Network Pharmacology and Molecular Docking Analysis of Chrysoeriol for Alzheimer's Disease

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Abstract. Alzheimer's disease is a brain disorder that slowly destroys memory and thinking skills and eventually the ability to carry out daily tasks. Chrysoeriol is a flavone compound that exhibits neuroprotective, anti-inflammatory, cholinesterase inhibitor and antioxidant properties. The potential targets of Alzheimer's disease were identified by Network Pharmacology. As a result of the analysis, 35 overlapping targets among 100 potential targets for chrysoeriol and 1255 for Alzheimer's disease were identified. Among the 35 overlapping targets, the Hub genes are highly enriched in the Biological Process, Cellular Component and Molecular Function were analyzed. Further the Molecular Docking analysis revealed that PTGS2, MMP2 and KDR have good binding affinity. It's shown that chrysoeriol has promising role in modulating key pathways involved in Alzheimer's disease pathogenesis, paving the way for further experimental validation and drug development.

Keywords: Alzheimer's disease, Chrysoeriol, Network pharmacology, Hub genes, Molecular docking

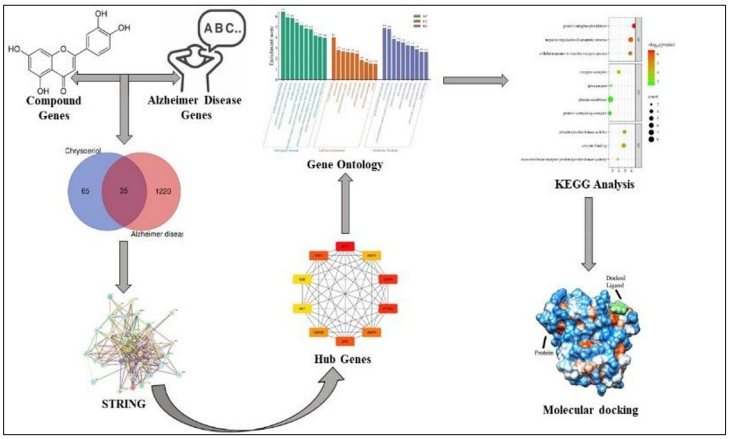

Fig. 1. Graphical abstract – Overall process

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1 Introduction

In 1907, Alois Alzheimer documented the case of a 51-year-old woman with rapidly deteriorating memory and cognitive abilities, marking the first recognized instance of what is now known as Alzheimer's disease. Her condition progressed over 4.5 years, ultimately leaving her bedridden, incontinent, and curled in a fetal position. Post-mortem analysis revealed a uniformly atrophied brain without macroscopic degeneration but with arteriosclerotic vascular changes. Using Bielschowsky's silver staining, Alzheimer identified neurofibrillary tangles and neuritic plaques in the cerebral cortex, key hallmarks of Alzheimer's disease. These plaques, primarily composed of amyloid, along with cerebrovascular amyloid containing beta/A4 protein and tau protein, distinguished the case from other known conditions [1]. Various plants have numerous medicinal properties, the plants can produce secondary metabolites, but they are not essential for plant growth and reproduction. Secondary metabolites can cure some diseases and have enormous pharmacological properties. Chrysoeriol is a flavone that is 30-O-methoxy. This chemically produced derivative is luteolin, a member of the flavonoid family. According to numerous research, chrysoeriol exhibits a variety of pharmacological effects that involve both biological, cellular and molecular modes of action. In many in-vitro investigations, chyrsoeriol has demonstrated notable neuroprotective, anti-inflammatory, Cholinesterase inhibitor and antioxidant properties. Shao et al. highlighted its neuroprotective properties in rats subjected to mid-brain artery blockage, showing improvements in neurological deficits and reduced ischemic damage. These effects were linked to the regulation of oxidative stress markers and inhibition of pro-inflammatory cytokines (TNF, IL-1β, IL-6) via the Wnt/β-catenin signaling pathway [2]. Similarly, Ruttanaphan et al. examined its insecticidal activity against Spodoptera litura, revealing its strong inhibition of carboxylesterases, glutathione S-transferase, and acetylcholinesterase, which disrupted detoxification and neurological functions in larvae [3]. In neurodegenerative disease models, Limboonreung demonstrated that chrysoeriol counteracts MPP+ induced toxicity in SH-SY5Y cells, suggesting a therapeutic role in Parkinson's disease. It preserved mitochondrial integrity and activated the PI3K/Akt pathway, mitigating apoptosis and cellular dysfunction [4]. Furthermore, Kim et al. investigated its effects on bone health, showing that chrysoeriol protects osteoblasts from oxidative damage by enhancing collagen production, alkaline phosphatase activity, and calcium deposition, while potentially involving estrogen-mediated mechanisms [5]. Wu et al. emphasized its anti-inflammatory efficacy in mouse models, reporting reductions in pro-inflammatory proteins (iNOS, COX-2), cytokines (IL-1β, IL-6, TNF-α), and signaling molecules like phospho-STAT3 and phospho-NF-κB. Its ability to suppress inflammation-related pathways and reduce oxidative markers such as malondialdehyde further supports its potential for treating inflammatory conditions [6]. Hopkins created network pharmacology, a novel in silico drug discovery technique, in 2008 to find possible molecular targets and active chemicals in a variety of herbal formulas or simple herbs. Based on systems biology, this tool combines several methodologies, including computer simulations, molecular network analysis, poly-pharmacology, and bioinformatics. This approach saves money, time, and energy in addition to speeding up medication discovery. In network pharmacology, genes linked to substances and illnesses are identified, a Protein Protein Interaction network is built and finally, the network is analyzed and visualized. To verify the interactions between the most active components and their possible targets, the network is lastly subjected to further validation. Thus, it is interesting to look into the mechanisms behind the therapeutic effects of flavonoids in Alzheimer's disease using network pharmacology [7].

