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Research Article

In Silico Molecular Docking and ADMET Analysis for Drug Development of Phytoestrogens Compound with Its Evaluation of Neurodegenerative Diseases

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Abstract

Neurodegenerative disease is one of the problems faced by postmenopausal women due to estrogen deficiency. Phytoestrogen compounds can be used as an alternative treatment for diseases caused by estrogen deficiency by binding to their receptors through the estrogen receptor (ER) dependent pathway. With *in silico* studies, this study aims to predict how phytoestrogen compounds will stop neurons from dying by using the dependent ER pathway. Genistein, daidzein, glycitein, formononetin, biochanin A, equol, pinoresinol, 4-methoxypinoresinol, eudesmin, α-amyrin, and β-amyrin compounds were prepared with ChemDraw Ultra 12.0. Then their pharmacokinetic and pharmacodynamic properties were examined using SwissADME. Geometry optimization of the compound was performed using Avogadro 1.0.1, and molecular docking of the compound to the ERα (1A52) and ERβ (5TOA) receptors was performed using AutoDock vina (PyRx 0.8). The interaction visualization stage was carried out with Biovia Discover Studio 2021, while the toxicity values of the compounds were analyzed using pkCSM and ProTox II. The results showed that the equol compound met the pharmacokinetic, pharmacodynamic, toxicity criteria, and had similarities with the native ligand 17β-estradiol. Equol compound inhibits neurodegeneration via an ER-dependent pathway by binding to ERα (1A52) and ERβ (5TOA) receptors.

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INTRODUCTION

Aging in the human body is characterized by a decline in physiological conditions, a rise in disease risk, and, eventually, mortality[1,2]. This phenomenon occurs due to progressive alterations in the body's metabolic and hormonal function caused by the failure of deoxyribonucleic acid (DNA) transcription, chronic inflammation, and the instability of the body's homeostasis of death[3,4]. This type of homeostasis can emerge due to a progressive decline in the hormone estrogen, also known as estrogen deficiency, which typically happens in postmenopausal women[5]. This situation will create

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neuroendocrine alterations that disrupt several systems. Cellular and metabolic processes cause neurodegenerative diseases[6,7].

Hormone replacement therapy, which is extremely effective and generally understood by the public, is a frequent therapy for overcoming health problems caused by estrogen shortages[8]. On the other hand, hormone replacement therapy has adverse effects that might lead to additional health concerns, such as hot flashes, cancer, ischemic stroke, and death[9,10]. Phytoestrogens are one of the medicines that have been made to work as well as hormone replacement therapy but with less risk[11].

Phytoestrogens are natural chemicals with the same structure, activity, and affinity as estrogens found in mammals[12]. Flavonoids, including genistein, daidzein, glycitein, formononetin, biochanin A, and equol, are among the most abundant classes of chemicals with phytoestrogen activity. In addition to flavonoid molecules, there are non-flavonoid compounds with phytoestrogen activity, such as pinoresinol, 4-methoxypinoresinol, eudesmin, α-amyrin, and β-amyrin[13-15]. According to current literature[13,16], the chemical is a polyphenolic compound with structural similarities to estradiol and estrogenic activity since it has a ring similar to estradiol and two hydroxyl groups with proper spacing between them.

Phytoestrogen chemicals have estrogenic activity after binding to their receptors (ER-dependent) and other pathways (ER-independent), allowing them to maintain brain homeostasis[17,18]. The ER-dependent pathway can deliver activity directly because one of its components binds to estrogen receptor α (ERα) and estrogen receptor β (ERβ)[19]. Many treatments for estrogen-related disorders can target the roles of ERα and ERβ these treatments show how important it is to understand ERα and ERβ mechanisms in order to get the most out of treatment[20].

Based on this rationale, it is required to do *in silico* studies on widely encountered phytoestrogen chemicals from the flavonoid and non-flavonoid categories. To understand the mechanism of phytoestrogen drugs via the ER-dependent pathway, neurotoxic activity in ER must be observed. *In silico* observations have advantages such as being quick and affordable in determining a compound's estrogenic activity[21].

