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Effect of Components of Gamma Oryzanol on Toll-Like Receptor 4: Receptor Structure-Based Pharmacophore, Hit Identification, and In Silico Evidence

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ABSTRACT

Introduction: Polycystic ovarian syndrome is a leading cause of female infertility. Inflammation has a central role in infertility. Persistent activation of Toll-like receptor 4 contributes to inflammation in PCOS. Gamma-oryzanol consists of esters of ferulic acid combined with phytosterols and triterpene alcohol derivatives of rice bran oil, and is known to have anti-inflammatory effects. However, the structural interaction of different gamma-oryzanol compounds with TLR4 remains unknown. **Objectives:** The study aimed to investigate gamma oryzanol compounds as hit compounds and inhibitors of Toll-like receptor 4 by developing a pharmacophore model through a receptor structure-based approach coupled with molecular docking studies with the Molecular Operating Environment (MOE) software. **Methods:** A structure-based pharmacophore model was generated from the co-crystalized structure of the TLR4–MD2 complex. Gamma-oryzanol derivatives were evaluated against the constructed pharmacophore model to identify potential hit compounds. The potential hit compounds that satisfied essential pharmacophoric features were subjected to molecular docking with TLR4. **Results:** The pharmacophore consisted of three characteristics: a hydrogen bond donor, a hydrogen bond acceptor, and a hydrophobic. Cycloartenyl ferulate, 24-methylenecycloartenyl ferulate, Campesteryl ferulate, and β-sitosteryl ferulate were found to be the hit compounds against the generated pharmacophore. The docking experiment showed that Cycloartenyl ferulate had the most potent binding interaction with TLR4 (7.9933), followed by 24-methylenecycloartenyl ferulate (-7.8580), Campesteryl ferulate (-6.1675), and β-sitosteryl ferulate (-5.9673). **Conclusion:** The present pharmacophore modeling and docking findings predict that gamma-oryzanol may bind with the TLR4 ligand binding domain, providing structural insights into their therapeutic potential role as a modulator of the TLR4-mediated inflammatory pathway. These findings provide a theoretical foundation for future in vitro and in vivo validation studies aimed at elucidating the mechanistic basis of gamma-oryzanol's anti-inflammatory activity in PCOS.

Keywords: Anti-inflammatory, Gamma oryzanol, Infertility, Polycystic ovarian syndrome, TLR4 antagonist

INTRODUCTION

Polycystic ovarian syndrome (PCOS) is a reproductive disorder that affects 4-20% of women in their childbearing age[1]. It is the primary cause of female infertility[2]. Literature reports that chronic low-grade inflammation is a central player in the pathogenesis of PCOS, and the Toll-like receptor 4 (TLR4) is a well-recognized pattern recognition receptor linked with this inflammation[3,4]. These receptors also have their role in innate immunity, cancer, and tissue homeostatic activities[5] and are expressed in various body tissues, including reproductive organs, where they recognize pathogenic as well as damage-associated patterns[6]. Recently, the therapeutic application of TLR4 inhibitors has caught attention due to their potential in treating various health conditions. Proteins have always been a primary target of interest, as they can bind various ligands, including drugs, as well as phytochemicals. In this context, computer-aided drug design (CADD), such as the generation of 3D structure-based pharmacophore and molecular docking methods, can aid in identifying the potential hit compounds, evaluating their interactions with the target receptor quickly, and developing efficient therapeutics for various health conditions[7]. Moreover, there has been growing interest in exploring the phytochemicals to find a safe anti-inflammatory therapeutic agent[8]. Gamma Oryzanol is a phytochemical derived from brown rice (Oryza sativa L.) with a broader safety profile and no major side effects. It consists of esters of ferulic acid combined with phytosterols and triterpene alcohol derivatives of rice bran and possesses antidiabetic, anti-inflammatory, antioxidant, and immune modulatory properties[9-12]. In a recent study, gamma-oryzanol exhibited TLR4-lowering activity[13,14]. However, which of the gamma oryzanol's main derivatives: β-sitosteryl ferulate, Cycloartenyl ferulate, Campesteryl ferulate, and 24-methylenecycloartanyl ferulate can potentially lower the levels of TLR4 is still unidentified, so the current study was designed to identify the active constituent responsible for the TLR4-reducing effect and the anti-inflammatory action of gamma oryzanol. We performed pharmacophore modeling and molecular docking experiments to investigate the manner of binding of β-sitosteryl ferulate, Cycloartenyl ferulate, Campesteryl ferulate, and 24-methylenecycloartanyl ferulate to TLR4 to explore the key structural attributes responsible for

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the inhibitory mechanism. The results of this study will contribute to a deeper understanding of the molecular basis underlying the inhibitory actions of these compounds and allow more accurate and quicker prediction of their biological activity. Moreover, the study provides significant guidance for the future design of potent and selective TLR4 inhibitors with promising therapeutic efficacy.

