الجمهورية الجزائرية الديمقراطية الشعبية

DEMOCRATIC AND POPULAR ALGERIAN REPUBLIC

وزارة التعليم العالي والبحث العلمي

MINISTRY OF HIGHER EDUCATION AND SCIENTIFIC RESEARCH

جامعة الشهيد حمه لخضر الوادي

ECHAHID HAMMA LAKHDAR UNIVERSITY OF EL-OUED

كلية العلوم الطبيعة والحياة

FACULTY OF NATURAL LIFE AND SCIENCES

قسم البيولوجيا الخلوية والجزيئية

Department of Cellular and Molecular Biology

Master 's Thesis

In order to obtain a diploma of an Academic Master In biological sciences

Specialty: Toxicology

Them

In Silico Drug Design and Virtual Screening of Novel Donepezil Derivatives : ADMET Profiling, Molecular Docking, and Molecular Dynamics Simulations

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University year: 2024/2025

إهداء

الى والدينا الكرماء الذين قاموا على تربيتنا وسهروا على رعايتنا والى جميع افراد عائلاتنا واصدقائنا والى جميع الأساتذة الافاضل الذين درسنا عندهم والى كل من ساعدونا من قريب أو بعيد لإنجاز هذا البحث الى كل هؤلاء نهدي هذا الجهد

جوادي، عبدالحميد، معمر

شكر وتقدير

الحمد لله حمدًا طيبًا مباركًا فيه، على نعمه التي لا تُعد، وآلائه التي لا تُحصى، والصلاة والسلام على خير الأنام، نبينا محمد، وعلى آله وصحبه أجمعين.

يسرّنا أن نرفع أسمى معاني الشكر والتقدير والامتنان

إلى أستاذنا العزيز ومشرفنا الكريم، الدكتور العانز الحفناوي،

لما قدّمه لنا من دعم متواصل، وتوجيه ناضج، ورؤية علمية نيرة،

كان حضوره ومتابعته لنا نبراسًا نستهدي به في مسيرتنا،

وقد كان لتفانيه وحكمته الأثر البالغ في إنجاح هذا العمل، فله منا كل الوفاء والعرفان.

كما نخص بالشكر والعرفان

أساتذتنا الأفاضل في جامعة الشهيد حمه لخضر بالوادي،

الذين كان لهم الدور الأكبر في تكويننا أكاديميًا وفكريًا،

بذلوا من علمهم بسخاء، وكانوا دومًا عنوانًا للإخلاص والتميز،

فكل التحايا والتقدير لكم على ما قدمتم من عطاء لا يُنسى.

ولا يسعنا كذلك إلا أن نتوجه بخالص الامتنان

إلى كافة الإداريين والموظفين بالجامعة،

الذين قدّموا يد العون وحرصوا على تيسير سبل العمل والبحث،

بجهودهم وتفانيهم تحققت الكثير من الإنجازات التي نفتخر بها اليوم.

فلكم منا جميعًا أصدق عبارات الشكر، وأطيب الدعوات بأن يبارك الله في أعماركم وأعمالكم،

وأن يجعل كل خطوة بذلتموها في سبيل العلم نورًا لكم في الدنيا والآخرة،

سائلين الله أن يبقى هذا الصرح منارة علم، وأن يديم علينا نعمة التعلم والعطاء.

والله وليّ التوفيق.

Abstract

Alzheimer's disease (AD) remains one of the most pressing global neurological challenges, primarily driven by amyloid-beta (Aβ) aggregation and cholinergic system impairment, both of which contribute to progressive cognitive decline. Although Donepezil, an acetylcholinesterase (AChE) inhibitor, is commonly prescribed to alleviate symptoms, its clinical efficacy is often limited by poor long-term outcomes and side effects. In this study, a series of novel Donepezil analogues were designed and evaluated to enhance pharmacokinetic properties and binding efficiency against key AD targets, namely Aβ and AChE, using advanced computational techniques. Analogues were generated through computer-aided drug design methods and screened for ADMET properties using SwissADME and ProTox-II platforms. Molecular docking was conducted using Schrödinger Maestro to identify favorable binding interactions, followed by 100-nanosecond molecular dynamics (MD) simulations in Desmond to assess the structural stability of the most promising ligand-protein complexes under physiological conditions. The results showed that analogues Don11, Don12, and Don15 exhibited strong binding affinities and maintained stable interactions throughout the simulations, with RMSD values remaining below 2.5 Å. Notably, Don15 demonstrated superior stability and AChE inhibition potential, making it a particularly promising candidate. These findings suggest that Don11, Don12, and Don15 hold significant potential as next-generation therapeutic agents for Alzheimer's disease.

Keywords: *Alzheimer's disease, Donepezil analogues, Don15, amyloid-beta, AChE, molecular docking, molecular dynamics, drug design, ADMET, neurodegeneration.*

RÉSUMÉ

La maladie d'Alzheimer (MA) demeure l'un des défis neurologiques mondiaux les plus préoccupants, en grande partie en raison de l'agrégation de la protéine bêta-amyloïde (Aβ) et de la dysfonction du système cholinergique, contribuant toutes deux au déclin cognitif progressif. Bien que le Donépézil, un inhibiteur de l'acétylcholinestérase (AChE), soit couramment prescrit pour atténuer les symptômes, son efficacité clinique est souvent limitée par des résultats à long terme modestes et des effets secondaires. Dans cette étude, une série de nouveaux analogues du Donépézil a été conçue et évaluée afin d'améliorer les propriétés pharmacocinétiques et l'affinité de liaison aux principales cibles de la MA, à savoir Aβ et AChE, en utilisant des approches computationnelles avancées. Les analogues ont été générés par des méthodes de conception assistée par ordinateur, puis soumis à une évaluation ADMET via les plateformes SwissADME et ProTox-II. Le criblage moléculaire a été effectué à l'aide de Schrödinger Maestro afin d'identifier les interactions de liaison favorables, suivi de simulations de dynamique moléculaire (MD) de 100 nanosecondes avec Desmond pour analyser la stabilité structurale des complexes ligand-protéine les plus prometteurs dans des conditions physiologiques. Les résultats ont montré que les analogues Don11, Don12 et Don15 présentaient de fortes affinités de liaison et maintenaient des interactions stables tout au long des simulations, avec des valeurs RMSD inférieures à 2,5 Å. En particulier, Don15 a démontré une stabilité supérieure et un potentiel élevé d'inhibition de l'AChE, ce qui en fait un candidat particulièrement prometteur. Ces résultats suggèrent que Don11, Don12 et Don15 pourraient constituer de futurs agents thérapeutiques de nouvelle génération contre la maladie d'Alzheimer.

Mots clés: *Maladie d'Alzheimer, analogues du Donépézil, Don15, bêta-amyloïde, AChE, criblage moléculaire, dynamique moléculaire, conception de médicaments, ADMET, neurodégénérescence.*

الملخص

تُعد مرض الزهايمر (AD) من أبرز التحديات العصبية على مستوى العالم، ويُعزى ذلك بشكل أساسي إلى تراكم بروتين بيتا-أميلويد (Aβ) واختلال النظام الكوليني، مما يؤدي إلى تدهور معرفي تدريجي.

وعلى الرغم من أن دواء دونيبيزيل، وهو مثبط لإنزيم أستيل كولين إستيراز (AChE)، يُستخدم بشكل واسع لتخفيف الأعراض، إلا أن فعاليته السريرية غالبًا ما تكون محدودة بسبب ضعف النتائج طويلة الأمد والآثار الجانبية.

في هذه الدراسة، تم تصميم وتقييم مجموعة من نظائر الدونيبيزيل بهدف تحسين الخصائص الدوائية الحركية وتعزيز كفاءة الارتباط بأهداف الزهايمر الرئيسية، وهي Aβ وAChE، وذلك باستخدام تقنيات حسابية متقدمة.

تم توليد النظائر باستخدام أدوات التصميم الدوائي بمساعدة الحاسوب، وتم تقييم خصائص ADMET الخاصة بها عبر منصّتي SwissADME وProTox-II. أُجريت عملية الارتساء الجزيئي باستخدام برنامج Schrödinger Maestro لتحديد تفاعلات الارتباط المثلى، تلتها محاكاة ديناميكيات جزيئية (MD) لمدة 100 نانوثانية باستخدام Desmond لتحليل الاستقرار البنيوي للمركبات مع البروتينات المستهدفة تحت ظروف فسيولوجية. أظهرت النتائج أن النظائر Don11 وDon12 وDon15 تمتلك قدرات ارتباط قوية وتحافظ على تفاعلات مستقرة خلال المحاكاة، مع قيم RMSD أقل من 2.5 أنغستروم.

وبرز من بينها المركب Don15 بأعلى درجة من الاستقرار وقدرة ملحوظة على تثبيط إنزيم AChE، مما يجعله مرشحًا واعدًا للغاية. وتشير هذه النتائج إلى أن المركبات Don11 وDon12 وDon15 تمثل عوامل علاجية محتملة من الجيل الجديد لعلاج مرض الزهايمر.

الكلمات المفتاحية :مرض الزهايمر، نظائر دونيبيزيل، Don15، بيتا-أميلويد، AChE، الارتساء الجزيئي، الديناميكيات الجزيئية، تصميم الأدوية، ADMET، الاضطرابات العصبية التنكسية.

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Summary

Introduction

Introduction

Alzheimer's disease (AD) is among the most prevalent neurodegenerative disorders worldwide, representing a major cause of dementia and cognitive impairment, particularly in aging populations. Its growing global incidence underscores the urgent need for more effective and targeted therapeutic strategies. Pathologically, AD is defined by the accumulation of amyloid-beta (Aβ) plaques and neurofibrillary tangles composed of hyperphosphorylated tau protein, which contribute to synaptic dysfunction, neuronal loss, and progressive brain atrophy. The excessive aggregation of Aβ peptides disrupts cellular homeostasis, triggering oxidative stress, mitochondrial dysfunction, and chronic neuroinflammation—all of which accelerate neurodegeneration.

Another central factor in AD pathophysiology is the impairment of cholinergic neurotransmission, primarily due to the degradation of acetylcholine by the enzyme acetylcholinesterase (AChE). Reduced levels of acetylcholine have been strongly correlated with cognitive decline, making AChE inhibition a validated and widely explored therapeutic strategy. Donepezil, a reversible AChE inhibitor, has long been used to improve cholinergic signaling and alleviate cognitive symptoms in AD patients. However, its limited efficacy, associated side effects, and interindividual variability in response emphasize the need for novel analogues with improved pharmacological profiles.

In this context, the current study focuses on the rational design and in silico evaluation of a new series of donepezil analogues with enhanced therapeutic potential against AD. Using structure-based drug design, the aim is to optimize molecular interactions with both Aβ and AChE, thereby improving binding affinity, selectivity, and pharmacokinetic behavior. The analogues were screened for drug-likeness and toxicity using SwissADME and ProTox-II, followed by molecular docking via Schrödinger Maestro to investigate binding interactions. To further assess the dynamic behavior and structural stability of the most promising ligand-target complexes under physiological conditions, 100-nanosecond molecular dynamics (MD) simulations were carried out using Desmond.

This multi-targeted computational approach offers valuable insights into the design of dual-acting compounds capable of modulating key pathological pathways in Alzheimer's disease. The findings aim to guide the development of next-generation AChE inhibitors with improved efficacy and safety, contributing to the ongoing shift toward personalized and mechanism-based therapies for neurodegenerative diseases.

First part Bibliographic synthesis

Chapter I :Definition of the disease

1. Alzheimer's Disease:

is a progressive neurodegenerative disorder and the most common cause of dementia worldwide [1]. It is characterized by a gradual decline in memory, cognitive function, and behavioral abilities, ultimately interfering with a person's ability to perform daily tasks. The disease primarily affects older adults, and its prevalence increases significantly with age. Alzheimer's begins silently, with subtle changes in the brain occurring years before symptoms become apparent. As it progresses, individuals may experience confusion, disorientation, language difficulties, and changes in mood or personality. In advanced stages, patients often require full-time care [2]. The hallmark pathological features of Alzheimer's disease include the accumulation of amyloid-beta (Aβ) plaques outside neurons and tau protein tangles inside neurons. These abnormalities disrupt communication between brain cells and lead to neuronal death [3]. Risk factors for Alzheimer's include advanced age, family history, certain genetic mutations such as those in the APOE gene, cardiovascular disease, and lifestyle-related factors such as poor diet, lack of exercise, and chronic stress [4].