MATERIALS AND METHODS

Materials

A Lenovo AIAQH4R personal computer was utilized as the tool. For *in silico* testing, the software includes Autodock Vina (PyRx 0.8), ChemDraw Ultra 12.0, Avogadro 1.0.1, and Biovia Discovery Studio 2021, as well as SwissADME for physicochemical property testing, pkCSM, and Protox II for toxicity testing. The 11 phytoestrogen compound's three-dimensional structures were created using the ChemDraw Ultra 12.0 software. The study discovered the following compounds: genistein, daidzein, glycitein, formononetin, biochanin A, equol, pinoresinol, 4-methoxypinoresinol, eudesmin, α-amyrin, and β-amyrin were all examples of phytochemicals. In addition to the 11 phytoestrogens substances, the structure of the native ligand (17-estradiol) and the protein (receptor) was created. A protein data database (www.rcsb.org) was used to figure out the three-dimensional crystal structure of the ERα (1A52) and ERβ (5TOA) receptors.

Methods

Preparation of samples[22]

Using the Biovia Discovery Studio 2021 application, the receptor was split into macromolecules and native ligands and saved in the Sybyl Mol 2. format. Biovia Discovery Studio 2021 is a free downloadable software. ChemDraw Ultra 12.0 was used to create a 3D structure from 11 compounds in mole format. This application is a product of http://www.cambridgesoft.com/. This software can be downloaded for free. Also, the compound was adjusted geometrically with Avogadro 1.0.1 to get a stable position using the MMFF94 method and saved in the Sybyl Mol 2 format19. This software can be downloaded for free.

Physicochemical examination

ChemDraw Ultra 12.0 was used to format each compound into a simplified molecular-input line-entry system (SMILES). The SMILES form was used to evaluate molecules' physical properties and compare them to compounds in IUPAC nomenclature[23]. The format was then copied one by one onto the SwissADME (http://www.swissadme.ch) and run to find out the compound's pharmacokinetics and pharmacodynamics in the form of the topological polar surface area (TPSA), molecular weight, log P, hydrogen bond acceptor (HBA), hydrogen bond donor (HBD), and the statement "Yes" or "No" in meeting Lipinski's rule of five parameters.

Molecules docking

Internal validation of the receptor and native ligand was performed first using AutoDock Vina (PyRx 0.8). Internal validation was used to determine the root mean square deviation (RMSD). The RMSD number is one of the characteristics that must be met to evaluate its applicability. The RMSD value reported in this study was less than 2.0, indicating that the application is suitable. This stage was completed by altering the grid box location where the ligand interacts with the target receptor to identify the coordinates of the receptor binding site, which was then saved using the csv menu[24]. Grid box determination for ERα (PDB ID 1A52) includes setting the location according to the grid center x = 90.4083, *y* = 14.0333, and *z* = 72.2361 with dimensions of 4.1 x 10.9 x 7.8 Å, and for ERβ (5TOA) location settings according to the grid center *x* = 19.8271, *y* = 43.3538, and *z* = 15.4885 with dimensions of 4.5 x 7.4 x 9.9 Å. Each chemical was docked to the ERα and ERβ receptors using AutoDock Vina (PyRx 0.8), and the interaction was visualized using Biovia Discovery Studio 2021 to analyze the distance between the pharmacophores and the bound amino acids. The molecular docking data of these compounds were compared to those of native ligands to see if they had similar interactions[25].

Toxicity test

The pkCSM (http://biosig.unimelb.edu.au/pkcsm/prediction) and ProTox II (http://tox.charite.de/protox II/) were used to predict LD_{50} values of each ligand using the SMILES format[26].