MATERIALS AND METHODS

Pharmacophore model generation

The Molecular Operating Environment (MOE) 2019 software was utilized to construct the receptor structure-guided pharmacophore model based on the x-ray crystallographic structure of TLR4/MD2. The pharmacophore query editor tool available in the MOE software was used to recognize different key pharmacophore features essential for binding and biological activity, such as hydrogen bond donor, hydrogen bond acceptor, hydrophobic, aromatic, and pi-interactions, and build a pharmacophore model after exploring the molecular interaction of the bound ligand within TLR4/MD2's active binding site[15,16]. Ten small molecules with known antagonistic activity against TLR4 validated the pharmacophore model. An in-house set of gamma oryzanol compounds was created in mdb. format. Then the in-house database was screened against the generated pharmacophore model, and hit compounds were identified. The threshold for hit identification was based on pharmacophore fit score with RMSD <1 Å and successful mapping of ligands to all pharmacophore features. Goodness of hit scoring was assessed by pharmacophore mapping, RMSD, and rScore.

Molecular Docking

All the hit compounds (CID: 5282164, CID: 9920169, CID: 15056832, CID: 9938436) were retrieved from the PubChem chemical repository in 3-dimensional sdf. format followed by energy minimization and preparation in MOE. The X-ray crystallographic structure of TLR4 was downloaded from the Protein Data Bank (PDB) database in pdb. format. The target protein was prepared by removing water and heteromolecules. The polar hydrogens were added, and energy was minimized following the standard protocol in the Molecular Operating Environment (MOE) 2019 software. After preparing the target protein, the active site was found using the active site finder feature of MOE. Dummy atoms were created and saved as shown in Figure 1. The hit compounds were docked with the active site of TLR4. For Placement, the triangular matcher method was used to generate ten poses for each ligand, and scored by London dG. For the refinement purpose, the induced fit refinement method was used; five poses were generated and scored by GBVI/WSA dG. For each ligand, the pose with the most negative (lowest) GBVI/WSA dG score was selected. The docking pose was validated by re-docking the co-crystallized ligand, achieving a RMSD ≤ 2Å.

Docking Analysis

Docking results were analyzed to evaluate ligand binding to the target protein, the involvement of interacting amino acid residues, and the nature of the binding interactions. MOE was used to analyze the docking results. The docking scores and Root Mean Square Deviation (RMSD) were considered the core parameters to evaluate this purpose. RMSD is an important metric to quantify the deviation of the atomic coordinates of the ligand relative to a reference structure in predicted docking poses. This parameter is essential for evaluating the reliability and precision of the docking results. Generally, a docking conformation with the most negative binding score and a RMSD value less than 2 Å is considered reliable.

RESULTS AND DISCUSSION

We generated a structure-based pharmacophore containing three features: one hydrogen bond donor, one hydrogen bond acceptor, and one hydrophobic feature (Figure 2). The purple ball represents the hydrogen bond donor; the cyan ball represents the hydrogen bond acceptor, and the green ball represents the hydrophobic feature.

The pharmacophore was used as a query to search for the compounds. In the screening of compounds against the generated pharmacophore, four hit compounds were identified with RMSD<1: 24-methylenecycloartenyl ferulate, Cycloartenyl ferulate, Campesteryl ferulate, and β-sitosteryl ferulate. The physicochemical properties of the hit compounds are represented in the Table. 1.

Based on the root mean square distance (RMSD) value <1, the hit compounds 24-methylenecycloartenyl ferulate, cycloartenyl ferulate, campesteryl ferulate, and β-sitosteryl ferulate were docked with TLR4, as shown in Table. 2.

The molecular docking results in Figure 3 and Figure 4 illustrate that the tested compounds were capable of direct binding to TLR4. Among all the ligands, Cycloartenyl ferulate had the most potent interaction with TLR4 (-7.9933) through two hydrogen bond interaction with Asp 395 (distance: 2.94, Energy: -3.7 Kcal/mol), and Gly 123 (distance: 3.51, followed by 24-methylenecycloartenyl ferulate (-7.8580) through two hydrogen bond interactions to Ser 120 (distance: 3.06, Energy: -1.0 Kcal/mol) and Lys 122 (distance: 3.26, Energy: -3.5 Kcal/mol), Campesteryl ferulate (-6.1675) through two hydrogen bonds with Arg 106 (distance: 3.15, Energy: -2.5 Kcal/mol) and Glu 154 (distance: 2.93, Energy: -3.1 Kcal/mol), and β-sitosteryl ferulate(-5.9673) through hydrogen bond interaction with Lys 341 (distance: 3.0, Energy: -1.5 Kcal/mol).

