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Defended on: ***22/06/2025.***

<u>In front of the jury</u>:

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Promotion: 2024-2025.

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Département de Génie des procédés

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MEMOIRE DE FIN D'ETUDE

Pour l'obtention du diplôme Master

En **Génie des procédés**

***Option :* Génie Pharmaceutique**

Présenté par : ***HADJ MOUSSA Warda Fazya***

Identification de nouveaux médicaments destinés aux malades atteints d'Alzheimer

Soutenu le : ***22/06/2025***

Devant le jury :

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« Identification of new drugs destined for Alzheimer's patients »

Abstract

The inhibition of acetylcholinesterase (AChE) continues to be a key therapeutic approach in Alzheimer's disease treatment. This study employs advanced computational methods to evaluate the AChE-inhibiting potential of hybrid derivatives combining donepezil and tacrine structures. Using robust 3D-QSAR modeling ($R^2 = 0.82$, $Q^2 = 0.738$), the analysis revealed significant structure-activity relationships (SAR), with steric factors playing a major role (46.05%) in influencing inhibitory potency. These findings facilitated the rational design of new derivatives, using a high-activity reference compound for structural refinement based on contour map analysis.

Molecular docking studies identified **compound D1** as the most promising candidate, demonstrating strong interactions with key AChE residues (TYR72, ASP74, and TRP286) and an excellent docking score of -11.70 kcal/mol. Further validation through MM-GBSA calculations confirmed its high binding affinity ($\Delta G \approx -52.43$ kcal/mol) and complex stability. In silico ADME/Tox assessments indicated favorable oral bioavailability, adherence to Lipinski and Veber drug-likeness rules, and minimal toxicity risks.

Density functional theory (DFT) analysis (B3LYP/6-311G) revealed a well-balanced HOMO-LUMO energy gap (3.85 eV), suggesting electronic stability and optimal reactivity. Molecular electrostatic potential (MEP) maps and density-of-states analyses further clarified charge distribution and orbital contributions relevant to target binding.

Overall, this study highlights the donepezil-tacrine hybrid scaffold as a versatile pharmacophore for AChE inhibition and identifies **D1** as a promising anti-Alzheimer's lead compound worthy of further experimental investigation.

Keywords: Alzheimer's disorder, Donepezil-tacrine hybrids, AChE inhibition, 3D-QSAR, molecular docking, MM-GBSA, ADME/Tox, DFT, electronic properties.

« Identification de nouveaux médicaments destinés aux malades atteints d'Alzheimer »

Résumé

L'inhibition de l'acétylcholinestérase (AChE) reste une approche thérapeutique clé dans le traitement de la maladie d'Alzheimer. Cette étude utilise des méthodes de calcul avancées pour évaluer le potentiel d'inhibition de l'AChE de dérivés hybrides combinant les structures

du donépézil et de la tacrine. En utilisant une modélisation 3D-QSAR robuste (R^2 = 0,82, Q^2 = 0,738), l'analyse a révélé des relations structure-activité (SAR) significatives, les facteurs stériques jouant un rôle majeur (46,05%) dans l'influence de la puissance inhibitrice. Ces résultats ont facilité la conception rationnelle de nouveaux dérivés, en utilisant un composé de référence à forte activité pour l'affinement structurel basé sur l'analyse des cartes de contours.

Les études de docking moléculaire ont identifié le composé D1 comme le candidat le plus prometteur, démontrant de fortes interactions avec les résidus clés de l'AChE (TYR72, ASP74 et TRP286) et un excellent score de docking de -11,70 kcal/mol. Une validation supplémentaire par des calculs MM-GBSA a confirmé son affinité de liaison élevée ($\Delta G \approx$ -52,43 kcal/mol) et la stabilité du complexe. Les évaluations ADME/Tox in silico ont indiqué une biodisponibilité orale favorable, le respect des règles de Lipinski et Veber en matière de ressemblance avec les médicaments et des risques de toxicité minimes.

L'analyse de la théorie de la fonctionnelle de la densité (DFT) (B3LYP/6-311G) a révélé un écart énergétique HOMO-LUMO bien équilibré (3,85 eV), ce qui suggère une stabilité électronique et une réactivité optimale. Les cartes du potentiel électrostatique moléculaire (MEP) et les analyses de la densité d'états ont permis de clarifier davantage la distribution des charges et les contributions orbitales pertinentes pour la liaison à la cible.

Dans l'ensemble, cette étude met en évidence l'échafaudage hybride donépézil-tacrine en tant que pharmacophore polyvalent pour l'inhibition de l'AChE et identifie D1 comme un composé phare anti-Alzheimer prometteur qui mérite des recherches expérimentales plus approfondies.

Mots-clés : Maladie d'Alzheimer, Hybrides donepezil-tacrine, Inhibition de l'AChE, 3D-QSAR, Docking moléculaire, MM-GBSA, ADME/Tox, DFT, Propriétés électroniques.

«تحديد أدوية جديدة مُخصصة لمرضى الزهايمر»

ملخص

يُعد تثبيط إنزيم الأستيل كولين إستراز (AChE) نهجًا علاجيًا محوريًا مستمرًا في علاج مرض الزهايمر. توظف هذه الدراسة طرقًا حسابية متقدمة لتقييم القدرة التثبيطية لمشتقات هجينة تجمع بين بنى دوائيتي دونيبيزيل وتاكرين. ومن خلال نمذجة3D-QSAR قوية (Q^2 = 0.738, R^2 = 0.82) ، كشفت التحليلات عن علاقات هامة بين البنية والنشاط(SAR) ، حيث لعبت العوامل الفراغية دورًا رئيسيًا بنسبة 46.05% في التأثير على قوة التثبيط.

مكّنت هذه النتائج من التصميم العقلاني لمشتقات جديدة، باستخدام مركب مرجعي عالي النشاط لتوجيه تحسين البنية استنادًا إلى تحليل خرائط الكنتور.

حددت دراسات الالتحام الجزيئي المركب **D1**كأكثر المرشحين الواعدين، إذ أظهر تفاعلات قوية مع الاحماض الامينية لإنزيم AChE الرئيسية (TYR72,ASP74, TRP286) وحقق درجة ارتباط ممتازة بلغت .**-11.70 kcal/mol** وقد تم تأكيد تقارب المركب واستقرارية معقده عبر حساباتMM-GBSA ، حيث بلغت طاقة الربط الحرة

($\Delta G \approx -52.43$ kcal/mol) ، مما يدل على تقارب وثبات عالٍ.

أظهرت التقييمات الحاسوبية لخصائص الامتصاص والتوزيع والتمثيل والإخراج والسمية (ADME/Tox) أن المركب يتمتع بتوافر حيوي فموي جيد، ويلتزم بقواعد "ليبينسكي" و"فيبر" للتشابه الدوائي، مع مستويات منخفضة من السمية المتوقعة.

أظهر تحليل نظرية دالة الكثافة (DFT) باستخدام المنهجية B3LYP/6-311G فجوة طاقة متوازنة بين المدار الأعلى المشغول (HOMO) والمدار الأدنى غير المشغول (LUMO) بمقدار 3.85 إلكترون فولت، مما يشير إلى استقرار إلكتروني وتفاعل مثالي.

كما أوضحت خرائط الجهد الكهروستاتيكي (MEP) وتحليلات كثافة الحالات الإلكترونية توزيع الشحنات والمساهمات المدارية ذات العلاقة بالارتباط مع الهدف البروتيني.

بشكل عام، تسلط هذه الدراسة الضوء على الهيكل الهجين لدونيبيزيل-تاكرين بوصفه فلكة دوائية متعددة الاستخدامات لتثبيطAChE ، وتُرشح المركب **D1**كقائد دوائي واعد ضد الزهايمر يستحق المزيد من الدراسة والتطوير التجريبي.

الكلمات المفتاحية:

مرض الزهايمر، مشتقات دونيبيزيل-تاكرين، تثبيطAChE ، 3D-QSAR، الالتحام الجزيئي، MM-GBSA، ADME/Tox، DFT، الخصائص الإلكترونية.

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Dedicate

To my dear parents

To my cherished siblings

To everyone I have loved

And to all who have supported me on this journey.

Last but not least

'In life, nothing is to be feared, everything is to be understood.'

Marie Curie

Abbreviation list

Aβ	Amyloid beta
ACh	Acetylcholine
AChE	Acetylcholinesterase
ACE	Angiotensin-Converting Enzyme
AD	Alzheimer's Disease/Disorder
ADME-Tox	Absorption, Distribution, Metabolism, Excretion – Toxicity
AICD	Amyloid Intracellular Domain
ALS	Amyotrophic Lateral Sclerosis
ANN	Artificial Neural Networks
APP	Amyloid Precursor Protein
BBB	Blood Brain Barrier
BuChE	Butyrylcholinesterase
CNS	Central Nervous System
CoMFA	Comparative Molecular Field Analysis
DFT	Density Functional Theory
GSK-3β	Glycogen Synthase Kinase-3 Beta
hAChE	Human Acetylcholinesterase
HOMO	Highest Occupied Molecular Orbital
IC_{50}	Median Inhibitory Concentration
LUMO	Lowest Unoccupied Molecular Orbital
MLR	Multiple Linear Regression
MM-GBSA	Molecular Mechanics - Generalized Born Surface Area
NDs	Neurodegenerative Diseases/Disorders
NFD	Neurofibrillary Degeneration

NMDA	N-methyl-D-aspartate
PD	Parkinson's Disease/Disorder
pIC_{50}	9-Log (IC_{50})
PLS	Partial Least Squares
QSAR/QSPR	Quantitative Structure-Activity/Property Relationships
SAR	Structure-Activity Relationship(s)
sAPP	Soluble Amyloid Precursor Protein
SP	Senile Plaque
SVM	Support Vector Machines

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General introduction

Memory and learning are essential cognitive functions that shape human behavior, allowing individuals to adapt to their environment, draw from past experiences, and anticipate future outcomes. However, these abilities can decline due to aging, traumatic brain injuries, psychiatric disorders (such as addiction, anxiety, depression, and schizophrenia), or chronic neurological conditions, most notably neurodegenerative diseases like Alzheimer's disease (AD) [1].

Alzheimer's disease represents a growing global health crisis with profound medical, social, and economic consequences. Recent estimates from the 2022 World Alzheimer Report indicate that more than 55 million people currently live with AD or related dementias—a number projected to nearly triple to 138 million by 2050 [2]. The progressive nature of the disease severely impacts patients' independence, often depriving them of a fulfilling later life [3].

To date, no cure exists for AD. Available treatments focus on symptom management and fall into two main categories: acetylcholinesterase (AChE) inhibitors and NMDA receptor antagonists [4]. Among the first-generation AChE inhibitors, tacrine was discontinued due to safety concerns, while donepezil emerged as a preferred option due to its enhanced selectivity and tolerability. Galantamine, another key drug in this class, not only inhibits AChE but also enhances cholinergic signaling by modulating nicotinic receptors [5].

Developing new AD therapies through traditional drug discovery is an expensive and lengthy process, often taking over a decade and billions of dollars, with high failure rates in late-stage trials. To overcome these challenges, computational approaches have become indispensable in early drug discovery. *In silico* methods enable rapid screening of large compound libraries, prediction of ADME/Tox properties, and optimization of lead molecules before synthesis [6]. These techniques significantly reduce costs and improve success rates by prioritizing compounds with favorable pharmacokinetics and minimal toxicity risks [7].

This investigation pursues three principal scientific aims:

- ✓ To systematically evaluate the structure-activity relationships governing molecular recognition through:
    - ❖ Comprehensive analysis of ligand-enzyme interactions

- ❖ Quantitative assessment of inhibitory potential
- ❖ Determination of key pharmacophoric features

- ✓ To rationally design and computationally characterize novel small-molecule inhibitors targeting acetylcholinesterase (AChE) with optimized binding affinities.
- ✓ To establish the drug development potential of lead compounds by:
  - ❖ Computational prediction of pharmacokinetic properties
  - ❖ Assessment of bioavailability parameters
  - ❖ Evaluation of toxicity profiles

This tripartite approach integrates molecular design, biological evaluation, and pharmaceutical profiling to advance therapeutic candidates for Alzheimer's disease treatment.

The dissertation adopts a systematic three-chapter organization designed to progressively develop and substantiate its scientific inquiry

- ✓ ***Chapter 1*** establishes the theoretical framework, providing: reviews neurodegenerative diseases, emphasizing AD pathology, and introduces tacrine-donepezil hybrid molecules as a therapeutic strategy.
- ✓ ***Chapter 2*** presents the computational methodology, encompassing: outlines the *in silico* methodology, including 3D-QSAR modeling and molecular docking studies.
- ✓ ***Chapter 3*** delivers the research outcomes through: the design of new hybrid compounds, supported by 3D-QSAR predictions, docking analyses, DFT-based electronic structure assessments, and ADME-Tox profiling.

The work concludes with a synthesis of key findings and their therapeutic implications, followed by proposed directions for future investigation.

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Chapter 01: Neurodegenerative diseases.

I. Introduction

The term ***neurodegeneration*** refers to neuronal loss marked by progressive neuronal cell death, accompanied by cognitive and motor dysfunctions depending on the brain area affected [1].

Neurodegenerative diseases (NDs) are serious health conditions that impact the brain and nervous system, making them among the most debilitating disorders affecting humans. In recent years, they have become the fourth leading cause of death globally, following heart disease, cancer, and stroke [2]. 50 million people worldwide are suffering from neurodegenerative diseases, and this number is projected to rise to 130 million by 2050 [3].

NDs are a vast and varied group of disorders that are classified as being primarily caused by the progressive loss of function or structural integrity of neurons and associated cell types in the nervous system. The pathogenesis of these disorders is still unclear, but researchers propose a complex interplay of genetic, epigenetic, and environmental factors. Until now, there have been no effective therapies developed to slow, halt, or prevent any NDs [4].

II. Different majors of neurodegenerative diseases

Among the most recognized neurodegenerative diseases are:

II.1 Parkinson's disease

Parkinson's disease (PD) was first described in 1817 by ***Dr. James Parkinson***, a British physician, who documented six cases of a disorder he referred to as the "shaking palsy" [5].

PD is a progressive neurodegenerative disorder that affects both movement and other body functions [6]. It causes slowness of movement, muscle stiffness, tremors, and walking difficulties (Figure 1) due to the loss of dopamine-producing neurons in the brain [7]. A key feature of PD is the buildup of abnormal protein clumps, called Lewy bodies, in nerve cells [8].

Besides movement problems, PD also leads to memory issues, mood changes, sleep problems, and difficulties with automatic body functions like blood pressure control [9]. There is no cure, but treatments such as medications, physical therapy, and deep brain stimulation can help manage symptoms [10].

Figure 1: Parkinson's disease symptoms [11]

II.2 Amyotrophic Lateral Sclerosis

Amyotrophic lateral sclerosis (ALS) was initially recognized as a motor neuron disorder in 1869 [12]. It is a fatal ND characterized by the progressive degeneration of upper and lower motor neurons, leading to muscle weakness, paralysis, and ultimately, respiratory failure [13].

