

Original Research Article

Molecular mechanism of isoflavone aglycoside compounds in suppressing endometriosis progression

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Abstract

Purpose: To assess the potential therapeutic efficacy of isoflavone aglycones in suppressing endometriosis progression through multi-pathway targeting.

Methods: Using molecular docking simulations, the interactions between genistein, daidzein, and glycitein, with estrogen receptor α (ER α), estrogen receptor β (ER β), Tumor Necrosis Factor- α receptor (TNF- α), Nuclear Factor-kappa Beta receptor (NF- κ B), and prostaglandin EP3 receptor were analyzed and compared to dienogest, a standard treatment.

Results: Results indicated that genistein and daidzein showed strong binding affinities to estrogen receptor β , with scores of -8.7 kcal/mol, surpassing dienogest (-8.5 kcal/mol). Glycitein exhibited the highest among other isoflavone aglycones binding affinity to the prostaglandin EP3 receptor (-9.2 kcal/mol), suggesting its potential in reducing inflammation and proliferation in endometriosis. Additionally, glycitein demonstrated the strongest interaction with NF- κ B (-6.4 kcal/mol), a key regulator of inflammation and cell survival in endometriosis.

Conclusion: The findings suggest that isoflavone aglycones, particularly genistein, daidzein, and glycitein, could serve as effective therapeutic agents for endometriosis by targeting multiple pathways involved in the pathogenesis of endometriosis. Further clinical studies are needed to validate their efficacy and safety in treating endometriosis while preserving reproductive function.

Keywords: Endometriosis, Genistein, Daidzein, Glycitein, Docking, In silico, Estrogen receptor

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INTRODUCTION

Endometriosis is a condition that occurs when tissue resembling the endometrium grows outside the uterine cavity and it affects more than 10% of women of reproductive age worldwide. Most patients with endometriosis experience pain in the pelvic region and face issues related to infertility [1]. The condition tends to improve after menopause in patients with endometriosis, indicating the role of estrogen hormones in the

pathogenesis of endometriosis. Estrogen interacts with all estrogen receptors α and estrogen receptors β in the body [2,3]. Estrogen receptor β plays a significant role in the local aromatization of endogenous estrogen by endometriosis cells, which is why estrogen receptor β is found in significant quantities in patients with endometriosis [4]. Higher levels of local estrogen produced by the endometriosis lesions are associated with inflammatory events, such as the release of interleukin-6 (IL-6),

interleukin-8 (IL-8), Cyclooxygenase-2 (COX2), and Prostaglandin E2 (PGE2). These various pro-inflammatory cytokines make endometriosis cells more resistant and able to survive the elimination process by the body's immune system [5].

The current recommended management of endometriosis includes gonadotropin agonists, contraceptive pills, and progestogen preparations such as dienogest. These treatment options cause several side effects, such as mood changes, weight gain, headaches, fatigue, bone density loss, and anovulation related to the suppression of ovulation and follicle formation, which can make it difficult for patients to conceive [1,6]. Therefore, better treatment is needed that can suppress endometriosis lesions while maintaining reproductive function. Isoflavones are nonsteroidal compounds derived from plants and have been shown to have estrogenic activity. These compounds share a similar structure with endogenous estradiol (E2), enabling isoflavones to bind to estrogen receptors and produce effects similar to estrogen hormones [7].

Previous studies have found that isoflavone compounds can reduce the risk of breast cancer, prostate cancer, and colorectal cancer, as well as improve the quality of life for menopausal women [8]. Isoflavones are primarily found in soybean plants and are classified into two types based on their glucose bonds when consumed: glycosides and aglycone isoflavones. Aglycone isoflavones are classified into 3 based on their chemical structures (genistein, daidzein, and glycitein) [9]. Aglycone isoflavones can be absorbed more quickly by the intestines without undergoing degradation by bacteria. Genistein, daidzein, and glycitein are aglycone isoflavones that have the ability to form strong molecular bonds with estrogen receptor β compared to estrogen receptor α . According to Sutrisno *et al* in 2024 [10], genistein works by inhibiting vascular endothelial growth factor A (VEGF-A), the estrogen receptor β - α ratio, NF- κ B, and prostaglandin receptors. In contrast, daidzein works by inhibiting the growth of endometriosis cells through the mechanism of inhibiting estrogen receptor β , IL-6, IL-8, COX2, and aromatase. Additionally, daidzein is believed to inhibit Tumor Necrosis Factor- α (TNF- α) induced by I κ B phosphorylation [7, 10]. Upon further examination, the potential of isoflavone aglycones as anti-endometriosis therapy is significant; however, existing studies has not yet comprehensively explained the mechanisms of interaction between genistein, daidzein, and glycitein with estrogen receptor α , estrogen

receptor β , prostaglandin EP3 receptor, TNF- α receptor, and NF- κ B receptor when compared to dienogest, the currently widely used therapy.