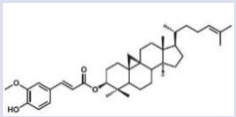
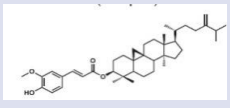
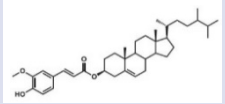
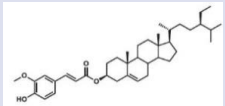
The pharmacophore modeling is a very handy tool for the discovery and development of lead compounds[17,18]. It is the primary step towards predicting the three-dimensional interaction between a receptor and a ligand and extracting the essential features required for their biological activity[19,20]. A structure-based pharmacophore model derived from the 3D structure of a target protein provides valuable insights into protein-

Table 1. Physicochemical properties of ligands

Compounds ID	MW (g/mol)	HBD	HBA	LogP
5282164	602.90	1	4	10.15
9920169	616.9	1	4	10.11
15056832	576.86	1	4	9.61
9938436	590.89	1	4	10.00

MW: Molecular weight; HBD: hydrogen bond donor; HBA: hydrogen bond acceptor

Table 2. Chemical Structure of Hit compounds and Docking scores

Hits	Compounds ID	Structure	RMSD	Docking Score (kcal/mol)
1	5282164		0.6921	-7.9933
2	9920169		0.6921	-7.8580
3	15056832		0.6921	-6.1675
4	9938436		0.6921	-5.9673

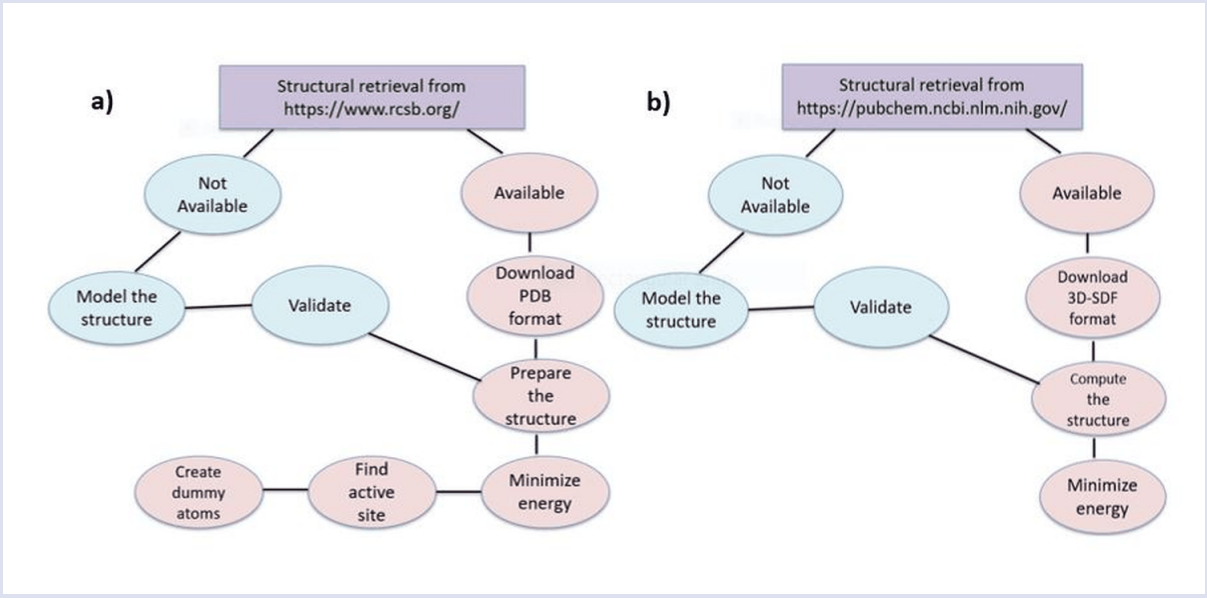


Figure 1. Preparation of structures for molecular docking; a) Preparation of target protein, b) Preparation of ligand