2 Materials and Methods

2.1 Toxicity prediction

The canonical SMILES of the flavonoids were obtained from the PubChem database, available at https://pubchem.ncbi.nlm.nih.gov/. The flavonoid's physicochemical characteristics, particularly their ADME qualities, were examined using SwissADME http://www.swissadme.ch/ and the OSIRIS tool was used to evaluate the other properties of the compound https://www.organic-chemistry.org/prog/peo/peo.jar [8].

2.2 Compound target prediction

Swiss target prediction http://www.swisstargetprediction.ch/ is an online database designed [1]to identify the target genes of the compounds by importing SMILES of the compounds into Swiss target prediction. The potential targets of the compound are identified [9].

2.3 Disease target prediction

The keyword "Alzheimer's disease" was used to pick prospective targets from GeneCards https://www.genecards.org/ and DisGeNET https://disgenet.com/. The Gene-Disease Association score is used to determine the genes highly linked to Alzheimer's disease genes. In this analysis, the GDA score which is greater than 0.1, is highly correlated to Alzheimer's disease genes. In GeneCards the relevance score is applied as the minimum value of 20. Two databases are subsequently combined removing the duplicate genes [10].

2.4 Intersection of similar targets

Using an online tool https://bioinformatics.psb.ugent.be/webtools/Venn/, combined the two sets of data compound-related genes and Alzheimer disease-related genes by Venn diagram, the overlapping genes are taken for further analysis.

2.5 PPI network and Hub genes analysis

The overlapped genes were imported into the STRING database http://stitch.embl.de/. A minimum interaction score of more than 0.4 was required to choose the human organism. Interactions were only considered substantial if they satisfied this requirement. Target proteins are represented by nodes in Protein Protein Interaction network, whereas interactions between proteins are represented by edges. The number of nodes that are directly connected to a node is known as its degree. A node with a higher degree was considered more significant [11]. After it was developed, this network was then loaded into Cytoscape https://cytoscape.org/ so that its structure could be seen and examined. The Cytoscape website can be accessed to get the CytoHubba software https://apps.cytoscape.org/apps/cytohubba. Using CytoHubba, the degree was computed to determine the primary targets. The top ten genes were chosen for further process and assigned to the role of core targets [12].

2.6 Gene Ontology and Kyoto Enrichment analysis of Genes and Genomes

Gene Ontology and Kyoto Enrichment analysis of Genes and Genomes were analyzed using the database DAVID https://davidbioinformatics.nih.gov/. In DAVID functional annotation tool was utilized to analyze Gene Ontology for three levels biological process, cellular component and molecular function. In this analysis, the terms are sorted by applying the significance level "p-value" ≤ 0.05 where the top 10 smallest values from each term are analyzed for Gene Ontology. Among the functional annotation tool Kyoto Enrichment analysis of Genes and Genomes to analyze the enrichment values of the hub genes [10]. These results are visualized using the tool https://www.bioinformatics.com.cn/en [13] [14].

