest in tamoxifen at 0.884, while it was 0.302 in ipriflavone and 0.297 in equol. Probability values for hepatotoxicity were found to be high for tamoxifen (0.996) and equol (0.884), while they ranged from 0.461 to 0.57 for other compounds.

Discussion

ERα plays a critical role in the prevention and treatment of osteoporosis because estrogen signaling via ERα both promotes bone formation by activating the Wnt signaling pathway in osteoblast precursors and reduces bone resorption by exhibiting pro-apoptotic effects in osteoclasts (26). Therefore, the presence of ERα is essential for maintaining bone mass and mechanical strength, making it a key target

in the treatment of osteoporosis and the maintenance of bone health (26). Today, synthetic SERMs have proven effective in preserving bone mineral density (17). Several compounds exhibit high interaction as ERα modulators. Tamoxifen, known to have a positive effect in the treatment of osteoporosis, especially in the postmenopausal period, was chosen as the reference compound in our study (28,29). The ligand binding site (LBD) within ERα strongly interacts with the ligand via hydrogen-coupled interactions with polar residues such as GLU353, ARG394, and HIS524, and Van der Waals interactions with hydrophobic residues such as LEU384, LEU387, MET388, LEU391, and PHE404. These residues play a key role in the binding of both the native agonist 17β-estradiol and agonist ligands such as DES (30). 4-Hydroxytamoxifen (OHT), while sharing the same ligand binding pocket, forms hydrogen bonds with ARG394 and GLU353, causing Helix-12 displacement and resulting in agonist conformation in endometrial cells or osteoblasts (31,32).

Findings from molecular docking analyses reveal that the interaction profiles of phytoestrogens and reference compounds toward the ERα binding pocket show a high degree of agreement with the known structural and functional characteristics of the receptor.

The docking results obtained in this study showed that compounds with high binding scores interact with critical polar and hydrophobic residues in the ERα ligand binding region. The formation of conventional hydrogen bonds by phytoestrogens such as genistein, coumestrol, and daidzein with polar residues known to play a key role in the literature, such as GLU353, ARG394, and HIS524, parallels the binding mechanism of the natural agonist 17β-estradiol and DES. These interaction patterns also show similarities to the binding of tamoxifen to the GLU353–ARG394 pair, which mediates agonist-like behavior in osteoblasts by affecting the position of Helix-12. The binding tendency of phytoestrogens to these critical residues reveals a binding pattern reminiscent of the molecular basis of tamoxifen's partial agonist effect on ERα. Furthermore, π-alkyl, alkyl, and π-σ interactions recorded with hydrophobic residues such as LEU384, LEU387, MET388, and LEU391 demonstrate that the ligands exhibit a conformation consistent with Van der Waals-weighted binding motifs in the ERα binding pocket. Since tamoxifen is also known to form strong aromatic interactions with similar hydrophobic core residues, these common binding contacts support the potential for phytoestrogens to form a tamoxifen-like stable conformation in ERα. The π-π stacking interactions observed with TRP383 stand out as significant contacts that enhance binding stability, particularly in aromatic compounds such as sophoraflavanone B and ipriflavone. Overall, the study shows that phytoestrogens can exhibit agonist-like binding modes in the LBD region of ERα, that the binding pattern exhibited by tamoxifen as a reference is partially replicated in many compounds, and that hydrogen bonds to the GLU353–ARG394 pair are particularly decisive for high affinity in ligands other than tamoxifen. The interactions of ligands other than tamoxifen were consistent with the residue types in binding reported in the literature. Unexpected residue bindings observed with tamoxifen were also seen in other ligands. These data suggest that phytoestrogen-based candidates can be evaluated as potential ERα modulators in the treatment of osteoporosis.

When ADMET analyses are examined in general, the conformity of all compounds to Lipinski rules indicates that ligands such as coumestrol, genistein, daidzein, and tamoxifen, which exhibit high binding scores with ERα, possess drug-like properties. When solubility and lipophilicity parameters are evaluated, tamoxifen shows a dominant lipophilic character with a high LogP value, while genistein and coumestrol present a more balanced solubility profile. Absorption and distribution data show that tamoxifen stands out with its significant BBB permeability, while equol and formononetin are more advantageous in terms of HIA. Metabolism results show that genistein, biochanin A, and coumestrol stand out as strong CYP inhibitors, indicating that these compounds have the potential for active metabolic interactions in terms of pharmacokinetics. Toxicity parameters are generally at acceptable levels, with tamoxifen and equol showing a tendency towards hepatotoxicity. These findings indicate that the structural and pharmacokinetic properties of the prominent ligands are evaluable in terms of ERα modulation. The findings of this study are consistent with the existing literature on ERα–ligand interactions, but offer a more detailed and holistic assessment of the binding mechanisms of phytoestrogens. In particular, it has been shown that the interactions of phytoestrogens with critical polar residues such as GLU353–ARG394–HIS524 and hydrophobic core residues form a molecular basis similar to the partial agonist binding model of tamoxifen. The contribution of π-π interactions observed with TRP383 to binding

stability highlights an aspect that has been limitedly addressed in the literature. The evaluation of docking results together with ADMET analyses strengthens the innovative contribution of the study by considering not only binding affinity but also drug similarity and pharmacokinetic suitability. In this respect, the study makes a significant contribution to the literature on the evaluation of phytoestrogens as potential candidates in the treatment of osteoporosis via ERα modulation.

Conclusions

This study demonstrates that the interactions exhibited by phytoestrogens at the ERα ligand binding site possess molecular characteristics consistent with known ERα agonists and selective estrogen receptor modulators. The findings indicate that interactions, particularly those occurring via critical polar and hydrophobic residues, suggest that phytoestrogens may exhibit agonist-like or partial agonist behavior on ERα. The observed binding similarities with tamoxifen, used as a reference compound, provide a molecular basis supporting the potential of these compounds to produce estrogen-like effects in bone tissue. Furthermore, the evaluation of binding tendencies in conjunction with pharmacokinetic and toxicological predictions demonstrates that phytoestrogens are viable candidates not only theoretically but also from a drug development perspective. In this respect, the study contributes to the development of alternative or complementary approaches in the treatment of osteoporosis through ERα modulation using phytoestrogens. Future in vitro and in vivo studies confirming the biological efficacy and tissue-specific effects of these compounds will allow for a clearer understanding of their clinical applicability.

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Author contribution

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