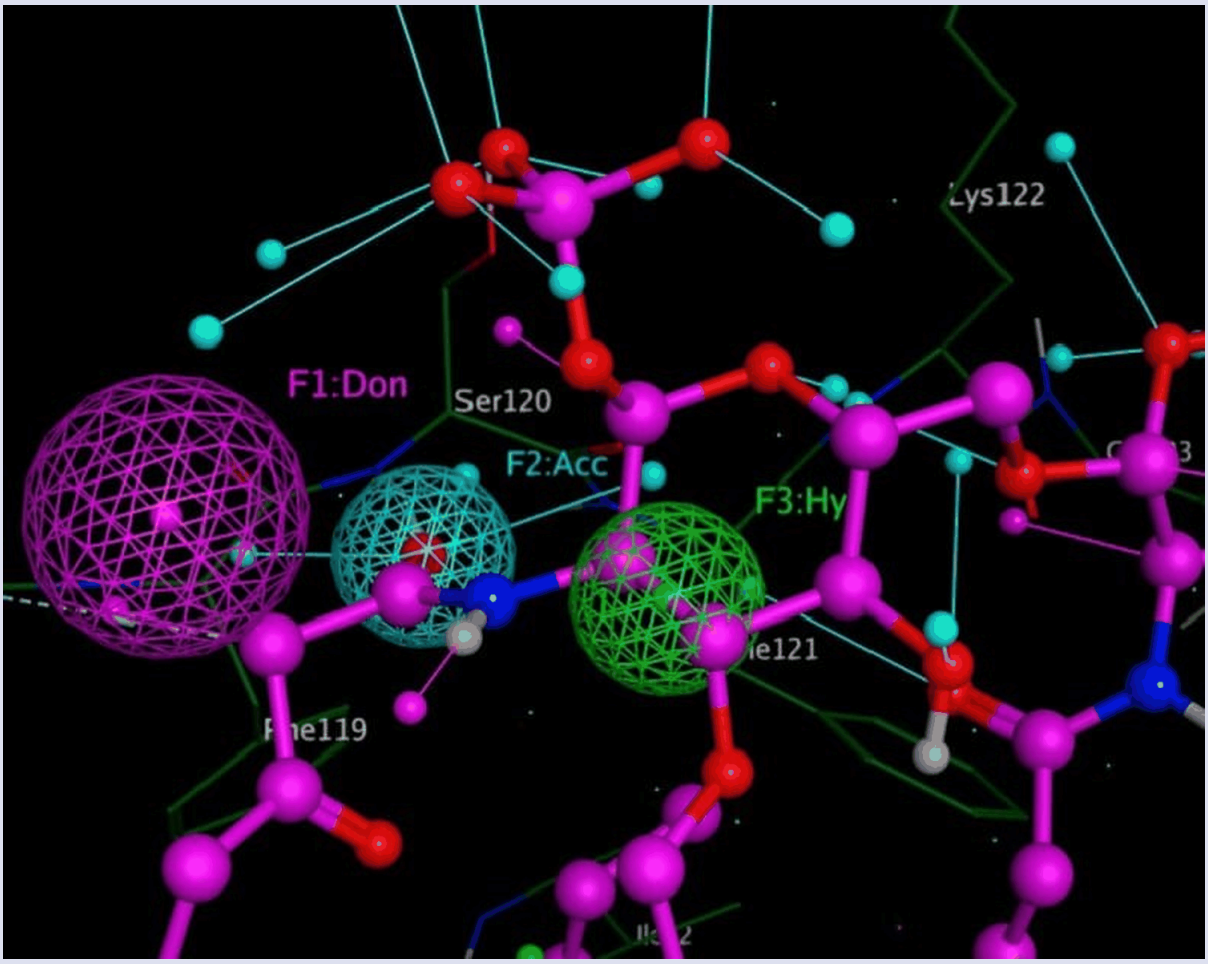


Figure 2. Structure-based pharmacophore model

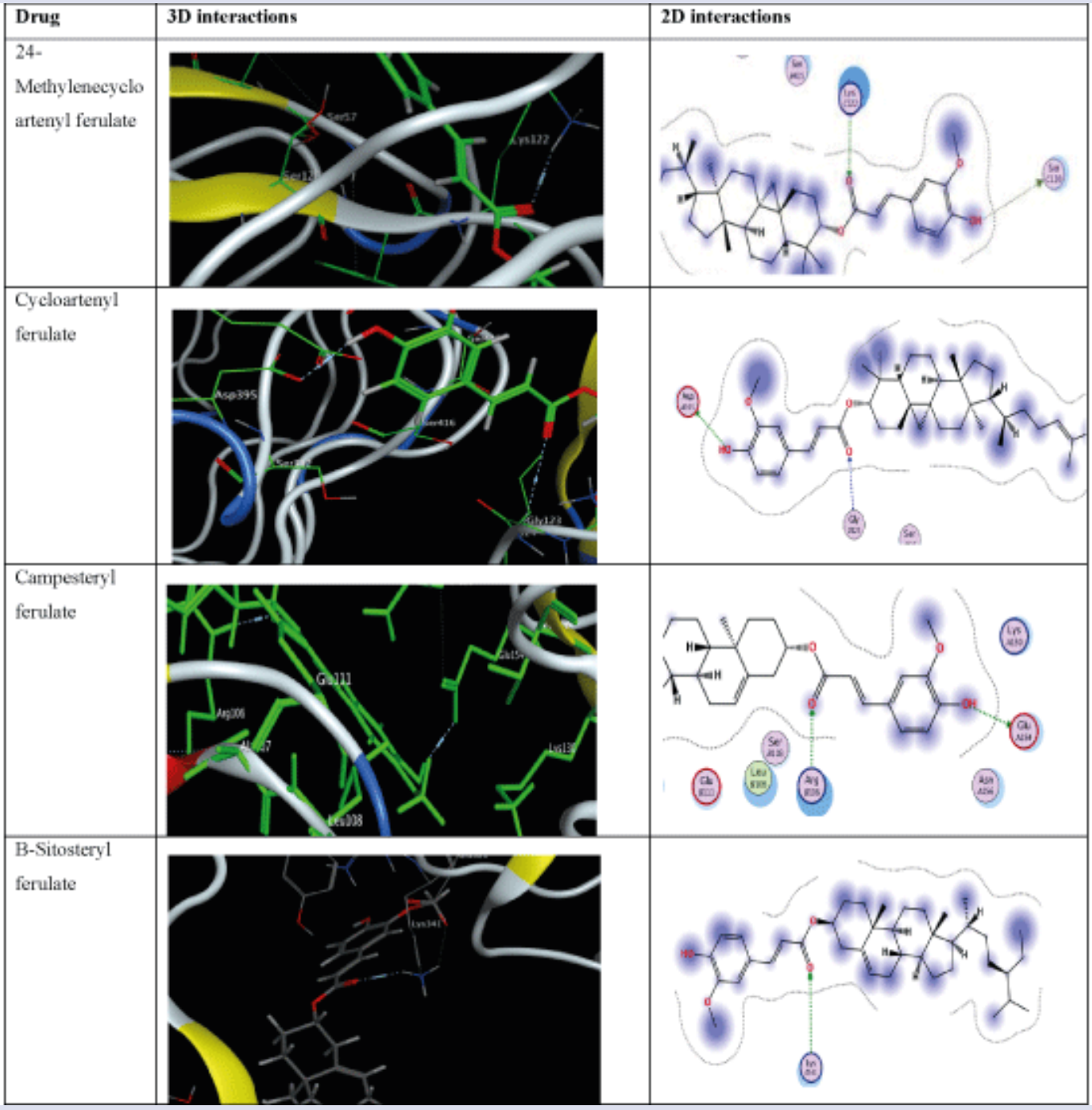


Figure 3. Interactions of ligands with TLR4

ligand interactions and the further development of ligand binding affinity[21,22]. Although the pharmacophore model constructed from already known inhibitors also allows the identification of key chemical features existing in experimentally known potent inhibitors[23-26]. Our study employed the structure-based pharmacophore modeling approach combined with molecular docking to predict the possible inhibitory compounds of TLR4 to set evidence for experimental studies. The pharmacophore modeling showed that the gamma oryzanol compounds fit the features extracted from the interaction of TLR4 with its ligands. The RMSD and rscore were used to identify the best-fit hit compounds. The docking result showed that it can bind with TLR4 to modulate its activity. Cycloartenyl ferulate had the most potent interaction with the TLR4 compared to 24-methylenecycloartenyl ferulate, Campesteryl ferulate, and β-sitosteryl ferulate. Emerging evidence suggests that TLR4 is a primary driver of inflammation in PCOS, leading to reproductive abnormalities, including infertility[27]. Experimental studies show that inhibiting TLR4 can improve various symptoms of PCOS. Thus, targeting the TLR4 pathway by specific phytochemicals and modulators could be a promising avenue to combat multiple abnormalities of PCOS[28,29]. The strong anti-inflammatory properties of gamma oryzanol support its therapeutic potential to suppress inflammation in PCOS. Different in vivo studies report their protective actions in male reproductive disorders and ovarian changes in PCOS[30-32]. To evaluate the potential for oral delivery and systemic exposure, the

