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Pharmacoinformatics based *in silico* Molecular Dynamics Simulation for Screening Phytochemicals as AMPK and INSR Modulators for Polycystic Ovarian Syndrome from Medicinal Plants

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ABSTRACT: Polycystic ovarian syndrome (PCOS) is a complex endocrinal and metabolic disorder of women of reproductive age women. PCOS is characterized by hyperandrogenism, irregular periods, cyctic ovaries, insulin resistance, hyperinsulinemia, obesity, and dyslipidemia resulting in an increased risk of diabetes and cardiovascular diseases. Due to its complexity and lack of systemic treatment, we have selected three plant materials *S. asoca* bark, *G. sylvestre* leaves, and *P. daemia* aerial parts, which are prominent and effective against any two or more major pathogenic pathways of PCOS to identify effective alternative drug molecules. The Phytoconstituents of the selected plant materials were retrieved from IMPPAT, Dr. Duke's public database, and also manually searched from articles. PubChem database was used to obtain the structures of the selected phytochemicals. Graph theoretical analysis was employed by exploitation of the KEGG pathway and finding out the significant PCOS pathways and the influential proteins such as AMPK and INSR. The retrieved compounds were docked against a selected target using Schrodinger Glide software, and ADMET studies were carried out in a web-based online tool. Compounds that possess superior molecular interaction and kinetic profiles were further selected for molecular dynamics studies. The identified 65 compounds were docked with the selected targets. A compound that has high docking score from each plant against each target was selected for further ADMET and molecular dynamic (MD) studies. These compounds with each target having docking scores in the range of (-11.09 to -1.14 kcal/mol) with AMPK and (-12.92 to -1.43 kcal/mol) with INSR. The selected compounds were further screened for ADMET studies through an online Swiss ADME and pkCSM web server. Based on the ADMET and docking results two compounds from each target that have higher docking scores, pharmacokinetic, and safety profiles were selected for MD studies. The MD results showed that β-sitosterol showed better intermolecular interaction and stability against AMPK and Gymnemoside E against INSR. These aforementioned findings interpreted that the β-sitosterol and Gymnemoside E have adequate potential as drug candidates for the treatment of PCOS. Further *in vivo* studies are needed to explore these constituents for clinical use.

Keywords: ADMET, AMPK, INSR, Docking, Molecular Dynamics, Polycystic Ovarian Syndrome.

INTRODUCTION

Polycystic ovary syndrome (PCOS) is a complex reproductive disorder of reproductive-age women associated with endocrinal and metabolic complications (Norman *et al.*, 2007). The ongoing therapeutic approaches are based on the individual desired outcomes of PCOS patients. The need for alternative medicine which is effective on both metabolic and endocrinal complications of PCOS is increasing toward systemic management (Hywood, 2012). Plant-based medicines are an effective alternative system of medicine because of their enormous phytoconstituents and synergistic actions (Ravishankar and Shukla 2007; Pachiappan *et al.*, 2022). Besides herbal medicines being an efficient source of medicine, based on that we have selected three plants that are effective in PCOS.

The selected plant materials are *Saraca asoca*, *Gymnema sylvestre*, and *Pergularia daemia* which are reported to possess the actions like a uterine tonic, antiandrogenic, hypolipidemic, insulin sensitivity improving the property and correct hormonal irregularity in PCOS (Pachiappan *et al.,* 2017; Pachiappan *et al.,* 2020; Ranjani *et al.,* 2023). Our study was aimed to screen the molecular interactions of phytoconstituents from the selected plants with key targets responsible for PCOS based on pharmacoinformatics information through *in silico* molecular docking and dynamic studies.

MATERIALS AND METHODS

Target Protein Preparation: Kyoto Encyclopedia of Gene and Genome (KEGG) pathway was constructed based on the graph theoretical analysis *Homo sapiens* (has: 04152) was selected as a species (Huang *et al.,* 2009; Sharma, 2022). The 5' adenosine monophosphate-activated protein kinase (AMPK) (**PDB: 2UV7)** and Insulin receptor (INSR) tyrosine kinase **(PDB: 5E1S)** were selected as the target. The X-ray crystal structures of the selected targets were retrieved from RCSB Protein Data Bank (http://www.rcsb.org/pdb). The downloaded targets were prepared for molecular docking using GLIDE software (version 11, Schrödinger, LLC, New York, 2016) and saved into Pdpqt format.

