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Prenylated Phytoestrogens as Selective Estrogen Receptor Modulators for Breast Cancer: A Molecular Docking and Dynamics Study

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Abstract

Background: Phytoestrogens share common structural features with human estradiol, which primarily contribute to the proliferation of breast tissue cells by binding Estrogen Receptor (ER) subtypes. The Selective Estrogen Receptor Modulators (SERMs) have many therapeutic implications, including Estrogen Receptor Positive Breast Cancer (ERPBC). Prenylated phytoestrogens have emerged as attractive flavonoid scaffolds for ERβ-selective agonists, with potential for effective utilisation in the development of anti-breast cancer agents. **Aim:** Exploring prenylated phytoestrogens as selective ERβ agonists for Estrogen Receptor positive breast cancer therapy. **Methods:** To investigate the SERM modulatory activity of prenyl phytoestrogens, molecular docking, Molecular Mechanics with Generalised Born Surface Area (MM-GBSA) and dynamic simulation approach. Prenylated phytoestrogens were retrieved from pubchem database and screened against ERα and ERβ. The pharmacokinetic parameters like Absorption, Distribution, Metabolism, Excretion and Toxicity (ADMET) predictions were done to analyse drug likeness, human oral absorption and toxicity. **Results:** In total, 215 prenylated phytoestrogens were retrieved from pubchem database by similarity searching, and six of them, namely, Gamma Mangostin, GancaoninL, Licocoumarone, 2,3-Dehydrokievitone and Xanthohumol, showed binding towards ERβ with a significant docking score comparable with that of the standard ERβ selective agonist genistein. Molecular dynamics studies indicated the stability of the top-scoring compound Gancaonin L, and the required mode of binding is significant. MM-GBSA implies good affinity of these compounds with ERβ. On in silico ADME prediction using Qikprop, all the compounds showed good human oral absorption, poor blood-brain barrier permeability and less toxicity. **Conclusion:** The present study highlights that the prenylated phytoestrogens may act as selective ERβ modulators based on their favourable in silico ADME profiles, high affinity, and specific binding mode. Further *in vitro* and *in vivo* validation will provide additional insights in developing ERβ modulators into potential anti-breast cancer agents.

Major Findings: The top-scoring compounds can serve as fundamental scaffolds for further exploration and development of novel SERMs.

Keywords: ADMET, ERβ, ERPBC, Molecular Docking, Molecular Dynamics, Prenylated Phytoestrogens

Abbreviations:# metab -Number of metabolites; #rotar -Rotatable bonds; #rtFG-Number of reactive functional groups; 4-HT -4-Hydroxytamoxifen ; ADMET-Absorption, Distribution, Metabolism, Excretion, Toxicity; Donor/acceptor -H-bond counts; ERPBC -Estrogen Receptor positive breast cancer; ERα-Estrogen Receptor alpha; ERβ-Estrogen Receptor beta; HOA-Human oral absorption; Lipinski RO5 -Rule of five; MM-GBSA-Molecular mechanics/generalised born surface area (MM/GBSA); MW-Molecular weight; NVT-number of particles, volume, and temperature; OPLS3e-Optimised potential for liquid simulations; PHOA -Percentage Human oral absorption; PSA-Polar surface area; QLogPo/w-Log of Octanol–water partition coefficient; QPlogBB - Brain/blood partition coefficient; QPlogHER - hERG (human ether-à-go-go-related gene); QPlogS - log of aqueous solubility; QPPCaco-Caco-2 cell permeability in nm/sec; RMSD -Root-mean-square deviation; RMSF -Root-mean-square fluctuation; SERM-Selective Estrogen Receptor Modulator.