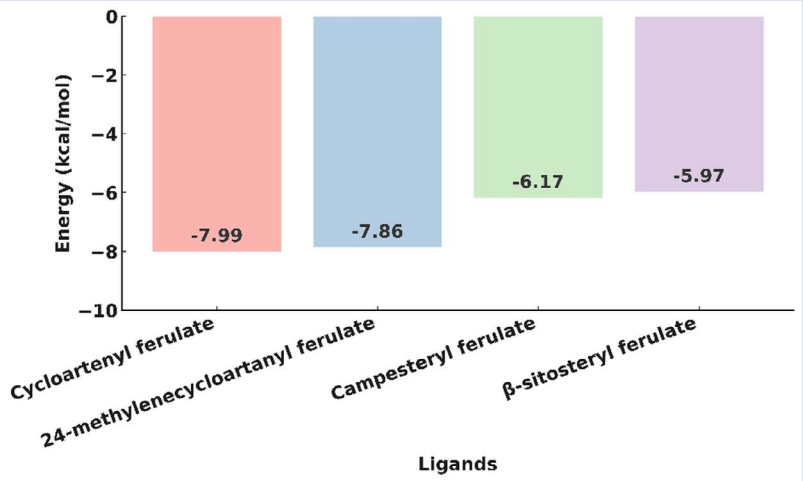


Figure 4. Comparison of binding energies of the ligands

drug-likeness of the primary gamma oryzanol compounds was assessed by Lipinski's Rule of Five (Ro5). It exhibited RO5 violations in terms of molecular weight and lipophilicity, which may contribute to its limited intestinal absorption and systemic bioavailability when administered orally in its native form[33]. However, RO5 mainly applies to synthetic molecules, and notable exceptions exist for natural compounds like gamma oryzanol and particularly for large natural compounds that utilize specific active transport mechanisms[34]. As triterpene ferulate, gamma oryzanol compounds are structurally similar to phytosterols, which are known to be absorbed by active, carrier-mediated transport in the gut[35], rather than depending solely on passive transport predicted by the Ro5, compensating for their limited aqueous solubility. This transport pathway is independent of the molecular size and lipophilicity constraints of Lipinski's rules. Moreover, its antioxidant and lipid-lowering activities have been consistently demonstrated in both in vitro and in vivo studies, supporting its therapeutic potential despite RO5 violations[36]. Its pharmacological activities often involve localized tissue interactions favored by its highly lipophilic nature, which could be particularly beneficial in managing reproductive disorders such as PCOS.

