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Original Article

In Silico Exploration of APOE4 Inhibitors: Molecular Docking and ADMET Profiling for Alzheimer's Therapy

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ABSTRACT

Background: Alzheimer's disease (AD), a prevalent neurodegenerative disorder, poses significant challenges to healthcare systems worldwide. The aggregation of Amyloid-β peptide and the presence of apolipoprotein E4 (apoE4) are recognized as central factors in AD pathogenesis. Despite the availability of FDA-approved drugs, there remains a dire need for more effective and targeted treatments to combat this disease. The advent of in silico methodologies offers a promising avenue for accelerating the drug discovery process, providing a cost-effective and efficient means to screen for potential therapeutic agents.

Objective: This study aimed to identify and evaluate potential inhibitors of apoE4 using in silico drug discovery methods. By targeting the interaction domain of apoE4, the study sought to discover compounds capable of reducing beta-amyloid aggregation, thus offering a novel approach to AD therapy.

Methods: Computational techniques, including the Lipinski Rule of Five and ADMET filtering, were employed to screen a dataset of 80 chemical inhibitors retrieved from the NCBI database. Molecular docking was performed to assess the binding affinities and orientations of the compounds to apoE4, utilizing Phyre2 for 3D structure prediction and AutoDock Vina for docking simulations. The selection criteria for lead compounds were based on their binding energy, hydrogen bonding interactions, and compliance with drug-like properties.

Results: Out of the initial 80 compounds screened, 15 were excluded based on the Lipinski Rule of Five, and an additional 43 were deemed unsuitable following ADMET analysis. Molecular docking identified two compounds, taxifolin and luteolin, exhibiting superior binding affinities to apoE4, with taxifolin showing the lowest binding energy of -7.4 kcal/mol and the highest number of hydrogen bonds in one of its conformations.

Conclusion: The study successfully leveraged in silico methods to identify taxifolin and luteolin as potent inhibitors of apoE4, highlighting the potential of computational drug discovery in developing novel AD therapies. These findings pave the way for future experimental validation and clinical trials to assess the therapeutic efficacy and safety of these compounds in treating Alzheimer's disease.

Keywords: Alzheimer's disease, apolipoprotein E4, in silico drug discovery, molecular docking, taxifolin, luteolin, ADMET, Lipinski Rule of Five.

INTRODUCTION

Alzheimer's disease (AD) is a degenerative brain disorder characterized by the inexorable progression of memory loss and cognitive decline. Central to the pathology of AD are the accumulation of beta-amyloid plaques and neurofibrillary tangles (NFTs), which have been extensively documented through post-mortem examinations of the neuro-cortex and hippocampus regions of affected brains. These studies have highlighted the presence of plaques consisting of beta-amyloid and NFTs comprised of tau proteins (1,2). The risk factors for AD are multifaceted, encompassing increased age, genetic predisposition through family history, prior severe head injuries, and lifestyle and conditions linked to vascular disease (3). The definitive diagnosis of AD, facilitated by microscopic examination, reveals the presence of amyloid plaques and NFTs within the brain tissue of those afflicted (4).

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To date, six inhibitors have received approval for the therapeutic management of Alzheimer's disease. Donepezil is sanctioned for use across all stages of AD, from mild to severe, while Rivastigmine and Galantamine are designated for the treatment of mild to moderate stages. Notably, Exelon is presented in a transdermal patch format, offering an alternative administration route that may be preferable for certain patients due to its ease of use through skin application. Despite their efficacy, these inhibitors are not without side effects, which may include nausea, vomiting, diarrhea, weight loss, and dizziness (5,6).