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1. Introduction

Phytoestrogens are a group of plant-derived molecules that possess estrogenic activity. They include flavonoids, stilbenes, and lignans, and share common structural features such as phenolic rings and hydroxyl groups with estradiol-like spacing[1,2]. Owing to their antioxidant activity and other beneficial effects, phytoestrogens are widely used not only as food supplements but also as adjuvant therapy in cancer and other diseases. Despite their relatively low estrogenic activity, phytoestrogens are commonly used by patients in gynaecology and menopause clinics. Phytoestrogens have become a central focus of research for their anticancer potential, particularly against hormone-dependent cancers, due to their ability to act as SERMs[3]. SERMs are chemical molecules that interact with ERs at either agonistic or antagonistic sites and exert modulatory activity[4]. Unlike pure agonists or antagonists, SERMs exhibit mixed agonist/antagonist behaviour depending on the target tissue and receptor subtype. The two ER subtypes, ERα and ERβ, play critical roles in regulating breast cancer cell proliferation. ERα promotes cell proliferation, whereas ERβ exerts an inhibitory effect. Estrogen Receptor–Positive Breast Cancer (ERPBC) patients respond favourably to treatment with ERα antagonists and/or ERβ agonists[5]. Therefore, ER is a key therapeutic target, and SERMs are considered a primary treatment strategy for ERPBC. But studies related to selective ERβ agonists without ERα activity in treating breast cancer are limited. Hence, exploring ERβ-selective agonists may contribute to the development of novel anti-breast cancer therapies for ER-positive breast cancer[6,7]. In recent years, prenylated flavonoids have gained attention as important flavonoid derivatives in anti-breast cancer drug development due to their favourable pharmacodynamics and pharmacokinetic interactions with estrogen receptors. Several studies have reported prenylated flavonoids as ERβ-selective derivatives[8-10]. Compared to unprenylated flavonoids, the presence of a prenyl moiety enhances lipophilicity, leading to improved bioavailability and absorption[11]. A large number of prenylated phytoestrogens in natural product databases and libraries remain unexplored, and this leads to research for investigating selective ERβ agonists with anticancer properties. In the early stages of drug discovery, computational tools such as structure-based virtual screening are widely employed to accelerate lead identification. Therefore, to bridge the gap between prenylated phytoestrogens and anti-breast cancer activity, the present study aims to perform an *in silico* screening of prenylated phytoestrogens to identify novel scaffolds that can be modified into therapeutic candidates for breast cancer.

2. Materials and Methods

2.1 Softwares

The *in silico* studies were performed in the Maestro workspace, Schrödinger.

2.2 Ligand Selection and Preparation

Similarity searching was done by giving Genistein, an ERβ agonist, as a search query to generate the compound library with the same scaffolds. Prenylated flavonoids were retrieved from this compound library generated by similarity searching. The 2D structures were imported and prepared using the Ligprep module of Schrödinger. LigPrep converts 2D structures into 3D by considering the structural properties such as tautomer, ionised form, conformations, and stereoisomers[12]. Transformation of 2D structures into low energy Three Dimensional (3D) structures was achieved by a module that generates possible stereoisomers, attaches hydrogens to the ligands, neutralises charged structures and determines the most probable ionisation state at the defined pH 7.0. Due to the flexibility of most of the ligands, necessary steps were taken to consider a range of thermally accessible conformational states with the intention of increasing the chances of finding something close to the putative binding mode. The conformational space was explored by the combination of Monte-Carlo Multiple Minimum (MCMM)/ Low Mode (LMOD) with a maximum of 1000 conformers per structure and 100 minimisation steps. Each minimised conformer was filtered by limiting 10 kJ mol^{-1} as a relative energy window, a value that sets an energy threshold relative to the lowest-energy conformer and 1.00Å as a minimum atom deviation. Conformers with a value higher than this threshold energy were discarded. All distances between pairs of corresponding heavy atoms were kept below 1.00 Å for two conformers to be considered identical.

2.3 Protein Preparation

The 3D crystal structures of the target proteins ERα (PDB ID: 3ERT), (PDB ID:1U3S), were downloaded from the Protein Data Bank (PDB) (www.pdb.org/pdb)[13,14]. They were imported to the Maestro workspace and prepared using the Protein Preparation Wizard of GLIDE (Grid-Based Ligand Docking with Energetic)[15,16]. Glide implements a mixture of rigid and flexible docking and keeps the ligand molecules flexible and the target protein like a rigid system. The protein preparation wizard has different options to assign the bond orders and to add possible missing hydrogen atoms in the PDB structure. Finally, the full energetic optimisation was done using the OPLS3 force field and by restricting the Root-Mean-Square Deviation (RMSD) of heavy atoms at 0.3 Å.

