Virtual Screening of Polyphenolic Compounds from Edible Plants for Potential Application in Alzheimer's Therapy

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Abstract: Alzheimer's disease is associated with reduced cholinergic neurotransmission, making the inhibition of acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) a crucial therapeutic approach. This study used molecular docking to assess two reference inhibitors and two natural flavonoids against both enzymes. The reference inhibitor donepezil showed strong selectivity for AChE, while luteolin demonstrated balanced binding affinity and notable stability with both enzymes. Apigenin exhibited modest binding affinity for both targets, whereas rivastigmine showed the lowest binding affinity and inhibition potency. Energy analysis revealed that Van der Waals forces, hydrogen bonds, and desolvation mainly drive complex stabilization, with electrostatic effects playing a lesser role. The results emphasize luteolin's potential as a dual AChE/BChE inhibitor and confirm donepezil's selectivity for AChE. These insights support the rational design of new Alzheimer's therapeutics, especially flavonoid-based compounds with optimized dual inhibition profiles.

Keywords: Alzheimer's disease, AChE and BChE inhibition, Flavonoids

1. Introduction

Alzheimer's disease (AD) is a progressive neurodegenerative disorder and the most common cause of dementia, characterized by cognitive decline, memory loss, and behavioral impairments. Despite decades of research, current therapeutic options provide only symptomatic relief, with no definitive cure available. One of the main pathological features of AD is the deficiency of cholinergic neurotransmission, mainly due to the breakdown of acetylcholine by the enzymes acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) in the brain. As a result, inhibitors of these enzymes have been widely used in clinical practice to reduce the cognitive symptoms of AD, with donepezil (DO) and rivastigmine (RI) being among the most commonly prescribed

drugs [1]. However, the clinical use of synthetic cholinesterase inhibitors is often limited by side effects and limited long-term effectiveness. This has increased interest in natural compounds, especially polyphenols from dietary sources, because of their multiple neuroprotective properties, including antioxidant, anti-inflammatory, and enzyme-inhibiting effects [2]. Among the many naturally occurring polyphenols, apigenin (AP) and luteolin (LU)—flavonoids found in edible plants such as parsley, celery, chamomile, and carrots—have shown promising anti-Alzheimer's activity in both *in vitro* and *in silico* studies [3,4]. In this study, we performed virtual screening of two polyphenolic compounds focusing on their binding affinity to AChE and BChE. Using DO and RI as reference ligands, and AP and LU as examined natural compounds, molecular docking simulations were performed to assess their potential as dual cholinesterase inhibitors. This approach aims to identify plant-derived compounds with high inhibitory activity, supporting the development of new, naturally based therapeutic agents for AD.

2. Methodlogy

The molecular docking simulations were used for *in silico* prediction of the inhibitory potency of AP and LU toward AChE and BChE receptors. All simulations are performed using the AutoDock 4.2 software [5]. The Protein Data Bank provided the three-dimensional (3D) crystal structures of AChE and BChE enzymes (PDB IDs: 4ey7 and 1p0i, respectively) [6,7]. The chosen proteins were prepared in the BIOVIA Discovery Studio 4.0 to be employed as receptors in molecular docking simulations. The detection of the pockets and cavities of the used 3D structures of enzymes is performed using AGFR (AutoGridFR) software, and the binding sites of the target proteins are defined. The grid boxes centers with dimensions -14.733 Å x -41.559 Å x 26.856 Å and 135.928 Å x 113.792 Å x 38.477 Å in -x, -y, and -z directions of AChE and BChE were applied to cover the proteins binding sites and allow ligands to move freely. The auto grid runs are done with a grid point spacing of 0.375 Å. BIOVIA Discovery Studio is used for the analysis of the results of molecular docking simulations and their visualizations.