RESULTS AND DISCUSSION

Validation of methods

The method was validated by anchoring the ERα receptor with PDB ID 1A52 and native ligand using AutoDock Vina (PyRx 0.8) and obtaining an RMSD of 1.761 Å. The RMSD result for the ERβ receptor with PDB ID 5TOA and native ligand using AutoDock Vina (PyRx 0.8) was 1.831 Å. An RMSD of less than 2.0 shows that the application is suitable for molecular anchoring processes that provide outcomes similar to experimental results[27-29]. **Figures 1** and **2** show the procedure validation findings.

Physicochemical examination

The SwissADME was used to forecast the pharmacokinetic and pharmacodynamic potentials, and it was discovered that only phytoestrogens from the flavonoid group satisfied the parameters and could be accepted by the body (**Table I**). Genistein, daidzein, glycitein, formononetin, biochanin A, and equol all meet the requirements of Lipinski's rule of five, so the body recognizes them. Lipinski's rule of five parameters with 500 g/mol molecular weight, hydrogen bond acceptors (HBA) ≤5, hydrogen bond donors (HBD) ≤5, and log P ≤5. A molecular weight of less than 500 g/mol suggests that the molecule can penetrate biological membranes. The log P value shows the compound's dissolved capacity in a liquid membrane. The hydrogen capacity of the H-acceptor and H-donor is shown, and the higher the value, the more energy is required for the absorption process[30]. Daidzein, formononetin, and equol have topological polar surface area (TPSA) values of 79 $Å^2$, indicating that these compounds can cross the blood-brain barrier and have an effect[31,32], indicating that these compounds require further processing to examine the outcomes of molecular docking with the estrogen receptor.