2.4 Grid Generation and Docking

The grid was generated by selecting the co-crystal ligand bound to the targets, and the default parameters of Glide were unchanged and applied. The default centre docking box is capable of accommodating ligands with a length of ≤20 Å. 10 **Å** was set as the *inner* grid *box size*. XP Scoring was carried out on the energy-minimised conformations, and rescoring was done using the Glide scoring function (G-Score)[17].

2.5 Docking Validation

The docking procedure was validated by splitting the native ligand from each target and re-docking into the active site using Glide by setting default grid parameters. It was done to ensure the inhibitor binds exactly to the active site cleft and must show less deviation compared to the actual co-crystallised complex. The RMSD of the actual co-crystallised complex and redocked complex was found to be 1.12 Å and 1.79 Å for ERα and ERβ, respectively, which is acceptable.

2.6 Molecular Mechanics/Generalised Born Surface Area (MM/GBSA) Calculations

To estimate the relative binding affinity of ligands to the targets, the Prime /MM-GBSA module was used by applying the optimised potential for liquid simulation (OPLS-AA) and the GB/SA method. The binding free energy ΔG bind was estimated as

$$\Delta G\ bind = \Delta E\ MM + \Delta\ G\ solv + \Delta G\ SA$$

where,

Δ E MM = difference in energy between the complex structure and the sum of the energies of the ligand and unliganded protein using the OPLS force field. ΔG solv = difference in the GBSA solvation energy of the complex and the sum of the solvation energies for the ligand and unliganded protein ΔG SA = difference in the surface area energy for the complex and the sum of the surface area energies for the ligand and unliganded protein[18].

2.7 Molecular Dynamics

2.7.1 System Preparation

To carry out the molecular dynamic simulations, Desmond Molecular Dynamics software from Schrödinger suite was used (Schrödinger Release 2022-3: Desmond Molecular Dynamics System, D. E. Shaw Research, New York, NY, USA, 2022[19,20]. The Ligand-target complex was first loaded system preparation was done with the System Builder application of Desmond. The simulations were run within an orthorhombic box full of explicit water molecules generated by the TIP3P mode[21]. Prior to the MD simulations, the steric clashes are avoided by minimisation of assemblies with the OPLS3e force field method. The OPLS-AA is an all-atom force field parameter for both proteins and organic small molecules, and further frameworks are not necessary.

2.7.2 MD Simulations

The completed setups were then loaded into Desmond's Molecular Dynamics interface, and simulations for50 ns runs were initialised[22]. The MD simulations were started with the relaxation protocol reported and without changing the default settings that include a multi-stage Molecular Dynamics (MD) simulation process by starting with a 12 ps-long NVT (Constant Substance, Volume, and Temperature) ensemble simulation at 10 K temperature; followed by two 12 ps-long NPT (Constant Substance, Temperature, and Pressure) ensemble simulations at 10 K temperature and 1.01325 bar pressure; and, finally, a 24 ps-long NPT ensemble simulation at 300 K temperature and 1.01325 bar pressure[23,24]. After the relaxation, all MD simulations were always carried out with NPT settings, where the pressure was 1.01325 bar, and the temperature was 300 K[25]. The recording interval of the trajectory was set to 1000 ps.

2.8 Drug Likeness Screening of Ligands

The Qikprop[26] module of Maestro, Schrödinger was used to predict the pharmacokinetic properties related to bioavailability, BBB permeability and toxicity were predicted and analysed[26-28]. They include the Lipinski rule of five, Human Oral Absorption (HOA), CNS activity, PSA, *etc*.

3. Results and Discussions

In recent years, prenylated flavonoids have been considered as important flavonoid derivatives in anti-breast cancer drug development due to their pharmacodynamic and pharmacokinetic effects. The present study aims to explore the SERM effects of prenylated flavonoids. To achieve this, the 2D structure of prenylated flavonoids was retrieved from pubchem database through similarity-based searching, and they were subjected to molecular docking, ADMET prediction and dynamic studies. On similarity-based searching, 215 prenylated flavonoids were retrieved. The ligand preparation yielded 431 entries, including stereoisomers.