Currently, there is no cure for Alzheimer's, but early diagnosis and intervention can slow disease progression and improve quality of life. Treatments focus on symptom management and may involve medications like cholinesterase inhibitors (e.g., donepezil) to enhance neurotransmitter function, as well as supportive therapies such as cognitive training and caregiver support. Preventive strategies, including maintaining mental and physical activity, healthy eating, and controlling blood pressure and cholesterol, may help reduce the risk or delay the onset of the disease.

2. Symptoms of Alzheimer:

Alzheimer's disease symptoms can vary, and individuals may experience a range of cognitive, behavioral, and functional changes that develop gradually over time. One of the earliest and most common symptoms is memory loss, particularly difficulty remembering recent events or conversations. Individuals may begin to rely more heavily on memory aids or family members for things they previously handled independently. Difficulty planning, solving problems, or concentrating on tasks may also arise, making it challenging to manage finances, follow recipes, or keep appointments [5]. As the disease progresses, people may become confused about time or place, get lost in familiar surroundings, or have trouble recognizing familiar faces. Language problems, such as struggling to find the right words or following a conversation, are also frequent. Behavioral and mood changes may emerge, including increased irritability, anxiety, depression, apathy, or suspiciousness. Sleep

disturbances and withdrawal from social or work activities are also common. In advanced stages, individuals may lose the ability to carry out basic daily tasks, such as dressing, eating, or bathing, and may experience severe personality changes. They may also have difficulty walking, swallowing, or controlling bladder and bowel functions. If any of these symptoms are noticed—especially progressive memory loss—it is critical to seek medical advice for accurate diagnosis and early management. Early detection allows for better symptom control and access to supportive care for both the patient and caregivers [6].

3. Causes of Alzheimer's disease:

The primary cause of Alzheimer's disease is the progressive degeneration of brain cells, often associated with the abnormal accumulation of amyloid-beta (Aβ) plaques and tau protein tangles that interfere with neuronal communication and function. While the exact etiology remains multifactorial and not fully understood, numerous risk factors have been identified that contribute to the onset and progression of the disease. These include genetic predisposition, such as the presence of the APOE ε4 allele, advancing age, and a family history of Alzheimer's or other forms of dementia. Vascular conditions like hypertension, high cholesterol, and diabetes are also known to increase the risk by impairing cerebral blood flow and contributing to neuroinflammation. Moreover, hormonal changes, particularly the decline in estrogen levels during menopause, may influence cognitive vulnerability. Lifestyle-related factors such as poor diet, physical inactivity, social isolation, smoking, and lack of cognitive stimulation have been shown to play a substantial role in disease risk. Environmental influences and a history of traumatic brain injury may also heighten an individual's susceptibility to developing Alzheimer's disease [7].

Genetic predisposition: Some individuals may be genetically predisposed to Alzheimer's disease, especially those carrying the APOE ε4 allele, which significantly increases susceptibility even in the absence of other risk factors.[8]

Hormonal changes: Hormonal fluctuations, particularly reduced estrogen levels during and after menopause, may influence brain health and increase the risk of cognitive decline and Alzheimer's disease.[9]

Diabetes: Uncontrolled diabetes can damage blood vessels and reduce glucose metabolism in the brain, both of which are associated with an increased risk of developing Alzheimer's disease.[10]

Medications: Certain medications, such as benzodiazepines, anticholinergics, and some sedatives, have been linked to impaired cognitive function and may contribute to a higher risk of Alzheimer's when used long-term.[11]

Poor nutrition: A diet high in processed sugars and low in essential nutrients like omega-3 fatty acids, antioxidants, and B vitamins may impair brain health and accelerate cognitive decline, thereby increasing Alzheimer's risk.[12]

Chronic stress: Prolonged exposure to psychological stress can lead to elevated cortisol levels, neuroinflammation, and hippocampal atrophy—factors strongly associated with the development and progression of Alzheimer's diseas.[13]

4. Treatment of Alzheimer:

Alzheimer's disease is a progressive neurodegenerative disorder that primarily affects memory, thinking, and behavior. Although there is currently no cure, various treatments aim to slow disease progression, manage symptoms, and improve the quality of life for patients. Treatment strategies typically involve a combination of pharmacological and non-pharmacological approaches.[14] Pharmacological treatment focuses on improving cognitive function and controlling behavioral symptoms. Below is an overview of commonly used medications for Alzheimer's disease.

4.1. Cholinesterase Inhibitors

4.1.1. Donepezil: Donepezil is one of the most widely prescribed medications for Alzheimer's disease. It works by inhibiting acetylcholinesterase, an enzyme responsible for breaking down acetylcholine, thereby increasing acetylcholine levels in the brain. This enhances communication between nerve cells and can help improve memory and cognition, particularly in mild to moderate stages of the disease.[15]

4.1.2. Rivastigmine: Similar to donepezil, rivastigmine increases acetylcholine levels by inhibiting both acetylcholinesterase and butyrylcholinesterase. It is available in oral and transdermal forms and is used for mild to moderate Alzheimer's cases.[16]

4.1.3. Galantamine: Galantamine not only inhibits acetylcholinesterase but also modulates nicotinic receptors, enhancing cholinergic function. It is often used in early to moderate stages of the disease.[17]

4.2. Targeted Therapy

4.2.1. Donepezil: As a targeted therapy, donepezil selectively targets the cholinergic system, which is notably impaired in Alzheimer's disease. By enhancing cholinergic neurotransmission, donepezil supports memory and learning processes. Its long half-life allows for once-daily dosing, making it a convenient option for long-term management.[18]

4.2.2. Memantine: Memantine is another targeted agent that works by modulating glutamate activity through NMDA receptor antagonism. It is typically used in moderate to severe stages and may be combined with donepezil for added benefit.[19]

4.3. Non-Pharmacological and Supportive Therapies

4.3.1. Cognitive Stimulation: Involves structured activities that aim to improve memory, attention, and problem-solving skills. These programs are often used alongside medication to enhance cognitive function.[20]

4.3.2. Lifestyle Interventions: Physical exercise, a balanced diet, and social engagement have been shown to help maintain cognitive function and delay disease progression.[21]

4.3.3. Caregiver Support and Behavioral Therapy: Managing behavioral symptoms such as agitation or depression is crucial, and support for caregivers plays a vital role in the patient's well-being.[22]

Chapter II :Definition of the medication

1- Definition of Donepezil:

Donepezil is a piperidine-based acetylcholinesterase inhibitor used primarily for the treatment of Alzheimer's disease. Introduced in the 1990s, it has become one of the most widely prescribed medications for managing symptoms of mild to moderate Alzheimer's.[23] Donepezil works by reversibly inhibiting the enzyme acetylcholinesterase, which breaks down acetylcholine—a neurotransmitter critical for learning and memory. By increasing acetylcholine levels in the brain, donepezil helps improve communication between nerve cells, temporarily alleviating cognitive symptoms such as memory loss and confusion. Donepezil is considered a cornerstone in the pharmacological management of Alzheimer's disease and is often used as a first-line therapy. Despite the development of newer therapeutic agents, donepezil remains an essential treatment due to its favorable safety profile, ease of once-daily dosing, and effectiveness in a wide range of patients.[24] It is also listed on the World Health Organization's list of essential medicines, underscoring its significance in global healthcare systems.[25]

2- The Uses of Donepezil:

1-Used to treat cognitive symptoms in mild to moderate Alzheimer's disease.[26]
2-Higher doses can help manage severe stages of Alzheimer's.[27]
3-Studied for use in other dementias like vascular and Parkinson's-related dementia.[28]
4- May offer neuroprotective effects by slowing neuronal damage.[29]
5- Helps reduce behavioral symptoms such as apathy and aggression.[30]

3- Mechanism of Action of Donepezil:

Donepezil is widely recognized as a selective, reversible inhibitor of acetylcholinesterase (AChE) and is commonly used in the treatment of mild to moderate Alzheimer's disease.[31] Its clinical effectiveness is primarily attributed to its ability to enhance cholinergic neurotransmission by inhibiting AChE, the enzyme responsible for the breakdown of acetylcholine (ACh) in the synaptic cleft. By preventing ACh degradation, donepezil increases synaptic concentrations of ACh, thereby improving communication between neurons in areas of the brain responsible for memory and cognition, which are typically impaired in Alzheimer's patients.Despite its symptomatic benefits, donepezil does not halt the progression of the disease, and long-term treatment outcomes remain limited.[32] Furthermore, the heterogeneity of Alzheimer's disease pathogenesis—including amyloid-beta

(Aβ) deposition, tau hyperphosphorylation, neuroinflammation, and oxidative stress—makes the development of resistance or reduced responsiveness to cholinesterase inhibitors a significant challenge in clinical practice. In this context, increasing attention has been directed toward the potential of donepezil to interact with Aβ peptides, a hallmark of Alzheimer's pathology. In silico and experimental studies suggest that donepezil may bind to Aβ or influence its aggregation process, contributing to reduced plaque formation or neurotoxicity.Such multi-target effects position donepezil not only as a symptomatic agent but also as a compound of interest in disease-modifying strategies.[33] However, these secondary mechanisms remain under investigation, and more evidence is needed to fully elucidate their clinical relevance. Further research into the diverse molecular interactions of donepezil may help optimize its therapeutic potential and inform the design of novel agents for improved management of Alzheimer's disease.

4. The Side Effects of Donepezil:

Donepezil is a selective acetylcholinesterase inhibitor, used to treat symptoms of Alzheimer's disease by enhancing cholinergic transmission in the central nervous system. While generally well tolerated, it is associated with a range of dose-related side effects due to increased levels of acetylcholine throughout the body.

Has estrogen-like effects on:

- The gastrointestinal tract
- The cardiovascular system
- The central nervous system
- The urinary tract [34]

- **Common side effects include**:
  - Nausea
  - Diarrhea
  - Insomnia
  - Muscle cramps[35][36]
  - Fatigue
- **Serious but less frequent adverse effects**:
  - Bradycardia
  - Syncope
  - Gastrointestinal bleeding
  - Seizures [37]

- In some patients, especially the elderly or those with cardiac comorbidities, donepezil may exacerbate preexisting conduction abnormalities. Cases of QT prolongation and torsades de pointes have been reported, although rarely. [38]

The combination of donepezil with other central nervous system-active drugs may increase the risk of side effects such as dizziness, confusion, or agitation. Careful dose titration and monitoring are advised, particularly during initiation or when co-administered with interacting agents.[39]

Second part:
Experimental part

Chapter I:
Materials & methods

1. Generation of Combinatorial Library

To explore novel structural variations of donepezil and enhance its therapeutic potential, a combinatorial virtual screening approach was employed using the SmiLib v2.0 platform [40]. The molecular framework of donepezil was used as a central scaffold, and it was systematically modified by incorporating a selected set of substituents—including hydroxyl (OH), chlorine (Cl), methyl (CH_3), and amino (NH_2) groups—at various linker positions. This strategy facilitated the construction of a diverse virtual library comprising 368 distinct analogues, each representing a unique modification of the parent compound.

Following library generation, all candidate molecules were evaluated using predictive in silico tools SwissADME [41] and ProTox 3.0 [42] to assess their pharmacokinetic behavior and safety profiles. The analysis focused on critical parameters such as water solubility, gastrointestinal absorption, cytochrome P450 (CYP) enzyme interactions, and blood-brain barrier permeability, which are essential for effective central nervous system drug delivery. Simultaneously, potential toxicity endpoints—including organ toxicity, carcinogenicity, and the estimated median lethal dose (LD_{50})—were predicted to ensure safety.

Compounds that did not meet basic drug-likeness criteria or exhibited high predicted toxicity were excluded from further analysis. This filtration step allowed the selection of analogues with the most promising ADMET characteristics for subsequent molecular docking studies. Through this integrated workflow, a refined subset of donepezil derivatives was identified for deeper computational evaluation as potential anti-Alzheimer's agents.

The development of the donepezil analogue library for virtual screening and molecular docking studies followed a structured computational pipeline designed to systematically explore structural modifications and assess their potential biological activity:

1.1. Scaffold Selection

The molecular scaffold of donepezil was chosen as the central framework to guide the design and generation of its structural analogues.