METHODS

Ligand preparation

Aglycone isoflavones are classified into 3 based on their chemical structures, which can be categorized into genistein, daidzein, and glycitein, which can be found in processed soy foods such as tofu, tempeh, and soy milk. Dienogest is a synthetic progestogen preparation that is currently the definitive treatment for endometriosis. The structures of genistein, daidzein, glycitein, and dienogest were obtained from the PubChem website (<https://pubchem.ncbi.nlm.nih.gov/>; Figure 1). The ligands: genistein, daidzein, glycitein, and dienogest were prepared using the USCF Chimera 1.14 program, displaying their 3D structures. The 3D structures were saved in PDB format.

Protein preparation

The 3D structures of estrogen receptor α (PDB ID: 1SJO), estrogen receptor β (PDB: 5TOA), TNF- α receptor (2PDB AZ5), NF- κ B receptor (2PDB AZ5), and prostaglandin EP3 receptor (PDB: 6AK3) were obtained from the RCSB PDB data center (<http://www.rcsb.org/>; Figure 2). These structures represent receptor structures with ligand-binding sites. The proteins were deposited on October 2, 2020, and published on November 18, 2020. The protein was determined using X-ray diffraction at a resolution of 1.83 Å. The protein was prepared by selecting the A chain group and blocking the other ligands. The molecular docking file archive was saved in *.pdb format. The protein was prepared using the USCF Chimera 1.14 program.

Molecular docking

Molecular docking was performed using the website <https://cadd.labshare.cn/cb-dock2/php/blinddock.php>. The docking results were visualized using the Discovery Studio Visualizer program (<http://discover.3ds.com/discovery-studio-visualizer-download>). The binding energy is expressed as a Vina score, and ligand interactions with amino acids are visualized in 3D and 2D using BioVia Discovery Studio 2024. These compounds were then created in 3D form and stored as ligands in the PDB (Protein Data Bank).

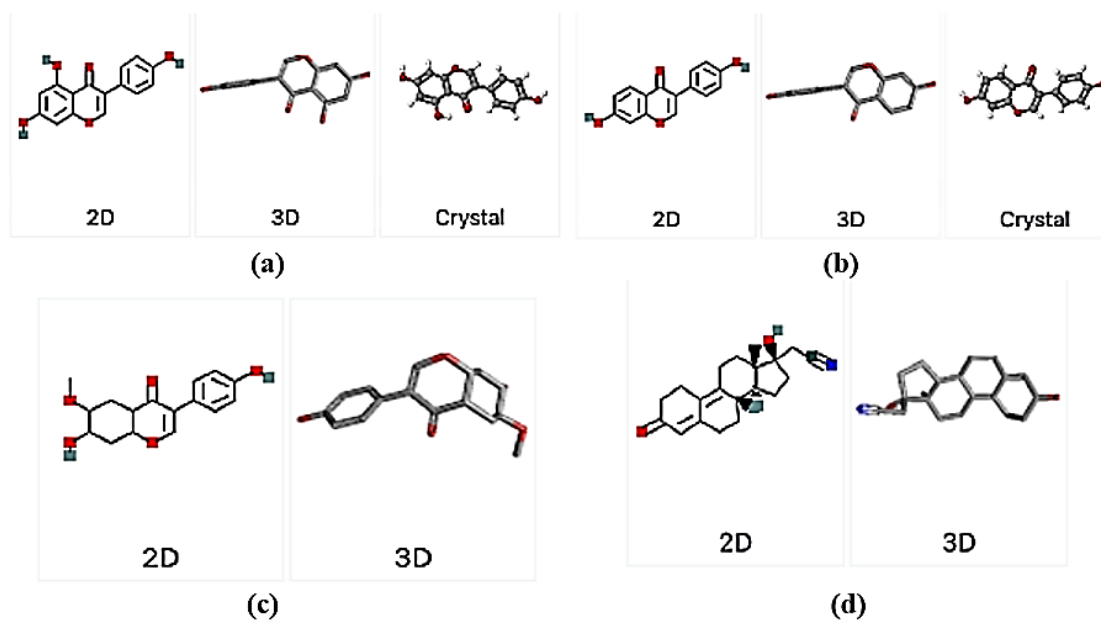


Figure 1: Structure of compounds (a) genistein (CID 5280961), (b) daidzein (CID 5281708), (c) glycitein (CID 73172674), (d) dienogest (CID 68861; PubChem: <https://pubchem.ncbi.nlm.nih.gov/>)