Though the computational analysis predicts its poor passive absorption, the compound's observed biological activities, structural class (phytosterols), and alternate transport mechanisms justify its continuous investigation as a valuable biologically active candidate, particularly when evaluated in the context of natural product-based drug discovery and novel delivery systems[37-40]. Many FDA-approved drugs violate Ro5 but have very good therapeutic potential, including many natural compounds, antibiotics, and anticancer drugs[41,42]. This virtual screening strategy, which integrates pharmacophore modeling, molecular docking, and key ligand target interactions, provides valuable mechanistic insights and can be a time-efficient and cost-effective method before extensive experimental validation. Though the computational modeling seems revolutionary, it has certain inherent limitations. It does not display the complexity of living systems, including the flexible nature of proteins, cellular permeability, the interaction of different proteins, and the effects of solvents. So, sometimes the in silico "hits" with promising binding scores flop and fail to decipher into therapeutic efficacy, warranting experimental validation. So, the subsequent studies employing cell-based assays and inflammatory pathway assessments are warranted to validate the predicted interactions and confirm the potential of gamma-oryzanol as a modulator of TLR4-driven inflammation in PCOS, for its use in future drug development as an anti-inflammatory therapy at a low cost. The integration of pharmacophore modeling with experimental validation could significantly advance safe, natural product-based drug discovery targeting inflammatory and metabolic pathways.

CONCLUSION

The present study was done to find the modulatory activity of gamma oryzanol compounds against TLR4. The structural key features of TLR4 required for its inhibition were extracted by employing the structure-based pharmacophore modeling using the 3-dimensional structure of TLR4. The results suggest that Cycloartenyl ferulate and 24-methylenecycloartenyl ferulate had the highest binding affinity for TLR4 and may serve as structural candidates for further exploration as TLR4 modulators. Though violations of Lipinski's Rule of Five were observed, these parameters are less restrictive for natural compounds, particularly phytosterol derivatives that utilize active transport mechanisms and local tissue absorption. The findings support gamma oryzanol's therapeutic promise, strengthened by its known antioxidant and lipid-modulating activities.

REFERENCES

1. Fahs, D.; Salloum, D.; Nasrallah, M.; Ghazeeri, G. "Polycystic ovary syndrome: pathophysiology and controversies in diagnosis." Diagnostics. 2023;13(9):1-13.
2. Asha Parvathi, V. "A successful management of polycystic ovarian syndrome (PCOS) induced infertility through ayurveda-a case report". World J. Pharm. Res. 2025;14(7): 1207-1215.
3. Abraham Gnanadass, S., Y. Divakar Prabhu, and A. Valsala Gopalakrishnan. "Association of metabolic and inflammatory markers with polycystic ovarian syndrome (PCOS): an update." Arch. Gynecol. Obstet. 2021;303(3):631-43.
4. Liu M.; Guo S.; Li X.; Tian Y.; Yu Y. and et al. "Semaglutide alleviates ovary inflammation via the AMPK/SIRT1/NF- κ B signaling pathway in polycystic ovary syndrome mice." Drug Des. Devel. Ther. 2024;18:3925-3938.
5. Tingstad, R.H.; Norheim, F.; Haugen, F.; Z. Feng Y.; S. Tunsjø H. and et al. The effect of toll-like receptor ligands on energy metabolism and myokine expression and secretion in cultured human skeletal muscle cells. Sci Rep. 2021 Dec 20;11(1):24219.
6. Muñoz-Caro, T.; J. Gibson, A; Conejeros, I.; Werling, D.; Taubert, A.; Hermosilla, C. and et al. The role of TLR2 and TLR4 in recognition and uptake of the apicomplexan parasite Eimeria bovis and their effects on NET formation." Pathogens. 2021 Jan 24;10(2):118.