Most people with ALS first notice weakness in their arms or legs (75% of cases), while others start with trouble speaking or swallowing [14]. The disease is linked to the abnormal buildup of a protein called TDP-43 in nerve cells, though some genetic forms involve other proteins like SOD1 and FUS [15].

II.3 Huntington's disease

In his paper ***"On Chorea."***, *Dr. George Huntington*, an American physician, has described Huntington's disease (HD), in 1872.

HD is a progressive ND that affects movement, cognition, and behaviour, eventually leading to severe disability and premature death [16]. It is an autosomal dominant genetic disorder caused by an expanded CAG trinucleotide repeat in the HTT gene, which results in the

production of mutant huntingtin protein (mHTT) [17]. This abnormal protein leads to the progressive loss of neurons, particularly in the caudate nucleus and putamen, regions of the brain essential for motor control.

HD symptoms (Figure 2) typically appear in mid-life but can manifest at any age. The disease begins with subtle changes in coordination, personality, and cognition, followed by the onset of chorea, characterized by involuntary, dance-like movements [18]. As HD progresses, patients experience rigidity, bradykinesia, cognitive decline, psychiatric disturbances, and difficulty swallowing [19].

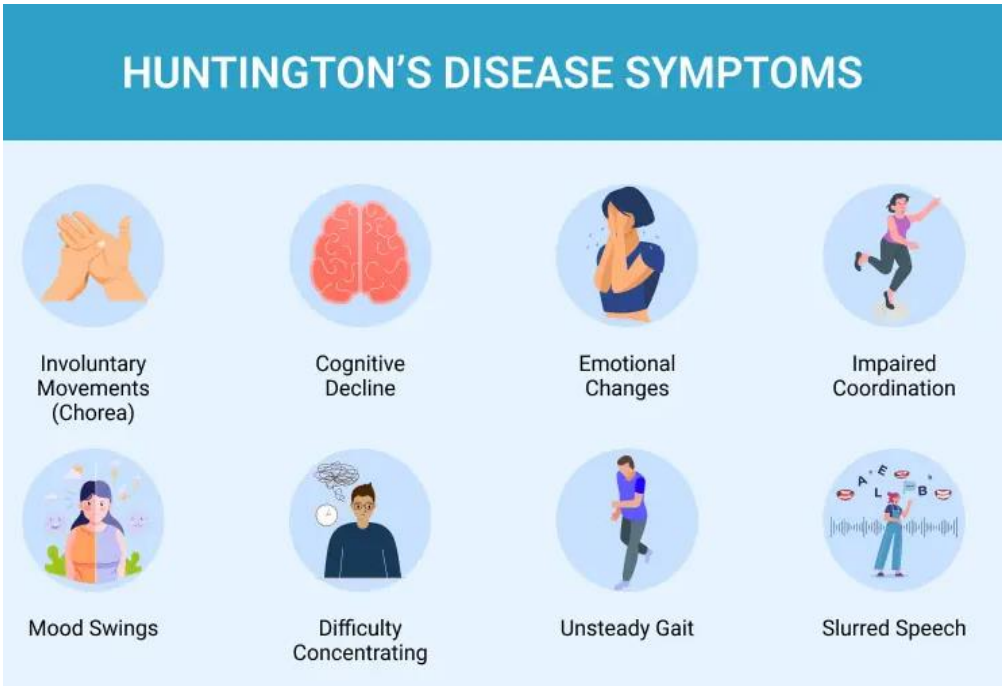


Figure 2: Huntington's disease symptoms [20].

II.4 Alzheimer's disease

In 1906, the German doctor Alois Alzheimer discovered a new disease, later named "Alzheimer's disease (AD)" [21].

AD is a ND characterized by a gradual decline in cognitive functions, leading to loss of independence. This deterioration is primarily associated with two types of brain lesions: neurofibrillary tangles, which develop inside neurons, and senile plaques, which accumulate outside nerve cells [22].

III. Alzheimer's disorder

III.1 Overview

Identified in 1906 by Alois Alzheimer (Figure 3), the disease was first described after the study of Auguste Deter, a patient suffering from severe cognitive impairment. Her autopsy revealed two characteristic lesions: amyloid plaques and neurofibrillary tangles. It was not until 1910 that Emil Kraepelin officially named the disease in honour of his colleague, Alzheimer.

It was only in the 1980s that the nature of these lesions was clarified: amyloid-β (Aβ) was identified as the main component of senile plaques (SP), and tau protein was recognized as responsible for neurofibrillary tangles. Further discoveries followed, including the association of the disease with hippocampal atrophy, cholinergic neuron loss, and synapse degeneration.

The 20th century thus marked major scientific advances that progressively improved the understanding of this disease's mechanisms [23].

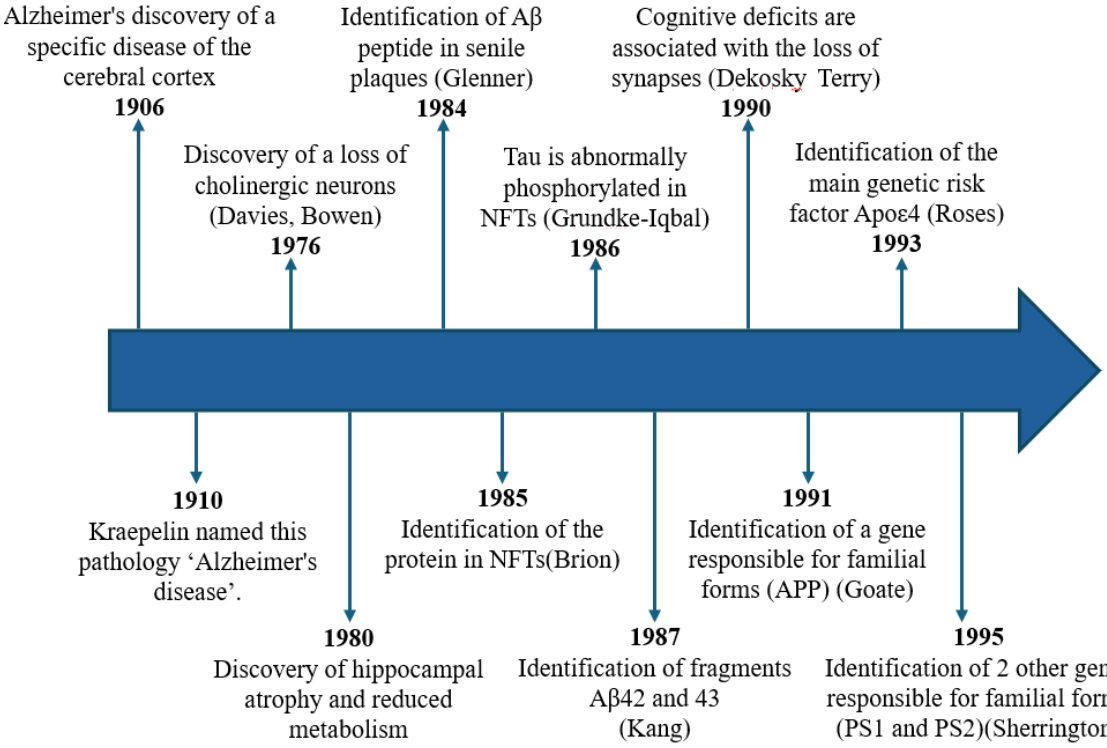


Figure 3: Main discoveries related to AD in the 20th century (non-exhaustive list) [23].

III.2 Symptoms of AD

AD is marked by cognitive decline, with memory loss being the primary symptom (Figure 4). Early signs include mild difficulty recalling recent events, which gradually extends to older

memories. Patients often experience disorientation, language impairments (aphasia), difficulty performing everyday tasks (apraxia), trouble recognising familiar people or objects (agnosia), and a lack of motivation or emotional response (apathy). Mood disturbances, anxiety, and aggression may also arise.

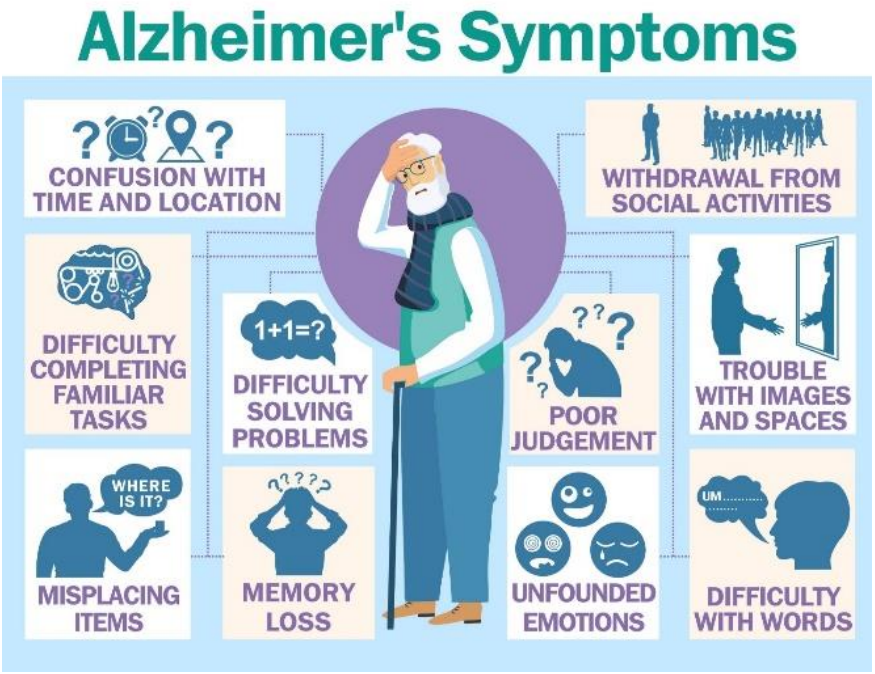


Figure 4: AD's symptoms [24].

AD progresses through three stages:

a. **Asymptomatic Phase (10–25 years before diagnosis):** Brain damage occurs without noticeable symptoms due to neural compensation.

b. **Mild Cognitive Impairment Phase (3–5 years before diagnosis):** Cognitive decline becomes evident but does not yet significantly affect daily life.

c. **Dementia Phase:** Symptoms worsen, leading to severe memory loss, behavioural changes, complete loss of independence, and full reliance on caregivers [25].

III.3 Neuropathological characteristics

Before the technological and scientific advancements of the 1980s, AD could only be definitively recognised after death. This diagnosis relied on detecting pronounced brain atrophy and microscopic features such as SP and neurofibrillary tangles during an autopsy [26].

III.3.1 Macroscopic lesions

During an autopsy, one of the first noticeable signs of AD is brain atrophy (Figure 5), indicating significant neuron loss.

However, this shrinkage is not uniform. It primarily affects the hippocampus, which is linked to cognitive decline, as well as the cerebral cortex, including the entorhinal cortex and amygdala. As the cortex shrinks, the brain's ventricles expand. Some of these changes also happen in other diseases. For example, hippocampal atrophy can occur in Parkinson's disease and vascular dementia [27].

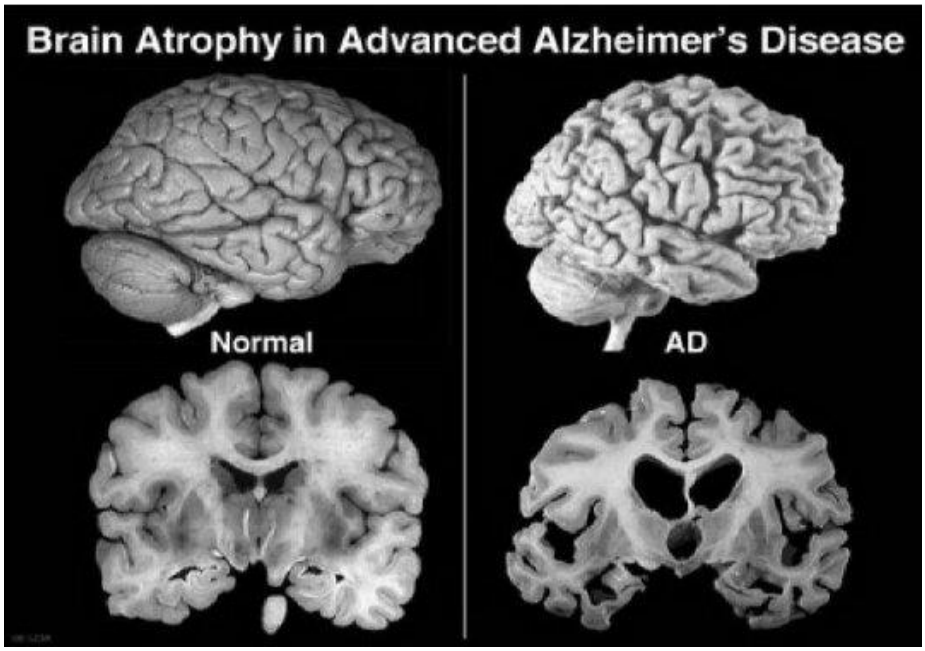


Figure 5: Brain Atrophy in Advanced AD [28].

III.3.2 Microscopic Features

III.3.2.1 Neurofibrillary degeneration

Neurofibrillary degeneration (NFD) occurs due to the intracellular aggregation of abnormally phosphorylated tau proteins. Under normal conditions, tau proteins play a crucial role in stabilizing microtubules, essential components of the neuronal cytoskeleton, particularly within axons. Their function is regulated by their phosphorylation state. In the provided image, NFD is indicated by a black arrow, while the adjacent oval structure represents a SP, identified using Bielschowsky silver staining [29].

The progression of NFD follows a characteristic spatial and temporal pattern that aligns with the clinical course of AD. It initially appears in the transentorhinal cortex, then spreads to the

hippocampus, temporal cortex, and progressively to polymodal and unimodal cortical areas, ultimately affecting the entire brain in the later stages of the disease [30].

III.3.2.2 Amyloid Plaques

Amyloid plaques develop as a result of the buildup of Aβ peptide, which is generated from the cleavage of the amyloid precursor protein (APP) (Figure 6). APP, a transmembrane glycoprotein, can be processed through two distinct pathways.

In the non-amyloidogenic pathway, APP is initially cleaved by α-secretase and then by γ-secretase, yielding non-toxic fragments such as P3 peptide, a soluble region (sAPP) and an amyloid intracellular domain (AICD). The γ-secretase complex, crucial for this process, is composed of presenilin, nicastrin, and two co-factors, with presenilin being vital for maintaining its stability.

In contrast, the amyloidogenic pathway involves the cleavage of APP by β-secretase (BACE1), producing a C99 fragment. This fragment is subsequently processed by γ-secretase, leading to the creation of the Aβ peptide. When Aβ accumulates in the extracellular space, it forms oligomers that eventually clump together into amyloid plaques, a key characteristic of AD [31].