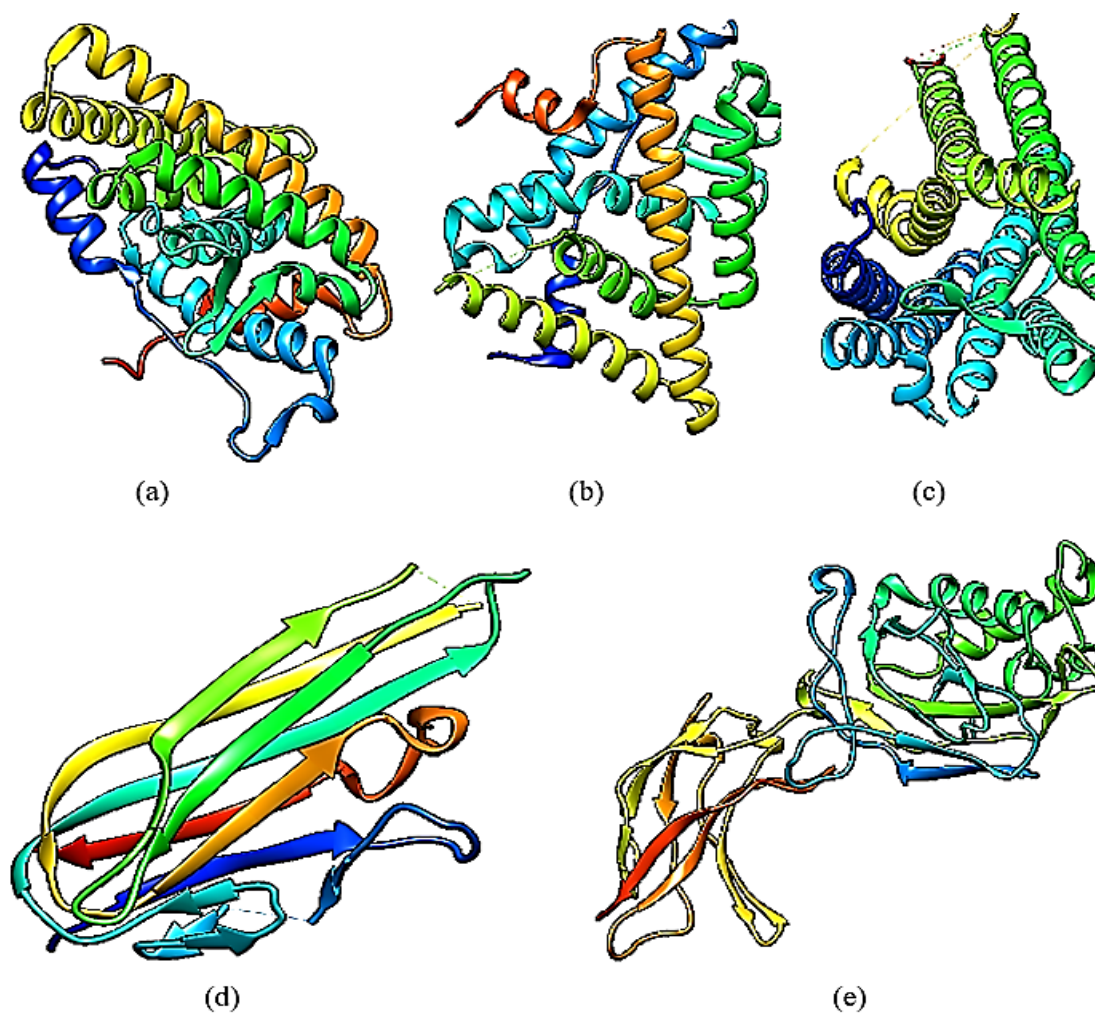


Figure 2: 3D structure of proteins: (a) estrogen receptor α , (b) estrogen receptor β , (c) prostaglandin EP3 receptor, (d) TNF- α receptor, (e) NF- κ B receptor

Proteins or receptors involved in endometriosis pathogenesis were tested against the ligands: genistein, daidzein, glycitein, and dienogest as control. These proteins or receptors include the TNF- α receptor, NF- κ B receptor, estrogen receptor α , estrogen receptor β , and prostaglandin EP3 receptor. The ligands and proteins were tested using the Autodock Vina scoring method [11, 12].

Statistical analysis

The binding affinity (Vina score) data obtained from the molecular docking simulations for each ligand (genistein, daidzein, glycitein, dienogest) against each target receptor (ER α , ER β , TNF- α , NF- κ B, prostaglandin EP3) were organized and analyzed using IBM SPSS Statistics version 29. Descriptive statistics were calculated for all ligand-receptor pairs and are presented as mean $\pm$ standard deviation (SD). The normality of the data distribution was confirmed using the Shapiro-Wilk test. To determine the statistical significance of the differences in binding affinities between the isoflavone aglycones and the control drug (dienogest) for each receptor, a one-way Analysis of Variance (ANOVA) was performed. This was followed by a post-hoc Tukey's Honestly Significant Difference (HSD) test for pairwise comparisons. A p-value of less than 0.05 ($p < 0.05$) was considered statistically significant.

RESULTS

Molecular docking using the SwissDock web server (<http://www.swissdock.ch/>) was used to predict molecular interactions between target proteins and ligands. Docking was performed between the target protein (receptor) and the ligands: genistein, daidzein, glycitein, and dienogest. The target proteins/receptors used include estrogen receptor α , estrogen receptor β , TNF- α receptor, NF- κ B receptor, and prostaglandin EP3 receptor. The results obtained were Vina scores and interactions between ligands and receptor protein amino acids. Molecular interactions between ligands and receptors were electrostatic, hydrophobic, and molecular bonds that contributed to the energy result (ΔG). Additionally, van der Waals forces were observed in all ligand-receptor interactions. The Vina scores for the interactions between genistein, daidzein, glycitein, and dienogest with the estrogen receptor α , estrogen receptor β , prostaglandin EP3 receptor, TNF- α receptor, and NF- κ B receptor are shown in Table 1.