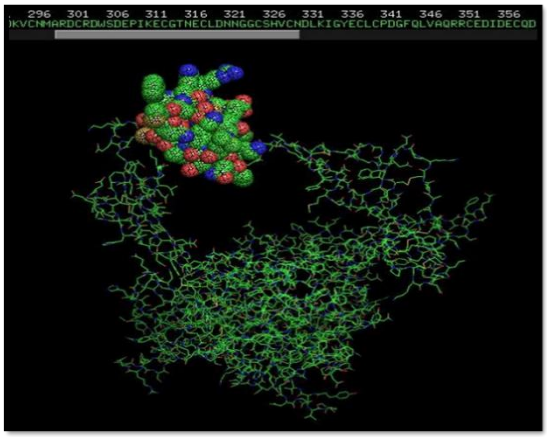
Recent studies have underscored the role of the apoe4 allele in exacerbating the toxicity or aggregation level of beta-amyloid in the brain, thus implicating apoe4 in the pathogenesis of AD. This discovery has paved the way for the exploration of inhibitory peptides capable of blocking or mitigating the activity of amyloid, offering a promising avenue for therapeutic intervention (7). The development of drugs for AD leverages both ligand-based and structure-based design strategies. Ligand-based drug design is informed by the properties of molecules known to bind to the biological target, facilitating the creation of a pharmacophore model that outlines essential structural features for effective binding (8). Conversely, structure-based drug design utilizes the three-dimensional structure of the biological target, acquired through techniques such as X-ray crystallography or NMR spectroscopy, to guide the development of therapeutic agents (9). In instances where an experimental structure is unavailable, homology modeling based on related protein structures can be employed (10).

Molecular docking represents a critical optimization challenge in drug design, aiming to determine the optimal orientation of a ligand as it binds to a target protein. This process acknowledges the inherent flexibility of both ligands and proteins, adopting an induced fit model to capture the dynamic nature of molecular interactions. A variety of software tools, including Autodock 4, Autodock Vina, and Patch Dock, facilitate this complex computational task (12). Furthermore, the ADMET screening process plays a pivotal role in the early-stage assessment of drug candidates, evaluating their absorption, distribution, metabolism, excretion, and toxicological profiles to ensure safety and efficacy (13,14). Targeting apoE4, a factor implicated in the accumulation of beta-amyloid, emerges as a strategic focus in disrupting the pathogenesis of AD at its nascent stages.

The overarching goal of current research endeavors is to innovate drugs that inhibit apoE4, thereby counteracting its contributory role in AD progression. This involves the meticulous screening of compounds through ADMET protocols and the utilization of three-dimensional structural analyses and molecular docking techniques to identify candidates with optimal binding properties. Through these advanced computational methodologies, the scientific community is poised to make significant strides in the development of targeted therapies for Alzheimer's disease, offering hope for effective management and potential reversal of this debilitating condition.

MATERIAL AND METHODS

In the pursuit of novel therapeutic strategies for Alzheimer's disease (AD), the most prevalent neurodegenerative disorder, the current research focused on the design of drugs targeting this condition due to its significant incidence and the current scarcity of effective treatments. The selection of the target protein was informed by the identification of ApoE, a 34-kDa protein comprising 299 amino acids, which is ubiquitously expressed in various brain cells, including astrocytes and microglia. Notably, the receptor-binding domain of ApoE is located within the amino acids 136-150 of the N-terminal region. Among the three human isoforms of ApoE (ApoE2, ApoE3, and ApoE4), differences at merely two amino acid positions (112 and 158) differentiate these isoforms. ApoE4, in particular, is associated with a significantly increased risk of AD, with its altered structural properties implicated in this enhanced vulnerability. The sequence of apoE4 was sourced from the National Center for Biotechnology Information (NCBI) in FASTA format, facilitating subsequent analyses.



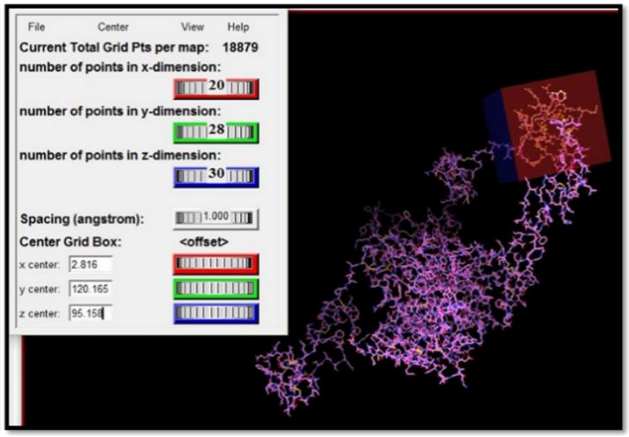


Figure 1 Dot surface showing active site of protein; Figure 2 Auto-grid dimension for ligand docking in apoE4

The 3D structure of apoE4 was generated using the Phyre2 online server, which employs hidden Markov Models for sequence alignment, thereby enhancing the accuracy of alignment and detection rates for

homology modeling. Following this, the active site of the protein was predicted using AutoDock Vina, which evaluates docking efficiency and active site prediction based on an energy-based scoring function. This process involved the use of PDB format files for both the target protein and potential inhibitors.