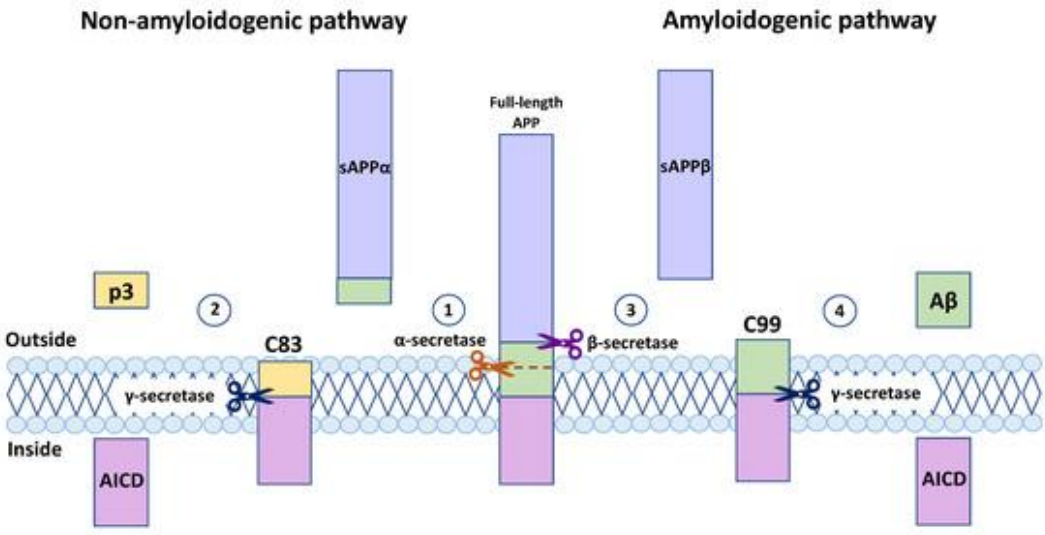


Figure 6: Amyloid precursor protein processing pathways [32].

III.3.3 Neuron and synapse loss

In AD, the accumulation of Aβ plaques triggers neuronal death in the nucleus basalis of Meynert, which decreases the production and release of acetylcholine (ACh). This process also elevates acetylcholinesterase (AChE) activity, disrupts muscarinic ACh signalling, and weakens cholinergic function. These disruptions indirectly impact N-methyl-D-aspartate

(NMDA) receptors, leading to excessive glutamate activity and contributing to further neuronal damage.

As plaques build up in regions such as the basal ganglia, temporal lobe, and neocortex, they impair memory and executive functions. Furthermore, the loss of neurons in the medial cortex and locus coeruleus reduces serotonin and norepinephrine levels. This decline in neurotransmitter activity results in symptoms like dysphoria and insomnia, stemming from altered serotonergic and adrenergic signalling in the brain [33].

III.4 Role of Glycogen Synthase Kinase-3β in AD

Glycogen Synthase Kinase-3 beta (GSK-3β) plays a crucial role in the progression of AD through multiple pathological mechanisms. One of its primary functions is the abnormal hyperphosphorylation of the tau protein, which contributes to the formation of neurofibrillary tangles, a key hallmark of the disease. GSK-3β participates in the amyloidogenic pathway by influencing the production of Aβ peptides through its regulation of presenilin 1 (PS1) and beta-secretase (BACE-1), enzymes critical for amyloid precursor protein (APP) cleavage. This dysregulation exacerbates Aβ accumulation, further promoting neurotoxicity. GSK-3β contributes to neuroinflammation by enhancing the release of pro-inflammatory cytokines such as IL-1, IL-6, and TNF-α, thereby amplifying neuronal damage. Its role extends to synaptic dysfunction, as it regulates key transcription factors involved in synaptic plasticity, memory formation, and cognitive functions. GSK-3β activity is also linked to neuronal degeneration due to its involvement in NMDA receptor hyperactivation, which leads to excessive calcium influx and neuronal death. Collectively, these findings underscore the significant role of GSK-3β in the neurodegenerative processes associated with AD [34].

III.5 Role of Butyrylcholinesterase in AD

Butyrylcholinesterase (BuChE), also referred to as pseudocholinesterase or non-specific cholinesterase, is a serine hydrolase responsible for hydrolysing choline esters. The presence of BuChE was first recognized in 1932 when an enzyme capable of catalysing the hydrolysis of choline esters was identified. This enzyme is widely distributed in the human brain, with high expression in white matter, glial cells, and certain neuronal populations. Its enzymatic activity extends to the hydrolysis of acetylcholine, long-chain acylated polypeptides, various esters, amides, and aromatic amines, as well as the detoxification of highly toxic synthetic organophosphates. While BuChE is associated with multiple physiological and pathological functions, including its involvement in AD, its role in cholinergic neurotransmission and immune modulation is particularly significant due to its ability to hydrolyse acetylcholine.

III.6 Role of AChE in AD

AChE has a significant impact on AD by breaking down Ach (Figure 7), a key neurotransmitter involved in memory and learning. Reduced cholinergic activity is a hallmark of AD and is associated with cognitive decline in patients [35].

AChE inhibitors, such as donepezil, rivastigmine, and galantamine, are commonly used to slow down ACh degradation and temporarily improve synaptic transmission. However, these treatments do not halt disease progression.

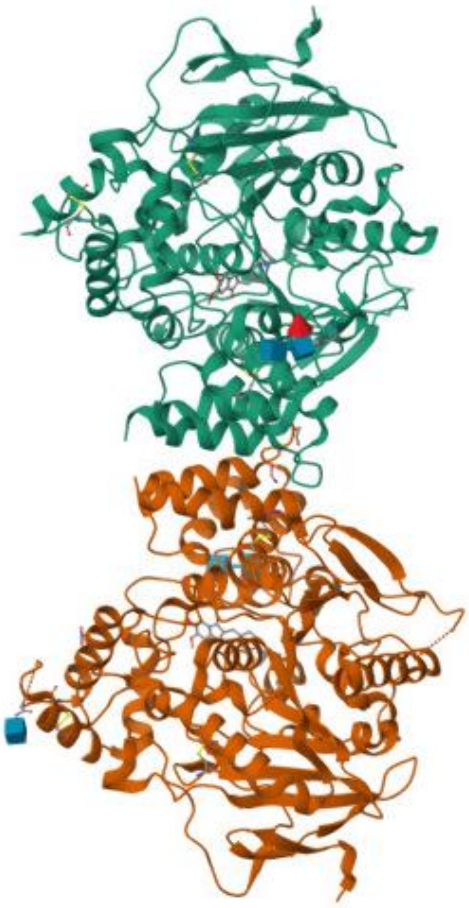


Figure 7: Crystal Structure of Recombinant Human Acetylcholinesterase in Complex with Donepezil (PDB code:4EY7) [36].

Beyond its enzymatic function, AChE also interacts with β-amyloid peptides, promoting their aggregation and the formation of amyloid plaques, which may accelerate neurodegeneration [37]. This dual role of AChE, in both cholinergic transmission and amyloid pathology, makes it a key target for developing new therapeutic strategies.

III.7 Treatment for AD

Current therapeutic approaches for AD primarily aim to slow disease progression and alleviate symptoms rather than provide a cure. Pharmacological treatments include AChE inhibitors (Figure 8), such as donepezil, galantamine, and rivastigmine, which enhance cholinergic neurotransmission, as well as memantine, an NMDA receptor antagonist, which helps regulate glutamate activity to mitigate cognitive decline. In recent years, the Food and Drug Administration (FDA) approval of monoclonal antibodies like aducanumab, which targets Aβ plaques, has sparked both optimism and controversy due to concerns about its clinical efficacy.

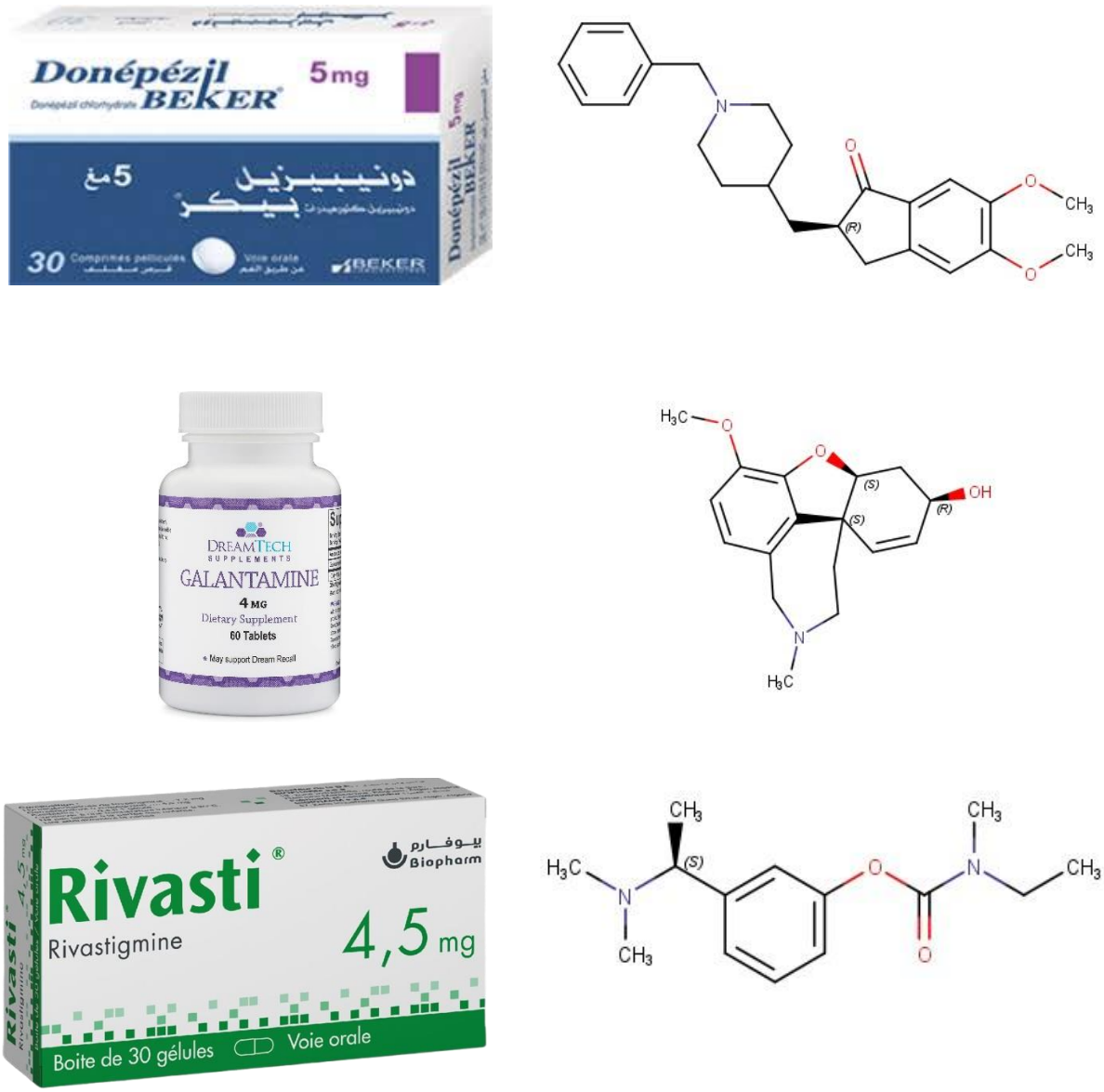


Figure 8: Current treatments used for AD.

Beyond conventional treatments, novel therapeutic strategies seek to modify disease progression by addressing key pathological mechanisms. Aβ-targeting therapies focus on reducing Aβ accumulation through secretase inhibitors, immunotherapies, and strategies that enhance its clearance.

Likewise, tau-targeted interventions are under investigation, given the strong correlation between tau pathology and neurodegeneration [38].

IV. Design of novel Donepezil-Tacrine hybrids as new agents against AD

Ningwei and colleagues [39] recognized that existing treatments for AD typically focus on a single factor, despite the disease's complex and multifactorial nature. To address this limitation, they aimed to design new multifunctional compounds capable of targeting several key pathological mechanisms including oxidative stress and neuroinflammation simultaneously. This approach was expected to enhance therapeutic effectiveness while minimizing potential neurotoxic side effects.

In order to achieve this purpose, Ningwei et al. designed and synthesised a series of novel compounds as possible multifunctional agents.

The molecules A1–A6, B1–B20 and C1–C8 (Figure 9) were synthesised through multi-step reactions involving commercially available reagents and key intermediates with variations in the final steps to introduce different structural features and substituents.

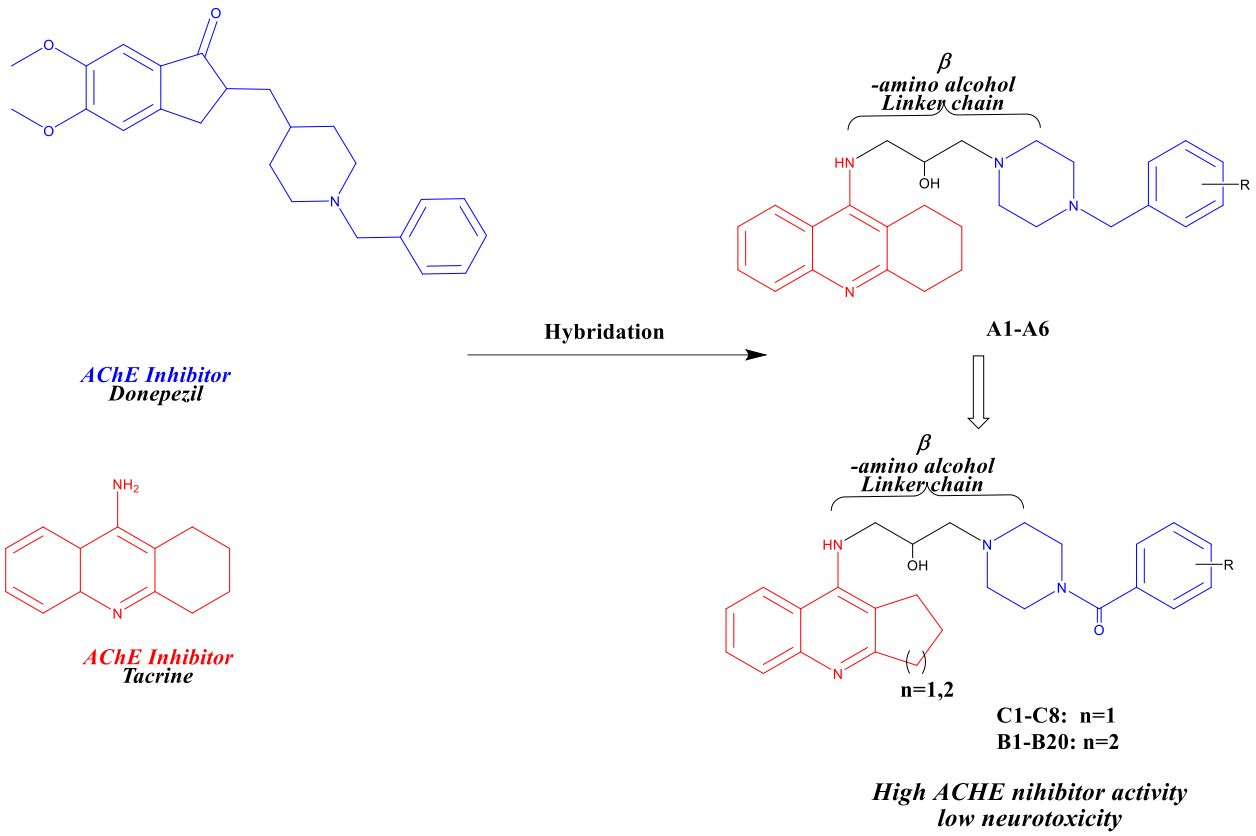
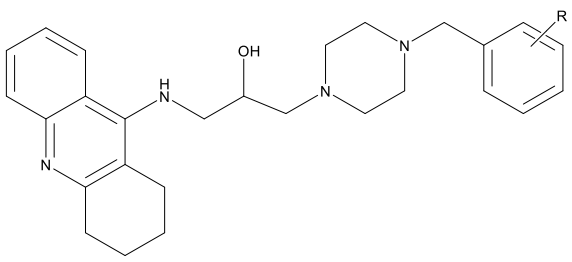


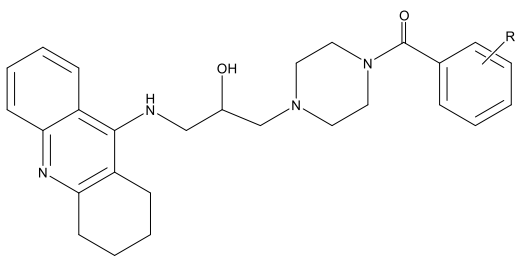
Figure 9: Design strategy of donepezil-tacrine hybrids.

