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In silico Drug Design of Natural Apolipoprotein E4 Inhibitors for Alzheimer's Disease Management

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Abstract

Background: Alzheimer's disease (AD) is the most common form of dementia among the elderly and one of the most difficult public health issues. The APoE4 allele is the main risk factor for late-onset sporadic AD.

Methods: This study chose phytochemicals from *Ginkgo biloba* because of their well-documented neuroprotective properties. A total of 258 *G. biloba* compounds were obtained from the LOTUS database and screened against the Apolipoprotein E4 (ApoE4) protein using the *in silico* method. Physicochemical and ADMET properties prediction assessment was conducted using the ADMETLab 3.0 web tool.

Result: The hit compounds quercetin, saccharic acid, acacetin, and L-tyrosine were discovered to strongly bind to ApoE4 protein and interact with key ApoE4 protein residues. In addition, these compounds had several amino acid interactions in common with the control compounds. Furthermore, these four compounds have distinct ADMET profiles, and the expected properties, such as solubility, permeability, and toxicity, fall within acceptable limits, making them potential drug candidates.

Conclusion: The compounds (quercetin, saccharic acid, acacetin, and L-tyrosine) can be used as ApoE4 inhibitors to manage AD.

Introduction

Alzheimer's disease (AD), the most common type of dementia among the aged population, is a neurological condition marked by cognitive deterioration. It affects about 13% of people over 65 and 45% of those over 85 [1]. AD is caused by an imbalance in amyloid-β (Aβ) peptide production and clearance, resulting in toxic oligomers, intraneuronal Aβ, and amyloid plaques. These aggregates harm synapses and cause neurodegeneration [2,3], and their toxicity is amplified by hyperphosphorylated tau, which produces neurofibrillary tangles [4]. Proteolytic breakdown of amyloid precursor protein (APP) produces Aβ, which contains 40-42 amino acids [5]. Familial early-onset AD (FAD) is mainly caused by mutations in APP, PSEN1, and PSEN2 genes, which produce Aβ and play a crucial role in the disease's etiology [6]. In contrast, approximately 95% of cases are sporadic late-onset Alzheimer's disease (LOAD), which is impacted by both hereditary and environmental factors. The apolipoprotein E (ApoE) gene is the most significant genetic risk factor for LOAD, influencing several disease pathways and providing a prospective treatment target [7].

APoE4 gene polymorphisms have a significant impact on the likelihood of LOAD. The APoE4 allele raises the risk, while APoE2 lowers it compared to the common APoE3 variant. APoE4 is highly linked to earlier and more widespread amyloid pathology. APoE isoforms influence tau pathology, neurodegeneration, microglial responses, and their effects on amyloid-β. APoE4 impairs brain homeostatic functions such as lipid transport, synaptic plasticity, glucose metabolism, and cerebrovascular function, contributing to its pathogenic nature [8].

Drug development is a process that aims to identify a small synthetic chemical or a large biomolecule for further investigation as a possible therapeutic candidate. It is a lengthy process that takes approximately 10 to 15 years and can cost up to 2.558 billion USD to bring medicine to market [9,10]. It is a multi-step process that includes identifying a potential therapeutic target, validating the drug target, discovering hits, optimizing lead compounds, and conducting preclinical and clinical research. Leading pharmaceutical corporations and research organizations have used computer-assisted drug discovery (CADD) tools in preliminary investigations to help speed up the drug development process, reducing costs and failures in the final stage [11,12]. This study aimed to find natural ApoE4 inhibitors to manage AD using the *in silico* tools.

Methods

Target retrieval and preparation

The Protein Data Bank (PDB ID: 6NCO) revealed the three-dimensional structure of ApoE4. The Discovery Studio Visualizer was utilized to optimize the docking preparation by removing co-crystallized ligand, water molecules, and heteroatoms from the protein, and the cleaned structure was saved in the ".pdb" format. The integration of hydrogen atoms into the protein and the assignment of Gasteiger charges to the structural component were performed using the AutoDock tool. The energy minimization process enhanced the protein's structure. Upon finalization of the structure, it was archived in the PDBQT format for utilization in further molecular docking based virtual screening.

Compound library preparation

This study selected phytochemicals from *G. biloba* due to their documented neuroprotective properties. A total of 258 compounds were obtained from the LOTUS database, an extensive resource for plant-derived natural products [13]. Ligand structures were acquired in SDF format, transformed to PDBQT format utilizing Open Babel, and refined for docking via energy minimization in PyRx. Rosuvastatin and ALZ-801 were chosen as positive controls for the virtual screening because they are well-known medications that have been shown to benefit Alzheimer's patients. We selected rosuvastatin and ALZ-801 as orthogonal, clinically grounded comparators that bracket the two principal ApoE4-relevant axes. Rosuvastatin serves as a lipid/lipoprotein functional benchmark, as statins enhance ABCA1-dependent cholesterol efflux and modulate lipoprotein handling central to ApoE lipidation. In contrast, ALZ-801 is an Aβ-oligomer pathway benchmark: tramiprosate binds soluble Aβ and prevents oligomer formation, and ALZ-801 is being advanced in APOE4-enriched clinical programs, including a dedicated Phase 3 in ε4/ε4 individuals [14-16].