2.7 Molecular docking analysis

The molecular docking analysis by utilizing PyRx, Swiss PDB viewer and Biovia Discovery Studio begins with preparing the target protein structure by removing ligands, water molecules and heteroatoms and cleaning up a protein in Biovia Discovery Studio and Swiss PDB viewer helps to minimize the energy and fixing mixing residues along with side chains of the protein. Import the protein then save the file in PDB format. Next, prepare the ligands by optimizing their geometry and keeping them in a suitable format, such as SDF. Import the prepared protein and ligands into PyRx and define the docking grid box around the protein's active site. Perform the docking simulation, where PyRx calculates binding affinities and generates possible binding positions. Analyze the highest negative binding energy of the proteins [15].

3 Result and Discussion

3.1 Result

- **Toxicity prediction and pharmacokinetics properties**

The compound has low toxicity risks as it is not flagged as mutagenic, tumorigenic, irritant and reproductive-effective. Its cLogP value (2.27) suggests moderate lipophilicity, and a solubility (-2.87) indicates good aqueous solubility. With a molecular weight of 300.26 g/mol and a Topological Polar Surface Area of 100.13 Å², it is likely to have high gastrointestinal absorption. The compound exhibits good drug-likeness and an acceptable drug score of 0.8, supporting its potential as a drug candidate.

- **Potential targets**

Obtained 100 Chrysoeriol-target genes from Swiss Target Prediction and 1255 Alzheimer's disease related targets from DisGeNET and GeneCards. Intersecting the two datasets, it results 35 potential targets of chrysoeriol is against to treat the Alzheimer's disease provided in Figure 2.

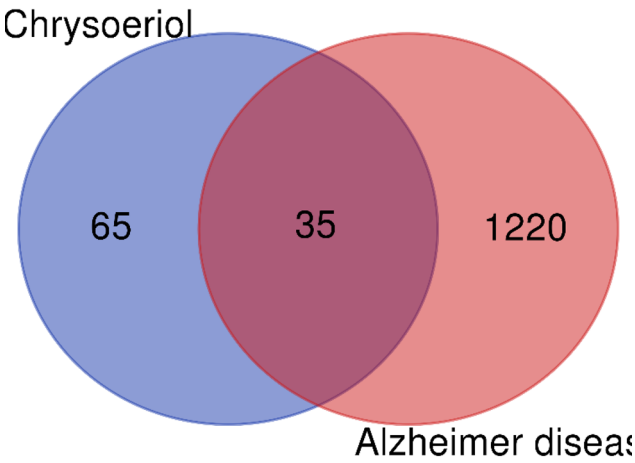

Fig. 2. Intersection of Chrysoeriol and Alzheimer's disease targets

- **Construction and analysis of PPI network and Hub genes**

A total of 35 overlapping targets were imported to the STRING database to identify Protein Protein Interaction networks. The PPI network was constructed, comprising 35 nodes and 196 edges, with an average node degree of 12.6 shown in Figure 3. For analysing Hub genes in Cytoscape software the CytoHubba tool was utilized, to identify the top 10 hub targets by degree and choosing the shortest path. This approach identifies hub genes based on their highest connectivity degree, implying that genes with greater connectivity are likely to serve as critical targets. Protein Protein Interaction network and the top 10 Hub genes are depicted in Figure 4.