The hybrid compounds synthesised were designed with different R groups to explore the structure-activity relationship (SAR) and enhance their inhibitory potential against Human acetylcholinesterase (hAChE). The choice of substituents such as methyl and halogens was guided by prior research highlighting their effect on biological activity and drug-like properties, and to evaluate this influence, they used Ellman's method to perform biological assays with the resulting inhibitory activities summarized and compared in Table 1.

Table 1: hAChE inhibitory activities of target compounds A1-C8. [39]

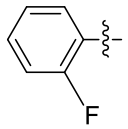
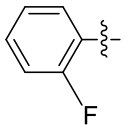
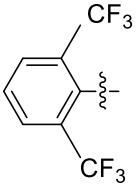
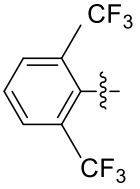
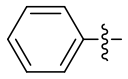
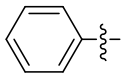
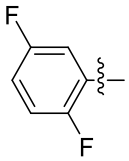
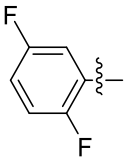
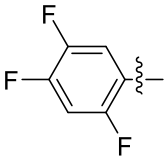
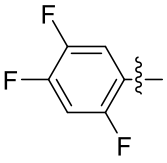
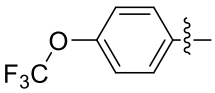
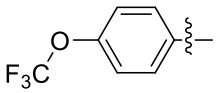
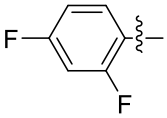
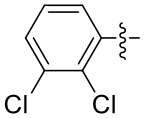


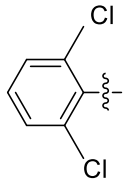
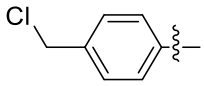
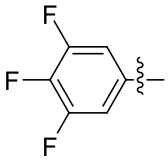
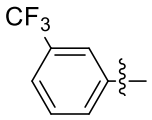
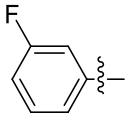
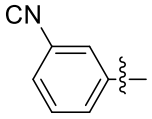
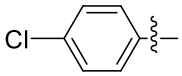
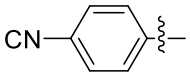
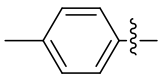
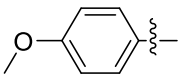
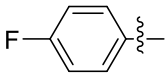
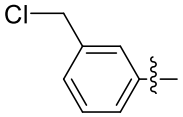
A1 – A6

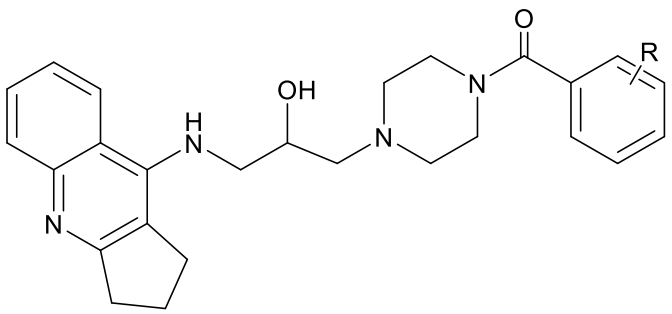


B1 – B20

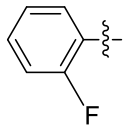
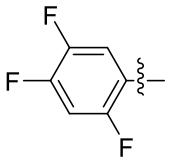
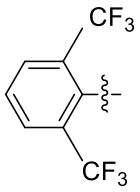
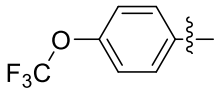
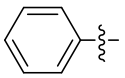
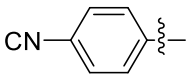
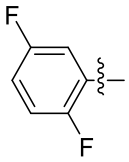
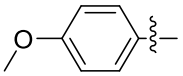
Compound	R	Inhibition@100 nM (%) hAChE	Compound	R	Inhibition@100 nM (%) hAChE

Tacrine	**-**	24.69±4.27	**Donepezil**	**-**	56.14±2.18
A1		23.23 ±0.2	**B1**		29.87 ±0.2
A2		8.03 ±0.1	**B2**		13.86 ±0.1
A3		11.8 ±0.3	**B3**		30.56 ±0.4
A4		13.33 ±0.3	**B4**		46.19 ±0.3
A5		36.40 ±0.2	**B5**		62.65 ±0.3
A6		10.70 ±0.3	**B6**		71.86 ±0.1
B7		58.15 ±0.3	**B14**		55.86 ±0.3

B8		4.07 ±0.1	**B15**		87.76 ±0.2
B9		64.5 ±0.1	**B16**		67.64 ±0.2
B10		18.3 ±0.3	**B17**		70.64 ±0.1
B11		67.73 ±0.2	**B18**		74.11 ±0.2
B12		56.53 ±0.2	**B19**		67.49 ±0.4
B13		70.8 ±0.3	**B20**		81.43 ±0.3



C1-C8

C1		2.85±0.3	**C5**		15.08±0.1
C2		4.91±0.3	**C6**		8.05±0.3
C3		4.67±0.3	**C7**		28.32±0.3
C4		8.01±0.2	**C8**		27.54±0.1

V. Conclusion

Neurodegenerative diseases, particularly Alzheimer's disease (AD), represent a profound global health challenge characterized by progressive neuronal degeneration and cognitive impairment. Despite significant advancements in understanding AD's pathological mechanisms such as amyloid-β plaque accumulation, neurofibrillary tangles, and cholinergic dysfunction current therapies remain palliative, targeting symptoms rather than halting disease progression.

The development of multifunctional compounds, exemplified by donepezil-tacrine hybrids, marks a promising shift toward addressing AD's multifactorial nature. These hybrids aim to simultaneously target key pathological pathways, including cholinergic deficits, oxidative stress, and neuroinflammation, offering potential advantages over single-target therapies. However, translating these innovations into clinically effective treatments necessitates further research to optimize their efficacy, safety, and pharmacokinetic profiles.

Collaborative efforts across disciplines, coupled with innovative therapeutic strategies, are essential to overcome the limitations of current approaches. Future research should prioritize elucidating novel molecular targets and refining drug design methodologies to develop disease-modifying therapies capable of altering AD's trajectory. Addressing these challenges will be critical to improving outcomes for the growing population affected by neurodegenerative disorders.

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Chapter 02: Methodology & Theoretical aspects

I. Introduction

Drug design, also called rational drug design, is the process of developing new drugs. It involves three main steps: identifying a biological target, understanding its structure and function, and then designing a drug molecule that interacts with the target in a beneficial way. Several factors are considered, such as the specificity of the target, the possibility of targeting a single target or several targets, and the minimum affinity required for binding to the target. Polypharmacology, i.e. the ability of a drug to bind to several targets, can improve the efficacy of the drug, but it can also lead to adverse effects. Drug design is a complex process that aims to find new treatments for specific diseases by interacting in a targeted way with biological targets [1].

Before initiating the drug design phase, various strategies of drug discovery are applied to identify potential candidate molecules (figure 1). One such strategy is discovery by chance, illustrated by the case of penicillin, whose antibiotic properties were first observed in vitro by Alexander Fleming in 1928 and later confirmed in vivo by Chain and Florey in 1940. Another approach is discovery based on empirical data, as exemplified by aspirin, derived from compounds found in willow leaves that were traditionally used for pain relief by ancient civilisations such as the Sumerians [2].

A more targeted method involves the discovery based on the knowledge of a physiological process or molecular target. For instance, understanding the role of the angiotensin-converting enzyme (ACE) in the renin-angiotensin system led to the development of ACE inhibitors. Researchers also rely on discovery from existing molecules, guided by structure–activity relationships (SAR), which explore how changes in a molecule's structure affect its biological activity. This approach also includes the concept of prodrugs, which are inactive compounds that must be metabolised in the body to release the active drug [3].

Screening and selection processes are used to evaluate large libraries of chemical compounds, even when their pharmacological properties are initially unknown. This high-throughput strategy (HTS) allows researchers to identify novel bioactive molecules that can then be further optimised through rational drug design.

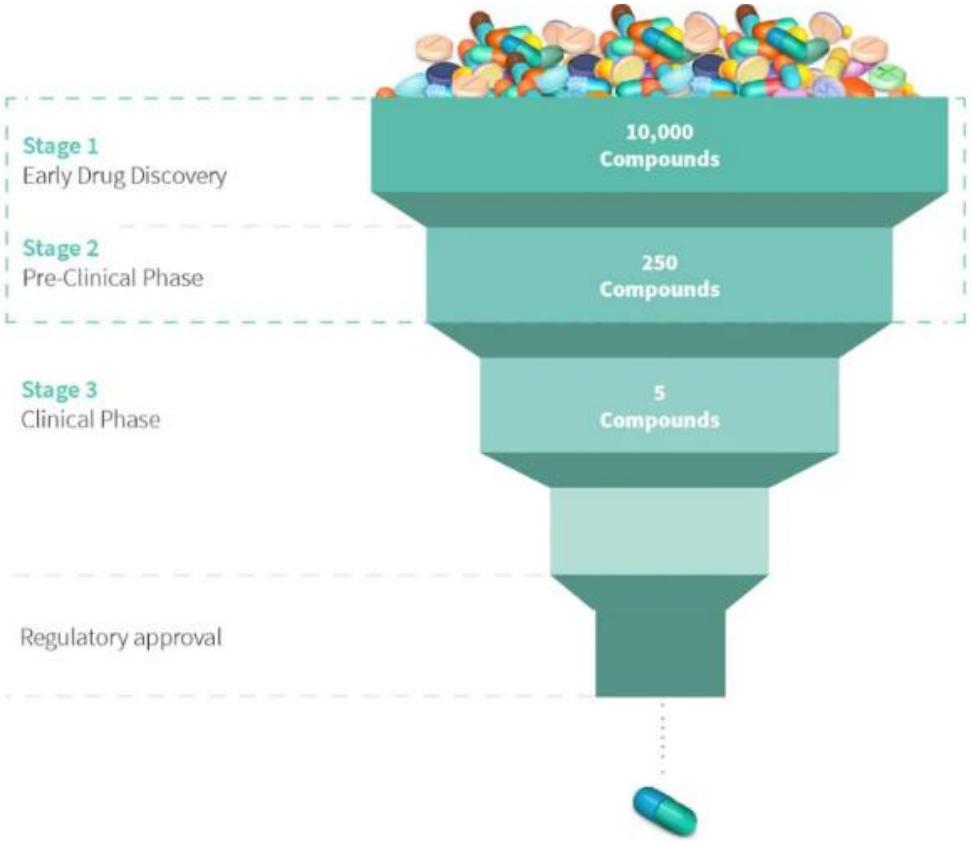

Figure 1: Stages of the Drug Discovery Process [4].

II. Quantitative structure-activity relationships

II.1 History

Quantitative Structure-Activity/Property Relationships (QSAR/QSPR) have gained significant importance in various sectors such as pharmaceuticals, chemicals, and cosmetics, particularly due to advances in computational resources. These methods are primarily applied to the rational design of new compounds and chemical entities [5]. While the term QSAR was formally introduced in the 1960s through the work of Corwin Hansch and Free & Wilson, who linked chemical structure to biological activity through mathematical models, the foundational concepts date back much earlier.

In the late 19th century, Alexander Crum-Brown and Thomas Fraser first explored the connection between molecular structure and physiological activity. Similarly, Richet identified the relationship between toxicity and physicochemical properties, while Meyer and Overton demonstrated a linear correlation between lipophilicity and biological activity in certain compounds. These early findings laid the groundwork for the development of QSAR as a formal scientific approach.

In recent years, the field of QSAR has advanced with the availability of extensive data and the development of powerful computational tools. Modern QSAR models play a crucial role in drug discovery by helping to prioritize molecules for testing, thereby reducing both time and costs in the development process. These models are also used to screen large chemical libraries and predict biological activity, toxicity and receptor interactions, making them a key component of contemporary drug development [6].

II.2 Definition

QSAR is a computational methodology that models how molecular structure influences biological activity and physicochemical properties through mathematical relationships [7]. This approach is based on the fundamental principle that a compound's biological effects are determined by its structural characteristics, enabling the prediction of various endpoints including pharmacological activity, toxicity and receptor binding affinity [8].

QSAR models employ statistical techniques to correlate molecular descriptors (quantitative representations of structural features) with experimental measurements of biological activity or chemical properties [9]. These models can be developed for specific chemical classes or more broadly applied to diverse compound sets. The methodology has become particularly valuable in pharmaceutical research and chemical safety assessment, where it helps predict compound behaviour while potentially reducing experimental testing requirements [10].

The modelling process establishes quantitative relationships between structural parameters and biological endpoints, with the goal of identifying key molecular features that influence activity [11] . By systematically analysing these structure-activity correlations, QSAR provides a powerful approach for compound optimization, hazard assessment and rational molecular design [12].

II.3 QSAR methodology

QSAR methodology (figure 2) is built on the idea that a compound’s biological activity can be explained by the combined influence of its structural or physicochemical characteristics. Traditional QSAR techniques, such as Free Wilson analysis, involve assigning values based on the presence or absence of specific chemical groups and linking these to biological responses. Hansch analysis, on the other hand, focuses on numerical descriptors like lipophilicity, electronic effects, and steric properties to model how compounds behave in biological systems. These classical methods usually apply linear regression to identify meaningful correlations. In more advanced approaches like 3D QSAR, specifically

Comparative Molecular Field Analysis (CoMFA), the 3D shapes of molecules are aligned, and their interaction energies with a hypothetical probe are measured across a spatial grid. These interaction patterns are then analysed using statistical techniques like partial least squares to create predictive models. Altogether, these QSAR methods help researchers understand how molecular features influence biological activity and support the design of more effective drug candidates [13].