Table 2 and Table 3 shows the ligand-receptor interactions, with the strongest interactions at the

estrogen receptor α observed for daidzein (Vina score -7.8 kcal/mol), genistein (Vina score -7.6 kcal/mol), and glycitein (Vina score -5.6 kcal/mol), as a comparison, the interaction between the dienogest and the estrogen receptor α yielded a Vina score of -5.9 kcal/mol. In the interaction between the estrogen receptor α and daidzein (Figure 4), van der Waals interactions were observed between the daidzein ligand and the estrogen receptor α at the LEU A:525, LEU A:346, MET A:343, HIS A:524, LEU A:428, MET A:388, and ARG A:394. Interactions between the estrogen receptor α and genistein (Vina score -7.6 kcal/mol) occurred in the O group at GL A:353 via conventional hydrogen bonding. As a comparison, in the dienogest ligand, van der Waals interactions were found in the LEU A:346, LEU A:525, MET A:343, HIS A:524, LEU A:384, GLU A:353, PHE A:404, MET A:388, and LEU A:428 groups. Pi Sigma bonds were observed at LEU A: 387, alkyl and pi-alkyl bonds are found in the ALA A:350, ILE A:424, LEU A:391, and PHE A:425 groups, as shown in Figure 3.

The interaction of the estrogen receptor β was found to be equally strong for daidzein and genistein (Vina score -8.7 kcal/mol). For glycitein, the Vina score was -8.3 kcal/mol, which was lower than that of dienogest with a Vina score of -8.5 kcal/mol. Different interactions were observed in the binding of glycitein to the estrogen receptor β (Figure 5), specifically at the residues GLY A:472, MET A:479, MET A:295, LEU A:490, THR A:229, LEU A:301, ARG A:346, PHE A:356, and MET A:336.

The analysis of binding affinities for the Tumor Necrosis Factor- α (TNF- α) receptor revealed a distinct pattern. The synthetic control drug, dienogest, demonstrated the strongest binding affinity with a Vina score of -6.5 kcal/mol. Among the isoflavone aglycones, genistein showed a very similar and high binding affinity to TNF- α (-6.4 kcal/mol). Statistical analysis ($p = 0.874$) confirmed that the binding potency of genistein was not significantly different from that of dienogest, indicating it is a comparably potent ligand for this receptor. In contrast, the binding affinities of glycitein (-5.7 kcal/mol) and daidzein (-5.6 kcal/mol) for the TNF- α receptor were notably weaker than both dienogest and genistein. TNF- α & dienogest interactions (Figure 6) occur via van der Waals interactions in the ASP A:45, ASN A: 46, GLU A:135, LEU A:26, ARG A:138, ASN A:137, GLN A:25, and GLY A:24. The results for the NF- κ B receptor indicate that glycitein had the strongest binding affinity among all tested ligands, with a Vina score of -6.4 kcal/mol. This binding was statistically significantly stronger ($p < 0.05$) than that of the

control drug, dienogest (-5.9 kcal/mol). The binding affinities for the Prostaglandin EP3 receptor revealed a dramatic and highly significant superiority of all three isoflavone aglycones over the standard treatment, dienogest. Glycitein exhibited the strongest binding of any ligand-receptor pair in the entire study, with an exceptional Vina score of -9.2 kcal/mol. It was closely followed by daidzein (-8.9 kcal/mol) and genistein (-8.6 kcal/mol). In stark contrast, the binding affinity of dienogest for the EP3 receptor was markedly weaker (-5.5 kcal/mol). The difference between each isoflavone and dienogest was highly statistically significant ($p < 0.001$).

DISCUSSION

Endometriosis is a common condition among women of reproductive age and is one of the leading causes of reduced quality of life for those affected. Endometriosis is estimated to affect 6 – 10 % of women of reproductive age, with over 50% of these women reporting pelvic pain and infertility issues [1]. Endometriosis is closely associated with estrogen hormone activity and prostaglandin compounds mediated by estrogen receptor α , estrogen receptor β , and prostaglandin receptors. Additionally, endometriosis is associated with chronic inflammation mediated by TNF- α , and endometriosis has the ability to proliferate and survive cells through NF- κ B mediation [13]. The results of this study indicate that most ligands have very strong interactions when bound to estrogen receptor β . Hariadi *et al* in 2018 [14] found that the compound genistein can strongly bind to estrogen receptor β (-213.62 kJ/mol). In this study, it was found that genistein and daidzein (Vina score -8.7 kcal/mol) interact more

with estrogen receptor β compared to dienogest (Vina score -8.5 kcal/mol).

A study conducted by Takaoka *et al* [7] found that daidzein-rich isoflavone aglycones (DRIA) inhibit the proliferation of ovarian endometriosis. In addition, DRIA can also inhibit the formation of endometriosis lesions in a mouse model of endometriosis. Mori *et al* [5] also stated that isoflavone compounds have partial agonist activity toward estrogen receptors, and supplementation with DRIA can suppress the production of proinflammatory cytokines and the expression of aromatase enzymes in endometriosis.