Ligands capable of inhibiting apoE4 were identified from the PubChem bioassay database, with 80 compounds deemed efficient in this capacity based on high throughput screening assays. These compounds were then subjected to a conversion process from SDF to PDB format using OpenBabel version 2.3.1, to ensure compatibility with most software utilized in this study.

To determine the drug-likeness of these compounds, the Lipinski Rule of Five was applied using an online server provided by SCFBio. This analysis facilitated the identification of compounds exhibiting properties consistent with oral drug viability. Furthermore, the ADME properties of the selected compounds were assessed using the FAF-Drugs2 package to evaluate their pharmacokinetics and pharmacodynamics, crucial for predicting the interaction of the drug within the body.

Molecular docking procedures were meticulously carried out for each chemical compound with the target protein using AutoDock Vina, setting a grid box within the active site of the protein and utilizing specific commands for docking. Visualization of the docked complexes was achieved through the PyMol molecular graphics system, enabling the detailed examination of bonding interactions between the target protein and the compounds.

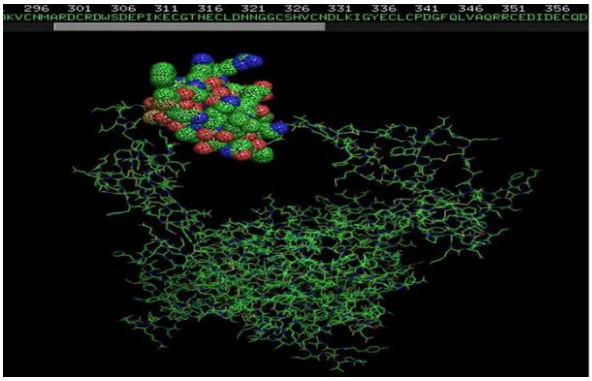
Compounds demonstrating a higher number of hydrogen bonds were advanced for lead identification, with their mutagenicity and toxicity predicted using TOXTREE software. This step was essential for excluding compounds with potentially harmful effects. The lead compound was selected based on a comprehensive evaluation of ADME properties, interaction quality, bond length, and binding energy, ensuring the identification of the most promising candidate for further development.

In adherence to ethical considerations, all procedures and methodologies employed in this research were conducted in accordance with the principles outlined in the Declaration of Helsinki, ensuring the integrity and ethical rigor of the study. This included the ethical treatment of data, the responsible reporting of findings, and the commitment to transparency throughout the research process. This study represents a significant step forward in the design of novel therapeutics for Alzheimer's disease, offering hope for effective treatment strategies in the future.

RESULTS

In this study, we embarked on a comprehensive approach to identifying potential therapeutic agents for Alzheimer's disease (AD) by focusing on the apoE4 protein, a significant risk factor for the condition. The absence of a known 3D structure for the target protein necessitated predictive modeling to elucidate its conformation. Utilizing the Phyre2 web server, we generated a 3D structure of apoE4 based on the intensive modeling mode. The reference amino acid sequence, obtained from the NCBI protein database, facilitated the identification of a human toll-like receptor as a homologue with maximal similarity. This template was instrumental in modeling the 3D structure of apoE4, with subsequent detailed analysis of the primary and secondary structure performed using PyMol v 1.3.

The active site of apoE4 was meticulously defined through the AutoDock Vina software, pinpointing specific amino acids that constitute the binding pocket essential for drug interaction. The identified amino acids, ranging from GLY 278 to ASP 308, were integral to the binding mechanism, providing a precise target for inhibitory compounds.