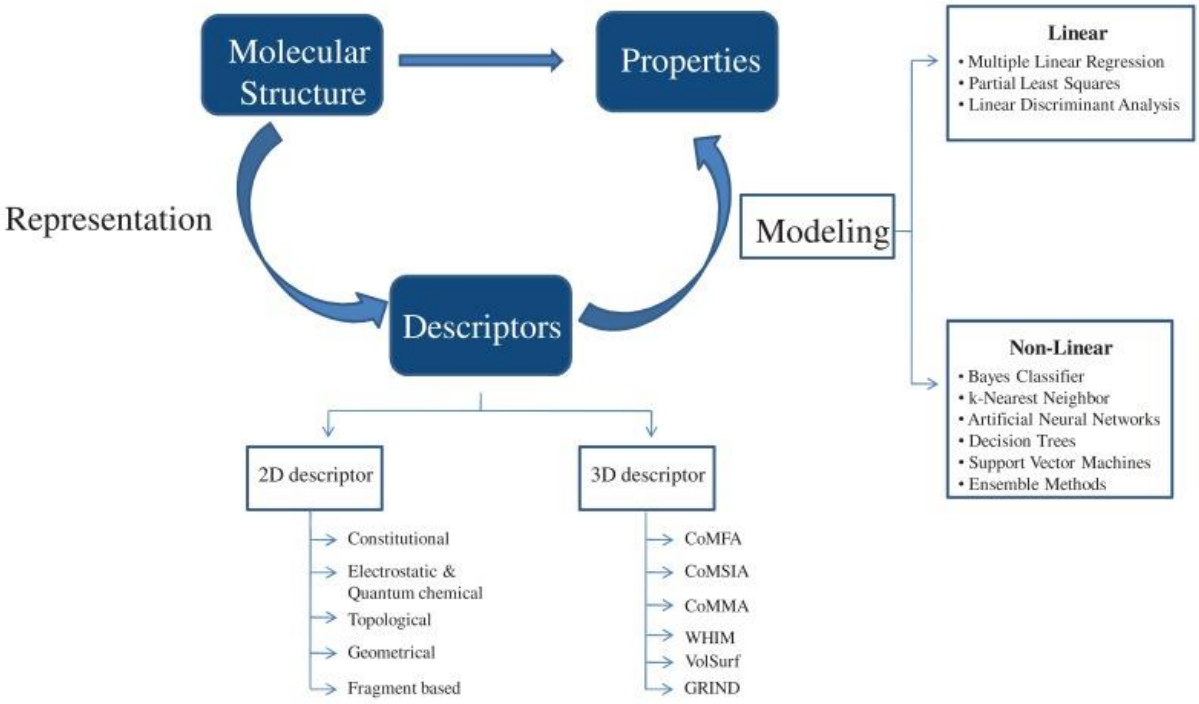

Figure 2: General methodology of QSAR [14].

QSAR establishes a mathematical relationship between molecular properties, known as descriptors, and biological activity. This relationship is expressed by the model:

$$[\text{Biological activity}] = f\,[\text{descriptors}]$$

Where the function (f) quantitatively maps descriptor values to the observed biological response.

II.4 Molecular descriptors

Molecular descriptors are the result of mathematical procedures that transform chemical information encoded within a molecular structure into a numerical representation, which their dimensionality can be used to identify the QSAR model type as following (figure 3):

Figure 3: Molecular descriptors used in QSAR modelling [15].

0D descriptors, which fall under the constitutional category, are obtained from molecular formulas and contain basic characteristics, like atom counts or molecular weight, without considering atom connectivity, while 1D descriptor depend on molecular substructures. Physicochemical and pharmacokinetic properties are frequently modelled using 2D descriptors, which are topological in nature and rely on molecular graphs to present atom connectivity. 3D descriptors, categorised as geometrical, are derived from the spatial 3D coordinates of atoms, and serve to capture molecular features such as size, shape, and atom distribution. The 4D models extend this approach by considering dynamic ensembles of molecular conformations to account for structural flexibility. In addition to these, electronic descriptors, such as HOMO/LUMO energies and dipole moments, are utilised to characterise the electronic structure and bonding properties of molecules. The utilisation of thermodynamic descriptors, including enthalpy of formation and molar refractivity, facilitates the correlation of molecular structure with energetic and chemical behaviour.

Finally, it is important to note that certain descriptors integrate multiple dimensions and are classified as hybrid, offering a more comprehensive representation of molecular properties [16].

II.5 Statistical methods

Statistics is the study of groups of similar objects by analysing their characteristics, called variables. A statistical model links the target variable to the weighted combination of these characteristics [17]. The main tools used to build such models include:

II.5.1 Multiple Linear Regression

Multiple linear regression (MLR) is a method used to understand the relationship between several factors and a response variable, assuming a linear relationship. It is simple to interpret but can be unstable in cases where the variables are dependent on each other.

II.5.2 Partial Least Squares

Partial Least Squares (PLS) is a statistical technique that allows to create models of the relationships between a set of predictor variables and one or more response variables. It projects the data into a hidden space to maximise the similarity between the explanatory variables and the response, which makes it effective for collinear data or large numbers of data.

II.5.3 Artificial neural networks

In QSAR studies, artificial neural networks (ANNs) are used with a wide range of architectures and techniques, including different ways to represent chemical structures, pre-process data, select relevant descriptors, train models, and interpret predictions. These components together define the computational methods applied in the field. The concept of a computational method is more specific than just the architecture or type of neural network, as it also includes how the model is trained and how results are handled, even if the underlying network structure is similar [18].

II.5.4 Support Vector Machines

Support Vector Machines (SVM) creates the optimal hyper planes for classification or regression, using kernel functions to handle non-linear separation. It is effective in high-dimensional spaces where the margin is clearly defined.

II.6 Model validation

Confirming the validity of QSAR models is a key but complicated part of statistical analysis. It is important to ensure that the model is statistically sound and can reliably predict the biological activity. Since every model is developed within a defined analytical scope, it must be interpreted and used strictly within that boundary [19]. Using the model outside its

intended scope should be done with caution, as the further you go from that scope, the risk of error increases.

To prevent mistakes during validation and use, it's important to define the model's limits, check its reliability, measure how well it predicts both known and new data, and make sure it's only applied within a specific chemical space [20].

II.6.1 Internal validation

The internal validation of a QSAR model is performed using the training dataset. The initial step involves the evaluation of the model's precision in replicating the characteristics of the training data. The second step uses cross-validation to assess the model's quality and robustness by simulating a situation in which it predicts new, unseen data. In this process, the training set is divided into two parts: a calibration set to build the model, and a validation set to test how well the model predicts data it wasn't trained on [21].

II.6.1.1 Leave-One-Out procedure

The method is based on the principle of eliminating one molecule at a time from the training set. In each round, a single molecule is removed to form the validation set, while the remaining (n-1) molecules are used to build the model. The process is repeated until every molecule has been used once as the validation set.

II.6.1.2 Leave-Many-Out procedure

It involves splitting the data set into multiple sections. One section is designated as an internal test set, while the remaining sections make up the training set. The trained model is then used to predict the properties of the molecules in each excluded group. This process is repeated p times, with p representing the total number of groups of molecules that are excluded [22].

II.6.2 External validation

The efficacy of a QSAR model is determined by its capacity to accurately predict the activity/property of compounds from an external test set, comprising compounds not utilised in the development of the model.

The objective of a suitable QSAR model is not only to predict the activity of the training set compounds, but also to predict the activities of the test molecules [23].

II.6.3 Statistical parameters

II.6.3.1 Determination coefficient R^2

The coefficient of determination, R^2, is a measure of the degree of relationship between the dependent variable (biological activity) and the independent variable (molecular descriptor). A value of R^2 close to 1 indicates a satisfactory fit of the regression model [24].

II.6.3.2 Prediction coefficient Q^2

The prediction coefficient Q^2 indicates how accurate the predictions are, as it reflects the predictive power of a regression model.

II.6.3.3 Test Fischer F

The Fischer test is utilised to substantiate the correlation between biological activity and the molecular descriptor, by quantifying the ratio between the variance of biological activity explicable and inexplicable by the regression model [25].

II.6.3.4 Standard Deviation SD

Is a measure of how dispersed the data is in relation to the mean. Low, or small, standard deviation indicates data are clustered tightly around the mean, and high, or large, standard deviation indicates data are more spread out. A standard deviation close to zero indicates that data points are very close to the mean, whereas a larger standard deviation indicates data points are spread further away from the mean.

III. Molecular Docking

III.1 An overview

Molecular docking is a technique in which a small organic molecule, known as a 'ligand', interacts with the active site of a macromolecule (protein), known as a 'receptor'.

The purpose of this technique is to determine the most effective method of attaching the ligand-receptor complex (figure 4), leading to the prediction of the "bioactive" conformation of the ligand within its receptor. Each relative conformation resulting from docking is associated with energy, defined as the 'Score'.

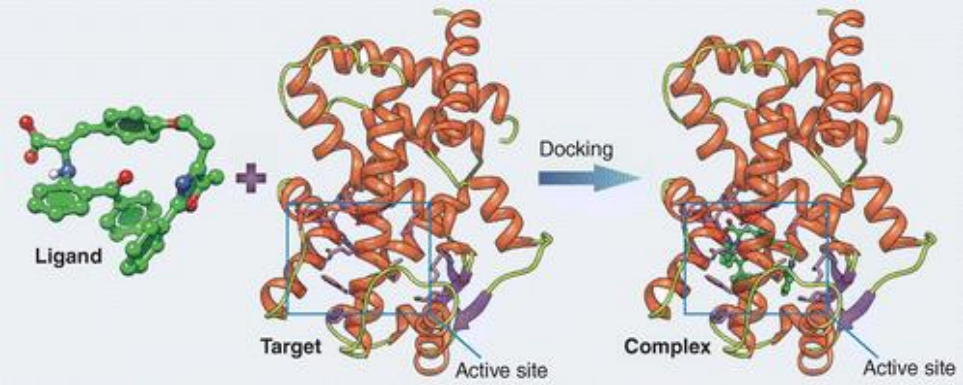

Figure 4: Illustration of the interaction between a molecule (ligand) and a protein (Target) [26].

The molecular docking simulation is based principally on the ligand-protein association, which can be considered rigid, flexible or semi-flexible.

III.1.1 Rigid docking

In rigid body docking, the protein and ligand are both held fixed, and the algorithm searches for the most favourable pose by systematically sampling all possible translations and rotations of the ligand within the binding pocket and scoring each position by energy. Any poses that clash with the active site are discarded, leaving only those that fit well. This method is very fast computationally and effectively filters out large or poorly complementary molecules before more detailed (and expensive) analyses because it only considers rigid movements [27].

III.1.2 Semi-flexible docking

The system here is divided into two regions: a flexible region which contain the ligand and flexible residues near the binding site and a rigid region which is the remaining protein structure. Semi-flexible docking approaches are widely used due to their efficiency, employing randomized search algorithms such as Monte Carlo simulations and genetic algorithms, which are categorized as stochastic methods.

These techniques balance computational accuracy and speed by allowing partial flexibility in the binding site while keeping the rest of the protein fixed [28].

III.1.3 Flexible docking

This approach indirectly accounts for protein flexibility by minimizing repulsive forces, enabling the ligand to interact more deeply with the protein surface. During enzymatic reactions, the protein dynamically adjusts its conformation to better accommodate the ligand,

fostering precise interactions while avoiding steric clashes. These adaptations enhance structural compatibility between the ligand and protein, optimizing conditions for hydrogen bond formation and improving binding efficiency [29].

III.2 Methodology

There are multiple steps in the process starting with target selection which are usually a protein structure can be gotten from protein data bank (PDB). Ligand preparation involves generating 3D conformations for small molecules and considering all forms as tautomer, to produce a chemically accurate structure. For protein preparation, the target structure must be refined by restoring all missing atoms as hydrogen while removing anything that is unnecessary like crystallographic solvents. The next step generates the receptor grid to represent the binding site and the related physicochemical properties (figure 5). There are a few docking models to use, mostly involving algorithms like rigid/flexible docking to provide a prediction for ligand poses that can be scored using binding energy or empirical scoring functions. After docking ligand interaction analysis identifies critical residues and interaction types (e.g. hydrogen bonds, π-π stacking) to validate binding modes.

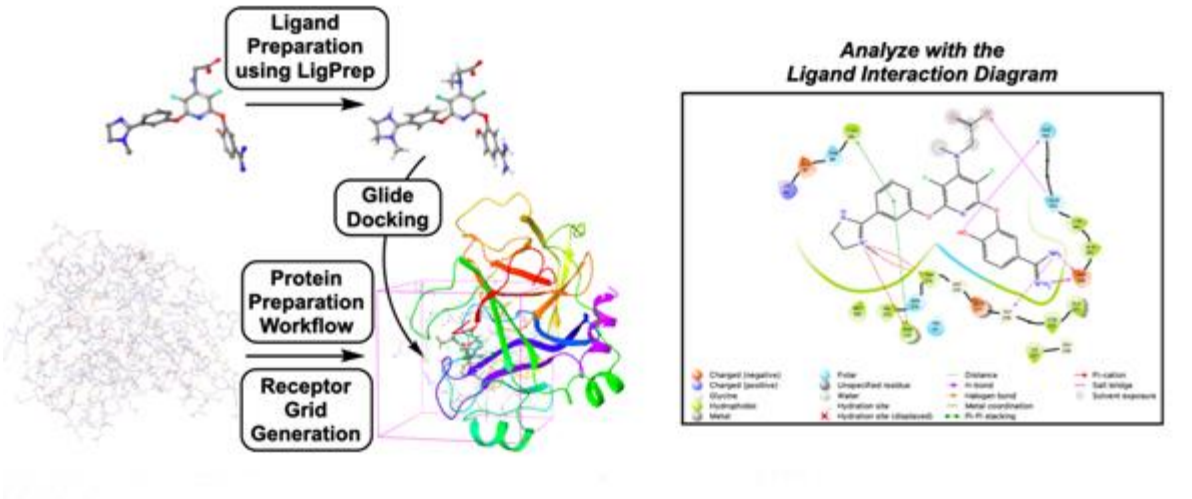

Figure 5: Molecular docking steps using Maestro Schrodinger [30].

III.3 Scoring

The docking score measures how strongly a ligand binds to a protein. It estimates the energy change (ΔG) when the free protein and ligand combine to form a complex, calculated as:

$$\Delta G = \Delta G_{complexe} - \Delta G_{ligand} - \Delta G_{protein}$$

This score reflects ligand's binding to its target, not biological activity. Scores should not be directly compared to experimental activity measurements but instead validated against

experimental binding data. Even if scores and experimental results disagree, this does not mean the scoring method is flawed, as experimental data often covers a narrow range compared to theoretical predictions.

Scoring functions have two main roles:

- Identifying the best-fitting ligand conformation.
- Ranking molecules to highlight those with the strongest predicted binding.

However, docking has limitations. Key challenges include modeling hydrogen bonds, energy changes, and water interactions during binding. Scoring functions simplify these complexities and cannot account for all molecular interactions, so results should be interpreted cautiously in drug discovery [31].