A literature review conducted by Pazzini *et al* [15] on the relationship between diet and endometriosis stated that phytoestrogen compounds (genistein and daidzein) found to be elevated in urine were associated with a reduced risk of severe endometriosis, with an adjusted odds ratio of 0.21 for genistein and 0.29 for daidzein. Genistein, daidzein, and glycitein have a diphenol structure that is stereochemically similar to endogenous estrogen (E2). Genistein, daidzein, and glycitein are phenolic compounds and phytoestrogens with potential antioxidant properties. Phenolic compounds can alter lipid peroxidation kinetics, thereby stabilizing cell membranes and preventing the diffusion of free radical compounds [16].

Lecomte *et al* [17] explain that phytoestrogen compounds can act similarly to selective estrogen receptor modulators (SERMs), which have the potential for dual effects, namely weak estrogenic or antiestrogenic properties and antiproliferative capabilities. Genistein and daidzein can also bind to the TNF- α receptor.

Table 1: Vina Scores for Ligand-Receptor Interactions (results are Mean $\pm$ SD)

Ligand/Receptor	Genistein (CID: 5280961)	Daidzein (CID: 528708)	Glycitein (CID: 5317750)	Dienogest (Control; CID: 68861)	P-value
Estrogen receptor α (1SJO)	-7.6 kcal/mol	-7.8 kcal/mol	-5.6 kcal/mol	-5.9 kcal/mol	<0.001*
Estrogen receptor β (5TOA)	-8.7 kcal/mol	-8.7 kcal/mol	-8.3 kcal/mol	-8.5 kcal/mol	0.012*
TNF- α receptor (2AZ5)	-6.4 kcal/mol	-5.6 kcal/mol	-5.7 kcal/mol	-6.5 kcal/mol	0.874
NF- κ B receptor (2AZ5)	-6.3 kcal/mol	-5.8 kcal/mol	-6.4 kcal/mol	-5.9 kcal/mol	0.021*
Prostaglandin EP3 receptor (6AK3)	-8.6 kcal/mol	-8.9 kcal/mol	-9.2 kcal/mol	-5.5 kcal/mol	<0.001*