Phytoconstituents retrieval and preparation from selected plant materials: The phytochemicals from the selected *Saraca asoca*, *Gymnema sylvestre*, and *Pergularia daemia* were retrieved from Dr. Duke's and Indian Medicinal Plants, Phytochemistry and Therapeutics (IMPPAT) phytochemical database, and also manually from standard articles with the search terms of concern plant names. PubChem database was used to obtain the PubChem ID, canonical smile, 2D and 3D structures of the selected phytochemicals (Choudhary *et al.,* 2021). The Schrödinger Suite *LigPrep* model was used to prepare the ligands.

Molecular Docking Studies: The intermolecular interactions between phytoconstituents and targets were examined by molecular docking studies. The GLIDE (Grid-based Ligand Docking with Energetics) module version software from Schrödinger, LLC, New York, NY, was used for the molecular docking studies (Schrodinger Inc.). The docking score and glide energy were used to select the ideal conformation. The docking score is based on the total energy from many processes, including lipophilicity, hydrogen bonding, metal contact, rotatable bond counts, and salvation. A force field based on the OPLS-2005 was used to determine the glide energy. Based on the lowest glide energy and lowest docking score, or both, the best compound was chosen (Parasuraman and Suresh, 2014; Patel, 2023).

***In silico* ADME studies:** *In silico* pharmacokinetic (ADME) parameter studies help to identify the integrity and efficacy of the selected compounds. The phytoconstituents that have a superior score in docking studies were further exploited for pharmacokinetic and physicochemical studies. The Swiss ADME web server (http://www.swissadme.ch/) was used for the pharmacokinetic (ADME) parameter studies (Wang *et al.,* 2019).

***In silico* toxicity studies:** *In silico* toxicity studies were carried out to explore the safety profile of the selected compounds. The toxicity studies were executed by using the pkCSM web server (http://biosig.unimelb.edu.au/pkcsm/) (Jia *et al.,* 2020).

Molecular dynamics: Molecular dynamic (MD) studies are useful to determine the molecular interaction between the selected phytoconstituents and targets. Through 100 ns molecular dynamic simulation, the complicated structure of the active site cavity was assessed. MD was carried out using the Desmond module of Schrödinger developed by the D.E. Shaw research group (Academic license, Version 2020-1) (Kunjiappan *et al.,* 2020). The MD was programmed to run for 100 ns at 310 K temperatures and 1.0 bar pressure with default settings for relaxation before simulation. The OPLS AA force field was used to indicate the receptor-ligand complex system. Through the web server "WebGRO for Macromolecular Simulations" (https://simlab.uams.edu/), the GROMACS simulation program was used to analyze the trajectory of root mean square deviation (RMSD) and root mean square fluctuation (RMSF).

RESULTS AND DISCUSSION

The systemic pharmacological method known as "*in silico* screening" is used during the drug discovery and development phase to explore hypotheses regarding drug-like molecules (Ekins *et al.,* 2007; Sudhakar *et al.,* 2018). Based on the pharmacoinformatics tool the AMPK/PRKAG2 and INSR target proteins were selected as key target proteins for polycystic ovarian syndrome. The AMPK-mediated signaling pathways in the ovary regulate both reproductive and metabolic complications of PCOS. The activation of the AMPK signaling pathway in the ovary alters the LH and FSH ratio, down-regulates the androgen receptor, and increases expression of GLUT4 leading to improves insulin sensitivity and promoting follicular development (Yang *et al.,* 2020; Shen *et al.,* 2014). The activation of INSR protein in the ovary also leads to improves insulin sensitivity by the translocation of GLUT4 in the ovary cytoplasm to the cell membrane leading to improve glucose uptake which promotes follicular development and ovulation (Unluhizarci *et al.,* 2021).

Molecular Docking Analysis: The molecular docking analysis was carried out for the 65 retrieved phytoconstituents with the target protein AMP-activated kinase (AMPK) (PDB: 2UV7) and INSR

Tyrosine Kinase (PDB: 5E1S) using the docking software GLIDE. The molecules were ranked according to the compounds that had more negative scores in the GLIDE scoring function. All the ligands were docked into the active site of the AMP-activated kinase and INSR Tyrosine Kinase intended proteins to explore the molecular binding interaction and affinity of phytoconstituents to targets. The molecular docking results were represented in Tables 1 & 2.