Docking-based virtual screening

AutoDock Vina, incorporated into the PyRx tool [17], was utilized for virtual screening to evaluate interactions between the ApoE4 protein and a selected compound library. The co-crystallized ligand was used as a reference to identify the active site of ApoE4. A docking grid was established around the binding pocket to identify critical residues implicated in ligand binding. The X, Y, and Z coordinates were set as 5.409167, -15.766500, and 13.083083, respectively. The docking results were examined to determine the essential interaction residues and binding affinities between the phytochemicals and the ApoE4 proteins. The compounds exhibiting the most advantageous

interaction profiles and binding energies were chosen for additional evaluation.

Physicochemical and ADMET properties prediction

The four hit compounds identified via virtual screening were analyzed comprehensively to ascertain their physicochemical properties and ADMET (Absorption, Distribution, Metabolism, Excretion, and Toxicity) profiles. This assessment was conducted using the sophisticated capabilities of the ADMETLab 3.0 web tool, which offers comprehensive insights into compound behavior and potential drug-likeness [18]. The ADMET assessment encompassed comprehensive predictions of absorption capacity, metabolic stability, tissue distribution, excretion patterns, and toxicity risks, yielding an in-depth understanding of the compounds' pharmacokinetic and safety profiles.

Results

This study aimed to identify natural compounds that could inhibit the ApoE4 protein, which is a key player in AD. Because natural compounds, particularly those derived from *G. biloba*, can protect neurons, this study investigated how they interacted with ApoE4 to identify potential therapeutic candidates. These drugs were used as standards to determine how well natural compounds bind. The LOTUS database was used to create a carefully curated library of 258 compounds representing bioactive components of *G. biloba*. To determine how well the binding works, docking-based virtual screening was performed using AutoDock Vina, which was included with the PyRx tool. Compounds were ranked based on their ability to bind to ApoE4, interact with key residues, and fit into the ApoE4 binding pocket. The results in Table 1 highlight the top ten compounds with higher binding affinities than the positive controls.

S. No.	Lotus ID	Compound name	Binding affinity (kcal/mol)
1.	LTS0004651	Quercetin	-6.9
2.	LTS0007607	Saccharic acid	-6.8
3.	LTS0020151	Acacetin	-6.7
4.	LTS0029981	L-tyrosine	-6.7
5.	LTS0005955	2-pentadecylbenzene-1,3-diol	-6.7
6.	LTS0008993	{6-[(6-{[2-(3,4-dihydroxyphenyl)-5,7-dihydroxy-4-oxochromen-3-yl]oxy}-4,5-dihydroxy-2-methyloxan-3-yl)oxy]-3,4,5-trihydroxyoxan-2-yl}methyl 3-(4-hydroxyphenyl)prop-2-enoate	-6.6
7.	LTS0001307	Bilobol	-6.5
8.	LTS0000701	Neoxanthin	-6.5
9.	LTS0005280	Grevillol	-6.4
10.	LTS0003899	(-)-shikimate	-6.3
11.	-	Rosuvastatin	-6.1
12.	-	ALZ-801	-5.4

Table 1: Top 10 screened compounds with their corresponding lotus ID, compound name, and binding affinities to ApoE4. Rosuvastatin and ALZ-801 are the positive controls.

A comprehensive binding interaction analysis was conducted to explain the interaction profiles of the four hit compounds: Quercetin, Saccharic acid, Acacetin, and L-Tyrosine. These compounds are extensively acknowledged natural products with established therapeutic properties.

Figure 1 illustrates the binding conformations of the control drug in conjunction with the highest-ranked natural compounds within the active site of the APoE4 protein. The molecular overlay emphasizes significant interactions with active site residues, such as Leu149, Asp153, Gln156, Trp24, and Trp26. Panel A depicts the structural alignment of the compounds in the binding pocket, whereas Panel B reveals the specific residues engaged in molecular interactions, including hydrogen bonding and hydrophobic contacts. The observations indicate the chosen natural compounds' robust and consistent binding affinity, implying their potential as effective APoE4 inhibitors.