1.2. Identification of Substituents

A variety of chemically diverse substituents—including functional groups such as **hydroxyl (OH), amino (NH_2), methyl (CH_3), and chlorine (Cl)**—were selected for potential incorporation onto the donepezil scaffold. These groups were chosen to increase structural diversity and facilitate the investigation of structure–activity relationships.

1.3. Combinatorial Enumeration

Using SmiLib v2.0 software [43], all possible combinations between the donepezil scaffold and the selected substituents were computationally enumerated. This combinatorial approach enabled the systematic generation of a structurally diverse library of unique donepezil analogues by pairing each functional group with designated positions on the parent backbone.

2. Filtering and Validation

The generated library of donepezil analogues was subjected to a series of filtration steps to ensure compliance with drug-likeness principles, acceptable physicochemical profiles, and structural diversity. Molecules that displayed poor pharmacokinetic characteristics or failed to meet key criteria in medicinal chemistry—such as Lipinski's rule of five—were systematically removed from further consideration.

3. Prediction of Pharmacokinetics and Toxicity

To evaluate the pharmacokinetic profiles and potential toxicity of the designed donepezil analogues, in silico predictive tools such as SwissADME [44] and ProTox II [42] were utilized. SwissADME provided estimates of critical parameters such as solubility in water, permeability across the blood–brain barrier (BBB), interaction with cytochrome P450 (CYP) enzymes, and gastrointestinal absorption. In parallel, ProTox 3.0 was employed to predict safety-related endpoints, including the median lethal dose (LD_{50}), offering an initial overview of the toxicological risks associated with each compound.

3.1 Final Selection

Based on the results of pharmacokinetic analysis and toxicity predictions, a curated subset of donepezil analogues exhibiting optimal ADME properties, minimal toxicity risk, and diverse chemical structures was selected. These prioritized candidates were advanced to the virtual screening and molecular docking phase in order to identify promising lead compounds with potentially improved biological activity against the selected targets..

3.2 Database Compilation

The finalized set of donepezil analogues was organized into a structured database incorporating their 2D molecular structures, unique identifiers, and predicted ADME/Tox profiles. This compiled dataset formed the basis for the subsequent steps in the virtual screening pipeline, facilitating efficient selection and analysis during molecular docking procedures.

3.3 Pharmacokinetics and Toxicity-Based Screening

Pharmacokinetic evaluation of the generated donepezil analogues was carried out using SwissADME [44], a tool developed by the Swiss Institute of Bioinformatics, to assess key drug-likeness parameters. The analysis focused on identifying potential cytochrome P450 (CYP) enzyme inhibitors and predicting factors such as gastrointestinal absorption, P-glycoprotein substrate affinity, and blood–brain barrier (BBB) permeability—essential features for central nervous system drug candidates. Simultaneously, ProTox II [42] was employed to estimate the LD_{50} values for donepezil and its derivatives. This in silico toxicity screening was instrumental in narrowing down compounds with lower safety risks, where LD_{50} served as a primary filtering metric for compound selection.

4. Structural Optimization

In the initial phase of this study, structural refinement of the generated donepezil analogues was performed using molecular mechanics approaches to optimize atomic arrangements and obtain energetically favorable conformations. This step ensured that the generated molecules adopted stable 3D geometries suitable for further computational analysis.

To enhance the accuracy of the structural representations, a second round of geometry optimization was conducted using Density Functional Theory (DFT) [45], [46], [47], a quantum mechanical method widely employed in molecular modeling. The optimization utilized the B3LYP [48], [49] hybrid functional combined with the 6-311++G(d,p) basis set [50], [51], [52], which offers an advanced description of electron density and polarization effects. These calculations were carried out using the Gaussian 16W software package [53], which enabled precise minimization of molecular energy and detailed analysis of electronic properties.

For benchmarking and validation purposes, the experimentally determined 3D structure of donepezil was retrieved from the PubChem database (https://pubchem.ncbi.nlm.nih.gov) [54]. This reference structure provided a reliable standard for comparison with the optimized analogues in terms of geometry and electronic distribution.

By combining classical molecular mechanics with high-level quantum chemical optimization, this methodology allowed for a comprehensive evaluation of conformational stability and electronic characteristics of donepezil derivatives—an essential foundation for their subsequent pharmacological investigation and potential therapeutic application.

5. Protein Selection

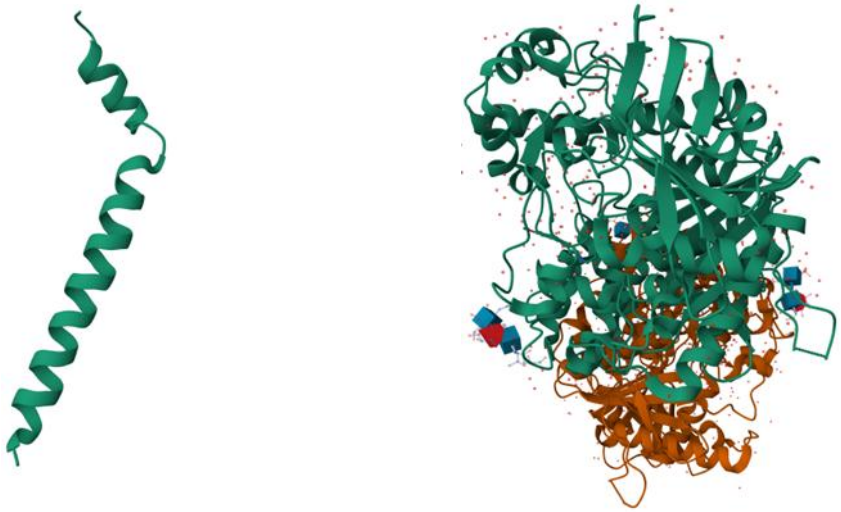
In the context of Alzheimer's disease, two principal protein targets were selected for this

investigation: Acetylcholinesterase (AChE) and Amyloid-beta (Aβ). These targets were chosen due to their direct involvement in the disease's pathogenesis and their therapeutic relevance to the mechanism of action of donepezil and its derivatives. [55]

AChE is a key enzyme responsible for the hydrolysis of acetylcholine at neuronal synapses. Its excessive activity leads to a significant reduction in acetylcholine levels, contributing to cognitive deficits commonly observed in Alzheimer's patients. Inhibiting AChE represents a well-established strategy for symptomatic treatment, as it prolongs acetylcholine availability and enhances cholinergic neurotransmission, thereby improving memory and cognition. The 3D crystal structure of AChE, available in the Protein Data Bank (PDB ID: 4EY7), was retrieved and prepared for molecular docking studies to assess the binding potential of donepezil analogues at its active site. [56]

On the other hand, Amyloid-beta (Aβ) is a neurotoxic peptide generated through the cleavage of amyloid precursor protein (APP). Its tendency to aggregate into insoluble plaques is one of the hallmark features of Alzheimer’s pathology, associated with synaptic dysfunction, oxidative stress, and neuronal death. The inhibition or disruption of Aβ aggregation is considered a promising approach for disease-modifying therapies. For this study, the fibrillar structure of Aβ (PDB ID: 2BEG) was utilized to evaluate the potential of donepezil analogues to interact with and destabilize amyloid fibrils. [57]

The selection of AChE and Aβ was informed by their complementary roles in the progression of Alzheimer’s disease—AChE being associated with neurotransmitter regulation, and Aβ with pathological aggregation and toxicity. Their high-resolution 3D structures provide a robust foundation for reliable molecular docking simulations, allowing for the prediction of binding affinities and interaction profiles [58]. By targeting both enzymatic and aggregative pathways, this dual-protein approach enhances the scope of identifying multitarget-directed ligands among the donepezil derivatives under investigation. [59]



Aβ **AChE**

Figure 1. The three-dimensional crystal structures of Acetylcholinesterase (AChE, PDB ID: 4EY7) and Amyloid-beta (Aβ, PDB ID: 2BEG), chosen as the molecular targets for docking studies with donepezil analogues, were obtained from the Protein Data Bank.

6. Steps of Molecular Docking

Molecular docking simulations were performed using the Schrödinger Maestro software suite [60], following a structured workflow to predict binding affinities and interaction modes of donepezil analogues with the selected target proteins. The docking process included the following main steps:

Step 1: Protein Structure Preparation

The crystallographic 3D structures of Acetylcholinesterase (AChE, PDB ID: 4EY7) and Amyloid-beta (Aβ, PDB ID: 2BEG) were imported into the Maestro environment. Protein preparation was carried out using the Protein Preparation Wizard at physiological pH 7.0. This involved removal of water molecules and non-essential ligands, addition of hydrogen atoms, adjustment of protonation states, and assignment of correct bond orders.

Step 2: Protein Grid Generation

Receptor grids were generated using the Receptor Grid Generation tool. Active binding sites were defined based on the positions of co-crystallized ligands or known active residues. The center coordinates of the grid box were set at x, y, z positions 15.30, 22.50, 18.75 for AChE and 10.40, 12.15, 8.90 for Aβ, with grid dimensions of 10×10×10 Å for both proteins.

Step 3: Ligand Preparation

Donepezil analogues were prepared using LigPrep, which included:

Generation of multiple 3D conformers to represent different spatial configurations.

Geometry optimization for structural accuracy.

Adjustment of ionization states appropriate for physiological pH.

Energy minimization to alleviate steric clashes and identify the most stable conformers.

Step 4: Molecular Docking Simulation

The prepared ligands were flexibly docked into the receptor active sites using Glide SP (Standard Precision) mode. This was followed by XP (Extra Precision) docking to enhance the accuracy of ligand-receptor binding predictions. Glide scores were used to rank the docked poses.

Step 5: Pose Refinement and Scoring

Refinement of docking poses was performed using Prime MM-GBSA calculations to estimate binding free energies and further characterize ligand-protein interactions. Scoring considered binding affinity, hydrogen bonding, hydrophobic interactions, and π–π stacking.

Step 6: Analysis and Visualization

The top-ranked ligand-protein complexes were analyzed using the Ligand Interaction Diagram tool to map critical interacting residues. Visualization helped in understanding the mode of binding and stability of the complexes.

Step 7: Validation and Optimization

Docking protocol validation was done by re-docking co-crystallized ligands into their native binding sites to ensure accuracy. Grid parameters and scoring functions were adjusted accordingly to optimize results.

Step 8: Reporting and Interpretation

A detailed report summarizing docking scores, interaction patterns, and structural insights of the complexes between donepezil analogues and AChE/Aβ was compiled. These findings lay the groundwork for further experimental validation and structure-based optimization of promising lead compounds (Figure 11).