IV. Principles of ADME/Toxicity

After entering the body, a drug must overcome the ADME/Tox barriers (Absorption, Distribution, Metabolism, Excretion, and Toxicity) to reach its therapeutic target effectively. Each of these physiological processes can reduce the drug's active concentration, making it essential for the compound to maintain sufficient levels to exert a meaningful biological effect. Focusing only on enhancing a molecule's affinity for its target may lead to failure in clinical settings if it’s pharmacokinetic and safety profiles are poor. Thus, successful drug development requires a balanced optimization of both target engagement and ADME/Tox characteristics [32]. These properties depend not only on the drug’s chemical structure but also on its interactions with transport proteins, metabolic enzymes, and plasma proteins. Since approximately 80% of marketed drugs are administered orally, it is particularly important to understand the dynamic and interdependent nature of ADME/Tox processes along the gastrointestinal tract. Computational tools, such as SAR models, play a crucial role in predicting how compounds will behave in the body, helping researchers prioritize candidates with favourable profiles and reduce late-stage failures [33].

IV.1 Toxicity

As the name implies, this filter evaluates the toxicity of a compound along with its metabolites. Today, toxicity and insufficient efficacy are the primary reasons for drug development failures. Various forms of toxicity are examined, including AMES mutagenicity,

hERG I channel inhibition, acute oral toxicity in rats (LD50), liver toxicity, and skin sensitization [34].

IV.2 Lipinski's Rule

Lipinski's Rule of Five is a widely used guideline for evaluating whether a chemical compound is likely to be orally bioavailable.

Derived from the analysis of drugs that successfully passed Phase II clinical trials, this rule helps identify compounds that may have poor absorption or permeability, rather than definitively labelling them as drug-like or not [35].

The rule focuses on key physicochemical properties that influence passive intestinal permeability and outline five main criteria:

- Molecular weight ≤ 500 g/Mol
- LogP (lipophilicity) ≤ 5
- Hydrogen bond donors ≤ 5
- Hydrogen bond acceptors ≤ 10
- Rotatable bonds ≤ 15

IV.3 Ghose rule

The Ghose Rule defines drug-likeness based on the quantitative and qualitative analysis of known drugs, aiming to identify compounds with favorable pharmacological profiles. This rule is widely used in medicinal chemistry for filtering drug-like candidates in virtual screening and combinatorial library design. The key criteria are:

- Quantitative Characterization:
    - Molecular weight between 160 and 480 g/Mol.
    - Log P (octanol-water partition coefficient) calculated between -0.4 and 5.6.
    - Molar refractivity between 40 and 130, reflecting molecular volume and polarizability.
    - Total number of atoms between 20 and 70.
- Qualitative Characterization:
    - Analysis of the occurrence of functional groups.

- Identification of important substructures, such as benzene rings and heterocycles, common in drug molecules.

These criteria were established through the profiling of the Comprehensive Medicinal Chemistry database, ensuring a practical definition of drug-like molecules based on empirical data [36].

IV.4 Veber rule

The rule of Veber, proposed by Veber et al. in 2002, provides criteria for predicting the oral bioavailability of drug candidates by focusing on molecular flexibility and polarity rather than molecular weight alone. Through the analysis of over 1100 compounds, the study found that good oral bioavailability is associated with the following key molecular properties:

- 10 or fewer rotatable bonds.
- Polar surface area (PSA) of 140 $Å^2$ or less.
- A total of 12 or fewer hydrogen bond donors and acceptors.

These properties were shown to strongly influence passive membrane permeability, which is essential for oral absorption. The study demonstrated that reducing molecular flexibility and limiting polar surface area enhances the probability of a compound being orally bioavailable, offering a practical guideline for drug design [37].

V. Conclusion

This chapter has outlined the fundamental computational methodologies employed in modern drug design, emphasizing their critical role in streamlining the discovery and optimization of therapeutic candidates. Quantitative Structure-Activity Relationship (QSAR) modeling, molecular docking, and ADME/Tox profiling serve as powerful tools to predict biological activity, elucidate ligand-receptor interactions, and assess pharmacokinetic properties, respectively. These approaches not only enhance the efficiency of drug development but also reduce reliance on costly and time-consuming experimental trials.

The integration of statistical validation techniques ensures the reliability and applicability of QSAR models, while advanced docking strategies provide insights into binding affinities and molecular interactions. Furthermore, adherence to drug-likeness rules (e.g., Lipinski's, Ghose, and Veber) aids in prioritizing compounds with favorable bioavailability and safety profiles.
Collectively, these computational frameworks bridge theoretical and experimental research, offering a robust foundation for rational drug design. Future advancements in machine learning and multi-scale modeling hold promise for further refining predictive accuracy and accelerating the development of novel therapeutics.

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Chapter 03:
Results & discussion

I. Introduction

Alzheimer's disease (AD) represents one of the most complex and devastating neurodegenerative disorders, characterized by progressive cognitive decline, memory loss, and functional impairment. Despite decades of research, current therapeutic strategies primarily AChE inhibitors and NMDA receptor antagonists offer only symptomatic relief without halting disease progression. The multifactorial nature of AD, involving amyloid-β aggregation, tau pathology, oxidative stress, and neuro-inflammation, demands innovative approaches that simultaneously target multiple pathological pathways.

Ningwei et al. [1] pursued the development of multifunctional therapeutic agents designed to exert simultaneous effects on multiple disease pathways. Their approach specifically aimed to attenuate oxidative stress and neuro-inflammation, two central drivers of neurodegeneration, in order to achieve improved therapeutic outcomes with reduced neurotoxic side, effects compared to traditional single-target drugs [2].

This chapter presents a comprehensive computational investigation of novel donepezil-tacrine hybrid derivatives designed to address these challenges. Leveraging advanced methodologies including 3D-QSAR modeling, molecular docking, ADME/Tox profiling, MM-GBSA binding free energy calculations, and density functional theory (DFT) analyses, we systematically evaluate the inhibitory potential, binding interactions, and drug-like properties of these compounds. The integration of these techniques enables the rational design of multifunctional agents with optimized AChE inhibition, improved pharmacokinetics, and reduced toxicity risks.

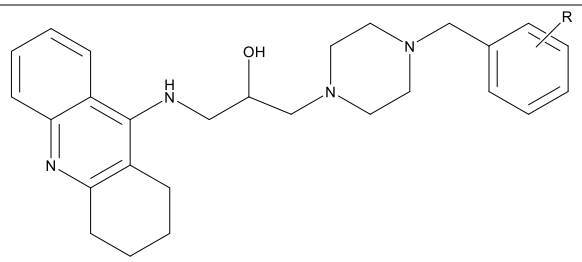
II. Materials and Methods

II.1 3D QSAR

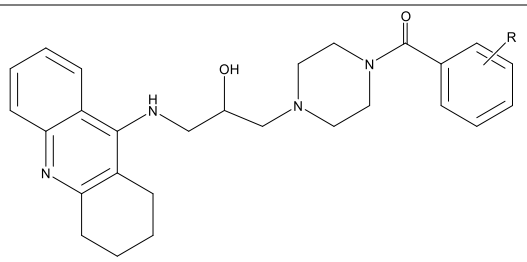
II.1.1 Data base

The study incorporated 34 compounds exhibiting AChE inhibitory activity [1]. The molecular structures of these AChE inhibitors, along with their respective pIC_{50} values, are compiled in Table 1. The IC_{50} values were then transformed into pIC_{50} values, which were used as the dependent variable in this study. The molecules are divided into 2 sets : training set and test set in the ratio (75:25).

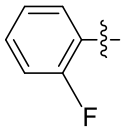
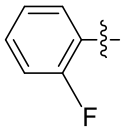
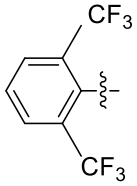
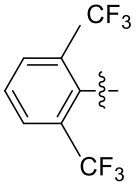
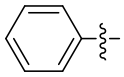
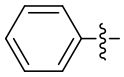
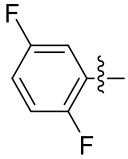
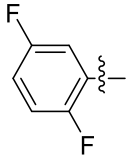
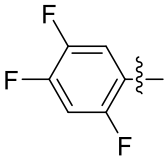
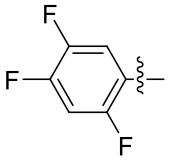
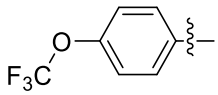
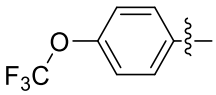
Table 1: The chemical structures and the observed activity (pIC50) data of the compounds.

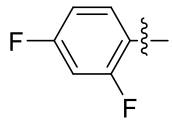
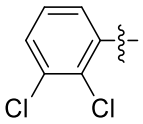
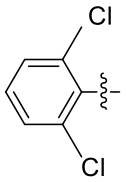
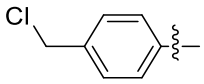
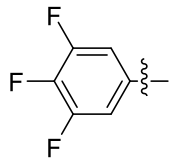
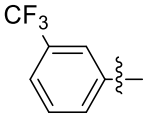
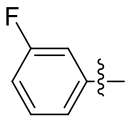
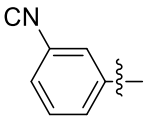
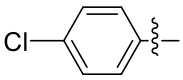
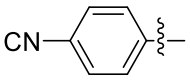
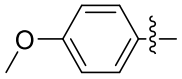
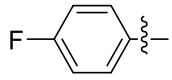
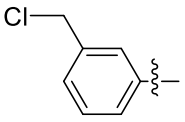


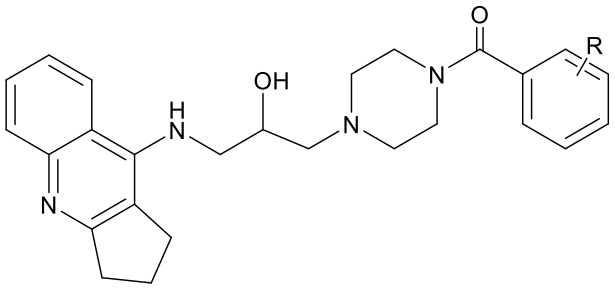
A1 – A6



B1 – B20

Compound	R	pIC_{50}	Compound	R	pIC_{50}
A1		7.63395079	**B1**		7.524764777
A2		8.095284455	**B2**		7.85823677
A3		7.928117993	**B3**		7.51484665
A4		7.875169851	**B4**		7.335452038
A5		7.438898616	**B5**		7.203078925
A6		7.970616222	**B6**		7.143512787

B7		7.235450281	**B14**		7.252899069
B8		8.390405591	**B15**		7.056703385
B9		7.190440285	**B16**		7.169796401
B10		7.73754891	**B17**		7.150949309
B11		7.169218924	**B18**		7.130123187
B12		7.247721015	**B19**		7.170760572
B13		7.149966742	**B20**		7.089215565



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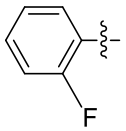
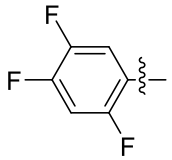
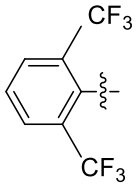
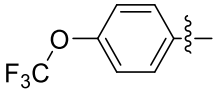
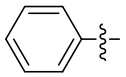
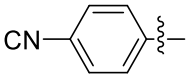
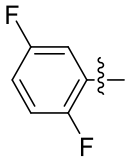
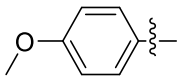
C1		8.54515514	**C5**		7.821598658
C2		8.308918508	**C6**		8.09420412
C3		8.330683119	**C7**		7.547906751
C4		8.096367484	**C8**		7.560036064

II.1.2 Optimisation and alignment

The molecules are optimised using the tool Ligprep which is a versatile ligand preparation tool integrated within the Maestro interface of the Schrödinger software suite [3], designed to efficiently generate high-quality small molecule structures for structure-based workflows such as virtual screening, molecular docking, and other computational modelling applications. It aims to produce accurate 3D representations of ligands by systematically expanding tautomeric forms, ionization states, ring conformations, and stereoisomers, ensuring comprehensive coverage of all relevant chemical states of a molecule [4]. Then the molecules are aligned using the Common Scaffold Alignment module (Maximum Common

Substructure). Since the molecule C1 is the most active (IC_{50}=2.85 nM), it is used as a reference for aligning the other molecules.

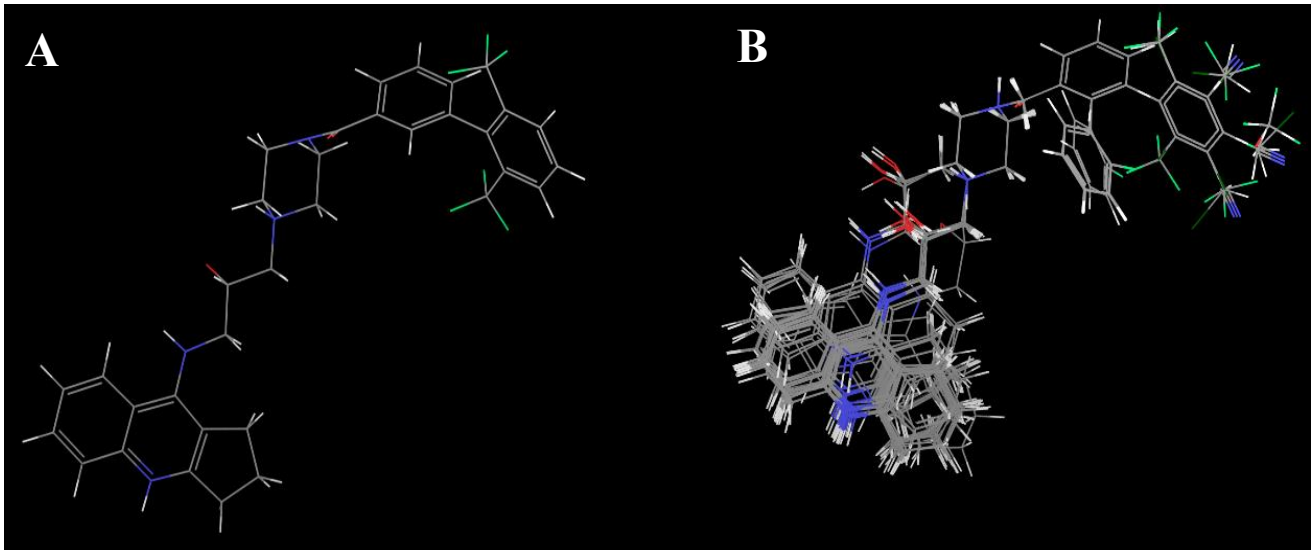


Figure 1: Molecules alignment. (A): Molecule C1; (B) Molecules alignment.

II.1.3 3D Field based QSAR

The 3D Field-Based QSAR method involves constructing a model based on five Gaussian fields that represent key molecular properties: hydrogen bond acceptors (HBA), hydrogen bond donors (HBD), steric, electrostatic, and hydrophobic interactions. These fields are mapped onto a three-dimensional grid surrounding the molecules in the training set. To ensure the relevance of the input data, variables with minimal variation were excluded. Model building was carried out using PLS analysis, where the Gaussian field descriptors served as independent variables and the biological activity (pIC_{50}) was used as the dependent variable. The predictive performance of the model was evaluated using LOO cross-validation, which provided the Q^2 value. Additional statistical parameters, such as R^2, standard error of estimate (SEE), and F values, were used to assess the quality of the model fit.