3.1 Molecular Docking Studies

The docking studies were done using Glide, Schrodinger, against ERα (PDB ID: 3ERT) and ERβ (PDB ID: 1U3S). The amino acid sequence of ligand binding domain of ERα and ERβ shares 59% similarity noted with remarkable differences. The residues Leu384 and Met421 present in ligand binding domain of ERα are replaced by Met336 and Ile373, respectively, in ERβ[3]. Six of the prenylated phytoestrogens demonstrated good binding affinity against ERβ with the XP GScore in the range of -13.008 to -5.686 (Figures 1 and 2). During this study, it was observed that all the compounds except Xanthohumol and Licocoumarone selectively bind to either of the ER subtypes (Figures 2 and 3). Out of all the flavonoids that were subjected for docking studies against ERβ, Gancaonin L showed a maximum of XP Gscore -13.008 against ER**β**. In addition to pi-pi stacking with PHE 356 at a distance of 5.23 Å, Gancaonin L showed two additional hydrogen bondings with GLU 305 and ARG 346 due to the free hydroxyl group at 2' and 3' positions of the phenyl ring at the distances of 1.92 and 2.86 Å, respectively. The target-ligand interaction distances are given in Table 1. Licocoumarone, 2,3-Dehydrokievitone and established hydrogen bonding with ARG346 and pi-pi stacking with PHE 356. Xanthohumol interacted with ERα and ERβ, with the docking score of -5.686 and -8.064. It established hydrogen bonding with GLU-305 and HIE-475. The binding mode of Gamma-Mangostin and ERβ was found to be hydrogen bonding with GLU305 and LEU339, and *pi*-pi stacking with PHE 356.

The docking analysis of prenylated flavonoids is comparable with the selective ERβ agonist Genistein, which establishes hydrogen bonding with GLU305, ARG346 and HIS475 and pi-pi stacking with PHE356. As both ERα antagonists and/or agonists are considered as therapeutic regimens to treat estrogen-positive breast cancer, the prenylated phytoestrogens were subjected to docking against ERα also. Mangostin binds with anti-estrogenic residues such as Asp351 and Thr347, whereas pomiferin and licocoumarone bind with Thr347 of ERα. Xanthohumol did not show any interaction with ERα despite having a docking score of -5.8. Mangostin, xanthohumol, pomiferin, and licocoumarone showed lesser binding affinity and interactions than the standard ERα antagonist 4-HT (Table 1) with ERα. Molecular docking studies revealed that the interactions made by the prenylated phytoestrogens are crucial for estradiol-like activity. Substantial studies have reported the effects of prenylated phytoestrogens on the selectivity and affinity of ER subtypes in recent years. Based on the previous research reports, prenylated flavonoids are considered to be more selective towards ERβ than ERα. To get additional insights on ERβ agonist activity, they are overlaid with Gensitein in the ERβ binding site. Figure 4 clearly indicates that Gancaonin L aligns very well with genistein, especially with the phenyl ring