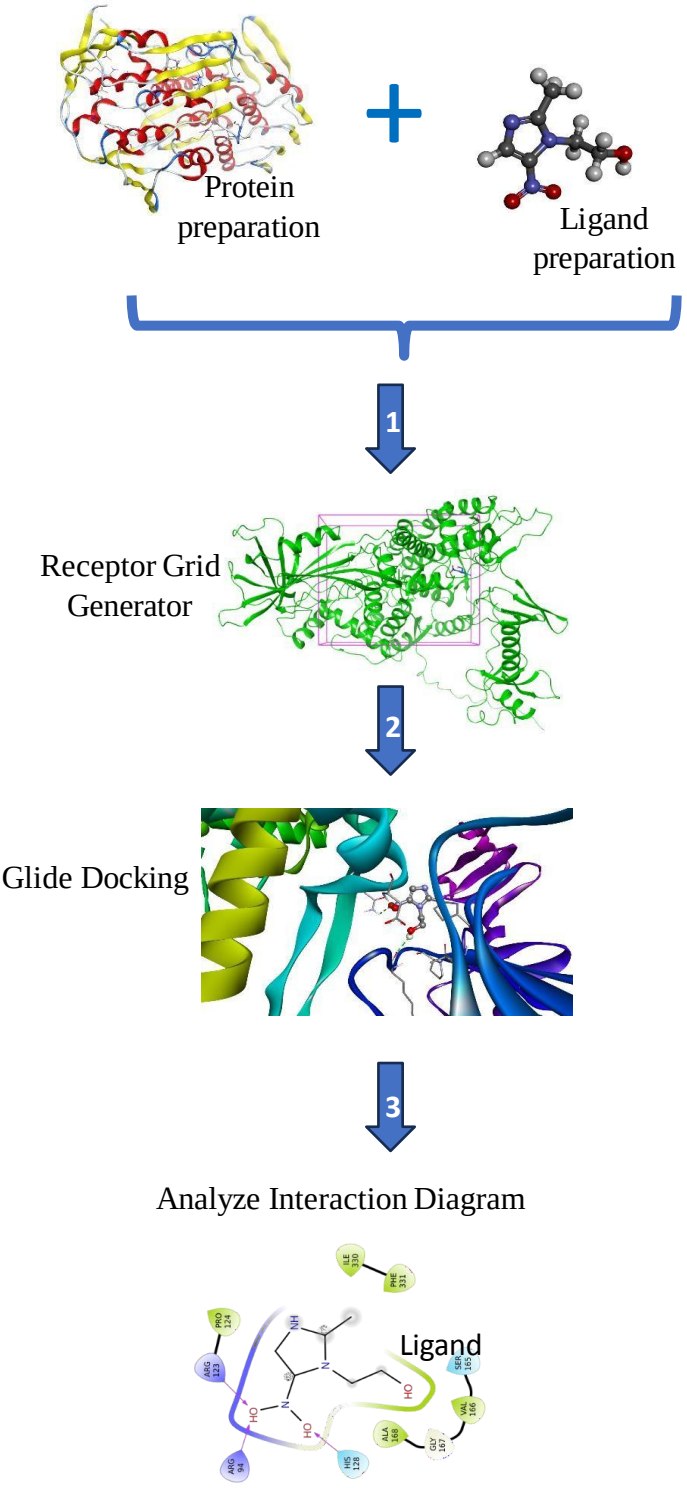


Figure 2. Molecular docking steps with Maestro

7. Steps of Molecular Dynamics Simulation:

To evaluate the dynamic behavior and stability of protein–ligand complexes under physiological conditions, Molecular Dynamics (MD) simulations were performed using the Desmond module integrated into Schrödinger Maestro. The simulations were run for 100 nanoseconds to analyze the conformational stability of donepezil analogues bound to Acetylcholinesterase (AChE, PDB ID: 4EY7) and Amyloid-beta (Aβ, PDB ID: 2BEG) targets. Key metrics such as Root Mean Square Deviation (RMSD), Root Mean Square Fluctuation (RMSF), and radius of gyration were monitored throughout the trajectories to assess stability and compactness of the complexes. The simulation process included the following steps, as illustrated in Figure 10.

Step 1: Complex Structure Preparation

Docked protein-ligand complexes from Glide (AChE-donepezil, AChE-donepezil analogues, Aβ-donepezil, and Aβ-donepezil analogues) were imported into the Maestro workspace and prepared using the Protein Preparation Wizard. The simulation pH was set to 7.0, and crystallographic water molecules were removed to prevent interference during solvation.

Step 2: Solvation and Neutralization

The prepared complexes were solvated using the System Builder tool with the following settings:

- Solvent Model: TIP3P water
- Box Type: Orthorhombic box with a 10 Å buffer region
- Force Field: OPLS4
- Ion Addition: Neutralization was achieved using Na^+ and Cl^- ions, with a final salt concentration of 0.15 M
- Ionization State: Adjusted to reflect physiological pH
- Energy Minimization: Performed to relieve steric clashes
- Equilibration: Conducted to stabilize solvent-protein-ligand interactions

The solvated and neutralized system is shown in Figure 12.

Step 3: Molecular Dynamics Simulation

Following equilibration, MD simulations were conducted under the following conditions:

- Simulation Duration: 100 nanoseconds

- Temperature: 300 K
- Pressure: 1 bar
- Recording Interval: 100 ps (total of ~1000 frames)
- Integrator: RESPA with default time steps
- Ensemble: NPT (constant Number of particles, Pressure, and Temperature)

The simulations were executed using the Desmond Molecular Dynamics Workflow. Default parameters were maintained unless otherwise specified.

Step 4: Simulation Interaction Analysis

Post-simulation, interaction profiles were extracted using the Simulation Interaction Diagram tool. Key interactions such as hydrogen bonds, hydrophobic contacts, salt bridges, and π–π stacking were identified and quantified for both AChE and Aβ complexes.

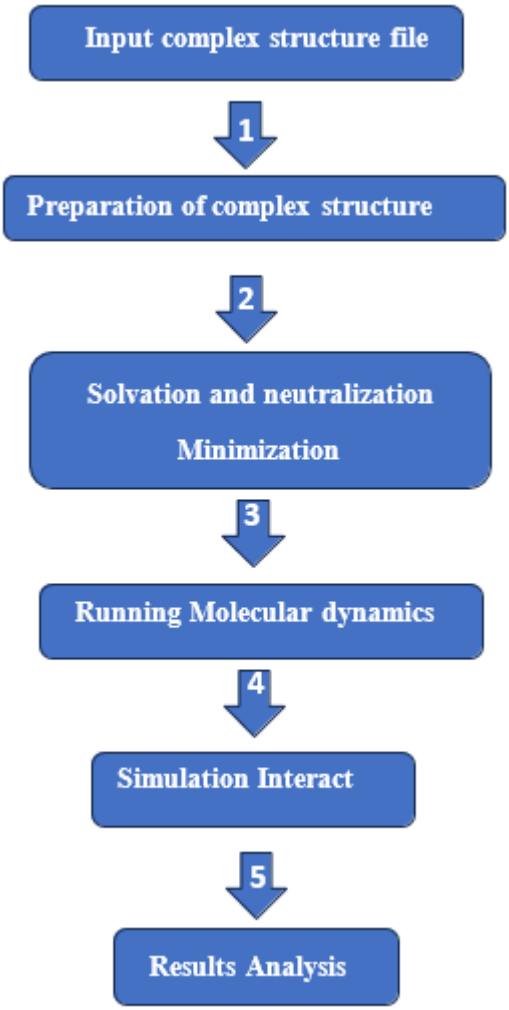


Figure 3. Molecular dynamics Simulation process

Step 5: Results Analysis

Output files from the Desmond MD simulation were analyzed to gain insights into the

dynamic behavior of AChE-donepezil, AChE-donepezil analogues, Aβ-donepezil, and Aβ-donepezil analogue complexes. Analyses focused on:

- Root Mean Square Deviation (RMSD): To assess the overall structural stability of the protein-ligand complexes over time.
- Root Mean Square Fluctuation (RMSF): To evaluate the flexibility of individual amino acid residues and detect regions of significant motion.
- Radius of Gyration (Rg): To monitor the compactness and folding behavior of the protein during the simulation.
- Hydrogen Bonding Profile: To determine the number and consistency of hydrogen bonds formed between the ligand and key residues at the active site.

These results were visualized using Maestro's Simulation Event Analysis and Interaction Diagram tools, providing clear depictions of time-dependent stability and binding behavior. The graphical outputs guided the selection of the most stable and biologically relevant conformations for further interpretation.

Figure 12 displays the solvation box around the donepezil-AChE complex during the MD simulation setup, illustrating the spatial distribution of water molecules and ions surrounding the system.

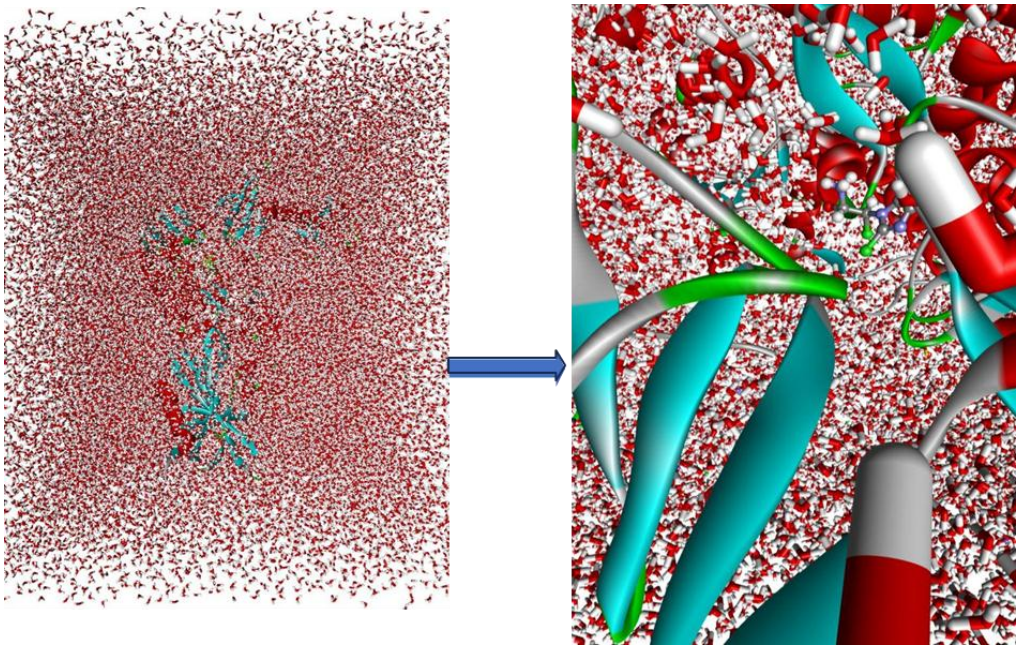


Figure 4. Solvation of AChE-donepezil complex for the MD simulation.

Chapter II:
Results & discussion

1. Virtual Screening

The process of generating a series of **donepezil** analogues began with the use of its core scaffold structure, as illustrated in Figure 5, serving as the foundational template. Functional groups including OH, CH_3 , NH_2 , and Cl were selected as principal substituents, complemented by empty linkers to enhance structural diversity. Simplified Molecular Input Line Entry System (SMILES) representations of both the scaffold and the selected functional groups were retrieved and integrated into the **SmiLib** software interface. This systematic enumeration strategy led to the creation of a diverse virtual library comprising **368 donepezil analogues** in SMILES format. Following library construction, the analogues underwent preliminary screening using the **ProTox 3.0** webserver to predict key toxicological parameters. The focus of this analysis was the estimation of the lethal dose (LD_{50}) and classification into toxicity classes (TC). Only molecules demonstrating a lower predicted toxicity—reflected by a higher TC and a lower LD_{50} compared to the parent compound donepezil—were selected for further investigation. Analogues that did not meet these safety criteria were excluded from subsequent analysis.

The selected low-toxicity analogues were then evaluated using the **SwissADME** platform to predict crucial pharmacokinetic parameters, such as gastrointestinal absorption, blood-brain barrier permeability, cytochrome P450 inhibition, and drug-likeness. This dual-stage screening—focusing on both toxicity and pharmacokinetics—was essential for identifying the most promising donepezil analogues with enhanced safety profiles and favorable pharmacological characteristics, positioning them as strong candidates for further in silico and experimental evaluation in the context of Alzheimer's disease therapy.

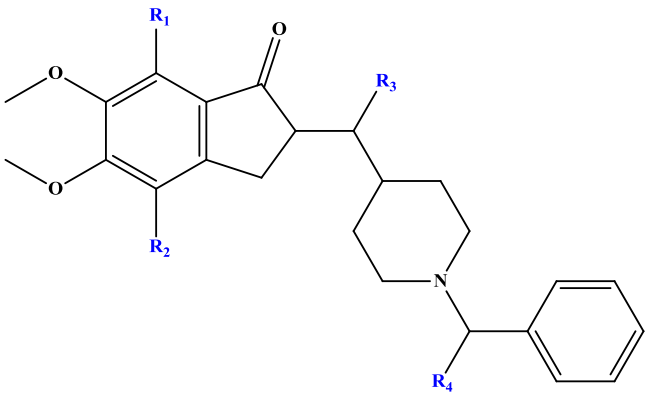


Figure.5. Scaffold structure used for the enumeration of donepezil analogues, (a) molecular structure presentation (b) SMILES presentation.

All generated molecules underwent a rigorous virtual screening process to evaluate their potential suitability as therapeutic agents targeting **Alzheimer's disease**, with a specific focus on their interaction with **Aβ and AChE proteins**—two key molecular targets implicated in the disease's progression. As part of this screening, a comprehensive toxicity evaluation was conducted using the **ProTox 3.0 webserver** [61]. This platform enabled the prediction of **toxicity class (TC)** and **lethal dose 50 (LD_{50})**, the latter referring to the dose required to cause death in 50% of a test population, thereby serving as a key indicator of acute toxicity.