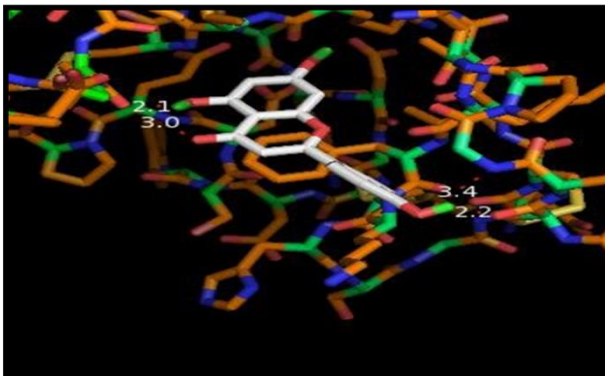


Figure 2 Dot surface of apoE4 active site in PyMol v 1; Figure 3 Apigenin interacting with apoE4

Upon applying the Lipinski Rule of Five, a pivotal step in assessing drug likeness, 65 out of 80 compounds initially retrieved complied with the criteria, underscoring their potential as orally active drugs. This filtration was critical in narrowing down candidates for further analysis. Subsequently, the ADME properties of these 65 compounds were evaluated, revealing 22 compounds with acceptable profiles. This assessment was crucial for understanding the physicochemical and pharmacokinetic properties essential for a drug's efficacy and safety.

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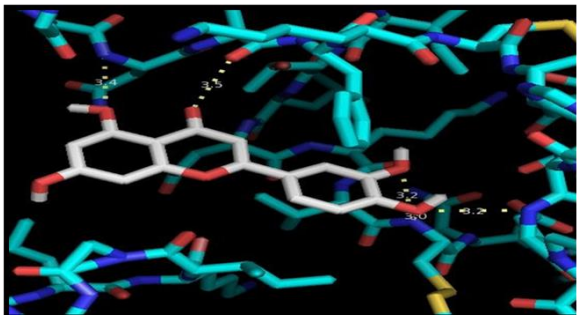
Molecular docking studies offered insight into the non-covalent binding affinities of the selected compounds to apoE4, using AutoDock Vina to generate nine binding conformations for each compound. The analysis of binding energies across these conformations highlighted the compounds' potential for stable interaction with the target protein. Notably, compounds such as Curcumin, Genistein, Daidzein, Luteolin, and Taxifolin demonstrated significant binding affinities, suggesting their potential as inhibitors of apoE4.



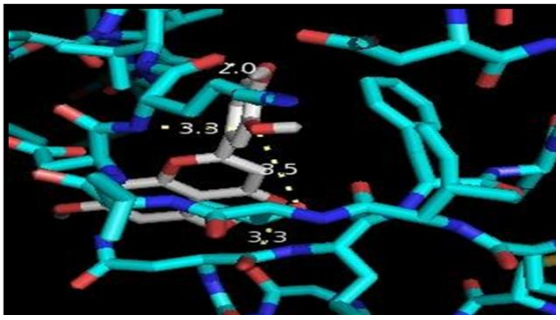
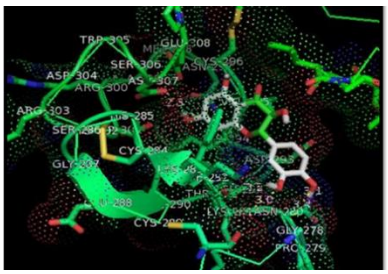
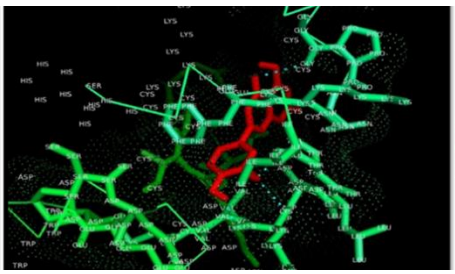


Figure 3 Luteolin interactions with apoE4; Figure 4 Luteolin binding to apoE4

Visualization of the docked complexes using PyMol facilitated a detailed examination of the interactions between apoE4 and the compounds, particularly focusing on hydrogen bonding, hydrophobic bonding, Van der Waals interactions, and ionic bonding. This comprehensive analysis revealed that the strength and number of these interactions play a crucial role in the stability and efficacy of the binding, with compounds exhibiting stronger hydrogen bonds considered more favorable due to their potential for higher inhibitory activity.