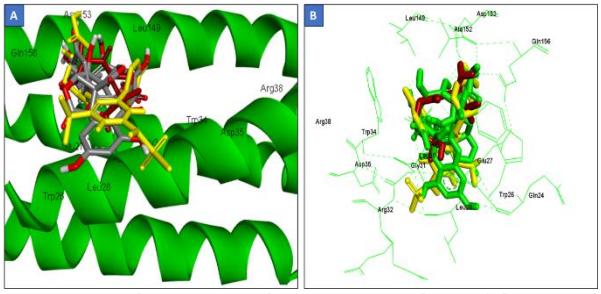

Figure 1: Binding poses and interaction analysis of the control drug and the top four hits. (A) Structural overlay showing the binding poses of the control (yellow and red) and top compounds—Quercetin, Saccharic acid, Acacetin, and L-Tyrosine within the ApoE4 active site. (B) A detailed interaction map depicts the residues involved in binding interactions, including key residues such as Leu149, Asp153, Gln156, Trp24, and Trp26.

The 2D interaction diagrams show the binding profiles of the control drugs Rosuvastatin and ALZ-801 and the four hits- quercetin, saccharic acid, acacetin, and L-tyrosine within the APoE4 protein's active site (Figures 2 and 3). The control drugs form strong hydrogen bonds with Glu27, Asp153, and Ala152, as well as π-π stacking interactions with Trp34 and hydrophobic interactions with Leu149 and Leu30, which effectively anchor them in the active site. The top four natural compounds have similar interaction patterns, demonstrating their efficacy in binding to the APoE4 target.

Quercetin forms hydrogen bonds with Asp153 and Ala152, π-π stacking interactions with Glu27, and hydrophobic contacts with Leu28, indicating strong stability within the active site. Saccharic acid forms several hydrogen bonds with residues Glu27, Asp153, and Ala152, as well as interactions with Trp34 and Leu149, which closely matches the control drugs' binding profile. Acacetin forms hydrogen bonds with

Glu27, Gln156, and Asp35 while also forming stabilizing hydrophobic interactions with Leu30 and Ala152. L-Tyrosine has strong hydrogen bonding with Asp153 and Glu27, π-π stacking with Trp34, and additional hydrophobic interactions with Leu30, making its interaction profile similar to the control drugs.

The comprehensive comparison reveals that these hits mimic the interactions of the control drugs with essential residues such as Asp153, Glu27, Ala152, Leu149, and Trp34, which are required for ligand stabilization.

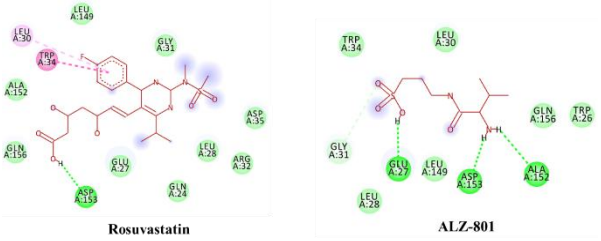

Figure 2: 2D interaction diagrams of the control drugs Rosuvastatin and ALZ-801 within the ApoE4 binding pocket. Green dotted lines represent conventional hydrogen-bond interactions, while pink dotted lines indicate hydrophobic and aromatic contacts, including π–π and π–alkyl interactions.

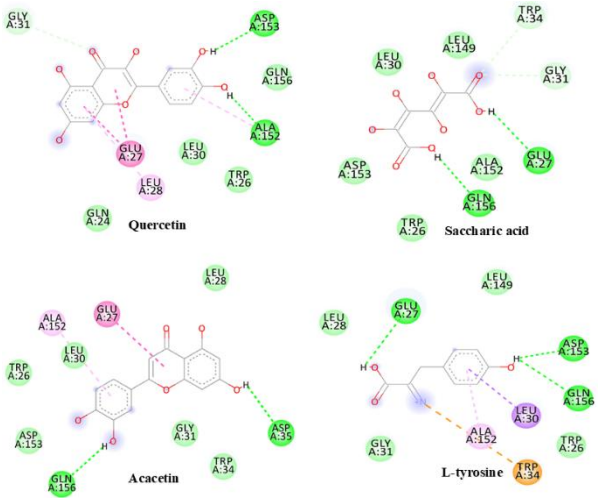

Figure 3: 2D interaction diagrams of the top four natural compounds, Quercetin, Saccharic acid, Acacetin, and L-tyrosine, within the ApoE4 binding pocket. Green dotted lines indicate conventional hydrogen-bond interactions, pink dotted lines represent hydrophobic and aromatic contacts, and the orange dotted line denotes an electrostatic π-related interaction between the ligand and residue.