Table 1: Molecular Docking Sore and Glide Energy Score of the Selected Phytoconstituents against AMPK.

Sr. No.	Plant name	Compound ID (CID)	Phytoconstituent	Docking Score kcal mol^{-1}	Glide energy kcal mol^{-1}
1.	*Saraca asoca*	**10813969**	**Isoquercitin**	**-10.04**	**-33.87**
2.		25203515	Kaempferol 3-O-beta-D-glucoside	-7.62	-32.38
3.		9064	Catechin	-4.69	-27.57
4.		255538	Epicatechol	-4.21	-30.96
5.		182232	(+)-Epicatechin	-4.21	-30.96
6.	*Gymnema sylvestre*	**441437**	**d-Quercitol**	**-10.04**	**-20.67**
7.		16091097	Gymnasmide	-10.04	-20.67
8.		265071	Kasuagamycin	-9.76	-35.34
9.		14264066	Gymnemic acid III	-4.13	-25.53
10.		101933150	Gymnemoside C	-3.49	-29.15
11.		91617872	Gymnemic acid II	-3.25	-26.54
12.		11953919	Gymnemic acid I	-3.23	-30.03
13.		17100	alpha-terpineol	-1.14	-17.13
14.	*Pergularia daemia*	**222284**	**β-sitosterol**	**-11.09**	**-46.06**
15.		5281643	Hyperoside	-8.46	-33.89
16.		441849	Calactin	-4.46	-27.65
17.		16142	Calotropin	-4.46	-27.65
18.		14134976	Calotropagenin	-3.46	-19.97
19.		12302397	Corotoxigenin	-2.82	-29.99
20.		441874	Uscharidin	-2.54	-28.54

Table 2: Molecular Docking Sore and Glide Energy Score of the Selected Phytoconstituents against INSR.

S. No.	Plant name	Compound ID (CID)	Phytoconstituent	Docking Score kcal mol^{-1}	Glide energy kcal mol^{-1}
1.	*Saraca asoca*	**10813969**	**Isoquercitin**	**-9.04**	**-46.06**
2.		44256718	Cyanidin 3,5-diglucoside	-8.3	-45.36
3.		1451039	Lyoniside	-6.76	-38.38
4.		25203515	Kaempferol 3-O-beta-D-glucoside	-6.41	-38.51
5.		440833	Leucocyanidol	-6.05	-33.04
6.		71629	Leucocyanidin	-6.05	-33.04
7.		9064	Catechin	-4.71	-32.89
8.		182232	(+)-Epicatechin	-4.71	-32.89
9.		255538	Epicatechol	-4.71	-32.89
10.	*Gymnema sylvestre*	**101933152**	**Gymnemoside E**	**-12.92**	**-51.08**
11.		101933153	Gymnemoside F	-11.85	-51.28
12.		6918768	Gymnemasaponin V	-11.51	-64.31
13.		21636600	Gymnemasaponin II	-9.85	-42.77
14.		101933151	Gymnemoside D	-9.79	-46.46
15.		21636602	Gymnemasaponin IV	-9.43	-49.95
16.		129465	Deacylgymnemic acid	-7.71	-40.01
17.		441437	d-Quercitol	-7.20	-18.60
18.		16091097	Gymnasmide	-7.20	-18.60
19.		6442217	Gymnemoside A	-6.51	-44.98
20.	*Pergularia daemia*	**5742590**	**Daucosterol**	**-9.67**	**-35.27**
21.		5281648	Hyperoside	-4.68	-31.56
22.		12302399	Coroglaucigenin	-4.25	-22.48
23.		14134976	Calotropagenin	-3.78	-27.48
24.		4418774	Uscharidin	-3.72	-33.11
25.		5280343	Quercetin	-3.67	-25.21
26.		44184	Calactin	-3.61	-34.14
27.		14134976	Calotropin	-3.61	-34.14
28.		12302397	Corotoxigenin	-2.80	-28.24
29.		92760	Uzarigenin	-2.39	-21.15