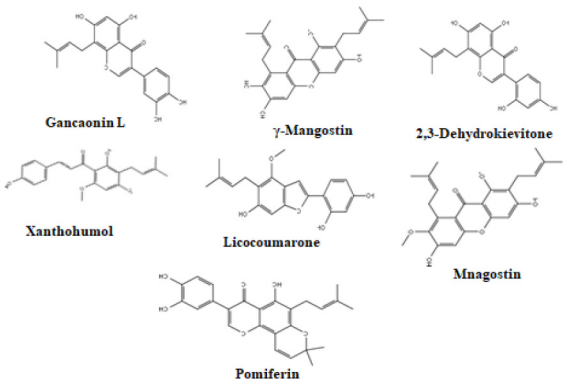


Figure 1. The structure of prenyl phytoestrogens interacts with ER**.**

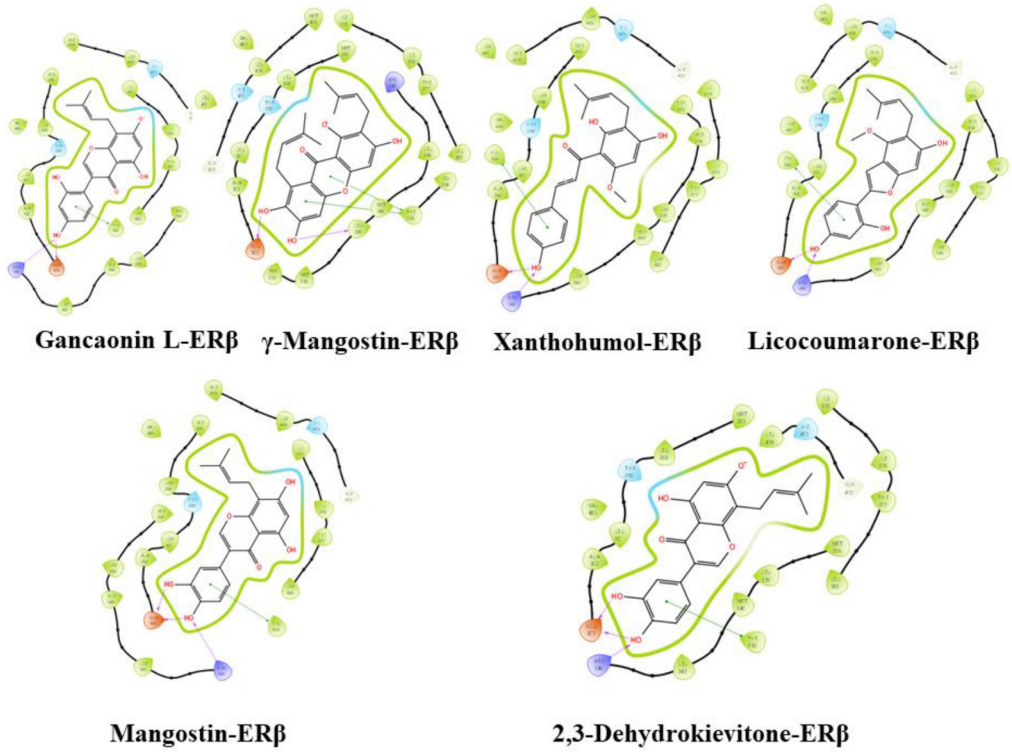


Figure 2. 2D Interaction diagram of prenylated flavonoid-ERβ complexes.

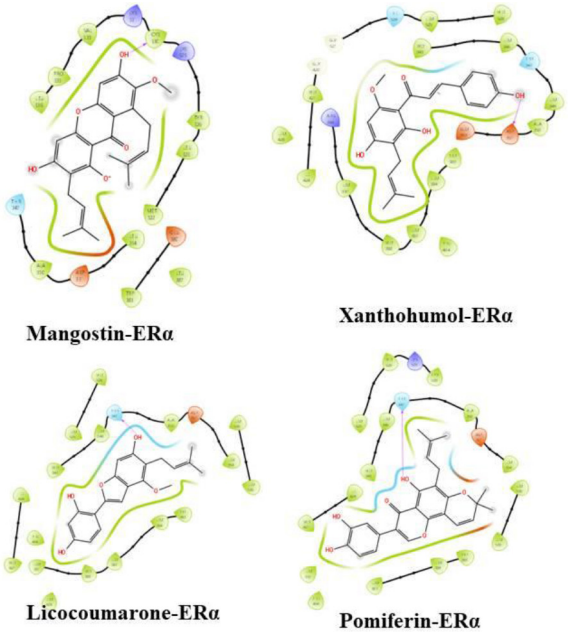


Figure 3. 2D Interaction diagram of prenylated flavonoids with Erα.

attached to the pyrone ring. Djiogue *et al.* revealed that prenylation at C-8 of the flavonone and flavone might improve the ERβ selectivity of the flavonoids[29] due to the ability of prenylated molecules to fit into the hydrophobic cavity that is present in ERs and enhance the ERβ agonistic activity[30-32]. We identified new prenyl phytoestrogens, namely Gancaonin L and licocoumarone, that have not yet been reported for their SERM activity in the present study. Since licocoumarone is a non-selective ligand towards ER subtypes, the study was further extrapolated to analyse the stability and ligand target interaction of top scored prenylated phytoestrogen, Gancaonin L-ERβ complex, through molecular dynamics studies.

3.2 Molecular Mechanics with Generalised Born and Surface Area (MM-GBSA)

In addition to docking studies, Molecular Mechanics with Generalised Born and Surface Area (MM-GBSA) scoring function was carried out to predict binding free energies for the prenylated phytoestrogens. As the MM-GBSA binding energies are approximate free energies of binding, a more negative value indicates stronger binding. The MMGBSA ΔG bind score of Gancaonin L, Licocoumarone and xanthohumol was found to be -95.19, -94.84 and -91.51, respectively, which are lower than the binding affinity of genistein and tabulated in Table 2.