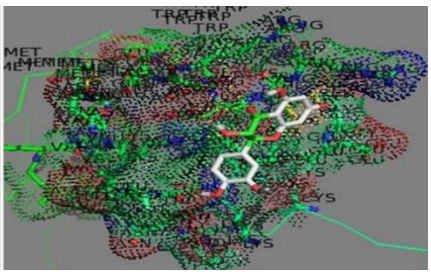
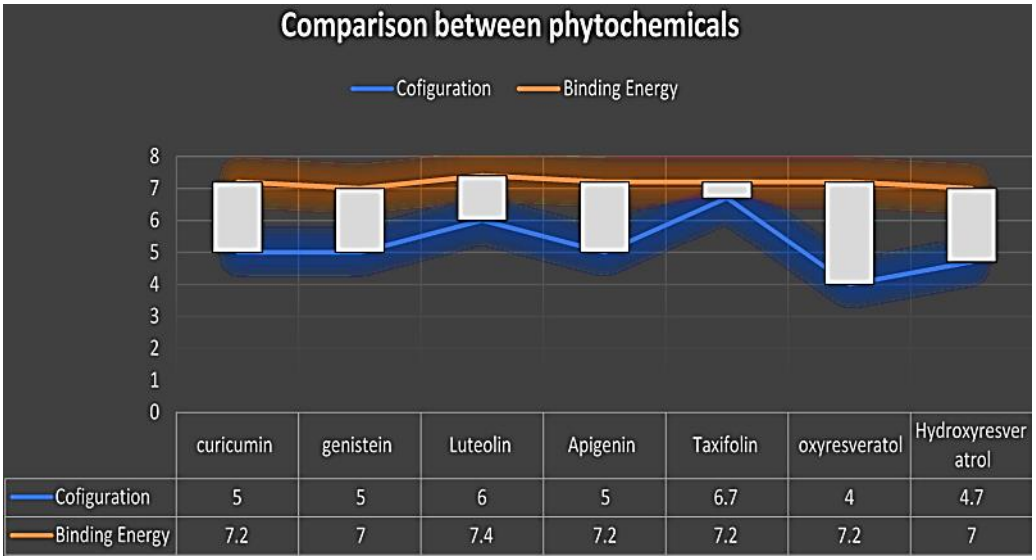


Figure 4 Taxifolin's 9 conformations with apoE4.; Figure 5 Luteolin's selective amino acid interactions with apoE4; Figure 6 Taxifolin's key amino acid interactions (278–308)

Taxifolin emerged as the lead compound after a rigorous selection process, distinguished by its low binding energy and a higher number of interactions, underscoring its promising inhibitory potential against apoE4.



	curicumin	genistein	Luteolin	Apigenin	Taxifolin	oxyresveratol	Hydroxyresveratrol
Cofiguration	5	5	6	5	6.7	4	4.7
Binding Energy	7.2	7	7.4	7.2	7.2	7.2	7

Figure 5 Binding energy and configuration comparison of top seven compounds

This selection was further validated by toxicity and mutagenicity assessments using TOXTREE software, which confirmed the non-toxic and non-mutagenic nature of the selected compounds.

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Table 1 Binding energies of compounds in kcal/mol, from auto dock Vina.

Compound	Mode, Kcal/mol								
	1	2	3	4	5	6	7	8	9
Curcumin	-7.3	-7.2	-7.2	-7.0	-6.9	-6.8	-6.7	-6.7	-6.5
Genistein	-7.6	-6.8	-6.6	-6.6	-6.4	-6.3	-6.3	-6.2	-6.2
Daidzein	-7.2	-6.8	-6.8	-6.6	-6.6	-6.4	-6.3	-6.3	-6.3
Luteolin	-7.6	-7.4	-7.2	-7.0	-7.0	-6.6	-6.5	-6.5	-6.3
Tangeretin	-6.6	-6.4	-6.0	-5.9	-5.7	-5.6	-5.6	-5.6	-5.5
Apigenin	-7.3	-7.3	-7.0	-6.9	-6.6	-6.4	-6.3	-6.3	-5.8
Quercetin	-7.0	-6.9	-6.6	-6.6	-6.4	-6.1	-5.8	-5.7	-5.5
Kaempferol	-6.8	-6.7	-6.4	-6.4	-6.4	-6.3	-6.2	-6.0	-5.9
Taxifolin	-7.4	-7.3	-7.1	-6.7	-6.6	-6.4	-6.3	-6.2	-6.1
Nobiletin	-6.5	-6.3	-6.1	-5.9	-5.9	-5.8	-5.7	-5.7	-5.5

The results of this study highlight the utility of computational methods in the early stages of drug discovery for AD, offering a foundation for further experimental validation. The identification of Taxifolin as a lead compound, characterized by favorable pharmacokinetic properties and potent interaction with apoE4, represents a significant step forward in the quest for effective AD therapeutics.