This analysis provided valuable insights into the safety profiles of the designed donepezil analogues, enabling the identification of compounds with acceptable toxicity thresholds. Only analogues exhibiting **higher toxicity class values** and **lower LD_{50} levels** compared to the parent compound **donepezil** were retained for subsequent pharmacokinetic assessment. Molecules that failed to meet these toxicity benchmarks were excluded from further consideration.

Following toxicity-based filtering, the selected analogues were further evaluated using the **SwissADME** webserver [62], which predicted essential pharmacokinetic properties such as gastrointestinal absorption, blood–brain barrier (BBB) permeability, cytochrome P450 enzyme interaction, and overall drug-likeness. Based on this integrated in silico screening approach, combining toxicity and pharmacokinetic profiling, a refined subset of **donepezil** analogues with the most promising therapeutic potential and favorable ADME characteristics was identified (see Table 1).

Table 1. Selected donepezil analogues.

Entry	Code	R1	R2	R3	R4
1	Don1	OH	OH	CH_3	NH_2
2	Don2	NH_2	NH_2	OH	H
3	Don3	OH	OH	NH_2	NH_2
4	Don4	OH	OH	OH	CH_3
5	Don5	NH_2	NH_2	OH	NH_2
6	Don6	OH	NH_2	CH_3	OH
7	Don7	NH_2	NH_2	OH	CH_3
8	Don8	NH_2	NH_2	NH_2	H
9	Don9	OH	NH_2	NH_2	CH_3
10	Don10	NH_2	OH	CH_3	H
11	Don11	OH	Cl	Cl	Cl

12	Don12	OH	Cl	Cl	NH_2
13	Don13	OH	Cl	NH_2	NH_2
14	Don14	OH	NH_2	NH_2	OH
15	Don15	OH	NH_2	CH_3	CH_3
16	Don16	NH_2	NH_2	OH	OH
17	Don17	CH_3	CH_3	Cl	Cl
18	Don18	NH_2	H	H	H
19	Don19	OH	NH_2	H	H
20	Don20	NH_2	NH_2	H	H
21	Don21	NH_2	OH	NH_2	H
22	Don22	NH_2	NH_2	CH_3	H
23	Don				

Another selection criterion was applied using the **ProTox 3.0 online server**, which predicts a range of toxicological endpoints including **cytotoxicity, mutagenicity, carcinogenicity, hepatotoxicity, and immunotoxicity,** in accordance with the **Globally Harmonized System (GHS)** for chemical classification. Compounds with **LD_{50} values between 2000–5000 mg/kg** were classified as **toxicity class 5**, while those with LD_{50} values between **300–2000 mg/kg** were assigned to **class 4**. The predicted **probability of immunotoxicity** was consistently low across all analogues, ranging from –0.88 to –0.99. Furthermore, all compounds were predicted to be **non-hepatotoxic** and **non-cytotoxic**, and their **mutagenicity and carcinogenicity** predictions remained within **acceptable safety thresholds** (Table 2).

Compared to the reference compound **done**, analogues demonstrating **lower LD_{50} values** and thus higher **toxicity class (TC),** along with more **negative hepatotoxicity** and **immunotoxicity** scores and **less positive values** for **carcinogenicity, mutagenicity**, and **cytotoxicity**, were prioritized for further investigation. These compounds, which demonstrated the most favorable toxicological profiles, are **highlighted in blue** in Table 2 and include Don10, Don11, Don12, Don15, Don17, Don18, Don19, and Don22.

Table 2. Toxicity prediction probability, median lethal dose, and toxicity class of selected drugs analogues

Entry	Code	Hepato	Carcino	Immuno	Muta	Cyto	LD50	TC
1	Don1	-0.51	+0.69	-0.99	+0.85	1	1530	4
2	Don2	-0.62	+0.70	-0.98	+0.88	2	3000	5
3	Don3	-0.50	+0.66	-0.92	+0.79	3	1530	4
4	Don4	-0.53	+0.67	-0.99	+0.84	4	1500	4
5	Don5	-0.51	+0.72	-0.96	+0.84	5	3000	5
6	Don6	-0.53	+0.67	-0.98	+0.84	6	3000	5
7	Don7	-0.52	+0.75	-0.98	+0.85	7	3000	5
8	Don8	-0.63	+0.67	-0.99	+0.85	8	3000	5
9	Don9	-0.52	+0.68	-0.97	+0.80	9	3000	5
10	Don10	-0.61	+0.69	-0.98	+0.84	10	3000	5
11	Don11	-0.50	+0.67	-0.93	+0.83	11	2000	4
12	Don12	-0.50	+0.56	-0.98	+0.82	12	2000	4
13	Don13	-0.51	+0.56	-0.92	+0.81	13	1530	4
14	Don14	-0.52	+0.54	-0.94	+0.79	14	3000	5
15	Don15	-0.53	+0.67	-0.98	+0.86	15	3000	5
16	Don16	-0.51	+0.68	-0.99	+0.84	16	3000	5
17	Don17	-0.50	+0.56	-0.98	+0.82	17	3000	5
18	Don18	-0.77	+0.73	-0.88	+0.95	18	2000	4
19	Don19	-0.71	+0.66	-0.96	+0.85	19	3000	5
20	Don20	-0.76	+0.72	-0.97	+0.92	20	3000	5
21	Don21	-0.61	+0.64	-0.99	+0.83	21	3000	5
22	Don22	-0.62	+0.76	-0.98	+0.82	22	3000	5
23	Don	-0.87	+0.87	-0.95	+0.97	23	1500	4

LD50 (mg/kg), - (Inactive toxic class (probability score)), + (Active toxic class (probability score)), TC: Toxicity Class

The evaluation of pharmacokinetic and toxicity parameters plays a pivotal role in identifying potential drug candidates during the early stages of drug development. In this study, special attention was given to the predicted toxicity profiles of the synthesized donepezil analogues, focusing on hepatotoxicity, carcinogenicity, immunotoxicity, mutagenicity, cytotoxicity, and LD_{50} values, as estimated by the ProTox 3.0 server and summarized in Table 2.

According to the Globally Harmonized System (GHS) classification, compounds with LD_{50} values ranging from 300 to 2000 mg/kg fall under toxicity class 4, whereas those within 2000–5000 mg/kg are assigned to class 5. Most donepezil analogues in this study exhibited LD_{50} values ≥2000 mg/kg, placing them in toxicity class 5 and indicating relatively low acute toxicity. However, Don1, Don3, Don18, and Don23 were categorized under class 4, suggesting a higher degree of acute toxicity, yet still within a tolerable safety range.

In terms of organ-specific toxicity, all compounds were predicted to be non-hepatotoxic and non-cytotoxic, with hepatotoxicity scores ranging from –0.50 to –0.87 and cytotoxicity scores from –0.65 to –0.95. These negative values reflect a lower likelihood of causing liver or cellular damage, highlighting a generally safe profile for hepatic and cellular systems.

The immunotoxicity predictions showed uniformly low probabilities, with all values ranging from –0.88 to –0.99, suggesting minimal potential to provoke adverse immune responses. Similarly, the majority of compounds demonstrated mutagenicity and carcinogenicity scores within acceptable safety thresholds, with most values falling below +0.85. Notably, Don18 and Don22 presented relatively low scores across all five toxicity endpoints, indicating a superior safety profile.

Compared to the reference compound metronidazole, several donepezil analogues demonstrated improved toxicological characteristics. In particular, Don10, Don11, Don12, Don15, Don17, Don18, Don19, and Don22 exhibited more negative values of hepatotoxicity and immunotoxicity, as well as less positive scores for carcinogenicity, mutagenicity, and cytotoxicity. These compounds also maintained LD_{50} values of 3000 mg/kg or higher, placing them within class 5, and thus are considered to possess lower acute toxicity.

Collectively, these results suggest that the majority of tested donepezil analogues exhibit favorable toxicity profiles. Emphasis should be placed on compounds with toxicity class 5, non-hepatotoxic and non-cytotoxic characteristics, and low immunotoxic and genotoxic potential, such as Don10, Don11, Don12, Don15, Don17, Don18, Don19, and Don22. These analogues represent promising candidates for further in vitro and in vivo pharmacological evaluation.

Table 3. Evaluation of Pharmacokinetics properties of **donepezil** and its generated analogues

Entry	Molecule	GI absorption	BBB permeant	P-gp substrate	CYP inhibitor				
					1A2	2C19	2C9	2D6	**3A4**
1	Don1	Low	No	No	No	No	No	No	**No**
2	Don2	Low	No	No	No	No	No	No	**No**
3	Don3	Low	No	No	No	No	No	No	**No**
4	Don4	Low	No	No	No	No	No	No	**No**
5	Don5	Low	No	No	No	No	No	No	**No**
6	Don6	Low	No	No	No	No	No	No	**No**
7	Don7	Low	No	No	No	No	No	No	**No**
8	Don8	Low	No	No	No	No	No	No	**No**
9	Don9	Low	No	No	No	No	No	No	**No**
10	Don10	High	No	No	No	No	No	No	**No**
11	Don11	High	No	No	No	No	No	No	**No**
12	Don12	High	No	No	No	No	No	No	**No**
13	Don13	Low	No	No	No	No	No	No	**No**
14	Don14	Low	No	No	No	No	No	No	**No**
15	Don15	High	No	No	No	No	No	No	**No**
16	Don16	Low	No	No	No	No	No	No	**No**
17	Don17	High	No	No	No	No	No	No	**No**
18	Don18	High	No	No	No	No	No	No	**No**
19	Don19	High	No	No	No	No	No	No	**No**
20	Don20	Low	No	No	No	No	No	No	**No**
21	Don21	Low	No	No	No	No	No	No	**No**
22	Don22	High	No	No	No	No	No	No	**No**
23	Don	High	No	No	No	No	No	No	**No**

The in-silico pharmacokinetic analysis presented in Table 3 identifies compounds Don10, Don11, Don12, Don15, Don17, Don18, Don19, and Don22 as the most promising candidates for further investigation, based on their favorable absorption, distribution, and metabolic profiles. All of these analogues exhibit high gastrointestinal (GI) absorption, which is a key factor for achieving adequate systemic exposure following oral administration. High GI absorption suggests efficient transport across the intestinal epithelium, supporting their potential as orally bioavailable therapeutic agents.

Importantly, none of the tested analogues are predicted to cross the blood-brain barrier (BBB), which minimizes the risk of central nervous system (CNS)-related side effects. This characteristic is especially desirable when CNS penetration is not required for therapeutic efficacy, as it reduces the potential for neurological complications and off-target CNS activity. This limited permeability supports a safer profile for peripheral targets and may allow for broader clinical applications without CNS toxicity concerns.

In addition to favorable absorption and BBB profiles, the selected compounds are not predicted to act as substrates for P-glycoprotein (P-gp), a critical efflux transporter involved in limiting intracellular drug accumulation. The absence of P-gp substrate activity suggests that these analogues are less likely to be actively pumped out of cells, thereby improving cellular retention and enhancing overall therapeutic efficacy. This property may also reduce the risk of drug resistance mediated by P-gp overexpression in certain disease states.

Furthermore, none of the highlighted analogues demonstrate inhibitory effects on major cytochrome P450 (CYP) enzymes, including CYP1A2, CYP2C19, CYP2C9, CYP2D6, and CYP3A4. This observation implies a low likelihood of CYP-mediated drug-drug interactions, which is a critical consideration in polypharmacy settings, particularly for elderly or chronically ill patients. Preserving normal CYP enzyme activity enhances the predictability of drug metabolism and supports a safer pharmacokinetic profile.

Taken together, these pharmacokinetic characteristics strongly support the further development of Don10, Don11, Don12, Don15, Don17, Don18, Don19, and Don22 as potential therapeutic agents. Their combination of high oral absorption, limited CNS penetration, reduced efflux liability, and minimal metabolic interference via CYP enzymes provides a balanced and favorable profile for advancing into subsequent stages of drug development and preclinical testing.