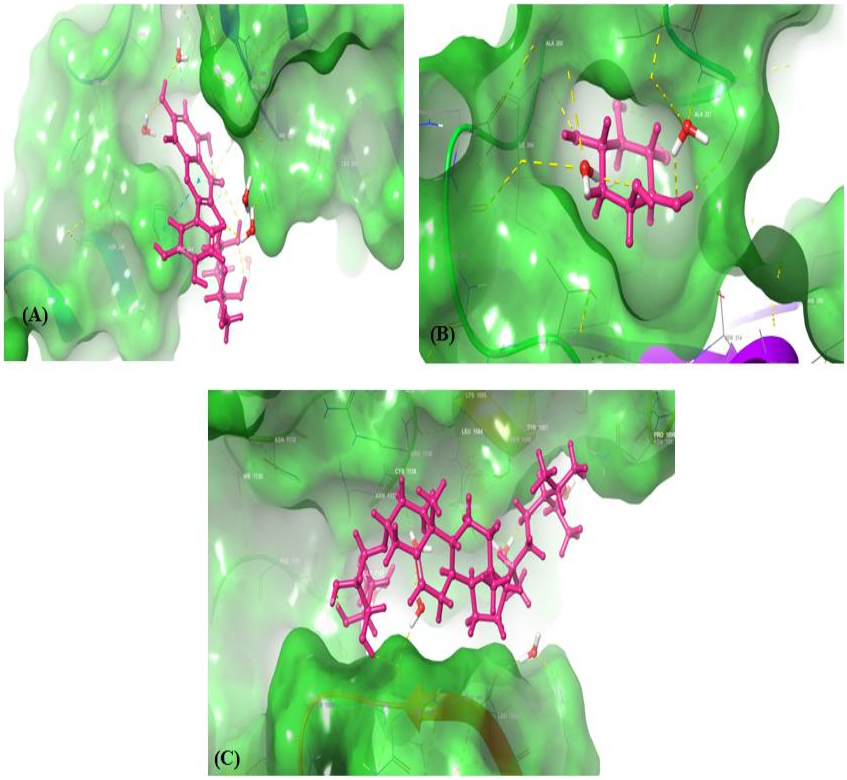

Fig. 1. Illustrated the 3D images of molecular interaction between the target AMPK and the compounds (A) Isoquercitin(CID: 10813969), (B) d-Quercitol (CID: 441437), and (C) β-sitosterol (CID: 222284)

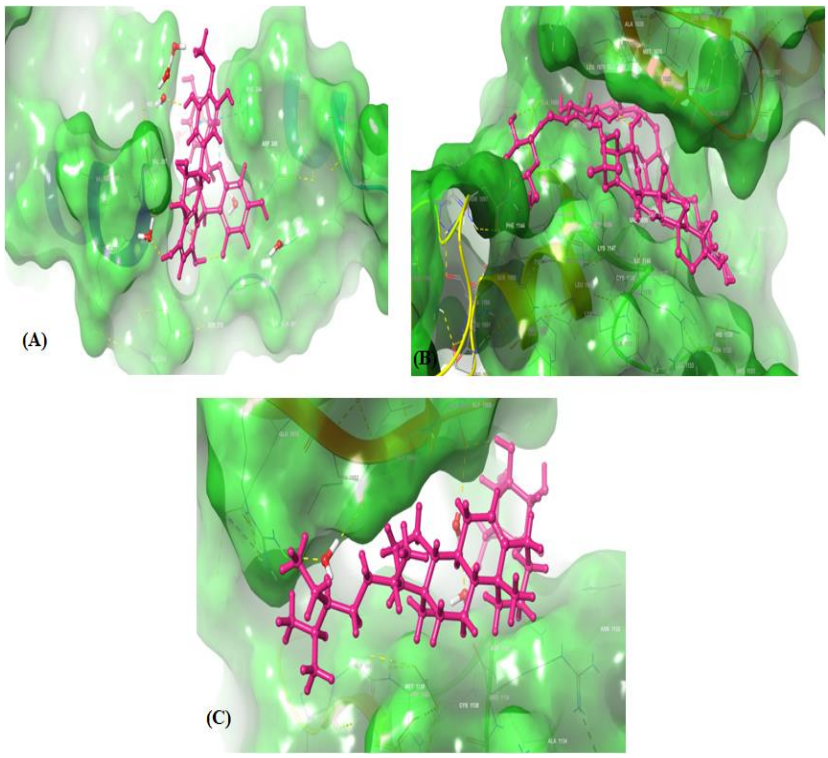

Fig. 2. Illustrated the 3D images of molecular interaction between the target INSR and the compounds (A) Isoquercitin (CID: 10813969), (B) Gymnemoside E (CID: 101933152), and (C) Daucosterol (CID: 5742590).