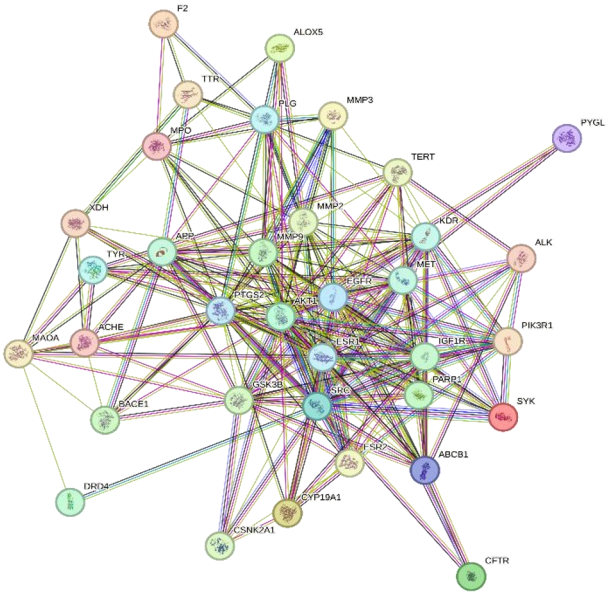

Fig. 3. Protein Protein Interaction network

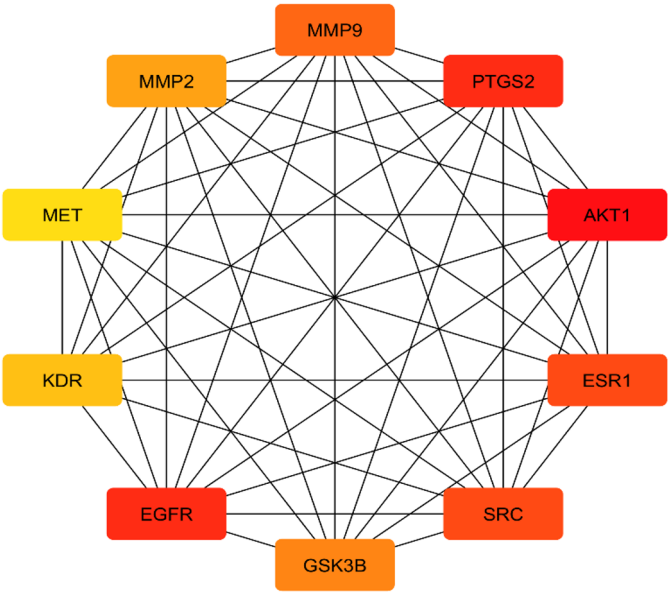

Fig. 4. Hub genes

- **Gene Ontology and Kyoto Enrichment analysis of Genes and Genomes**

From top 10 Hub genes further mapped into the GO analysis and KEGG pathway with DAVID for enrichment on target genes. In Gene Ontology enrichment analysis, dividing the genes into three categories Biological Process, Cellular Components, and Molecular Function, the enrichment values represent the heights of the bars are depicted in Figure 5. Important terms are identified in this analysis include Biological Process: negative regulation of apoptosis and response to oxidative stress; Cellular Component: receptor complex and plasma membrane; and Molecular Function: protein tyrosine kinase activity and enzyme binding. These findings lies under Alzheimer's pathologies are oxidative stress, synaptic dysfunction, and altered protein signaling. The Figure 6 depicts enrichment analysis from KEGG pathways related to Alzheimer's disease- associated genes in the Kyoto Encyclopedia of Genes and Genomes. It divides all genes into different categories such as biological processes, cellular components and molecular functions for better visualization. Also, it creates a color gradient depicting significant terms which are determined from the highest by −log10(p-value); red depicts maximum significance. The size of the bubbles shows the number of genes involved. Bigger the bubbles, more is the count of genes. Major processes include protein autophosphorylation, negative regulation of apoptosis, and response to reactive oxygen species, cellular components like receptor complexes and postsynapse, and molecular functions such as protein tyrosine kinase activity and enzyme binding. These terms emphasize pathways important for neuronal function and survival, both of which are perturbed in Alzheimer's disease.