Table 1. Docking scores, mode of binding and distances of prenylated phytoestrogens-ER complexes

S. No.	Name	Pubchem ID	ERα		ERβ		Distance	Binding ratio ERβ/ERα
			Docking score	Amino acid interaction	Docking score	Amino acid interaction		
1	Gancaonin L	14604077	-	-	-13.008	GLU-305 ARG-346 PHE- 356	1.84;1.92 1.99 5.23	-
2	Licocoumarone	503731	-5.871	THR-347	-11.915	GLU305, ARG 346, PHE 356	1.84 1.97 5.33	2.0
3	Xanthohumol	639665	-5.686	ASP-351	- 8.246	HIE-475 PHE 356	2.07 5.39	1.5
4	2,3-Dehydrokievitone	5746354	-	-	-11.887	GLU-305 ARG 346 PHE-356	1.82 2.07 4.11	-
5	*gamma-Mangostin*	5464078	-	-	-7.965	GLU-305 LEU -339 PHE-356	2.49 1.78 4.96;5.30	-
6	Genistein	5280961	-	-	-11.330	GLU-305 ARG 346 PHE-356 GLY- 472	1.86 2.09 5.22 2.13	-
7	Mangostin	5281650	-10.478	THR-347 ASP-351	-	-	1.96	-
8	Pomiferin	4871	-7.449	THR-347	-	-	2.07	-
9	4-hydroxy tamoxifen	449459	-11.949	ARG 394 GLU353 THR347 ASP351	-	-	2.09 1.63	-
10	DB07236	5287551	-	-	-12.335	GLU-305 ARG 346 PHE-356	2.58 1.92 5.12 and 5.14	-

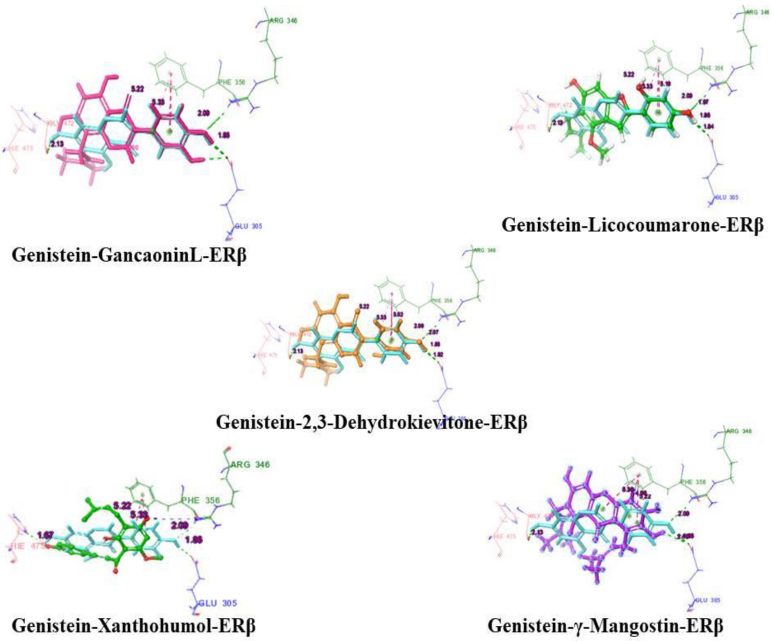


Figure 4. Overlay of prenylated flavonoids with genistein-ERβ complex.

3.3 Molecular Dynamics Simulation Studies

The *in silico* validation was done by molecular dynamic studies using Desmond, Schrodinger to analyse the interactions established and stability of the target-ligand complex. Generally, RMSD and RMSF are used to evaluate the stability of the target-ligand complex. The RMSD of the GancaoninL-1U3S complex for a 50 ns timeline was found to be 2.4 Å, which is equal to the RMSD of the protein alone and shows that the interactions made by Gancaonin L favour the stability (Figure 5). The Root-mean-square fluctuation (RMSF) is a plot of fluctuations of each residue versus the simulation timeframe, and the stability is inversely proportional to RMSF. The RMSF of gancaoninL-1U3S complexes was found to be in the range of 0.8 to 4.5 Å. The simulation interaction diagram and ligand contact histogram obtained from MD studies were used to analyse the ligand target interaction from the perspective of ERβ agonist activity of Gancaonin L. It illustrates water-mediated hydrogen bonding of Gancaonin L with Glu305 and Hie475. All the hydrogen bonding was made for more than 80% of the time frame, which indicates the compactness of Gancaonin L in the binding site.