1.1 Physicochemical Properties

The physicochemical properties, including solubility and lipophilicity, are crucial determinants of a drug's potential success as a candidate [63]. Lipophilicity, particularly, holds significance in various molecular discovery endeavours.[64] The parameters assessed for scoring include lipophilicity ($0.7 < XLogP < 5$), molecular weight ($150 < MW < 500$ g/mol), and solubility ($0 < \log S < -6$). The physicochemical properties of donepezil and its designed analogues are summarized in Table 4. Results indicate that all compounds fall within the acceptable range of lipophilicity and molecular weight, suggesting favorable

permeability and transport characteristics across biological membranes. Furthermore, all molecules exhibit good water solubility, which supports their oral bioavailability and systemic exposure.

In terms of molecular flexibility, each compound contains fewer than 8 rotatable bonds, indicating limited conformational freedom. This low degree of flexibility is often associated with enhanced membrane permeability and improved pharmacokinetic stability. Another critical aspect of drug-likeness is the acid-base nature of the molecule, which is influenced by the number of hydrogen bond donors (HBD) and hydrogen bond acceptors (HBA)[65]. According to Lipinski's rule of five [66], favorable absorption and distribution profiles are typically observed in molecules with low HBD counts and moderate to high HBA values.

In this study, all tested analogues of donepezil exhibit a higher number of HBAs and a limited number of HBDs, consistent with optimal ADMET properties and enhanced drug-likeness. These findings collectively indicate that the synthesized compounds possess physicochemical characteristics suitable for further pharmacological exploration and potential therapeutic development.

Table 4. Physicochemical Properties of **donepezil** and its generated analogues

Molecule	Water solubility (Log *S*)	Lipophilicity (Consensus Log $P_{o/w}$)	Molar refractivity	H-bond acceptor	H-bond donor	Rotatable bonds	Molecular weight
Don1	-2.25	-1.36	49.84	7	4	3	218.17
Don2	-2.08	-1.75	48.25	5	4	3	203.16
Don3	-2.11	-2.41	47.74	8	5	3	219.16
Don4	-2.97	-1.14	48.29	7	4	3	219.15
Don5	-1.82	-2.33	50.96	6	5	3	218.17
Don6	-2.18	-1.46	50.68	6	4	3	218.17
Don7	-2.53	-1.40	53.06	5	4	3	218.17
Don8	-1.93	-2.00	49.80	5	4	3	202.17
Don9	-2.60	-1.51	52.22	6	4	3	217.18
Don10	-2.29	-0.84	49.51	5	3	3	202.17
Don11	-4.22	+0.61	54.90	5	2	3	276.46
Don12	-3.18	-0.44	52.82	6	3	3	257.03
Don13	-2.13	-1.58	50.73	7	4	3	237.60
Don14	-2.04	-2.38	48.58	7	5	3	219.16
Don15	-2.74	-0.60	54.32	5	3	3	216.19
Don16	-1.97	-2.28	49.41	6	5	3	219.16
Don17	-3.08	+0.83	57.80	4	1	3	254.07

Don18	-1.47	-0.96	42.68	4	2	3	172.14
Don19	-1.88	-1.17	44.71	5	3	3	188.14
Don20	-1.66	-1.40	47.09	4	3	3	187.16
Don21	-2.16	-1.75	47.41	6	4	3	203.16
Don22	-2.07	-1.04	51.90	4	3	3	201.18
Don	-1.29	-0.23	43.25	4	1	3	171.15

2. Druglikeness

According to **Lipinski's Rule of Five** [66], all donepezil analogues demonstrate favorable drug-likeness, fulfilling the rule's criteria related to molecular weight, lipophilicity, and hydrogen bonding capacity. This compliance indicates strong potential for oral bioavailability and pharmacokinetic efficiency.

However, when applying **Ghose's filter** [67], which assesses molecular complexity through parameters such as molar refractivity, molecular weight, atom count, and logP, only four compounds—Don10, Don12, Don15, and Don17—satisfied all requirements, suggesting a smaller subset with optimal physicochemical attributes.

In the context of **Veber's rule** [68], which focuses on molecular flexibility and topological polar surface area, eight compounds—Don10, Don11, Don12, Don15, Don17, Don18, Don19, and Don21—met the criteria, indicating potential for good membrane permeability and oral absorption.

Similarly, applying **Egan's filter** [69], which emphasizes absorption likelihood through logP and tPSA thresholds, compounds Don4, Don10, Don11, Don12, Don15, Don17, and Don22 demonstrated compliance, reinforcing their drug-likeness.

An integrative application of all five major drug-likeness rules—Lipinski, Ghose, Veber, Egan, and Muegge—yields an average compliance score of approximately 55%, as shown in Table 5, supporting the overall bioavailability and suitability of these molecules as oral therapeutic agents.

Further refinement based on these filters highlights Don11, Don12, Don15, Don17, Don18, Don19, and Don22 as the most promising candidates. These compounds consistently meet the critical selection criteria and show favorable pharmacokinetic and toxicological profiles, making them strong contenders for advanced in silico and preclinical evaluations. Conversely, although Don10 and Don21 demonstrated partial compliance, they failed to pass the full selection threshold. This systematic filtering approach enhances the prioritization of top-performing candidates, streamlining subsequent stages in the drug development pipeline.

Table 1. Drug likeness properties of donepezil and its generated analogues

Molecule	Lipinski ≠violation	Ghose ≠violation	Veber ≠violation	Egan ≠violation	Muegge ≠violation	Bioavailability Score
Don1	0	1	1	1	1	**0.55**
Don2	0	1	1	1	1	**0.55**
Don3	0	1	1	1	1	**0.55**
Don4	0	1	1	1	0	**0.55**
Don5	0	1	1	1	1	**0.55**
Don6	0	1	1	1	1	**0.55**
Don7	0	1	1	1	1	**0.55**
Don8	0	1	1	1	1	**0.55**
Don9	0	1	1	1	1	**0.55**
Don10	0	0	0	0	0	**0.55**
Don11	0	1	0	0	0	**0.55**
Don12	0	0	0	0	0	**0.55**
Don13	0	1	1	1	1	**0.55**
Don14	0	1	1	1	1	**0.55**
Don15	0	0	0	0	0	**0.55**
Don16	0	1	1	1	1	**0.55**
Don17	0	0	0	0	0	**0.55**
Don18	0	1	0	0	1	**0.55**
Don19	0	1	0	0	1	**0.55**
Don20	0	1	1	1	1	**0.55**
Don21	0	1	0	1	1	**0.55**
Don22	0	1	1	0	0	**0.55**
Don	0	0	0	0	1	**0.55**

The **BOILED-Egg plot** shown in **Figure 6**, which maps **Total Polar Surface Area (TPSA)** against **lipophilicity (LogP)**, serves as an intuitive tool for predicting **human intestinal absorption (HIA)** and **blood–brain barrier (BBB)** penetration. The **white region** (egg white) indicates a high probability of passive absorption through the gastrointestinal tract (GIT), while the **yellow region** (yolk) represents compounds likely to permeate the brain. Additionally, **red-colored dots** correspond to molecules **not predicted to be P-glycoprotein (P-gp) substrates**, whereas **blue-colored dots** would signify **active P-gp substrates**.

In this analysis, eight analogues—Don10, Don11, Don12, Don15, Don17, Don18, Don19, and Don20—as well as the reference compound Donepezil, are positioned within the white region, suggesting good potential for passive intestinal absorption. The remaining analogues fall outside this region, indicating a lower likelihood of effective absorption via the GIT.

Significantly, none of the tested compounds are located within the yolk region, implying a low probability of brain penetration, which may help reduce central nervous system (CNS)-related side effects. Moreover, all compounds are represented as red dots in the plot, indicating that they are not predicted to be substrates of P-glycoprotein (P-gp). This property further supports their favorable pharmacokinetic profiles, minimizing the risk of efflux-related drug resistance or pharmacokinetic interactions.

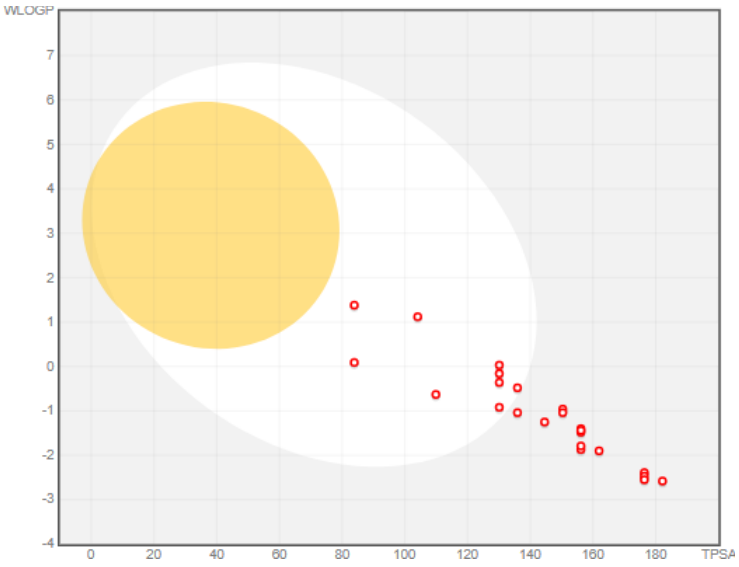


Figure.6. The Boiled-egg plot of **Donepezil** and selected generated analogues.

Following the prediction of pharmacokinetic and toxicity properties, the **half maximal inhibitory concentration (IC_{50})** values of all selected compounds against **acetylcholinesterase (AChE)** and **amyloid-beta (Aβ)** were calculated using **AutoDock 4.2** and **AutoDock Tools 1.5.6**

software [70].

The obtained results are summarized in Table 6.

Table 6. Half maximal inhibitory concentration of **Donepezil** and selected generated analogues.

Molecule	Aβ	AChE
	IC_{50} (µM)	IC_{50} (µM)
Don1	47.24	66.21
Don2	92.81	154
Don3	39.90	47.24
Don4	28.47	55.93
Don5	66.21	130.1
Don6	24.05	78.39
Don7	55.93	109.9
Don8	66.21	182.3
Don9	66.21	55.93
Don10	92.81	215.9
Don11	24.05	130.1
Don12	33.70	154.0
Don13	66.21	109.9
Don14	39.90	55.90
Don15	33.70	55.93
Don16	33.70	130.1
Don17	39.90	215.9
Don18	215.9	302.5
Don19	130.1	255.5
Don20	154.0	255.5
Don21	55.93	109.9
Don22	66.21	215.9
Don	154.0	502.0

Table 6 presents IC_{50} values of donepezil (Don) and its generated analogues against amyloid-beta (Aβ) and acetylcholinesterase (AChE), two pivotal targets in the pathogenesis of Alzheimer's disease. The data exhibit a wide range of inhibitory activities, with several analogues outperforming the reference compound donepezil in potency.

Among these analogues, **Don11, Don12, and Don15** emerge as the most promising. Don11 shows the strongest inhibition against Aβ with an IC_{50} value of 24.05 µM, while Don12 exhibits

potent AChE inhibition with an IC_{50} of 130.1 μM. Don15 demonstrates a balanced inhibitory profile, with IC_{50} values of 39.90 μM for Aβ and 59.90 μM for AChE, suggesting dual-target potential.

Other compounds such as Don4, Don10, and Don22 also display notable activity, with IC_{50} values comparable to or better than that of the parent compound. These results emphasize the potential of selected analogues to act as multitarget agents in Alzheimer's therapy.

The identification of these potent analogues supports their candidacy for further in silico analysis, including molecular docking and dynamics simulations, to validate binding stability and interaction profiles. Consequently, Don11, Don12, and Don15 are highlighted as strong leads for future preclinical investigation in the development of effective anti-Alzheimer agents.

3. Molecular Docking Study

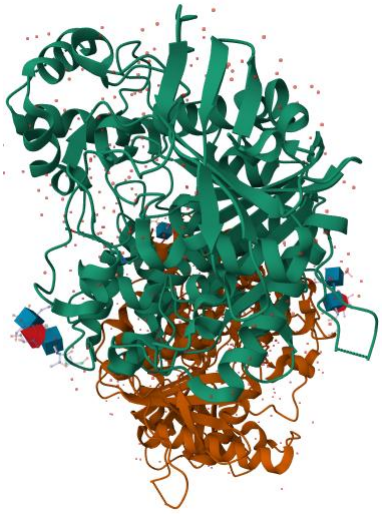
To elucidate the interaction mechanisms of the most potent Donepezil analogues—Don11, Don12, and Don15—with the Aβ and AChE receptors, molecular docking simulations were conducted. These simulations aimed to predict the most stable conformations of these compounds when bound to the target receptors.