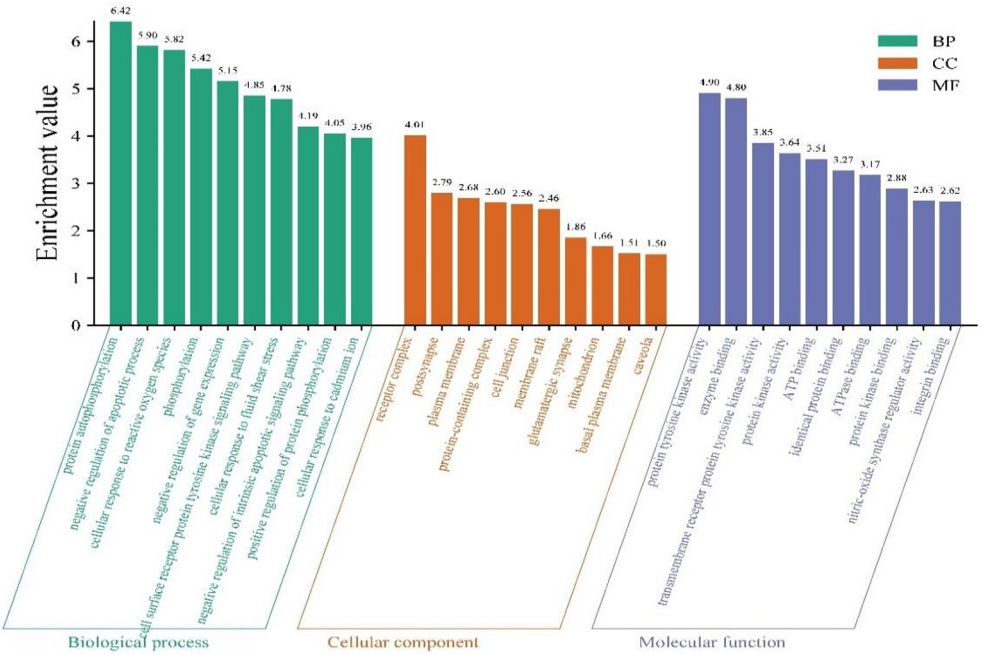

Fig. 5. Gene ontology – Biological Process, Cellular Component and Molecular Function

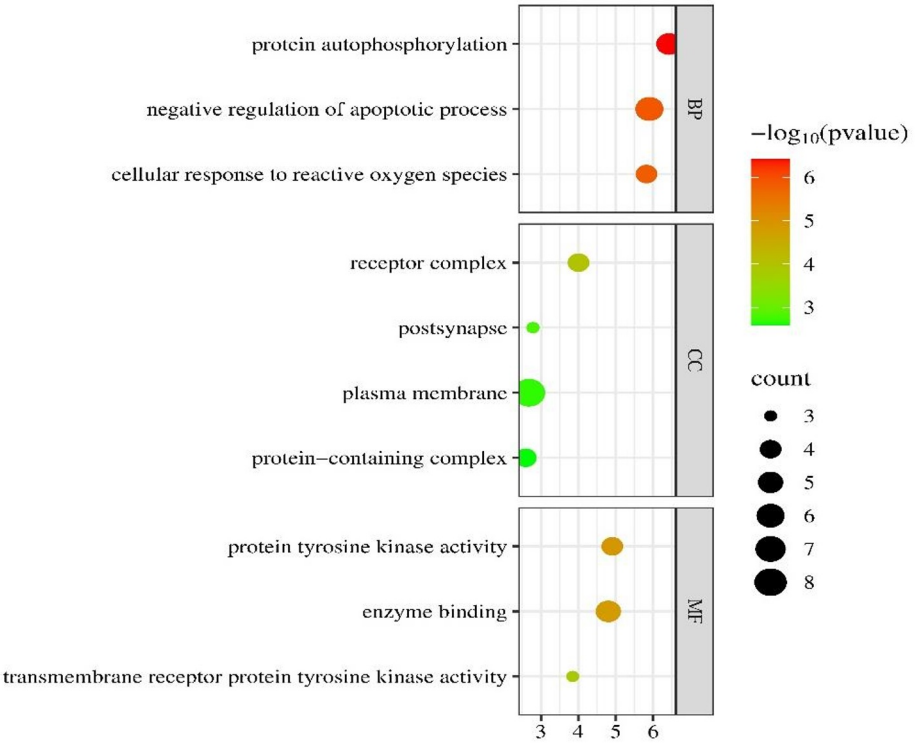

Fig. 6. Kyoto Enrichment analysis of Genes and Genomes pathway

- **Molecular docking analysis**

Table 1. Binding energy of Chrysoeriol and its potential targets

Rank	Name	Score	PDB ID	Chrysoeriol (kcal/mol)	Rivastigmine (kcal/mol)
1	AKT1	29	1UNQ	-7.3	-5.9
2	EGFR	25	8A27	-9.3	-6.6
2	PTGS2	25	5F19	-11.3	-8
4	ESR1	23	8APS	-7.8	-5.8
4	SRC	23	6C4S	-8.3	-5.9
6	MMP9	22	4XCT	-10	-7.6
7	GSK3B	20	1O6L	-10	-7.5
8	MMP2	19	7XJO	-10.8	-8
9	KDR	18	3VO3	-10.3	-7.8
10	MET	17	4R1V	-9.9	-7.2

Molecular docking analyses are used to identify the binding affinities between the 10 targets and compound Table 1. Among these 10 targets, PTGS2 has the strongest binding affinity (-11.3 kcal/mol) when docked with chrysoeriol and compared to rivastigmine. This result shows that chrysoeriol may effectively interact with these targets, which play significant roles in Alzheimer's pathology, thus offering valuable insights for future therapeutic strategies.