* $p < 0.05$ vs Dienogest

Table 2: Molecular docking of ligands with receptor proteins

Ligand	Receptor proteins	Interactions
Genistein	Estrogen α	Gugus O $\rightarrow$ GL A:353 (ikatan hidrogen konvensional); LEU A:384, LEU A:346, LEU A:525, MET A:343, HIS A:524, PHE A:425, LEU A:428, MET A:388, dan ARG A:394 (van der Waals); LEU A:387 (Pi-Sigma), PHE A:404 (Pi-Pi T-shaped), ILE A:424, MET A:421, LEU A:391, dan ALA A:350 (Pi-Alkyl)
	Estrogen β	Gugus O $\rightarrow$ GLU A:353(ikatan hidrogen konvensional); LEU A:525, MET A:343, HIS A:524, PHE A:425, LEU A:428, LEU A:346, LEU A:384, ARG A:394, dan MET A:388 (van der Waals); LEU A:387 (Pi Sigma), ALA A:350, LEU A:391, ILE A:424, dan MET A:421 (Pi-Alkyl). PHE A:404 (Pi-Pi T-shaped)
	TNF α	Gugus O $\rightarrow$ THR A:107, TYR A:114, dan THR A:206 (ikatan hidrogen konvensional). LEU A:59, GLY A:102, GLN A:103, THR A:106, PHE A:133, LEU A:329, MET A:58, ARG A:333, dan SER A:51 (van der Waals). MET A:137 (Pi Sulfur), TRP A:207 (Pi-Pi T-shaped); PRO A:55 (Pi-Alkyl)
	NF- κ B	THR P:102, GLN P:204, HIS P:108, GLY P:104, GLU P:207, VAL P:212, dan TYR P:241 (van der Waals); ASP P:209(<i>attractive charge</i>); MET P:208 (Pi-Sulfur), ASN P:103 (Amide-Pi Stacked)
	Prostaglandin EP3	Gugus O $\rightarrow$ TYR A:114, THR A:206 dan THR A:107 (ikatan hidrogen konvensional); LEU A:59, GLY A:102, GLN A:103, THR A:106, SER A:336, PHE A:133, LEU A:329, ARG A:333, MET A:58, dan SER A:51 (van der Waals). VAL A:110 (Pi-Sigma). MET A:137 (Pi-Sulfur). TRP A:207 (Pi-Pi T-Shaped); PRO A:55 (Pi-Alkyl)
Daidzein	Estrogen α	LEU A:525, LEU A:346, MET A:343, HIS A:524, LEU A:428, MET A:388, ARG A:394 (van der Waals), GLU A:353(ikatan hidrogen), LEU A:387 (Pi-Sigma), ILE A:424, LEU A:391, ALA A:350 dan MET A:42 (Pi-Alkyl), PHE A:404 (Pi-Pi T-shaped)
	Estrogen β	ILE A:376, GIY A:472, MET A:295, ILE A:373, LEU A:380, MET A:340, MET A:479, LEU A:490, THR A:299, MET A:336, LEU A:301, GLU A:305, dan ARG A:346 (van der Waals), LEU A:339 (ikatan konvensional hidrogen), LEU A:298 (ikatan senyawa karbon-hidrogen), PHE A:356 (Pi-Pi T-shaped), LEU A:343 (Pi Alkyl)
	TNF α	GLN A:25, ILE A:136, PRO A:139, LEU A:26, SER A:133, dan ILE A:83 (van der Waals), ASN A:46, ASP A:45, dan GLN A:47(ikatan hidrogen konvensional), GLU A:135(<i>attractive charge</i> & Pi-Anion)
	NF- κ B	VAL P:61, TYR P:60, GLY P:64, ARG P:59, HIS P:67, GLY P:68, PHE P:56, ILE P:142, GLY P:141, dan ARG P:57 (van der Waals)
	Prostaglandin EP3	GLY A:102, VAL A:332, PHE A:133, TRP A:207, GLN A:103, MET A:58, ARG A:333, TYR A:114, dan LEY A:329 (van der Waals), THR A:107 dan THR A:206 (ikatan hidrogen konvensional), SER A:336 (Pi-Donor ikatan hidrogen), VAL A:110 dan THR A:106 (Pi-Sigma), PRO A:55 (Pi-Alkyl)
Glycitein	Estrogen α	GLU A:353, LEU A:349, PHE A:404, ILE A:424, MET A:421, HIS A:524, MET A:343 (van der Waals), ARG A:394 (<i>unfavourable Donor-Donor</i>), ALA A:350, LEU A:387, LEU A:391, MET A:388, LEU A:384, LEU A:525 (Alkyl)
	Estrogen β	GLY A:472, MET A:479, MET A:295, LEU A:490, THR A:229, LEU A:301, ARG A:346, PHE A:356, MET A:336, MET A:340 (van der Waals), HIS A:475 dan GLU A:305 (ikatan hidrogen konvensional), LEU A:339 (Pi-Sigma), ILE A:376, LEU A:476, LEU A:343, ALA A:302, ILE A:373 (Alkyl & Pi-Alkyl)
	TNF α	LEU A:29, ASN A:30, GLN A:27, ARG A:44, ASN A:46, dan ASP A:45 (van der Waals), TRP A:28 (ikatan hidrogen konvensional), LEU A:43 (Pi-Sigma)
	NF- κ B	HIS P:67, GLY P:68, ARG P:57, PHE P:56, ILE P:142, ARG P:59, GLY P:141, GLY P:64, TYR P:60 (van der Waals), VAL P:61 (ikatan hidrogen), VAL P:115 (Pi-Sigma), LEU P:143 dan PRO P:65 (Alkyl & Pi-Alkyl)
	Prostaglandin EP3	GLN A:339, GLY A:102, GL A:103, MET A:58, ARG A:333, LEU A:329, TYR A:114, PRO A:204, PHE A:133, VAL A:332, SER A:336 (van der Waals), THR A:107 (ikatan hidrogen konvensional), THR A:106 dan VAL A:110 (Pi-Sigma), MET A:137 dan PRO A:55 (Alkyl & Pi-Alkyl), TRP A:207 (Pi-Pi T-shaped)

Table 3: Molecular docking of ligands with receptor proteins (*continued*)

Ligand	Receptor proteins	Interactions
Dienogest	Estrogen α	LEU A:346, LEU A:525, MET A:343, HIS A:524, LEU A:384, GLU A:353, PHE A:404, MET A:388, dan LEU A:428 (van der Waals),
	Estrogen β	LEU A:380, GLY A:472, MET A:479, MET A:295, THR A:299, LEU A:490, LEU A:301, ARG A:346, PHE A:356, MET A:336, dan MET A:340 (van der Waals), GLU A:305 dan HIS A:475 (ikatan hidrogen konvensional), LEU A:339 (Pi-Sigma), ILE A:376, ILE A:373, LEU A:476, ALA A:302, LEU A:298, LEU A:343 (Alkyl & Pi-Alkyl)
	TNF α	ASP A:45, ASN A:46, GLU A:135, LEU A:26, ARG A:138, ASN A:137, GLN A:25, dan GLY A:24 (van der Waals), ILE A:136 (ikatan hidrogen konvensional), PRO A:139 (Alkyl)
	NF- κ B	ASN P:139, LYS P:80, GLY P:69, PRO P:71, PHE P:56, GLY P:68, HIS P:67, dan ARG P:57 (van der Waals), TYR P:82 dan ARG P:59 (ikatan hidrogen konvensional)
	Prostaglandin EP3	VAL A:110, THR A:107, PRO A:55, MET A:58, ARG A:333, GLN A:103, SER A:336, ALA A:335, TRP A:295, PHE A:140, dan GLY A:102 (van der Waals), THR A:106, GLN A:339 dan GLY A:141(<i>unvafourable bump</i>), MET A:137 (Alkyl)