The molecular interaction energy includes intermolecular hydrogen bonding as well as electrostatic energy and van der waals energy was calculated for each minimized compound. The docking score varied from -1.14 Kcal/mol to -11.09 Kcal/mol against AMPK and -1.43 Kcal/mol to -12.92 Kcal/mol for INSR. A potent molecule from each target was selected from the docked molecules, based on their docking score against both the targets and the ligand interaction was shown in Fig. 1 and 2.

Based on the compounds from each plant that have superior docking and glide scores were further selected for *in silico* ADMET studies. The compounds selected that have higher molecular interaction with the AMPK target are Isoquercitin (-10.04 kcal/mol), d-Quercitol (-10.04 kcal/mol), and β-sitosterol -11.09 kcal/mol). The compounds with INSR are Isoquercitin (-9.04 kcal/mol), Gymnemoside E (-12.92 kcal/mol), and Daucosterol (-9.67 kcal/mol).

Pharmacokinetic Parameters of the Selected Phytoconstituents

The compounds that possess better docking scores against the targets were further screened for *in silico* pharmacokinetic studies to explore their pharmacokinetic profile. β-sitosterol has 90.94%, Daucosterol has 79.68%, Isoquercitin has 48% and d-Quercitol has 34.97 % of intestinal absorption. Gymnemoside E does not possess intestinal absorption. Hence the compounds that have higher intestinal absorption possess better bioavailability. Daucosterol and β-sitosterol metabolized by CYP3A4 and CYP1A2, Isoquercitin metabolized by CYP1A2, and Quercitol metabolized by CYP3A4 and CYP1A2. Based on the aforementioned findings, it can be interpreted that the identified bioactive molecules have adequate potential as drug candidates for the treatment of PCOS.

Toxicity Profile of the Selected Phytoconstituents

The selected compounds were further screened for *in silico* toxicity studies to explore their safety profile using pkCSM web-based server. The carried-out skin sensitization test, hepatotoxicity, AMES toxicity study, oral acute and chronic toxicity studies results showed that the screened compounds are nontoxic and safer drug candidates.

Among these selected compounds the compounds β-sitosterol and Isoquercitin complex with AMPK, and Gymnemoside E and Daucosterol complex with INSR target proteins for molecular dynamic studies, this selection is based on the compounds which have better molecular interaction, physiochemical and safety profile.

Molecular Dynamics Studies

Two molecules for each protein, AMPK, and INSR were chosen for molecular dynamics based on molecular docking scores and better anticipated pharmacokinetic parameters. Gymnemoside E and Isoquercitin in complex with INSR Tyrosine Kinase, as well as β-sitosterol and Isoquercitin in complex with AMP-activated kinase, underwent a 100 ns molecular dynamic simulation. To understand the fluctuations and stability of the protein-ligand complex, root mean square deviation (RMSD) and root mean square fluctuation (RMSF) of receptor atoms were used in a molecular dynamic trajectories study.

The Isoquercetin-associated AMPK complex showed variations in backbone RMSD ranging to 52 Å. For β-sitosterol associated AMPK complex showed variations in backbone RMSD ranging to 7.2 Å, the stable conformation was attained from the period 100 ns with no considerable deviations. Gymnemoside E-associated INSR complex showed variations in backbone RMSD ranging to 16Å and isoquercetin-associated INSR complex showed variations in backbone RMSD ranging to 11Å.

Molecular docking studies revealed that β-sitosterol has better molecular interaction and stability with AMPK target protein and Gymnemoside E exhibited better molecular interaction and stability with INSR. Hence these finds revealed that the phytoconstituents β-sitosterol and Gymnemoside E have ample potential drug candidates for PCOS through activating AMPK as well as INSR-mediated signaling pathways in the ovary.

CONCLUSION

Based on these *in silico* studies the phytoconstituents β-sitosterol and Gymnemoside E were identified as significant drug candidates for polycystic ovarian syndrome through activating AMPK and INSR target protein medicated pathway improves follicular development and ovulation by altering hyperandrogenism and insulin resistance in PCOS ovary. Further scope full *in-vitro* and *in-vivo* studies are required to explore these phytoconstituents as better drug candidates for PCOS.

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REFERENCES

Choudhary, N., Choudhary, S., Kumar, A. & Singh, V. (2021). Deciphering the multi-scale mechanisms of *Tephrosia purpurea* against polycystic ovarian syndrome (PCOS) and its major psychiatric comorbidities: studies from network pharmacological perspective. *Gene 773*, 145385.