3.4 ADMET Prediction

3.4.1 Drug Likeness and Lipinski Rule of Five

#stars is a score indicating how many computed pharmacokinetic and physicochemical properties of a molecule fall outside the optimal range for 95% of known drugs. A low #stars value (recommended 0-5) suggests a molecule is more likely to be "drug-like" and has a better predicted ADMET profile, while a high value indicates the molecule is less "drug-like". The ADMET prediction by qikprop, Schrodinger suggests that all the compounds showed drug-likeness as the descriptors of the qikprop module, like lower #stars, lower MW, moderate logP, Donor/acceptor H-bond counts and Rotatable bonds were found to be within the recommended range (Table 3).

Table 2. MM-GBSA dG binding score of prenylated phytoestrogens flavonoid-ERβ complexes

Title	MMGBSA dG Binding Score
Gancaonin L	-95.19
Licocoumarone	-94.84
Xanthohumol	-91.51
2,3-Dehydrokievitone	-47.01
Genistein	-71.23

3.4.2 Rule of Three

For any molecule to have oral absorption, intestinal membrane permeability is an essential parameter. The rule of three of the prenylated phytoestrogens was predicted to analyse the Gut-blood barrier permeability. The compounds showed QPPCaco permeability in the order of mangostin > gammamangostin > pomiferin > 2,3-Dihydrokievitone > Xanthohumol > licocoumarone > GancaoninL. When HOA and PHOA are observed, mangostin, gamma mangostin, licocoumarone and xanthohumol showed excellent oral absorption with 100%, 100%, 93.874% and 95.829%, respectively. But gamma-mangostin showed high PHOA despite a lower HOA score. As QPlogS is a measure of aqueous solubility of a compound, the higher the value reduces the permeability. The results reveal that gamma-mangostin has poor solubility (–6.027), and other compounds are considerably permeable and have good bioavailability. All the prenyl phytoestrogens except gamma-mangostin satisfy the rule of three and indicate their good permeability (Table 4).

3.4.3 CNS Activity

To find out the ability of prenylated phytoestrogens to affect CNS activity, QPlogBB and QPPMDCK were predicted and analysed (Table 5). QPlogBB descriptor is used to predict the ability of BBB permeability, and the acceptable range is from -3.0 to 1.2. QPPMDCK is used to predict the BBB permeability, and the poor BBB permeability is indicated by a QPPMDCK value of <25 and good BBB

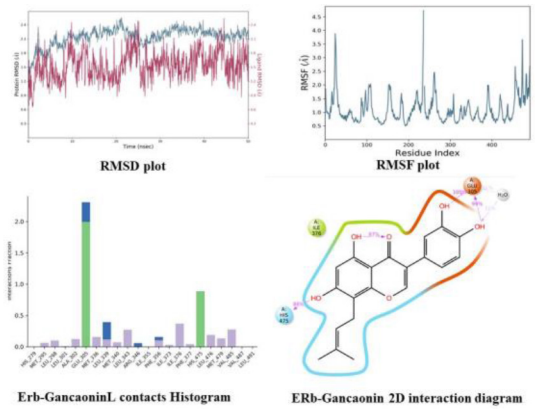


Figure 5. Molecular dynamic simulation parameters of GancaoninL-ERb complex.