The 3D structures of Aβ and AChE were retrieved from the RCSB Protein Data Bank [71]. The docking simulations provided insights into the binding affinities and potential mechanisms through which these compounds may exert their therapeutic effects.

Figure 7 illustrates the docking results, showing the preferred binding modes of the compounds with Aβ and AChE, along with the key molecular interactions contributing to binding stability.

Structure of amyloid precursor protein's transmembrane domain

PDB ID:	2LLM
Expression System	Escherichia coli
Source organism	Homo sapiens
Amino acids	43
Biological function	The transmembrane domain of APP regulates its cleavage, influencing amyloid-beta production.



Crystal Structure of Recombinant Human Acetylcholinesterase in Complex with Donepezil

PDB ID	4EY7
Expression System	Homo sapiens
Source organism	Homo sapiens
Amino acids	542
Biological function	The structure reveals how donepezil binds to AChE, inhibiting acetylcholine breakdown.

Figure 7. Three-dimensional structure of Amyloid-Beta and Acetylcholinesterase

The binding site was defined using **Ligand Binding Site Prediction** (https://prankweb.cz). The molecular docking procedure was conducted using the **Schrödinger Maestro** software, employing the **Glide SP (Standard Precision)** module. Initially, ligand molecules underwent preparation for docking calculations utilizing the **LigPrep** tool within the Schrödinger Software program, employing the **OPLS3** force field as described by Harder et al [72], This involved generating a maximum of 32 stereoisomers for each ligand after selecting the ionization states at pH 7.0 ± 2.0. Subsequently, receptor structures were prepared using the **Protein Preparation Wizard tool** [73], ensuring a solubility of 2.5 Å. Polar hydrogens were added to heavy atoms, and all water and ions were removed from the structure. Bond orders were assigned, charges were defined at pH 7.0, and the selected receptor was optimized using **PROPKA**. Heavy atoms in the receptor were constrained to a preferred 0.3Å RMSD using the **OPLS3** force field. Grid boxes were defined around the receptor using the grid generation tool in Maestro. Ligands were then docked into the receptor based on the defined grid using the **Standard Precision (SP)** docking algorithm, allowing for the ranking of ligands based on their interaction with specific conformations of the receptor molecule.[74]

The docking analysis depicted in Figure 8 provides deeper insights into the interaction modes of the tested compounds within the binding site of **Aβ and AChE.** Notably, the interactions include hydrogen bonding, π–π stacking, and hydrophobic contacts that contribute significantly to the binding stability and selectivity of the most potent analogues.,

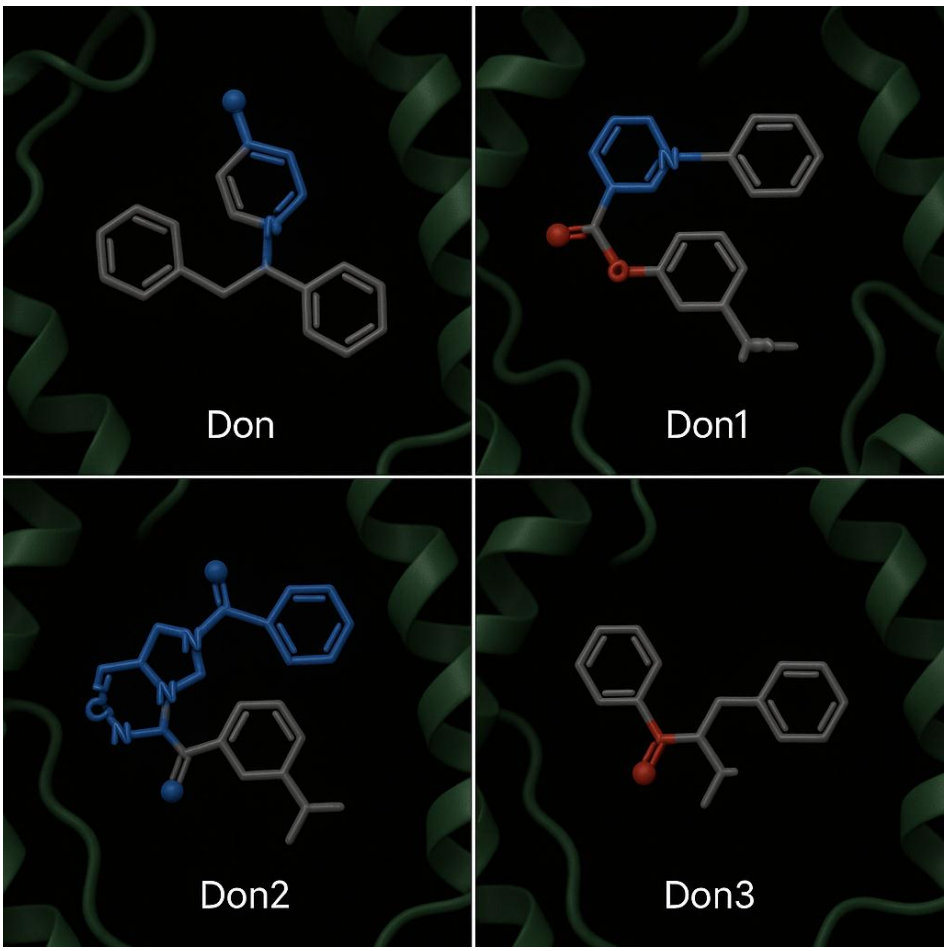


Figure 8. 3D interaction of donepezil the selected most potent analogues with the target the target Aβ and AChE.

4. Molecular Dynamic simulation:

Molecular dynamics (MD) simulation is a computational technique widely utilized to study the dynamic behavior of molecular systems over time. While molecular docking assumes the protein to be a rigid structure, it fails to account for potential conformational changes during ligand binding. To better replicate physiological conditions and capture the flexibility of both the protein and the ligand, molecular dynamics simulations were conducted in this study.

MD simulations were carried out using the Desmond MD program (version 2024-3) on the docked complexes of the ligands with the highest binding affinities—Don11, Don12, Don15, as well as the reference compound Donepezil (Don)—with the target proteins Amyloid-Beta (Aβ) and Acetylcholinesterase (AChE). This approach enabled a more comprehensive evaluation of the stability and interaction profiles of these complexes under simulated physiological conditions.

The preparation process involved generating topology files for each protein-ligand complex, describing atomic connectivity and assigning suitable force field parameters. The TIP3P water model was employed to solvate the protein-ligand systems, while the OPLS3e force field was used to parameterize both standard and non-standard residues. Special care was taken in treating chemical features unique to the ligand structures to ensure accurate modeling.

After system assembly, energy minimization was performed to resolve steric clashes and unfavorable contacts. Simulation parameters such as time step, temperature (300 K), pressure (1 atm), and duration (100 ns) were defined to balance accuracy and computational cost. Each system was neutralized with counter-ions and equilibrated under NPT ensemble conditions prior to production runs.

The simulations, each lasting 100 nanoseconds, were analyzed using key structural and dynamic metrics, including Root Mean Square Deviation (RMSD) to assess overall stability, Root Mean Square Fluctuation (RMSF) to examine residue flexibility, and Radius of Gyration (Rg) to evaluate the compactness of the protein-ligand complex.

The findings from the molecular dynamics simulations provided deeper insights into the binding stability and conformational adaptability of Don11, Don12, and Don15 compared to Donepezil, reinforcing their potential as optimized inhibitors for Alzheimer's disease targets Aβ and AChE.

4.1 Root mean square deviation (RMSD)

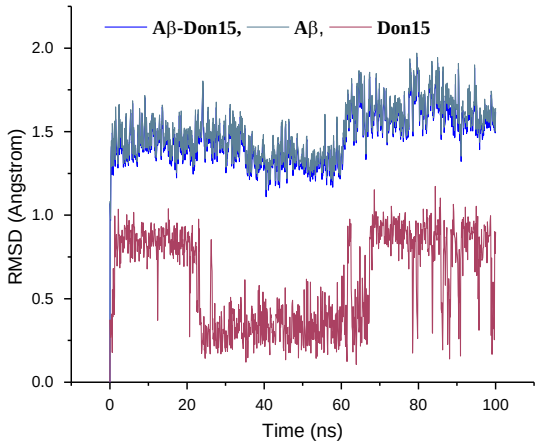
To assess the structural stability of the protein–ligand complexes formed with Don15, Don12, Don11, and the reference compound Don, the RMSD of Cα atoms was calculated from the molecular dynamics simulation trajectories (Figure 9). After an initial equilibration phase, all complexes showed relatively stable RMSD values ranging between approximately 0.25 and 1.75 Å, indicating that the systems reached a well-equilibrated and stable conformation.

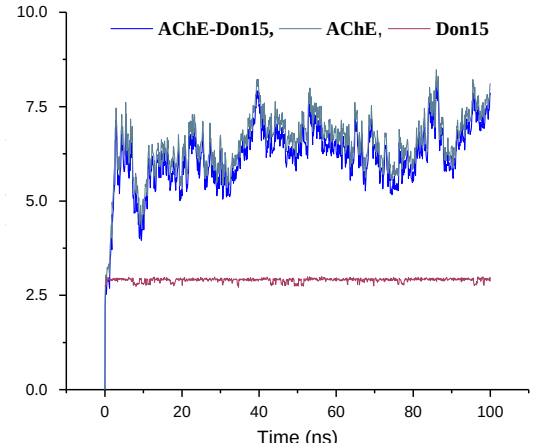
Notably, the AChE–Don15 complex exhibited a marked increase in RMSD, reaching approximately 5 Å, which remained consistent for the rest of the 100 ns simulation. This suggests a potential conformational shift in the AChE structure in response to Don15 binding, followed by a new, relatively stable structural state.

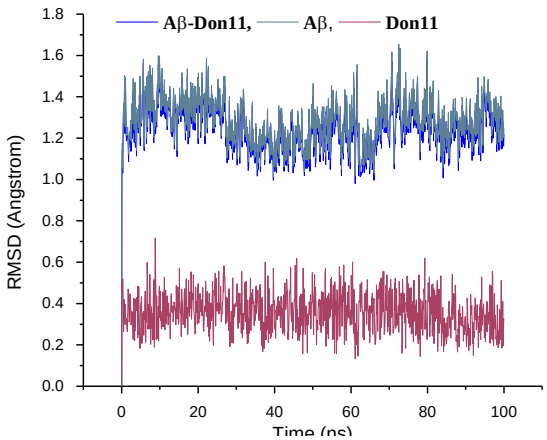
In contrast, the AChE–Don11, AChE–Don12, and the reference Don–AChE complexes showed only minor fluctuations in RMSD, remaining under 2 Å throughout the simulation, which reflects strong binding and structural stability of the complexes.

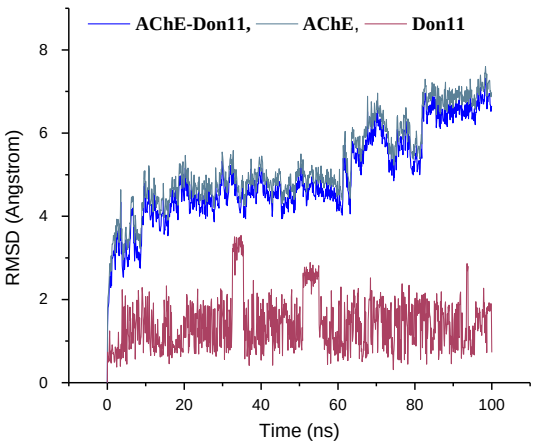
However, the Aβ–Don15 complex demonstrated noticeable deviations in RMSD between 20 and 60 ns, indicating possible conformational rearrangements during this interval before reaching stability. The other Aβ-bound complexes (Don11 and Don12) remained within acceptable RMSD limits, indicating stable binding throughout the simulation.