II.2 Design of new compounds

Due to its high biological activity, the compound C1 is chosen as the reference structure in order to create a set of molecules focusing the modifications on the R group. These changes aim to enhance binding affinity, improve pharmacokinetic properties and reduce potential toxicity. The newly designed compounds are then evaluated using a 3D QSAR model to predict their biological activity and identify promising candidates for further development.

II.3 Molecular docking

Molecular docking was performed to analyse the interactions between the designed compounds and the target protein. The protein's crystal structure of AChE was obtained from the Protein Data Bank (PDB) (PDB ID: 4EY7) and selected as the receptor, then was prepared using Schrodinger's Protein Preparation Wizard. During the process of protein preparation minimisation, the OPLS4 force field was employed to refine the structure and resolve any steric clashes. After preparation, a receptor grid was generated to define the active site for docking. The ligands were then docked into this site using the Ligand Docking tool to evaluate binding conformations, interaction energies and key molecular contacts. This process facilitated the identification of promising compounds with strong predicted affinities for the target.

II.4 ADME/Tox and drug likeness prediction

In the course of drug discovery process, it is of the crucial importance to conduct early ADME/Tox (absorption, distribution, metabolism, excretion, and toxicity) profiling.

These proprieties were calculated using SwissADME website [5] and for toxicity pkCSM website [6]. Pharmacokinetic parameters, which facilitate prediction of a drug's behaviour within the body, were also considered as part of the ADME analysis to optimise drug-likeness and efficacy.

II.5 MM-GBSA approach

The MM-GBSA (Molecular Mechanics - Generalized Born Surface Area) approach is used to calculate the binding free energies between ligands and receptors. It calculates important energy values for the free receptor, free ligand, and their complex in the best possible states. From these, the binding energy (ΔG Bind) and the strain energies for both the receptor and the ligand are calculated. The strain-free binding energy (ΔG Bind NS) excludes conformational strain, offering a clearer view of interaction strength [7].This is depicted by the following equation, which uses the OPLS4 force field and the VSGB solvent model.

Here, the binding free energy for each complex was determined using the following equation:

$$\Delta G_{bind} = G_{complex} - (G_{protein} + G_{ligand})$$

where G_{ligand} represents the free energy of the ligand, $G_{protein}$ is the target protein's free energy value, $G_{complex}$ corresponds to the free energy of the ligand-protein complex, and ΔG_{bind} indicates the resulting binding free energy [8].

II.6 Density Functional Theory (DFT) calculation

The Gaussian input files for the molecules C1 and D1 were generated using the GaussView 5 program [9], and their quantum chemical calculations were carried out with the Gaussian 09W package [10]. Geometry optimisations were performed at the DFT/B3LYP/6-311G basis set level of theory. The highest occupied molecular orbital (HOMO) and lowest unoccupied molecular orbital (LUMO) energies, as well as the energy gaps, were calculated to evaluate the electronic properties of the optimised structures. Furthermore, the total density of states (TDOS) and the partial density of states (PDOS) were computed using Multiwfn (the Multifunctional Wavefunction Analyser) [11] in order to examine the contribution of individual elements to the molecular orbitals.

III. Results and discussion

III.1 3D QSAR

PLS regression was applied to investigate the structure–activity relationship of the compounds under study. This statistical technique effectively minimises the difference between experimental and predicted biological activities. The determination coefficient (R^2=0.82), which exceeds the commonly accepted threshold of 0.6 [12], demonstrates a strong correlation between the molecular descriptors and the biological activity which is expressed as pIC_{50}. The model's statistical validity is further supported by a high Fisher test value F=103.9 and a low standard deviation of error SD= 0.1831, indicating good internal consistency and predictive accuracy.

The percentage contributions of the molecular fields to the model were as follows: steric (46.05%), hydrophobic (23.96%), electrostatic (12.80%), hydrogen bond acceptor (11.37%), and hydrogen bond donor (5.79%). These results suggest that steric and hydrophobic interactions are the primary factors influencing biological activity, while hydrogen bonding and electrostatic effects play a lesser, though still relevant, role. Model validation was conducted using the LOO cross-validation approach [13], which involves systematically excluding one compound at a time from the dataset and reconstructing the model. The cross-validated coefficient Q^2=0.738> 0.5, confirms the model's robustness and satisfactory predictive performance.

Table 2: Observed and calculated activities.

Molecule	Observed pIC_{50}	Calculated pIC_{50}	Residual
A1	7.63395079	7.822	-0.18805
A2	8.095284455	8.105	-0.00972
A3	7.928117993	7.774	0.154118
A4	7.875169851	7.685	0.19017
A5	7.438898616	7.520	-0.0811
A6	7.970616222	7.708	0.262616
B1	7.235450281	7.275	-0.03955
B2	8.390405591	7.617	0.773406
B3	7.190440285	7.097	0.09344
B4	7.73754891	7.605	0.132549
B5	7.169218924	7.293	-0.12378
B6	7.247721015	7.256	-0.00828
B7	7.149966742	7.284	-0.13403
B8	7.524764777	7.458	0.066765
B9-	7.85823677	7.826	0.032237
B10	7.51484665	7.503	0.011847
B11	7.335452038	7.357	-0.02155
B12	7.203078925	7.195	0.008079
B13	7.143512787	7.401	-0.25749
B14	7.252899069	7.347	-0.0941
B15	7.056703385	7.079	-0.0223
B16	7.169796401	7.211	-0.0412

B17	7.150949309	7.153	-0.00205
B18	7.130123187	7.007	0.123123
B19	7.170760572	6.984	0.186761
B20	7.089215565	7.229	-0.13978
C1	8.54515514	8.079	0.466155
C2	8.308918508	8.537	-0.22808
C3	8.330683119	8.160	0.170683
C4	8.096367484	7.968	0.128367
C5	7.821598658	7.903	-0.0814
C6	8.09420412	8.102	-0.0078
C7	7.547906751	7.616	-0.06809
C8	7.560036064	7.687	-0.12696

Contour map analysis (Figure 2) is commonly used to identify molecular fragments that are favourable or unfavourable for biological activity. The activity of molecules is influenced by various physicochemical properties, including hydrophobic, steric, electrostatic characteristics, as well as hydrogen bond donor and acceptor capabilities. These properties vary from one molecule to another depending on their specific substituents, which can alter the overall physicochemical profile and, consequently, impact biological activity. Among the studied compounds, **C1** exhibited the highest biological activity and was therefore selected as the reference structure for generating the contour map.

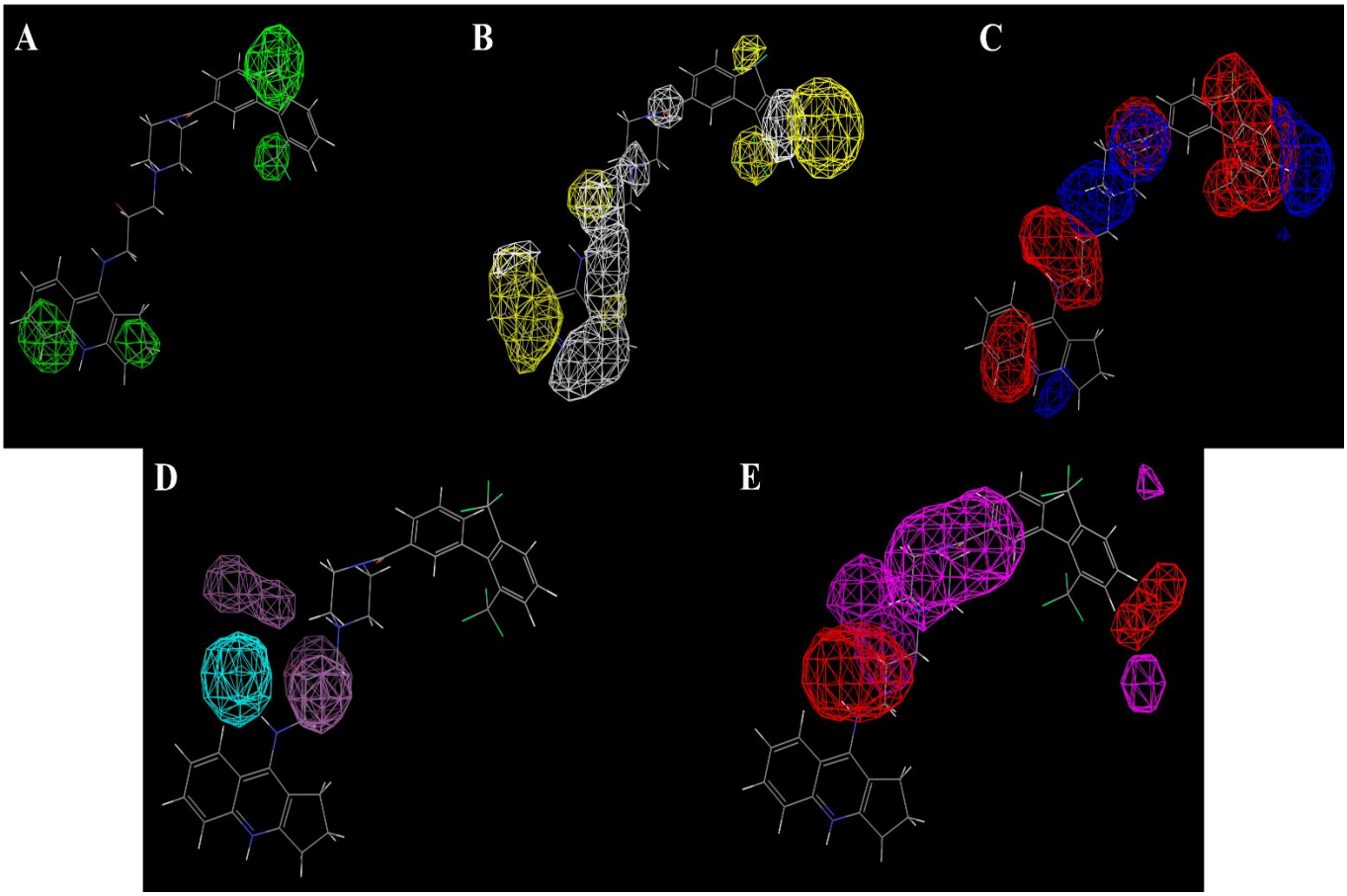


Figure 2: Gaussian distributions: (A) steric, (B) hydrophobic, (C) electrostatic, (D) H-bond donor and (E) H-bond acceptor.

The steric field is the principal factor contributing to activity with 46.05%, indicating that incorporating bulky groups in green regions enhances target binding and molecular fit. Conversely, areas absent of green contours imply that introducing additional larger substituents in these positions would be neutral or unfavourable.

The hydrophobic field shows that yellow regions are favourable for hydrophobic substituents, such as alkyl groups. In contrast, grey regions support the presence of hydrophilic or polar groups, which could enhance solubility or favour beneficial polar interactions with the target environment.

Red areas in the electrostatic field favour electronegative atoms such as O or N, which facilitate favourable electrostatic interactions with the positively charged regions of the target. Meanwhile, blue regions are favourable for electropositive groups, such as protonated amines or electron-deficient moieties, as their presence is predicted to enhance biological activity.

The HBD field indicates that cyan regions are favourable for hydrogen bond donors, such as NH or OH groups. Their strategic placement could strengthen key hydrogen bonding interactions with the biological target. Purple regions in this field indicate areas where donor

groups may still be tolerated or neutral. Conversely, the HBA field demonstrates that purple regions are favourable for hydrogen bond acceptors, such as O or N atoms capable of donating lone pairs. In contrast, red regions in this field are unfavourable for such functionalities, likely due to steric hindrance or desolvation penalties.

Finally, these contour maps are a valuable resource for the rational design of more potent and selective novel compounds

III.2 Design of new compounds

A rational modification of the R group was conducted based on steric field analysis. A total of twenty derivatives were constructed by introducing variations at the R position to optimise molecular interactions within the active site using MarvinSketch [14]. The steric field revealed that the incorporation of bulky substituents near the R position can enhance biological activity, provided they are placed in regions where such volume is favourable. Guided by this, larger groups were suggested to R in order to strengthen the binding affinity through improved steric complementarity with the target. However, careful attention was paid to the overall molecular weight of the newly designed compounds to ensure that it remained around or below 500 g/mol, thus ensuring respect for Lipinski's rule of five and the maintenance of desirable drug-like properties.

III.3 Molecular docking

Among the twenty compounds evaluated for anti-Alzheimer activity as AChE inhibitors, compound D1 exhibited the highest binding affinity with the enzyme, with a docking score of -11.696 kcal/mol. Analysis of the D1-AChE complex revealed several key interactions within the active site. D1 formed a hydrogen bond with TYR72 at a distance of 8.30 Å, corresponding to a hydrophobic interaction, as well as a hydrophilic hydrogen bond with ASP74. A significant π-cation interaction with TRP286 was also observed at 5.88 Å, contributing to hydrophobic stabilisation of the complex . Additionally, a hydrogen bond with a structural water molecule (H_2O) at 6.72 Å further supported the ligand’s orientation and binding stability within the pocket.

These interactions indicate a strong and specific binding mode for compound D1. The combination of high docking affinity and diverse non-covalent interactions identifies D1 as the most promising candidate for AChE inhibition.

The compound C1 have a docking score of -9.674 kcal/mol and formed a hydrogen bond with a structural water molecule , a salt bridge between NH and GLU292 and π-cation interaction

with TRP286. Compound D1 exhibits superior AChE inhibition properties compared to C1 due to its enhanced binding affinity and diverse interactions.

III.4 ADME/Tox and drug likeness prediction

Comprehensive evaluation of ADME/Tox characteristics and pharmacokinetic parameters at an early stage of drug discovery is crucial for predicting in vivo performance, improving drug-like properties and identifying potential safety or efficacy issues prior to synthesis and experimental testing. Accordingly, the five highest scoring docked compounds underwent detailed profiling of ADME/Tox properties. Their compliance with established drug-likeness filters, including Lipinski's Rule of Five, Veber's Criteria and Ghose's Guidelines, was also assessed to determine the potential for oral bioavailability and suitability as lead candidates (Table 3).

Table 3: Drug-likeness, and Medicinal Chemistry Parameters of compounds D1, C1.

Mol	MW (g/mol)	HBA	HBD	Rotatable Bonds	SA	Lipinski	Veber
D1	507.07	6	2	8	4.48	Yes	Yes
C1	524.63	5	2	8	4.27	Yes	Yes

Whereas, **MW:** molecular weight; **Rotatable Bonds :** the number of single non-ring bonds around which the molecule can freely rotate; **SA**: Synthetic Accessibility

Table 4: Predicted ADME/Tox parameters for the compounds D1, C1.

	Model	Unit	D1	C1
Absorption	Intestinal absorption (human)	Numeric (% Absorbed)	88.85	91.166
Distribution	BBB permeability	Numeric (log BB)	-0.589	-1.037
Metabolism	CYP3A4 substrate	Categorical (Yes/No)	Yes	Yes
	CYP2D6 inhibitor	Categorical (Yes/No)	Yes	No

Excretion	Total Clearance	Numeric (log ml /min/kg)	1.312	0.787
Toxicity	AMES toxicity	Categorical (Yes/No)	No	Yes

III.4.1 Absorption

Poor intestinal absorption is typically defined as a value below 30%. However, compound D1 displays predicted human intestinal absorption with 88.85%, suggesting excellent oral bioavailability.