7. Muchtaridi, M., D. Dermawan, and M. Yusuf. "Molecular docking, 3D structure-based pharmacophore modeling, and ADME prediction of alpha mangostin and its derivatives against estrogen receptor alpha." J Young Pharm. 2018;10(3):252-259.
8. Sabalingam, S. "In-vitro approaches to evaluate the anti-inflammatory potential of phytochemicals: A Review." J. Drug Deliv. 2025 Jan 1;15(1):187-92.
9. Radda, M. I.; Omar, N.; Ahmad, R.; Jalil, R. A.; Ishak, W. R. W., and et al. "Gamma-oryzanol: a novel promising supplement for diabetes mellitus." Univ Med. 2025 Mar 9;44(1):90-100.
10. Ali, M.A.; Chew, S. Efficacy of exogenous natural antioxidants in stability of polyunsaturated oils under frying temperature. J. Food Meas. Charact. 2023 Feb;17(1):408-29.
11. Zeini, S., N. Davoodian and S. A. Mousavi. "Gamma-oryzanol attenuates lipopolysaccharide-induced cognitive impairment by modulation of hippocampal inflammatory response and glial activation in mice." J. Neuroimmunol. 2024 Feb 15;387:578292.
12. Sari-Aslani K, Davoodian N, Zeini S, Mousavi SA, Eftekhar E. "γ-oryzanol ameliorates the oxidative stress and inflammatory response in a mice model of LPS-induced liver injury." Disease and Diagnosis. 2024 Sep 5;13(3):107-113.
13. Alam, N.; Ding, X.; Fu, Y.; Jia, L.; Ali, S. and et al. "Oryzanol ameliorates MCD-induced metabolic dysfunction-associated steatohepatitis in mice via gut microbiota reprogramming and TLR4/NF-κB signaling suppression." Am J Physiol Gastrointest Liver Physiol. 2025 May 1;328(5):G578-593.
14. Juricic, H.; Cuccioloni, M.; Bonfili, L.; Angeletti, M.; Uberti, D. and et al. "Biochemical, Biological, and Clinical Properties of γ -Oryzanol." Antioxidants. 2025 Sep 9;14(9):1-18.
15. Ahmad, I.; Khalid, H.; Perveen, A; Shehroz, M.; Nishan, U., and et al. Identification of novel quinolone and quinazoline alkaloids as phosphodiesterase 10A inhibitors for Parkinson's disease through a computational approach. ACS omega. 2024 Mar 26;9(14):16262-16278.
16. Khalid, H.; Sattar, F.; Ahmad, I.; Junior, V. F.; Nishan, U., and et al. Computer-assisted discovery of natural inhibitors for platelet-derived growth factor alpha as novel therapeutics for thyroid cancer. Front. Pharmacol. 2025 Jan 9;15:1512864.
17. Ishfaq, M.; Shah, S. W.; Li, S.; Bilawal, A.; Shah, Z., and et al. QSAR and pharmacophore modeling in computational drug design. Comput. Methods Med. Chem. Pharmacol. Toxicol. Elsevier. p. 99-118; 2025.
18. Ghosh, R.; Roy, S.; Rakshit, G.; Singh, NK.; Maiti, N. J. "Pharmacophore Modeling in Drug Design." Comput. Methods. Ratio. Drug Des. Wiley Online Library. 167-194; 2025.
19. Baei, B.; Askari, P.; Askari, F. S.; Kiani, S. J.; Mohebbi, A. "Pharmacophore modeling and QSAR analysis of anti-HBV flavonols." PloS one. 2025 Jan 13;20(1):e0316765.
20. Georrge, J. J.; Mishra, S. K.; Chhetri, T.; Roy, S.; Gurung, K. Trends of Pharmacophore Modelling in Drug Discovery. Mol. Model. Dock. Tech. Drug Discov. Des. IGI Global Scientific Publishing. p. 505-534;2025. DOI: 10.4018/979-8-3693-5598-5.ch017
21. Khalid, H.; Sattar, F.; Ahmad, I.; Junior, V. F.; Nishan, U., and et al. "Computer-assisted discovery of natural inhibitors for platelet-derived growth factor alpha as novel therapeutics for thyroid cancer." Front. Pharmacol. 2025 Jan 9;15:1512864.
22. Jin, X.; Wang, Y.; Chen, J.; Niu, M.; Yang, Y., and et al. "Novel dual-targeting inhibitors of NSD2 and HDAC2 for the treatment of liver cancer: structure-based virtual screening, molecular dynamics simulation, and in vitro and in vivo biological activity evaluations." J. Enzyme Inhib. Med. Chem. 2024 Dec 31;39(1):2289355.
23. Banat, R.; Daoud, S.; and Taha, M. O. "Ligand-based pharmacophore modeling and machine learning for the discovery of potent aurora A kinase inhibitory leads of novel chemotypes." Mol. Divers. 2024 Dec;28(6):4241-4257.
24. Ranade, S. D.; Alegaon, S. G.; Khatib, N. A.; Gharge, S.; Kavalapure, R. S. "Quinoline-based Schiff bases as possible antidiabetic agents: ligand-based pharmacophore modeling, 3D QSAR, docking, and molecular dynamics simulations study." RSC med. chem. 2024;15(9):3162-3179.