DISCUSSION

In the realm of modern drug discovery, the adoption of computational methodologies, often referred to as "in silico" techniques, has revolutionized the identification and development of new therapeutic agents. This approach stands in stark contrast to the traditional, more labor-intensive processes, offering rapid and cost-effective alternatives that leverage the vast capabilities of computational tools for drug design. The term "in silico," possibly derived from silicon's pivotal role in computer technology, denotes the virtual environment where these drug discovery processes occur, encompassing activities from ligand identification to the blocking of specific biological targets (15).

This study focused on Alzheimer's disease (AD), a neurodegenerative disorder characterized by the aggregation of Amyloid-β peptide (Aβ) and associated with mutations in the amyloid precursor protein (APP) and presenilin genes (17). AD's complexity is further highlighted by its symptoms, ranging from apathy to cognitive disabilities, underscoring the urgent need for effective therapeutics. Apolipoprotein E4 (apoE4) has emerged as a significant risk factor and a promising target for drug development due to its unique pathological conformations and detrimental role in various neurological disorders (19, 20).

The primary objective of this research was to identify chemical inhibitors capable of efficiently binding to apoE4's active domain, thereby mitigating beta-amyloid aggregation. Leveraging publicly available computational tools and databases, such as NCBI, this study utilized in silico techniques to evaluate the drug-like properties of various inhibitors. The application of the Lipinski Rule of Five and ADMET filters was instrumental in refining the selection process, reducing the initial set of compounds based on their pharmacokinetic and pharmacodynamic properties (21, 24). Notably, molecular docking emerged as a crucial step in assessing the binding orientations and energies of potential drug candidates to apoE4, with the goal of identifying compounds that exhibit strong and energetically favorable interactions (25).

The analysis revealed a subset of compounds demonstrating significant binding affinities, characterized by the presence of hydrogen bonds, hydrophobic interactions, and van der Waals forces. Such interactions are pivotal in stabilizing the ligand-protein complex, with hydrogen bonding being particularly indicative of strong binding potential (26, 27). Among these, two compounds distinguished themselves based on their hydrogen bonding profiles, leading to the selection of a lead compound after thorough comparison of their ADMET properties, toxicity profiles, and binding efficiencies.

Despite the promising findings, this study is not without limitations. The reliance on computational predictions necessitates experimental validation to confirm the efficacy and safety of the identified lead compounds. Moreover, the in silico models, while powerful, may not fully capture the complexity of biological systems, potentially overlooking critical interactions or effects. Future research should, therefore, include extensive in vitro and in vivo testing to corroborate the therapeutic potential of these compounds.

In conclusion, the integration of in silico techniques in drug discovery presents a transformative approach to identifying novel therapeutics for complex diseases like AD. This study underscores the efficacy of computational tools in narrowing down potential drug candidates, with the lead compounds, taxifolin and luteolin, emerging as promising inhibitors of apoE4. Their progression to

clinical trials could mark a significant milestone in the development of targeted treatments for Alzheimer's disease. However, the translation of these findings into clinical applications will require a comprehensive evaluation of their pharmacological profiles and therapeutic indices, alongside a consideration of their cost-effectiveness and specificity in addressing the multifaceted challenges of AD.

CONCLUSION

The convergence of in silico methodologies with traditional drug discovery processes represents a pivotal advancement in the quest for effective Alzheimer's disease (AD) therapies, highlighting the potential of computational tools to streamline the identification of novel inhibitors targeting apolipoprotein E4 (apoE4). This study's findings, particularly the identification of taxifolin and luteolin as promising lead compounds, underscore the critical role of computational pharmacology in accelerating the development of targeted treatments for AD. By demonstrating the capacity to rapidly evaluate and refine potential therapeutic agents based on their pharmacokinetic and pharmacodynamic profiles, in silico approaches offer a promising pathway to enhance the precision and efficiency of drug development. The healthcare implications of these findings are significant, suggesting a future where the integration of computational techniques could substantially reduce the time and cost associated with bringing new, effective treatments to market, thereby offering hope to millions of individuals affected by Alzheimer's disease and potentially other neurodegenerative disorders.

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