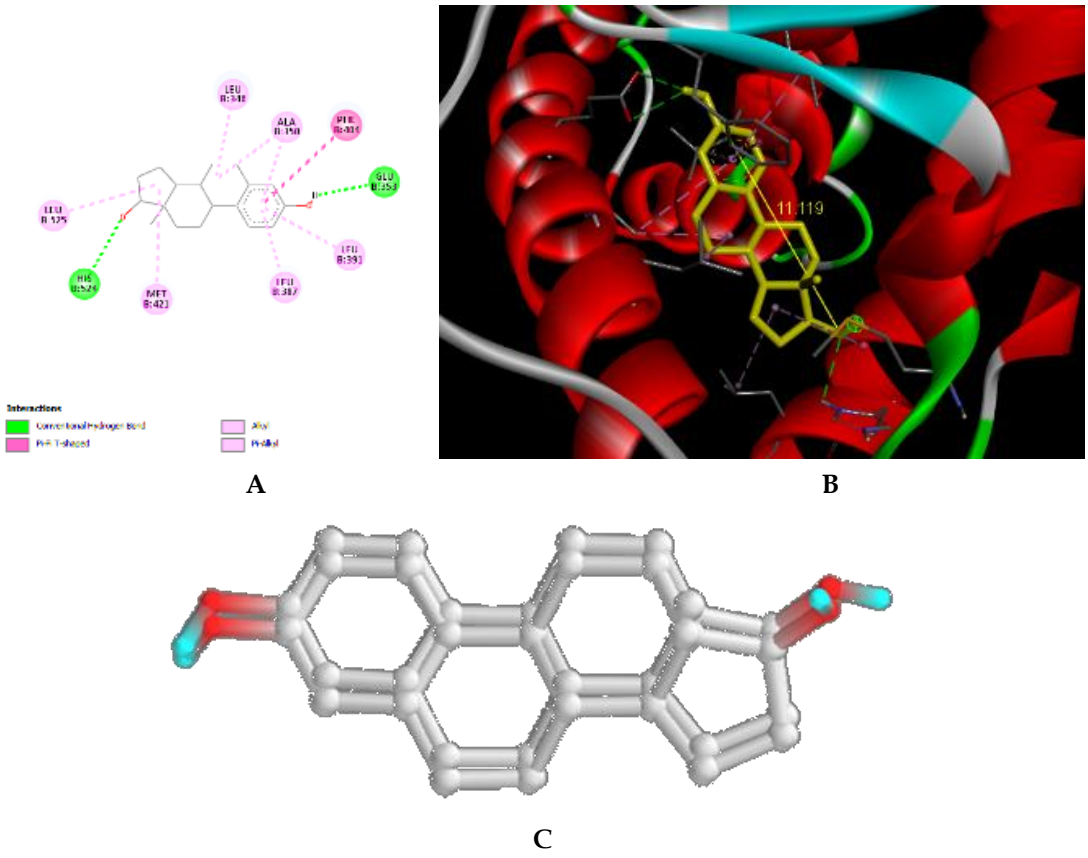

Figure 1. The results of the validation of the internal ligand method with ERα (**A**), 2D (**B**), and 3D (**C**) overlay of crystallographic ligand.

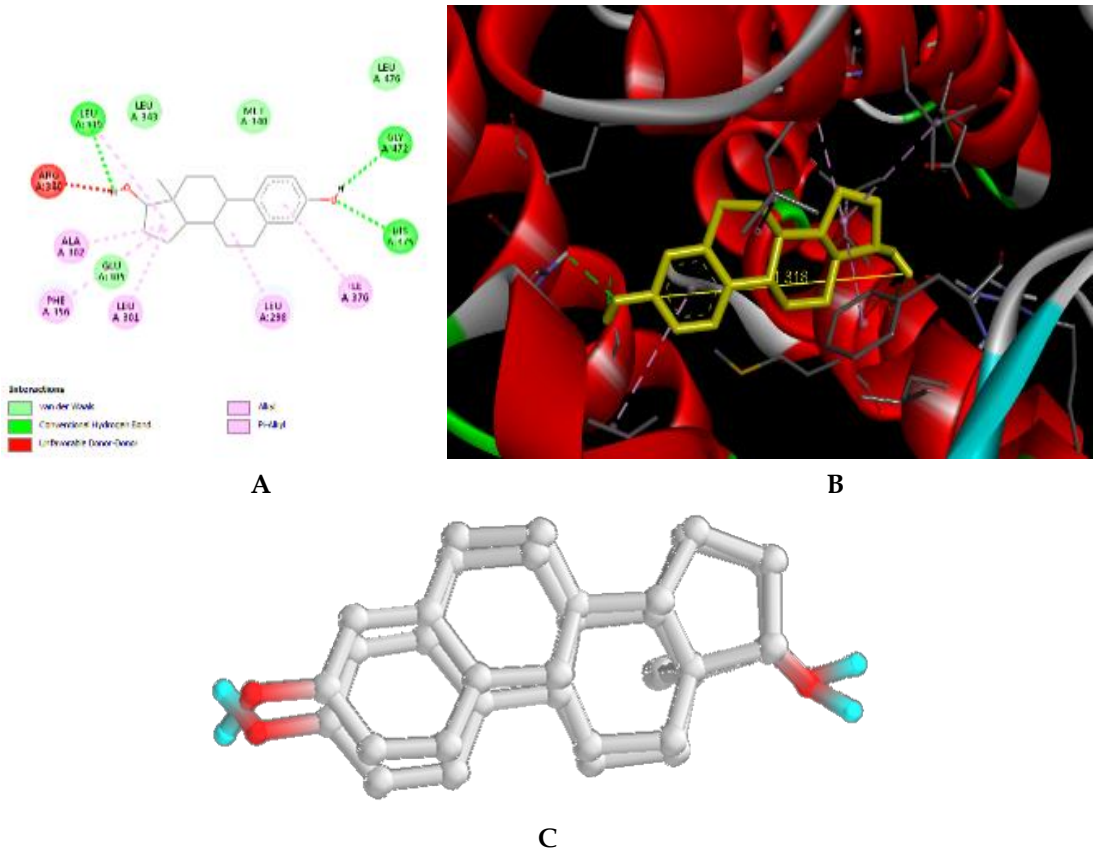

Figure 2. The results of the validation of the internal ligand method with ERβ (**A**), 2D (**B**), and 3D (**C**) overlay of crystallographic ligand.