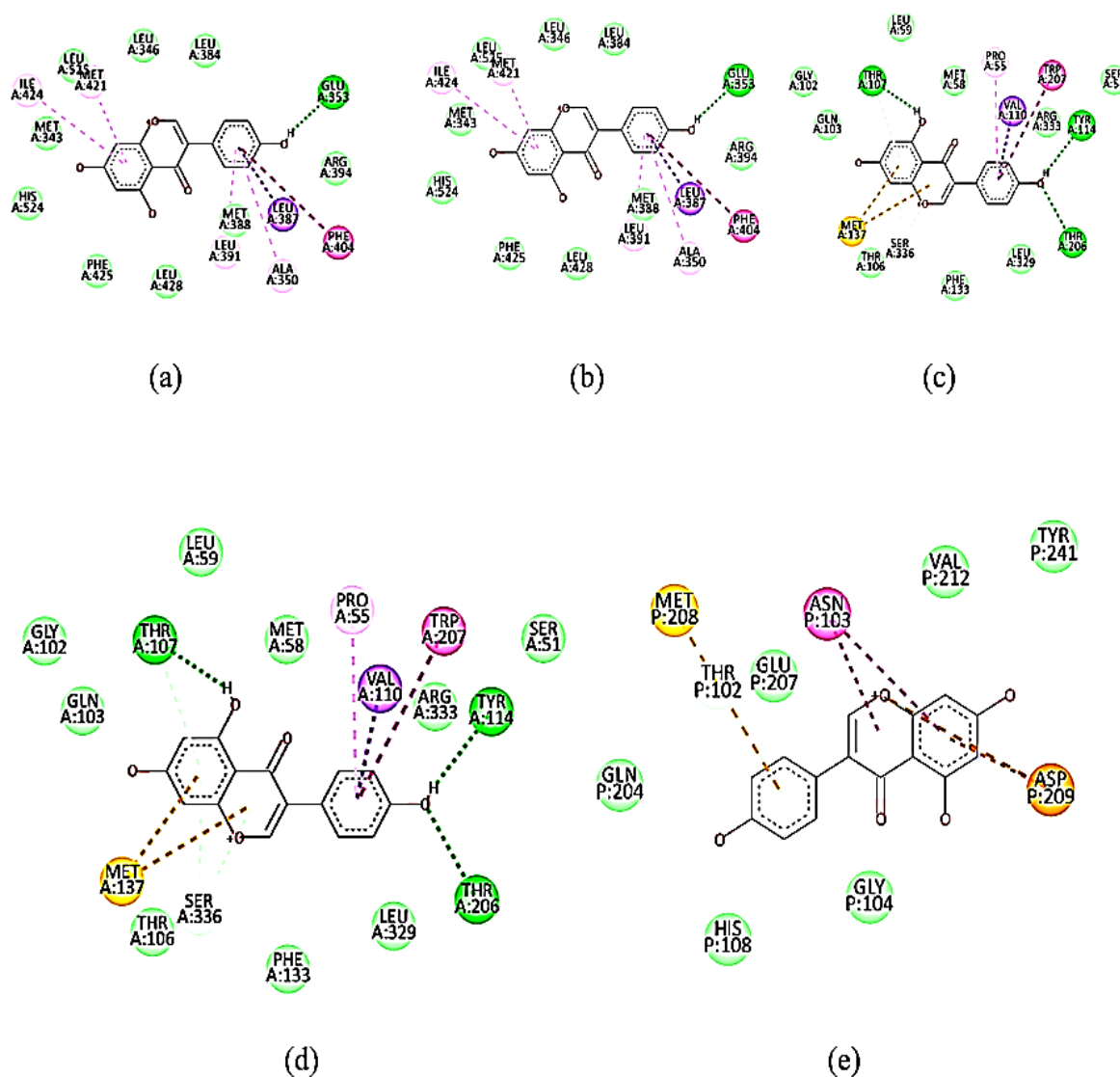
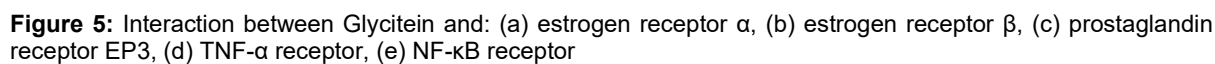
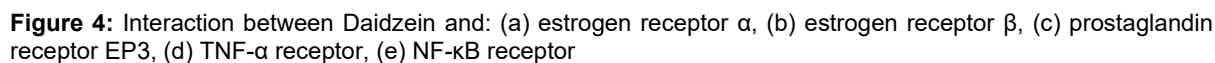


Figure 3: Interaction between Genistein and: (a) estrogen receptor α , (b) estrogen receptor β , (c) prostaglandin receptor EP3, (d) TNF- α receptor, (e) NF- κ B receptor