3. Results and Discussion

The evaluation of the inhibitory potency is commonly based on two important parameters, the free energy of binding (ΔG_{bind}) and the inhibitory constant (K_i). The AutoDock calculates the values of both of these parameters. The energy that is released during the formation of contacts between a ligand and a target protein is presented by the value of ΔG_{bind}. The value of ΔG_{bind} is a summary of the values of Final Intermolecular Energy (FIE), Final Total Internal Energy (FTIE), Torsional Free Energy (TFE), and Unbound System's Energy (USE). As regards the value of the inhibitory constant, it is calculated by the following equation:

$$K_i = \exp(\Delta G_{bind} / RT) \quad (1)$$

where R is the gas constant (R = 1.99 cal/mol·K), and T is the value of the room temperature (298.15 K).

Table 1. The important thermodynamical parameters from molecular docking simulations of the corresponding protein-ligand complexes. The inhibition constant values (Ki) are presented in μM. Energy values are presented in kcal/mol.

Complex	ΔG_{bind}	K_i	FIE	vdW + Hbond + desolv Energy	Electrostatic Energy	FTIE	TFE	USE
AChE-DO	-11.44	0.01	-13.23	-13.13	-0.10	-1.03	+1.79	-1.03
AChE-RI	-7.69	2.32	-9.18	-8.51	-0.67	-0.82	+1.49	-0.82
AChE-AP	-8.50	0.58	-9.70	-9.51	-0.19	-0.89	+1.19	-0.89
AChE-LU	-8.61	0.49	-10.10	-9.87	-0.23	-2.09	+1.49	-2.09
BChE-DO	-8.98	0.26	-10.77	-10.67	-0.10	-1.56	+1.79	-1.56
BChE-RI	-7.15	5.75	-8.64	-7.52	-1.12	-0.66	+1.49	-0.66
BChE-AP	-8.00	1.36	-9.20	-8.81	-0.39	-0.92	+1.19	-0.92
BChE-LU	-7.89	1.65	-9.38	-8.94	-0.44	-2.18	+1.49	-2.18

The compounds with the lowest ΔG_{bind} and K_i values have a high binding affinity to the targeted protein and a significant inhibitory potency. In addition, the compounds that possess lower values of K_i require less quantity of compound to inhibit the function of the receptor. The results presented in Table 1 imply a correlation between the values of G_{bind} and K_i. More precisely, the lower values of ΔG_{bind} are followed by the lower values of K_i, and vice versa. Results from Table 1. indicate that DO displayed the highest binding affinity toward AChE, significantly higher than both natural compounds and RI. This strong affinity correlates with its well-established potency as a standard AChE inhibitor. Among the natural compunds, LU showed slightly better binding afiinity than AP, while RI exhibited the lowest inhibitory potency. The results indicate that hydrophobic and hydrogen-bonding interactions are the primary determinants of binding strength, while electrostatics play a minor role in complex stabilization. Additionally, DO exhibited the strongest binding affinity to BChE, outperforming both natural ligands and RI. AP showed slightly higher affinity than LU, while RI had the lowest inhibitory potency. Overall, the binding energies for BChE are higher than those observed for AChE, suggesting reduced binding efficiency of these ligands toward BChE. While DO remains the most potent BChE binder in absolute affinity, LU demonstrates notable thermodynamic stability and favorable steric accommodation. Compared to AChE results, all ligands show reduced binding affinity toward BChE, pointing to a degree of enzyme selectivity—especially for DO and LU.

4. Conclusions

Molecular docking analysis revealed a distinct difference in ligand affinity toward AChE and BChE. DO showed the highest binding to AChE and was highly selective over BChE, making it ideal for targeted AChE inhibition. In contrast, LU displayed a balanced affinity for both enzymes along with high thermodynamic stability in BChE, indicating strong potential for developing dual inhibitors. AP exhibited moderate

binding to both enzymes. Additionally, RI showed the weakest performance and limited potential for further optimization within this ligand set. Energy analysis indicated that hydrophobic interactions, hydrogen bonding, and desolvation are the main contributors to complex stabilization, with electrostatic forces playing a minor role. These findings offer a valuable foundation for designing new compounds with improved affinity and selectivity, especially focusing on optimizing flavonoid scaffolds for dual AChE and BChE inhibition.

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