Table I. Physicochemical analysis of phytoestrogen compounds analyzed.

Compound name	MW ≤500 g/mol	Log P ≤5	HBA ≤5	HBD ≤5	Lipinski's Rule of Five	TPSA (Å²)
Flavonoid						
Genistein	270.24	2.04	5	3	Yes	90.90
Daidzein	254.24	2.24	4	2	Yes	70.67
Glycitein	284.26	2.30	5	2	Yes	79.90
Formononetin	254.24	2.24	4	2	Yes	70.67
Biochanin A	284.26	2.44	5	2	Yes	79.90
Equol	242.27	2.58	3	2	Yes	49.69
Non-flavonoid						
Pinoresinol	358.39	2.26	6	2	Yes	77.38
4-Methoxypinoresinol	372.41	2.70	6	1	Yes	66.38
Eudesmin	386.44	3.06	6	0	Yes	55.38
α-amyrin	426.72	7.06	1	1	Yes	20.23
β-amyrin	426.72	7.20	1	1	Yes	20.23

Molecules docking

Internal validation studies with 17β-estradiol ligand showed an average RMSD value of 1.761 Å for ERα receptors with PDB ID 1A52 and 1.831 Å for ERβ receptors with PDB ID 5TOA, indicating that they can be used for docking simulations because they have met the RMSD value requirements of <2.0[33]. Simulated compounds are then screened for prospective compounds that can be used as estrogen replacements. **Tables II** and **III** show the analysis results on the interaction between amino acids and the pharmacophore distance of the selected flavonoid compounds. Based on these findings, the three flavonoid compounds were found to be agonists of the 1A52 and 5TOA proteins. The amino acids bound by each chemical were used to categorize the compounds. The simulation results show that the chosen flavonoid compounds can function as agonists against ERα and ERβ and produce estrogenic effects (**Figure 2**).

Table II. Molecular docking of compounds against ERα receptors.

Compound name	Binding affinity (kcal/mol)	RMSD (Å)	Amino acid residues	Pharmacophore distance (Å)
Internal ligand				
17β-estradiol	-8.8	1.761	Glu353 (Hydrogen bond) His 524 (Hydrogen bond)	11.119
Compounds				
Daidzein	-7.53	1.877	Glu353 (Unfavorable donor-donor) His524 (Unfavorable acceptor-acceptor)	12.311
Formononetin	-7.3	1.034	Glu353 (Hydrogen bond) His524 (Hydrogen bond)	12.108
Equol	-7.38	1.611	Glu353 (Hydrogen bond) His524 (Unfavorable donor-donor)	10.271

Table III. Molecular docking of compounds against ERβ receptors.

Compound name	Binding affinity (kcal/mol)	RMSD (Å)	Amino acid residues	Pharmacophore distance (Å)
Internal ligand				
17β-estradiol	-9.6	1.831	Leu339 (Hydrogen bond) Gly472 (Hydrogen bond) His475 (Hydrogen bond)	11.318
Compounds				
Daidzein	-8.53	1.861	Leu339 (Hydrogen bond) His475 (Hydrogen bond)	12.308
Formononetin	-8.67	1.793	Leu339 (Hydrogen bond) Gly472 (Hydrogen bond) His475 (Unfavorable bump)	12.263
Equol	-7.1	1.330	Leu339 (Hydrogen bond) Gly472 (Hydrogen bond)	10.160

Toxicity test

Toxicity experiments were carried out on drugs with agonist interactions to the ERα and ERβ utilizing the ProTox II. Toxicity testing is classified into several types. To predict the toxicity level of the substances, the toxicity class of LD_{50} is applied. According to the Globally Harmonized System of Classification and Labelling of Chemicals (GHS), six toxicity classes exist. Class I (LD_{50} ≤5 mg/kg) is deadly if swallowed, class II (5 <LD_{50} ≤50 mg/kg) is fatal if swallowed, class III (50 <LD_{50} ≤300

mg/kg) is toxic if eaten, class IV (300 <LD_{50} ≤2000 mg/kg) is harmful if swallowed, class V (2000 <LD_{50} ≤5000 mg/kg) is hazardous if swallowed, and class VI (LD_{50} >5000 mg/kg) is non-toxic[34]. The higher the LD_{50} value, the less dangerous the substance is to the body, and vice versa[35]. The results showed that three chemicals were in classes IV and V, which means they were not very dangerous.