The radar plots illustrate the ADMET characteristics of the four leading compounds evaluated with ADMETLab 3.0. The graphs juxtapose the anticipated properties of each compound (represented by yellow lines) with the lower limits (green area) and upper limits (blue area), which delineate the acceptable thresholds for drug-likeness and pharmacokinetic characteristics (Figure 4). The analysis indicates that all four compounds display unique ADMET profiles. Specific anticipated properties, including solubility, permeability, and toxicity, reside within the acceptable parameters delineated by the green lower limit and blue upper limit regions, whereas a few exhibit minor deviations, suggesting potential avenues for optimization. The radar plots facilitate a rapid visual assessment of each compound's advantages and disadvantages, offering insights into their pharmacokinetic appropriateness and potential as drug candidates.

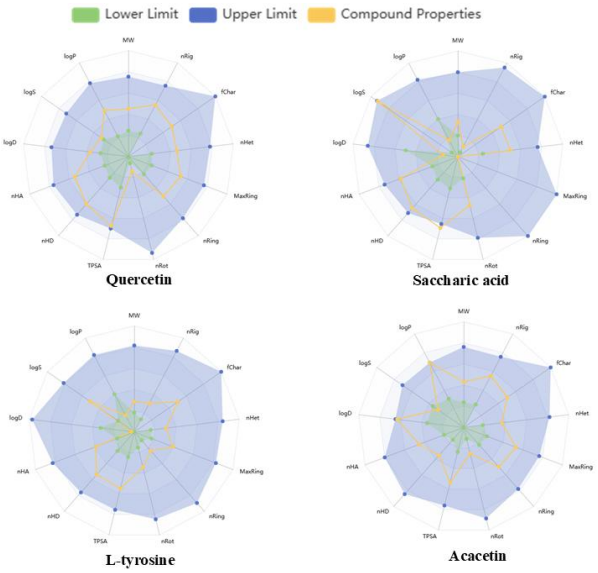

Figure 4: Radar plots for ADMET analysis of the top four compounds using ADMETLab 3.0.

Discussion

AD, the most common form of dementia [19], accounts for 60-70% of cases and affects more than 27 million people globally [20]. It causes considerable difficulties on patients' families and society due to its high emotional and financial expenses. The APoE ε4 allele is the main genetic risk factor for late-onset sporadic AD. Having one ε4 allele increases the risk 2-3 times while having two ε4 alleles increases it 10-15 times [21].

Rosuvastatin and ALZ-801 were used as positive controls because they are well-known medications that have been shown to benefit patients with Alzheimer's. In this study, ApoE4 residues Trp34, Leu30, Gln156, Ala152, Asp153, Leu149, Glu27, Leu28, and Gly31 were important for binding with Rosuvastatin and ALZ-801. Interestingly, the top four hit compounds, quercetin, saccharic acid, acacetin, and L-tyrosine, were also found to bind to the majority of these ApoE4 residues.

A higher negative binding affinity value indicates that the ligand-protein complex has a strong binding to each other [22-25]. Accordingly, the top four hit compounds, quercetin, saccharic acid, acacetin, and L-tyrosine, have higher negative binding affinity values than the controls (rosuvastatin and ALZ-801), indicating that these compounds strongly bind to the ApoE4 protein.

The diverse secondary metabolites found in many plant sections provide medicinal plants with their therapeutic abilities. Flavonoids, polyphenols, alkaloids, and glycosides can help prevent neurodegeneration and improve cognitive performance. Natural compounds derived from plants or marine organisms have anti-Alzheimer effects by targeting β-amyloid, Beta-secretase 1, and Acetylcholinesterase via distinct pharmacological pathways [26]. Natural compounds originating from plants, animals, and fungi have been intensively investigated for their therapeutic potential in diseases, including neurodegenerative disorders (NDs) [27]. These chemicals may benefit NDs by modifying microglia, but questions about their efficacy, molecular targets, physicochemical qualities, and safety remain due to a lack of empirical evidence. *G. biloba* is believed to have originated in Zhejiang province, China [28]. Standardized *G. biloba* extract derived from dried leaves treats memory loss and dementia, including AD [29,30]. Clinical investigations have shown that cognitive performance improves in the elderly and Alzheimer's patients [31-34]. In this study, bioactive compounds from *G. biloba* were found to bind strongly to the ApoE4 protein, suggesting they can be used as ApoE4 inhibitors to treat AD.

This study screened the bioactive compounds in *G. biloba* against the ApoE4 protein. The hit compounds, quercetin, saccharic acid, acacetin, and L-tyrosine, strongly bind to the ApoE4 protein and interact with key ApoE4 protein residues. In addition, these four compounds have distinct ADMET profiles, making them potential drug candidates. These compounds can be used as ApoE4 inhibitors to manage AD. However, additional experimental validation is required to optimize them as ApoE4 inhibitors.

Author Contributions

Alnowihi SM: Methodology, data curation, and writing – original draft.

AlBishi HM: Data analysis, interpretation of results, critically revised manuscript.

Alshubaily FA: Supervision, project administration, and writing – review, and editing.

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Conflict of Interest

No conflict of interest

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