III.4.2 Distribution

The predicted blood brain barrier (BBB) permeability (logBB) value of compound D1 is -0.589 . This result indicate moderate to low passive diffusion across the BBB. While the compound is unlikely to cross the BBB efficiently (logBB > 0.3), its value is not low enough to suggest complete exclusion from the brain (logBB < -1). This suggests limited, though not negligible, brain exposure an attribute that may be beneficial for drugs intended for peripheral targets, but which may require further optimisation for central nervous system (CNS) applications.

III.4.3 Metabolism

Compound D1 is predicted to be substrates of CYP3A4, indicating it is primarily metabolised by CYP3A4 and may be less affected by genetic variability. Furthermore, the inhibition profile indicates that while CYP2D6 inhibition is observed in compound D1,

III.4.4 Excretion

The predicted total clearance of D1 value (log (CLtot) is 1.048 suggest high elimination rates, generally higher than the control (0.787). This implies efficient excretion, which reduces the risk of accumulation but may require dose adjustments to maintain therapeutic levels.

III.4.5 Toxicity

Toxicity predictions indicated that compound D1 poses no AMES toxicity, suggesting a low risk of mutagenicity. This result reinforces its safety profile from a genotoxicity perspective and supports its suitability for further biological evaluation. The absence of predicted mutagenic potential highlights D1 as a promising candidate for subsequent in vitro and in

vivo investigations, aligning with favourable ADME/Tox characteristics observed during simulation of human metabolic behaviour.

III.5 MM-GBSA

The MM-GBSA binding free energy components, including van der Waals energy (vdW), lipophilic energy (Lipo), Coulombic energy (electrostatic), covalent binding contributions, and solvation energy (solv GB), were calculated (Table 5).

The total binding free energy (ΔG Bind) values for the compounds D1 and C1.

Table 5: The relative binding-free energies (kcal/mol) obtained by Prime MM–GBSA.

Compound	ΔG_{Bind}	ΔG_{Lipo}	$\Delta G_{coulomb}$	$\Delta G_{covalent}$	ΔG_{solv}	ΔG_{vdW}
D1	-52.43	-32.95	-28.20	11.87	44.05	-43.24
C1	-49.83	-33.71	-37.38	3.54	75.98	-50.73

Among the compounds, D1 demonstrates a notable binding affinity of -52.43 kcal/mol, principally driven by substantial van der Waals (-43.24 kcal/mol) and lipophilic (-32.95 kcal/mol) interactions. Electrostatic (Coulombic) energy (-28.20 kcal/mol) further supports binding, indicating key polar or charged contacts within the binding site. Despite opposing contributions from solvation (44.05 kcal/mol) and covalent (11.87 kcal/mol) energies, these are outweighed by the dominant non-covalent forces that stabilise the ligand-receptor complex.

The MM-GBSA results demonstrate that strong van der Waals and lipophilic interactions are pivotal in stabilising binding, while electrostatic interactions enhance specificity. Though desolvation penalties oppose binding, they are effectively compensated by positive interactions, confirming that compound D1 exhibit high binding affinities.

III.6 Density Functional Theory (DFT) calculation

The electronic properties of compounds C1 and D1 were investigated using DFT calculations at the B3LYP/6-311G theoretical level. The optimised molecular structures were then used to calculate the HOMO and LUMO energies and the corresponding energy gaps. These values provide valuable insight into the molecules' chemical reactivity and electronic characteristics. Visual representations of the HOMO and LUMO orbitals for both compounds are presented in figure 4.

For compound C1, the HOMO energy is -4.1685 eV, the LUMO energy is -1.1520 eV and the energy gap is 3.0165 eV. Compound D1, in comparison, exhibits HOMO and LUMO energies of -5.5955 eV and -1.7469 eV respectively, corresponding to an energy gap of 3.8486 eV.

The energy gap (ΔE_{gap}) is an indicator of a chemical compound's reactivity and stability. A smaller energy gap, as observed in compound C1, suggests higher electronic reactivity and greater potential to facilitate electronic transitions, enhancing its interaction with biological targets. However, the larger energy gap of compound D1 indicates a more chemically stable and less reactive nature.

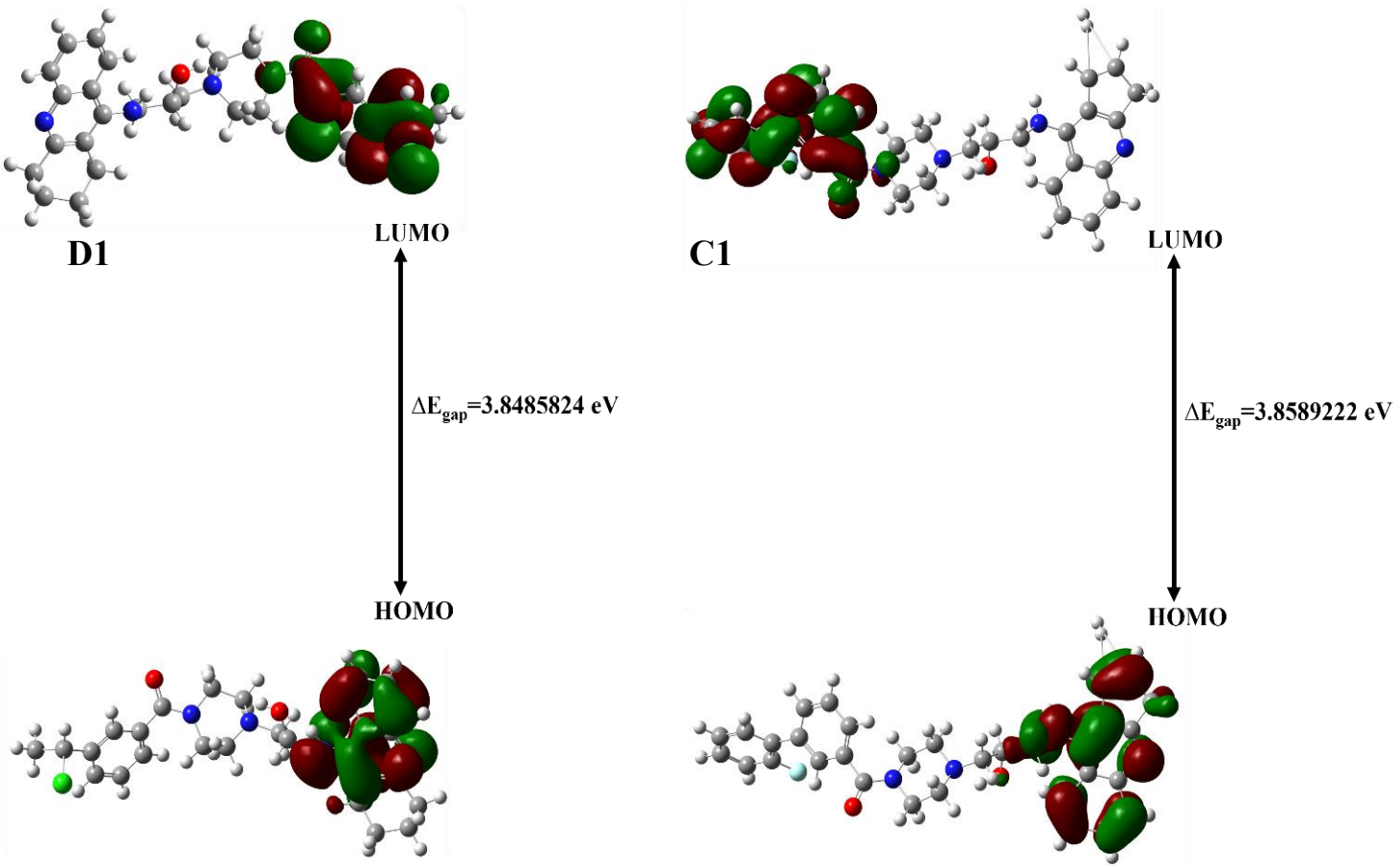


Figure 3: The geometries of the HOMO and LUMO orbitals, along with the value of the energy gap values.

Molecular electrostatic potential (MEP) maps of compounds D1 and C1 show how electrostatic charge is distributed across each molecule. They highlight potential sites for electrophilic and nucleophilic interactions. Red regions (electron-rich) are mainly located around nitrogen and oxygen atoms, indicating zones favourable to electrophilic attack. In contrast, blue regions (electron-poor) mark areas favourable to nucleophilic attack, typically near hydrogen atoms or areas of low electron density.

A key structural difference between the two compounds lies in their halogen substituents: D1 contains chlorine and C1 contains fluorine. Both halogens are positioned near coloured electrostatic surfaces and influence local polarity due to their electronegative nature. Yellow regions represent intermediate potentials and indicate moderately polar zones.
Despite the variation in halogens, both compounds exhibit broadly similar electrostatic profiles, with reactive regions associated with heteroatoms. However, the specific halogen present may affect the molecule's reactivity and binding interactions subtly.

The DOS analysis reveals significant electronic differences between C1 and D1 that help to explain their different bioactivities. While both compounds exhibit significant PDOS contributions near the frontier orbitals (−4 to 0 eV), D1 exhibits a slightly broader HOMO–LUMO gap (3.85 eV versus 3.84 eV), indicating greater thermodynamic stability and reduced inherent reactivity.
Fragments 2 and 3 in D1 show localised PDOS peaks near the HOMO region (~−5.6 eV), suggesting optimised orbital alignment for interaction with AChE. In contrast, C1 displays more dispersed PDOS signals, which is consistent with greater reactivity but reduced target specificity. Additionally, the deeper-energy PDOS fragment (1) of D1 (−16 to −8 eV) suggests better stabilisation of core electrons, which correlates with its superior metabolic stability in ADME predictions.
Together, these electronic features account for D1's stronger docking affinity of −11.7 kcal/mol compared to C1's −9.7 kcal/mol, as well as its enhanced drug-like profile, thereby reinforcing its potential as the more promising therapeutic candidate.

IV. Conclusion

This comprehensive computational study explored the potential of novel donepezil-tacrine hybrid derivatives as multifunctional agents for Alzheimer's disease (AD) treatment. By integrating advanced techniques such as 3D-QSAR modeling, molecular docking, ADME/Tox profiling, MM-GBSA binding free energy calculations, and DFT analyses, the research systematically evaluated the inhibitory potential, binding interactions, and drug-like properties of these compounds.

The 3D-QSAR model demonstrated a strong correlation (R^2 = 0.82) between molecular descriptors and biological activity (pIC_{50}), with steric (46.05%) and hydrophobic (23.96%) interactions identified as the primary contributors to AChE inhibition. The model's robustness was confirmed by cross-validation (Q^2 = 0.738), ensuring its reliability for predicting the activity of newly designed compounds. Guided by steric field analysis, twenty derivatives were rationally designed, with modifications focused on the R group to optimize binding affinity while maintaining compliance with Lipinski's Rule of Five.

Molecular docking revealed that compound D1 exhibited the highest binding affinity (-11.696 kcal/mol), forming critical interactions with AChE residues (TYR72, ASP74, and TRP286) and structural water molecules. This superior performance compared to the reference compound C1 (-9.674 kcal/mol) underscores D1's potential as a more effective AChE inhibitor.

ADME/Tox predictions further highlighted D1's favourable pharmacokinetic profile, including high intestinal absorption (88.85%), moderate BBB permeability (logBB = -0.589), and low toxicity risks (no AMES toxicity or hERG inhibition). MM-GBSA calculations reinforced these findings, with D1 exhibiting strong binding free energy (-52.43 kcal/mol), driven by van der Waals and lipophilic interactions.

DFT analyses provided additional insights into D1's electronic properties, revealing a HOMO-LUMO gap (3.85 eV) indicative of chemical stability. The MEP and DOS analyses identified key electrophilic and nucleophilic regions, further elucidating D1's interaction potential with biological targets.

In summary, this study identifies D1 as a promising lead compound for AD therapy, combining potent AChE inhibition, favourable drug-like properties, and low toxicity. Future work should focus on synthesizing and experimentally validating D1's efficacy in vitro and in vivo to advance its development as a multifunctional anti-AD agent. The integrated

computational approach employed here offers a robust framework for rational drug design, accelerating the discovery of novel therapeutics for complex neurodegenerative disorders

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General conclusion

This comprehensive study integrates computational and theoretical approaches to explore novel donepezil-tacrine hybrid derivatives as multifunctional agents for Alzheimer's disease (AD) treatment. By combining insights from neurodegenerative disease pathology (Chapter 1), advanced drug design methodologies (Chapter 2), and computational evaluations (Chapter 3), the research provides a robust framework for developing next-generation AD therapeutics. Below is a detailed synthesis of the key findings and their implications.

Alzheimer's disease is a multifactorial disorder characterized by amyloid-β plaques, neurofibrillary tangles, cholinergic dysfunction, and neuroinflammation. Current therapies, such as acetylcholinesterase (AChE) inhibitors (e.g., donepezil) and NMDA antagonists (e.g., memantine), offer symptomatic relief but fail to halt disease progression. The limitations of single-target therapies underscore the need for multifunctional agents that address AD's complex pathology. The design of donepezil-tacrine hybrids by Ningwei et al. represents a promising strategy, targeting AChE inhibition while mitigating oxidative stress and neuroinflammation.

The study employed a suite of computational tools to optimize drug design:

- ✓ QSAR Modeling: A 3D-QSAR model (R^2 = 0.82, Q^2 = 0.738) identified steric (46.05%) and hydrophobic (23.96%) interactions as critical for AChE inhibition, guiding the rational design of 20 derivatives.
- ✓ Molecular Docking: Compound D1 exhibited the highest binding affinity (-11.696 kcal/mol), forming key interactions with AChE residues (TYR72, ASP74, TRP286) and structural water molecules.
- ✓ ADME/Tox Profiling: D1 demonstrated favorable pharmacokinetics (88.85% intestinal absorption, logBB = -0.589) and low toxicity (no AMES mutagenicity or hERG inhibition), complying with Lipinski's and Veber's rules.
- ✓ MM-GBSA/DFT Analyses: D1's binding energy (-52.43 kcal/mol) and electronic properties (HOMO-LUMO gap = 3.85 eV) confirmed its stability and target specificity.

While computational results are promising, translational steps are essential:

- ✓ Experimental Validation: Synthesis and in vitro/in vivo testing of D1 to confirm AChE inhibition and safety.

- ✓ Disease-Modifying Potential: Investigations into D1's effects on amyloid-β aggregation and tau phosphorylation.
- ✓ Clinical Adaptability: Refinement of BBB permeability and metabolic stability for CNS delivery.

Finally, this work exemplifies the power of integrating computational drug design with neurodegenerative disease biology. By leveraging QSAR, docking, and ADME/Tox profiling, the study identifies D1 as a lead compound with balanced efficacy and safety. The methodologies outlined here not only advance AD research but also provide a template for tackling other complex diseases. Collaborative efforts between computational and experimental researchers will be pivotal in translating these findings into clinically viable therapies, addressing the urgent global need for effective AD treatments.