The pkCSM was employed to estimate the values of hepatoxicity, skin sensitization, and Ames toxicity, whereas the ProTox II was used to forecast the toxicity class of compound LD_{50}. Hepatotoxicity is one type of toxicity used to discover hazardous substances in the liver[36]. Skin sensitization is a hypersensitivity reaction triggered by reactive substances that penetrate the stratum corneum layer of the skin[37]. Ames toxicity is used to determine various chemicals' mutagenic and carcinogenic potency[38]. **Table IV** shows that equol compounds are non-toxic for hepatotoxicity, cutaneous sensitization, and Ames toxicity. The findings of the toxicity test indicate that the equol chemical is not harmful.

Table IV. Toxicity test results for daidzein, formononetin and equol.

Compound name	Hepatotoxicity*	Skin sensitization*	Ames toxicity*	LD_{50} (GHS class)**
Daidzein	No	No	Yes	IV
Formononetin	No	No	Yes	V
Equol	No	No	No	IV

* pkCSM; ** ProTox II

The findings of this study reveal that equol molecules can replace estrogen in the body in neurodegenerative disorders. Equol (4',7 isoflavandiol) is a metabolite of daidzein that is an isoflavone derivative. Equol ($C_{15}H_{14}O_3$) is a non-polar isoflavones phenolic chemical that may be responsible for its physiological activity[39-41]. Equol has an asymmetric carbon atom at the C3 position, which gives rise to the *R*(-)- and *S*(-)-equol enantiomers. Equol is more stable, easier to absorb, and has a lower clearance than daidzein, its precursor molecule. It is also more estrogenic than other isoflavones or isoflavone-derived metabolites[40,42]. This equol molecule exhibits estrogenic action similar to 17β-estradiol and works via the ER-dependent pathway by binding to ERα and ERβ. ERα and ERβ are membrane-associated proteins found in synaptic terminals, dendritic spines, dendritic shafts, axons, and glial cell processes[43,44].

Neuroinflammation is the most common cause of neurodegenerative disorders[45-47]. These phytoestrogen chemicals will bind to the ER and substitute estrogen, inhibiting neuroinflammation via four mechanisms: (1) suppression of IK activation; (2) inhibition of IB phosphorylation; (3) direct inhibition of NF-κB activation; and (4) induction of HLA-B27 gene downregulation. This causes the expression of MHC II to go down and Arg1 to go up. This prevents neuroinflammation and neuronal cell death through necrosis or apoptosis[48].

This NF-κB activation causes M1 polarity in microglia cells and the production of inflammatory cytokines (TNF, IL-1, IL-6, and others)[49-52]. This increased synthesis of inflammatory cytokines will impact the production of brain-plasticity-related molecules such as brain-derived neurotrophic factor (BDNF) and glial cell line-derived neurotrophic factor (GDNF)[53,54]. This disease causes mitochondrial malfunction and apoptosis of dopaminergic neuron cells through mediating mitogen-activated protein kinase (MAPK)/extracellular signal-regulation kinase (ERK) signaling[55].

CONCLUSION

Equol has estrogenic effects like 17β-estradiol and works by binding to ERα and ERβ, which is part of the ER-dependent pathway.

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AUTHORS' CONTRIBUTION

All authors have an equal contribution to carrying out this study.

DATA AVAILABILITY

None.

CONFLICT OF INTEREST

The authors declare there is no conflict of interest in this research.

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