Ekins, S., Mestres, J. & Testa, B. (2007). In silico pharmacology for drug discovery: applications to targets and beyond. *British journal of pharmacology 152*(1), 21-37.

Huang, D. W., Sherman, B. T. & Lempicki, R. A. (2009). Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. *Nature protocols 4*(1), 44-57.

Hywood, A. (2012). Phytotherapy for polycystic ovarian syndrome. *Australian Journal of Herbal Medicine 24*(3), 81-86.

Jia, C. Y., Li, J. Y., Hao, G. F. & Yang, G. F. (2020). A drug-likeness toolbox facilitates ADMET study in drug discovery. *Drug discovery today 25*(1), 248-258.

Kunjiappan, S., Sankaranarayanan, M., Kumar, B. K., Pavadai, P., Babkiewicz, E., Maszczyk, P. &

Panneerselvam, T. (2020). Capsaicin-loaded solid lipid nanoparticles: Design, biodistribution, in silico modeling and in vitro cytotoxicity evaluation. *Nanotechnology 32*(9), 095101.

Norman, R. J., Dewailly, D., Legro, R. S. & Hickey, T. E. (2007). Polycystic ovary syndrome. *The Lancet*, *370*(9588), 685-697.

Pachiappan, S., Matheswaran, S., Saravanan, P. P. & Muthusamy, G. (2017). Medicinal plants for polycystic ovary syndrome: A review of phytomedicine research. *Int J Herb Med. 5*(2), 78-80.

Pachiappan, S., Ramalingam, K. & Balasubramanian, A. (2021). Combined effects of *Gymnema sylvestre* and *Pergularia daemia* on letrozole-induced polycystic ovarian syndrome in rats. *Asian Pacific Journal of Reproduction 10*(2), 68-73.

Pachiappan, S., Ramalingam, K. & Balasubramanian, A. (2020). A review on phytomedicine and their mechanism of action on PCOS. *Int. J. Cur. Res. Rev. 12*(23), 81-85.

Parasuraman, P. & Suresh, R, P. (2014). Balancing anti-amyloid and anti-cholinesterase capacity in a single chemical entity: In silico drug design. *Int J Pharm Pharm Sci. 6*(2), 571-575.

Patel, A. K. (2023). A Molecular Docking Study of Indirubin against Hyperlipidemia. *Biological Forum–An International Journal 15*(2), 246-255.

Ranjani, N., Kannan, R., Kokila, D. & Bhuvaneswari, V. (2023). Ethnobotanical Survey of Medicinal Plants used by Malayali Tribesin Palamalai Hills Salem District, Tamil Nadu, India. *Biological Forum–An International Journal 15*(1), 222-229.

Ravishankar, B. & Shukla, V. J. (2007). Indian systems of medicine: a brief profile. *African Journal of Traditional, Complementary and Alternative Medicines 4*(3), 319-337.

Sharma, P. (2022). An Overview of Cancer Research using Bioinformatics. *International Journal of Theoretical & Applied Sciences 14*(2), 52-56.

Shen, M., Zhang, Z., Ratnam, M. & Dou, Q. P. (2014). The interplay of AMP-activated protein kinase and androgen receptor in prostate cancer cells. *Journal of cellular physiology 229*(6), 688-695.

Sudhakar, P., Pushkalai, S. P., Sabarinath, C., Priyadharshini, S. & Haripriya, S. (2018). Molecular docking and synthesis of 1, 2, 4-triazin analogue of diclofenac as potential ligand for Parkinson's. *Research journal of Pharmacology and Pharmacodynamics 10*(1), 8-12.

Unluhizarci, K., Karaca, Z. & Kelestimur, F. (2021). Role of insulin and insulin resistance in androgen excess disorders. *World Journal of Diabetes 12*(5), 616-620.

Wang, Y., Liu, H., Fan, Y., Chen, X., Yang, Y., Zhu, L. & Zhang, Y. (2019). *In silico* prediction of human intravenous pharmacokinetic parameters with improved accuracy. *Journal of chemical information and modeling 59*(9), 3968-3980.

Yang, W., Wang, L., Wang, F. & Yuan, S. (2020). Roles of AMP-activated protein kinase (AMPK) in mammalian reproduction. *Frontiers in Cell and Developmental Biology 8*, 593005.

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