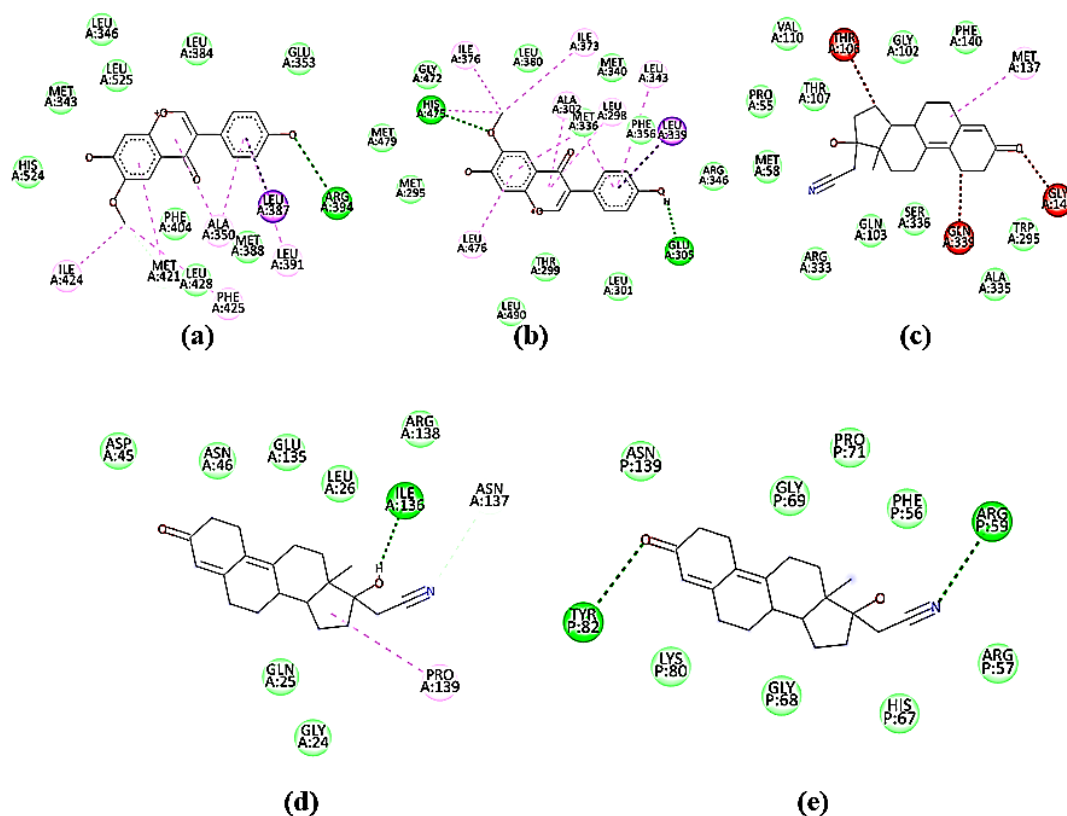


Figure 6: Interaction between Dienogest and: (a) estrogen receptor α , (b) estrogen receptor β , (c) prostaglandin EP3 receptor, (d) TNF- α receptor, (e) NF- κ B receptor

In a study conducted by Bao *et al* [18], it was found that exposure to daidzein can inhibit TNF- α , which induces cellular migration and invasion of breast cancer cells without estrogen receptors. In this study, the potential of genistein (Vina score -6.4 kcal/mol) to interact with the TNF- α receptor was found to be nearly identical to that of dienogest (Vina score -6.5 kcal/mol).

In this study, glycitein (Vina score -6.4 kcal/mol) amongst the ligands was found to have the strongest interactive potential with the NF- κ B receptor compared to genistein (Vina score -6.3 kcal/mol), daidzein (Vina score -5.8 kcal/mol), and dienogest (Vina score -5.9 kcal/mol). NF- κ B is believed to play a crucial role in the survival and proliferation of ectopic endometriosis cells. In a study by Golabek *et al* [19], it was mentioned that NF- κ B is a transcription factor that plays a key role in the development of endometriosis cells through the activation of anti-apoptotic genes and the induction of endometriosis cell proliferation. Glycitein significantly reduces mitochondrial transmembrane potential ($\Delta\Psi$ m) and increases mitochondria-mediated apoptosis in gastric cancer cells, as well as causing cell cycle arrest in the G0/G1 phase [20]. Glycitein can also activate mitogen-activated protein

kinase (MAPK) and inhibit signal transducer and activator of transcription 3 (STAT3) and NF- κ B [20].

This study found that all isoflavone aglycones studied bind tightly to the prostaglandin EP3 receptor compared to dienogest. The most potent interactive potential was observed with exposure to glycitein, daidzein, and genistein in that order. Glycitein, daidzein, and genistein that bind to the prostaglandin receptor (EP3) can reduce inflammatory levels in patients with endometriosis. The prostaglandin EP3 receptor functions to regulate cell proliferation, migration, and invasion [21].

Limitations of this study

In silico computational prediction studies are based on mathematical calculations using physical theoretical rules, and as such, there are rare chances of obtaining false positive results.

CONCLUSION

Isoflavone aglycone compounds (glycitein, daidzein, and genistein) have the ability to interact strongly with several receptors involved

in the pathogenesis of endometriosis. The results of this study may position these isoflavone aglycone compounds evaluated in this study as potential treatment for endometriosis after undergoing clinical trials and being proven effective in treating the condition.

DECLARATIONS

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Conflict of interest

No conflict of interest is associated with this work.

Contribution of authors

This work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by them. Yosef DC Salan, Aditya Rahman, Iwan D. Putra, Hardyan Sauqi conceptualized the study and Yakob Togar, Sandra Nathasa, Muhammad A. Wicaksono and Eko Suhartono performed the molecular docking simulations.

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