Table 3. Drug likeness descriptors of prenylated phytoestrogens

Name of the compound	#stars	Mol MW	QPlogPo/w	Donor HB	Accpt HB	# rotor	Lipinski RO5
Gancaonin L	0	354.359	2.694	3	4.5	7	0
Licocoumarone	0	340.375	3.634	3	3.5	6	0
Xanthohumol	1	396.439	3.852	3	4.5	8	0
2,3-Dehydrokievitone	0	354.402	4.04	2	4	10	0
Gamma-Mangostin	0	354.359	2.785	3	4.5	7	0
Mangostin	0	410.466	4.686	2	4.5	8	0
Pomiferin	0	420.461	4.659	2	4.5	6	0

Table 4. Rule of three parameters of prenylated phytoestrogens

Name of the compound	QPPCaco	Oral Absorption		QPlogS	# metab	PSA	Rule of three
		HOA	PHOA				
Gancaonin L	77.043	2	76.49	-4.872	7	116.818	1
Licocoumarone	89.941	2	78.227	-4.91	7	117.814	1
Xanthohumol	182.3	1	89.964	-6.027	10	111.915	2
2,3-Dehydrokievitone	261.718	3	93.874	-5.605	7	93.197	1
Gamma-Mangostin	456.811	3	95.829	-5.341	8	77.782	1
Mangostin	481.237	1	100	-6.605	10	98.307	2
Pomiferin	263.257	1	100	-6.992	6	99.804	1

Table 5. CNS and toxicity descriptors of prenylated phytoestrogens

Name of the compound	BBB permeability			Toxicity		
	CNS	QPlogBB	QPPMDCK	QPlogHERG	# metab	#rtFG
Gancaonin L	-2	-2.127	30.977	-5.695	7	0
Licocoumarone	-2	-2.056	36.619	-5.738	7	0
Xanthohumol	-2	-1.877	78.587	-5.652	10	0
2,3-Dehydrokievitone	-2	-1.819	116.171	-6.021	7	1
Gamma-Mangostin	-2	-1.247	212.112	-5.755	8	0
Mangostin	-2	-1.409	224.397	-5.655	10	0
Pomiferin	-2	-1.589	116.909	-6.152	6	0

permeability is indicated by >50. As depicted in Table 5, the CNS activity, QPlogBB, QPPMDCK and PSA indicate the poor BBB permeability of prenylated phytoestrogens. #rtvFG is a descriptor used to evaluate the different functional groups that can interfere with the results of *in vitro* assays and lead to instability, reactivity or toxicity problems *in vivo*. #rtvFG of all the compounds are within the recommended range except gamma-mangostin, which has one reactive functional group (#rtFG=1), which may affect stability or reactivity. QPlogHERG, another toxicity descriptor in Schrödinger's QikProp, predicts a molecule's potential to block the hERG (human ether-à-go-go-related gene) potassium ion channel. All the compounds showed low toxicity as the HERG inhibition is found to be below -5.6.

4. Conclusion

In order to explore the anti-ERPBC effects through SERM activity of prenylated phytoestrogens, similarity-based virtual screening, MM-GBSA and Molecular dynamic studies were done. On Molecular Docking of 215 prenylated phytoestrogens obtained from similarity searching against estrogen receptors, three of

the prenylated phytoestrogens showed ERβ selectivity, two of the prenylated phytoestrogens showed ERα selectivity, and two of them showed both ERα and ERβ binding affinity. But ERα affinity shown by the prenylated phytoestrogens is not comparable with the standard ERα selective antagonist in terms of docking score and interactions. The docking score, affinity and stability of prenylated phytoestrogens with ERβ were significant and comparable with genistein, a known ERβ agonist. This study highlights that Gancaonin L, the top-scoring prenylated phytoestrogen, showed excellent docking score and stability with ERβ, and it is less explored for anti-breast cancer potentials. The pharmacokinetic parameters of prenylated phytoestrogens were found to be within the recommended range and may have better oral bioavailability than the parent flavonoids. So, this study provides a basic deep insight into the pharmacodynamics and pharmacokinetic effects of prenylated phytoestrogens, and structural refinement of prenylated phytoestrogens may yield new therapeutic candidates for ERPBC.

5. Limitations

However, the SERM activity and pharmacokinetic properties of prenylated phytoestrogens reported here have to be validated with *in vitro* and *in vivo* experimental studies.

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Authors Contribution

S. E. Maida Engels - Conceptualisation, methodology, software, validation, formal analysis, data curation, writing-original draft preparation, writing-editing; Muthiah Ramanathan - Conceptualisation, investigation, supervision, review; K. Narayanasamy and S. Kalpana – Resources. All authors have read and agreed to the published version of the manuscript.