3.2 Discussion

The present study highlights the potential of chrysoeriol as a therapeutic agent for Alzheimer's disease, supported by its favorable pharmacokinetic profile, target specificity, and molecular interactions. The compound demonstrated low toxicity, moderate lipophilicity, and high gastrointestinal absorption, satisfying the criteria for drug-likeness. These properties align with the ideal characteristics of oral drugs, supporting its candidacy for further development. The identification of 35 intersecting targets between chrysoeriol and Alzheimer's disease-related genes underscores the compound's multifaceted therapeutic potential. The constructed PPI network revealed a dense interaction landscape, with 196 edges and top hub genes, including AKT1, EGFR, ESR1, GSK3B, KDR, MET, MMP2, MMP9, PTGS2 and SRC. These hub genes are known to play crucial roles in pathways implicated in oxidative stress, inflammation, and apoptosis key pathological process in Alzheimer's disease. Gene Ontology and KEGG pathway enrichment analyses provided further insights, emphasizing the compound's influence on biological processes such as negative regulation of apoptosis and response to oxidative stress, molecular functions like protein tyrosine kinase activity, and cellular components such as receptor complexes. These pathways are intricately linked to neuronal survival, synaptic integrity, and inflammation regulation in Alzheimer disease pathophysiology. Molecular docking results confirmed the strong binding affinities of chrysoeriol with critical targets, notably PTGS2, which exhibited the highest binding energy (-11.3 kcal/mol). This suggests a robust interaction that could inhibit PTGS2 activity, a key mediator of neuroinflammation. Comparisons with rivastigmine, an FDA-approved drug for AD, showed superior binding energies for chrysoeriol across multiple targets, further reinforcing its therapeutic potential. These

findings collectively highlight chrysoeriol's capability to modulate multiple pathological mechanisms associated with Alzheimer's disease. Its neuroprotective and anti-inflammatory properties, demonstrated in previous invitro and invivo studies, align with the molecular interactions observed in this analysis. Future studies should validate these computational findings through in vitro and in vivo experimentation to establish a more comprehensive understanding of chrysoeriol's therapeutic efficacy and mechanisms of action in Alzheimer's disease.

4 Conclusion

Chrysoeriol shows strong potential as a therapeutic agent for Alzheimer's disease, with favorable pharmacokinetic properties. Network pharmacology analyses identified 35 key targets associated with Alzheimer's disease, which are involved in critical pathways like beta-amyloid response and acetylcholine regulation. Molecular docking studies revealed that strong binding affinities, with chrysoeriol while compared with rivastigmine. These findings provide a robust foundation for further experimental validation and development as a multi-target therapeutic strategy for Alzheimer's disease.

Acknowledgement

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List of Abbreviations:

TNF-α: Tumor Necrosis Factor-alpha; IL-1β: Interleukin-1 beta; IL-6: Interleukin-6; Wnt/β-catenin: Wnt Signaling Pathway and Beta-catenin; MPP+: 1-Methyl-4-phenylpyridinium; SH-SY5Y: Human Neuroblastoma Cell Line; PI3K/Akt: Phosphoinositide 3-Kinase/Protein Kinase B Pathway; iNOS: Inducible Nitric Oxide Synthase; COX-2: Cyclooxygenase-2; STAT3: Signal Transducer and Activator of Transcription 3; NF-κB: Nuclear Factor Kappa B; TPSA: Topological Polar Surface Area; SMILES : Simplified Molecular Input Line Entry System; ADME : Absorption, Distribution, Metabolism, and Excretion; PPI : Protein Protein Interaction; OSIRIS : Open Source Interactive Regression and Interpretation System; DAVID : Database for Annotation, Visualization, and Integrated Discovery; KEGG : Kyoto Enrichment analysis of Genes and Genomes; STRING : Search Tool for the Retrieval of Interacting Genes/Proteins; PDB : Protein Data Bank; SDF : Structure Data File: AKT1 : Alpha serine/threonine kinase1; EGFR : Epidermal Growth Factor Receptor; ESR1 : Estrogen Receptor 1; GSK3B : Glycogen Synthase Kinase:3 Beta; KDR : Kinase Insert Domain Receptor; MET : Mesenchymal Epithelial Transition; MMP2 : Matrix Metallopeptidase 2; MMP9 : Matrix Metallopeptidase 9; PTGS2 : Prostaglandin-Endoperoxide Synthase 2; SRC : Proto:oncogene tyrosine:protein kinase SRC; FDA : Food and Drug Administration; AD : Alzheimer's Disease

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