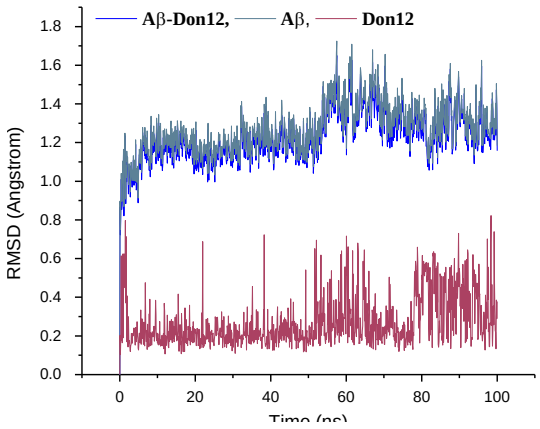
Overall, these RMSD analyses suggest that Don11 and Don12 form more stable interactions with both AChE and Aβ under physiological simulation conditions, while Don15 may induce greater structural alterations, particularly with AChE, which could influence its biological activity.

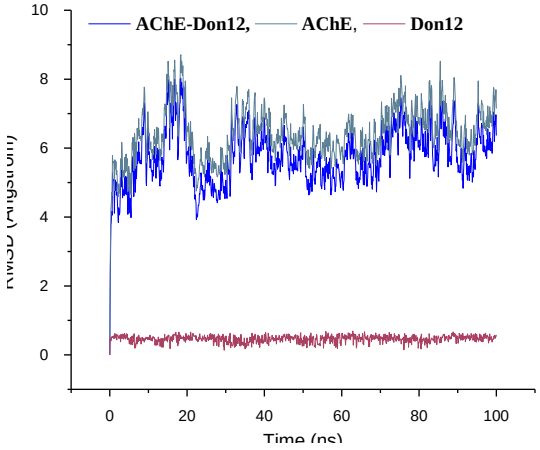












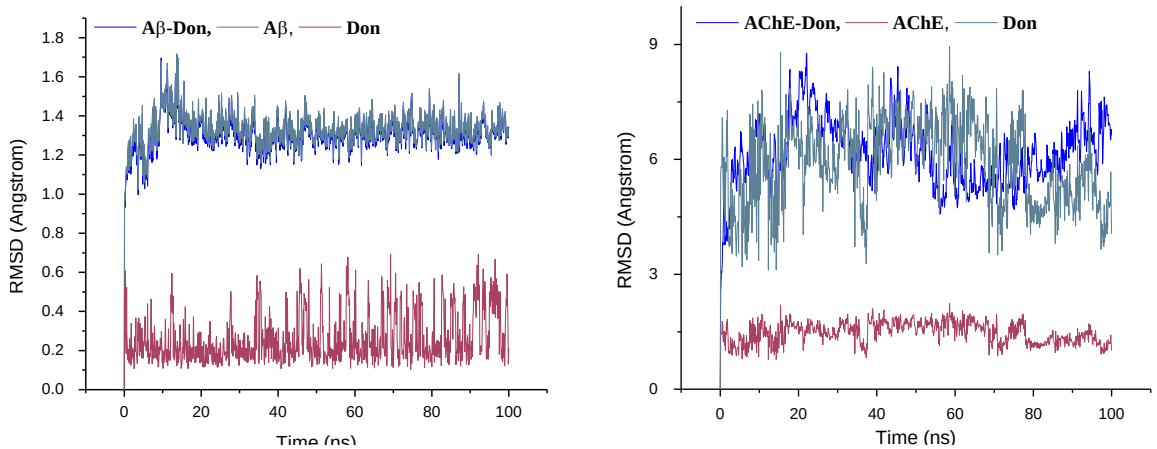


Figure 9. The RMSD plots of Aβ and AChE complexes during 100 ns simulation of Don15, Don12, Don11, and the control Don.

4.2 Root mean square fluctuation (RMSF):

To evaluate the dynamic behavior of the protein when bound to the ligands, we calculated the root mean square fluctuations (RMSF) values. These values provide insights into the flexibility and movement of individual protein residues throughout the entire simulation duration [75].

Upon analysing the RMSF values for the protein Aβ, we observed that the majority of protein residues exhibited minimal fluctuations, measuring less than 2 Å, throughout the simulation. This suggests that these residues maintained a relatively rigid and stable conformation in the presence of the ligands Don11, Don12, and Don15. However, the loop regions of the protein demonstrated higher RMSF values, peaking at approximately 3.4 Å, Figure 10.

Similarly, the analysis of Root Mean Square Fluctuation (RMSF) values for the AChE protein revealed that most protein residues experienced minimal fluctuations, measuring also less than 2 Å, throughout the simulation period. This indicates that these residues maintained a relatively rigid and stable conformation in the presence of the Don11, Don12, and Don15 ligands. However, the loop regions of the protein exhibited higher RMSF values, reaching a peak of approximately 12.2 Å, as depicted in Figure 10.

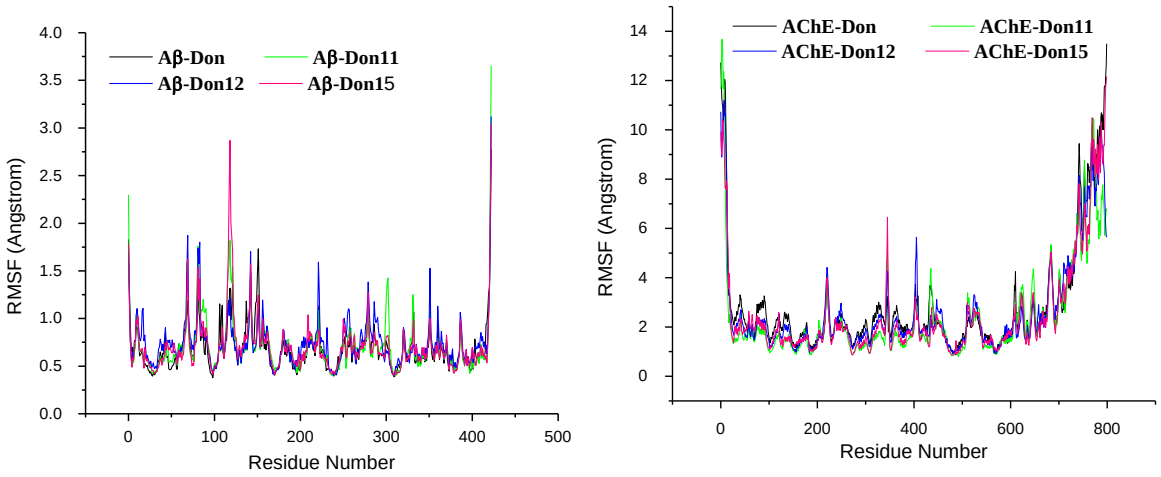


Figure 10. The fluctuation of protein residues of Aβ and AChE during simulations as determined by RSMF values.

The RMSF analysis indicates that the protein-ligand complex remained stable overall, with most residues adopting a rigid conformation. The elevated RMSF values observed in the loop regions suggest that these areas experienced more pronounced fluctuations and potentially engaged in dynamic interactions with the ligands. In summary, the RMSF values support the notion of a stable protein-ligand complex, as the majority of protein residues displayed minimal fluctuations, while the loop regions exhibited relatively higher flexibility.

4.3 Radius of gyration :

To evaluate the structural compactness of the Aβ and AChE proteins in complex with the compounds Don11, Don12, and Don15 in addition to the reference drug donepezil, we conducted an analysis of the radius of gyration (Rg). The Rg values provide insights into the overall compactness of the protein structure, where lower values indicate a more compact structure and higher values suggest unfolding events during the simulation [76]. The Rg plots of the Aβ-ligands complexes revealed that the Rg values remained stable within the range of 2.68 to 2.82 Å throughout the simulation, except for compound Don15 which have higher values of Rg of 2.92 Å in the range of simulation from 0 to 20 ns and from 70 to 100 ns, Figure 11. This indicates that the enzyme structure maintained its compacted conformation when bound to Don11, Don12, and Don15 compounds. The consistent Rg values further support the notion of structural stability during the simulation period.

In a parallel manner, the Radius of Gyration (Rg) plots for the AChE-ligand complexes depicted consistent Rg values within the range of 2.68 to 2.91 Å across the simulation duration, as illustrated in Figure 11. This observation suggests that the enzyme structure

retained its compact conformation while interacting with the Don11, Don12, and Don15 compounds. The stability in Rg values throughout the simulation period further corroborates the structural integrity and stability of the enzyme-ligand complexes.

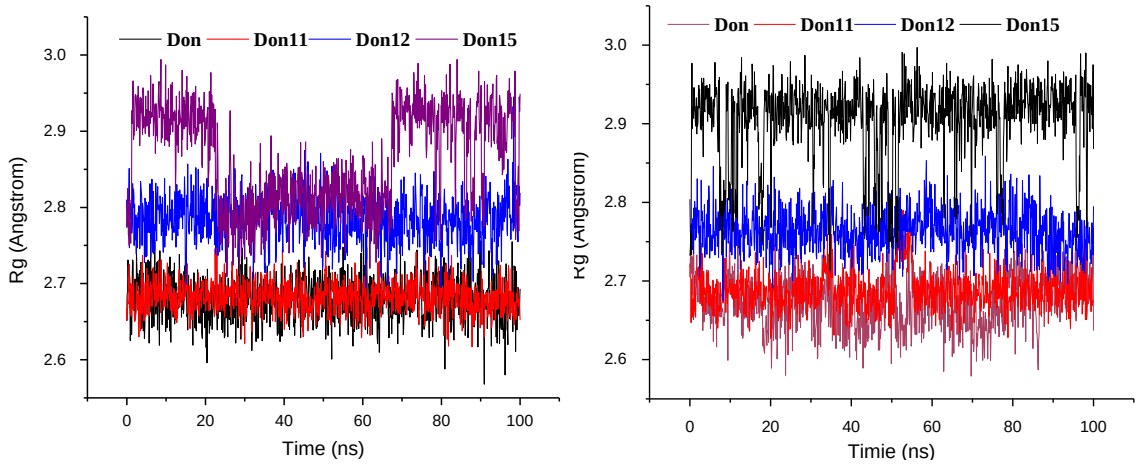


Figure 11. The Rg plots of Aβ-Don, Aβ-Don11, Aβ-Don12, Aβ-Don15 complexes as a function of simulation time

Conclusion

Conclusion

Studying the effectiveness of therapeutic compounds in Alzheimer's disease (AD) treatment is a crucial step toward developing more efficient strategies against this complex and progressive neurodegenerative disorder. Alzheimer's disease is the most common form of dementia, primarily affecting the elderly population, and is characterized by memory loss, cognitive decline, and behavioral changes. The pathological hallmarks of AD include the accumulation of amyloid-beta (Aβ) plaques and the loss of cholinergic neurons, particularly associated with reduced levels of the neurotransmitter acetylcholine. One of the key therapeutic targets in AD is acetylcholinesterase (AChE), an enzyme responsible for the degradation of acetylcholine in the synaptic cleft. Inhibiting AChE helps to increase acetylcholine levels in the brain, thereby improving cognitive function. Donepezil, a widely approved AChE inhibitor, is frequently used for the symptomatic treatment of mild to moderate AD. In addition to AChE, recent studies have explored the interaction of Donepezil and related compounds with amyloid-beta peptides, which are directly implicated in neurotoxicity and plaque formation in AD. In this study, a series of Donepezil analogs (Don11, Don12, Don15) were computationally designed and evaluated using in silico molecular docking and molecular dynamics simulations to assess their potential efficacy and binding behavior with both AChE and Aβ. The docking results indicated strong binding affinities of these analogs to the active sites of both target proteins, suggesting potential dual-modulatory effects. Molecular dynamics (MD) simulations were further performed to investigate the structural stability and interaction persistence of the protein-ligand complexes under simulated physiological conditions. The Root Mean Square Deviation (RMSD) and other trajectory analyses confirmed that some analogs, particularly Don11 and Don12, formed stable complexes with AChE and Aβ, indicating their promise as multitarget therapeutic candidates. In conclusion, this study highlights the potential of novel Donepezil derivatives as effective agents for Alzheimer’s disease through dual inhibition of AChE and modulation of Aβ aggregation. The integrative use of computational modeling, docking, and MD simulations offers valuable insights into the design of next-generation anti-Alzheimer’s drugs that could improve efficacy while minimizing side effects, thus contributing to the advancement of personalized neurodegenerative therapies.

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