25. Saravanan, V.; Chagaleti, B. K.; Packiapalavesam, S. D.; Kathiravan, M. "Ligand based pharmacophore modelling and integrated computational approaches in the quest for small molecule inhibitors against hCA IX." RSC Adv. 2024;14(5):3346-58.
26. Yuliantini, A.; Ocktavyanie, S.; Febrina, E.; and Asnawi, A. "Virtual Screening Using a Ligand-based Pharmacophore Model from Ashitaba (Angelica keiskei K.) Isolates and Molecular Docking to Obtained New Candidates as α-Glucosidase Inhibitors." Trop. J. Nat. Prod. Res. 2024 Jan 1;8(1);p5811.
27. Yang Q, Wan Q, Wang Z. Curcumin mitigates polycystic ovary syndrome in mice by suppressing TLR4/MyD88/NF-κB signaling pathway activation and reducing intestinal mucosal permeability. Scientific reports. 2024 Dec 2;14(1):29848.
28. Wu H, Yang M, Yan C, Liu M, Wang H, Zhang W. Tenascin C activates the toll-like receptor 4/NF-κB signaling pathway to promote the development of polycystic ovary syndrome. Molecular Medicine Reports. 2024 Jun 1;29(6):1-2.
29. Wang K, Li Y. Signaling pathways and targeted therapeutic strategies for polycystic ovary syndrome. Frontiers in endocrinology. 2023 Oct 19;14:1191759.
30. Kuang YY, Xiong MQ, Cai JX. Clinical efficacy of gamma-oryzanol combined with Femoston for perimenopausal syndrome. World Journal of Clinical Cases. 2024 Aug 6;12(22):4992.
31. Alizadeh M, Moshtagh S, Amir SA, Jeddi M, Tahmasebzadeh S, Radman G, Bagheri A, Bagheri Y, Shahabinejad N. Anti-oxidant and anti-apoptotic effects of gamma-oryzanol on male reproductive function in chronic restraint stress in rats. Avicenna Journal of Phytomedicine. 2025 Jan;15(1):890.
32. Lisnawati L, Poeranto S, Endharti AT, Santoso MI. Antioxidant and anti-Inflammatory Activity of γ-Oryzanol Compared to Rice Bran Oil to Repair Ovarian Histological Structure from One Push Transfluthrin Exposure Effect. Open Access Macedonian Journal of Medical Sciences. 2022 Jan 1;10(B):1-2.
33. Radda MI, Omar N, Ahmad R, Jalil RA, Ishak WR, Zin AA, Romli AC. Gamma-oryzanol: a novel promising supplement for diabetes mellitus. Universa Medicina. 2025 Mar 9;44(1):90-100.
34. Young RJ, Flitsch SL, Grigalunas M, Leeson PD, Quinn RJ, Turner NJ, Waldmann H. The time and place for nature in drug discovery. Jacs Au. 2022 Oct 14;2(11):2400-16.
35. Sulaiman A, Sulaiman A, Sert M, Khan MS, Khan MA. Functional and Therapeutic Potential of γ-Oryzanol. Functional Foods: Phytochemicals and Health Promoting Potential. 2021 Nov 10:259.
36. Juricic H, Cuccioloni M, Bonfili L, Angeletti M, Uberti D, Eleuteri AM, Abate G, Cecarini V. Biochemical, Biological, and Clinical Properties of γ-Oryzanol. Antioxidants. 2025 Sep 9;14(9):1099.
37. Malik AQ, Hailat W, Bouchekara HR, Javaid MS. Gamma oryzanol loaded microspheres with improved bioavailability. African Journal of Pharmacy and Pharmacology. 2018 May 8;12(17):202-7.
38. Jumnongprakhon P, Nitjapol A, Lonlab K, Nudmamud-Thanoi S, Chomchalao P, Tiyaboonchai W. From Formulation to Function: γ-Oryzanol Solid Dispersion Development and Its Neuroprotective Effects on the Depression Model. ACS omega. 2025 Sep 26.
39. Ito J, Kumagai N, Suzuki A, Shoji N, Parida IS, Takahashi M, Nakagawa K. Effect of microemulsion system on water dispersibility and bioavailability of γ-oryzanol. Bioscience, Biotechnology, and Biochemistry. 2025 Apr;89(4):633-7.
40. Sulaiman A, Sulaiman A, Sert M, Khan MS, Khan MA. Functional and Therapeutic Potential of γ-Oryzanol. Functional Foods: Phytochemicals and Health Promoting Potential. 2021 Nov 10:259.

41. Roskoski Jr R. Rule of five violations among the FDA-approved small molecule protein kinase inhibitors. Pharmacological research. 2023 May 1;191:106774.

42. Lohit N, Singh AK, Kumar A, Singh H, Yadav JP, Singh K, Kumar P. Description and in silico ADME studies of US-FDA approved drugs or drugs under clinical trial which violate the Lipinski's rule of 5. Letters in Drug Design & Discovery. 2024 Jun 1;21(8):1334-58.

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