Design, Synthesis, And Biological Evaluation of Mechanism Based Potent AChE and BChE Inhibitors Exhibiting Multifunctional Properties For The Management of Alzheimer's Disease

Thesis submitted in partial fulfillment for the
Award of Degree

Doctor of Philosophy

By

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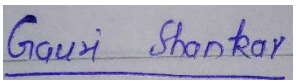

Date: 24/01/2025 Gauri Shankar

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List of Abbreviations

Abbreviations	Full forms
AD	Alzheimer's disease
ACh	Acetylcholine
*h*ACh	Human acetylcholine
MW	Molecular weight
MTT	3- (4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium
PAS	Peripheral anionic site
ROS	Reactive oxygen species
TMS	Tetramethylsilane
BChE	Butyrylcholinesterase
BBB	Blood-Brain Barrier
CAS	Catalytic active site
$CDCl_3$	Deuterated chloroform
DMSO-d_6	Deuterated dimethyl sulfoxide-d_6
DPZ	Donepezil
DTNB	5,5'-dithiobis-2-nitrobenzoic acid
DPPH	2,2-diphenyl-1-picrylhydrazyl
RIV	Rivastigmine
FA	Ferulic acid
MABs	Monoclonal antibodies
JNK	Janus kinase
HRMS	High-resolution mass spectrometry
HOBt	*N*-hydroxybenzotriazole
IC_{50}	The inhibitory concentration required to kill 50% of the population
IL-6	Interleukin-6
IL-β	Interleukin-beta
MMP	Mitochondrial membrane potential
TLR4	Toll-like receptor 4-Nucleotide-binding domain, leucine-rich containing
NLRP3	family, pyrin domain–containing-3
CAT	Catalase
MDA	Malondialdehyde
SOD	Superoxide dismutase

List of Symbols

Symbols	Meaning
α	Alpha
β	Beta
δ	Delta
ºC	Degree Celsius
Å	Angstrom
mg	Milligram
μg	Micro gram
μM	Micromole
mmol	Millimole
ml	Millilitre
μL	Microliter
h	Hour
s	Singlet
nm	Nanometer
µm	Micrometer
mm	Millimeter
cm	Centimeter
ppm	Parts per million
rpm	Revolutions per minute
Kcal	Kilocalories
Hz	Hertz
MHz	Megahertz
J	Coupling constant
d	Doublet
t	Triplet
q	Quartret
m	Multiplet
dd	Doublet of doublet
m/z	Mass-to-charge ratio
%	Percent
pH	Potential of hydrogen
≤	Less than or equal
<	Less than
>	More than
±	Plus or minus

Preface

Alzheimer's Disease (AD) is an age-related neurodegenerative disorder that accounts for over 80% of dementia cases in older adults worldwide. It is characterized by a low level of acetylcholine (ACh), an increase in oxidative stress, accumulation of metals, deposition of amyloid-beta (Aβ) plaques, and neurofibrillary tangles, leading to the progressive loss of memory and cognitive functions. Despite decades of research on the etiology of the disease and substantial efforts by the pharmaceutical industry to develop effective therapies, no treatment has been found to cure AD or significantly inhibit its progression. Currently, four drugs- donepezil (DPZ), galantamine, rivastigmine (RIV) (acting on the cholinergic pathway), and memantine (acting on the NMDA receptor) are approved by the USFDA. Aducanumab and Lecanemab are monoclonal antibodies (MABs) recently approved for treating AD. These drugs target amyloid-beta (Aβ) plaques, which are believed to play a central role in the pathophysiology of AD. As we know, AD is a multifactorial disease; therefore, targeting single drugs is insufficient for comprehensive treatment. Although combination therapies offer dosing flexibility, they pose considerable challenges, such as the risk of drug-drug interactions, complicated treatment protocols, and lower patient compliance. The development of multiple drugs substantially increases research and development costs. In the case of AD therapies, which encompass preclinical studies and clinical trials, these costs frequently surpass $2 billion, primarily due to the high attrition rates associated with drug development. In contrast, multifunctional ligands offer a more efficient therapeutic strategy by targeting multiple pathological pathways simultaneously, reducing the need for multiple agents. This approach not only simplifies clinical trial design and regulatory approval processes but also potentially lowers overall development costs, providing a more holistic solution to the multifactorial nature of AD.

Rivastigmine (RIV), a second-generation pseudo-irreversible cholinesterase inhibitor, is a widely used therapeutic agent for AD. However, several limitations restrict its efficacy as a comprehensive treatment. RIV shows selective inhibition of butyrylcholinesterase (BChE; IC_{50} = 91 ± 0.40 nM) while only moderately inhibiting acetylcholinesterase (AChE; IC_{50} = 6630 ± 0.76 nM), making it less effective in the earlier stages of AD where AChE activity predominates. Additionally, RIV provides symptomatic relief without targeting the pathological hallmarks of AD, such as oxidative stress, amyloid beta (Aβ), and tau protein aggregation, inflammation, and neurodegeneration. Its covalent mechanism of action through carbamylation of the enzyme's active site serine residue further limits its safety profile by causing pseudo-irreversible inhibition and potential off-target effects. To address these drawbacks, we designed two series of multifunctional molecules to enhance therapeutic efficacy.

The first series of molecules focused on improving RIV's limitations by replacing its amine group with aromatic and heterocyclic moieties to enhance interactions with AChE and BChE while modulating Aβ and tau aggregation. The carbamate moiety of RIV was replaced with amidoxime to enhance antioxidant properties and achieve a better balance between hydrophobicity and hydrophilicity. This series showed significant improvement in AChE inhibition, antioxidant properties, and modulation of Aβ and tau aggregation, but BChE inhibition remained suboptimal compared to RIV. In the second series, insights from the literature on carbamate-based selective BChE inhibitors were utilized to design potent inhibitors with additional therapeutic benefits. Substituted tryptamine fragments replaced the N-ethyl-N-methylamine group of RIV to improve multifunctional activity, including inhibition of AChE and BChE, antioxidant effects, and modulation of Aβ aggregation. Chirality was optimized by synthesizing the molecules' racemic and enantiomeric versions, with S-configured analogs

exhibiting superior potency. Compounds such as **15e** and **16e** demonstrated enhanced inhibitory activity and antioxidant and neuroprotective properties, offering a disease-modifying therapeutic approach. These rationally designed molecules aim to overcome the limitations of RIV and provide a comprehensive strategy to target multiple pathological aspects of AD.

The present study is divided into six chapters:

Chapter 1: This chapter overviews AD, including its pathophysiology and the current treatments.

Chapter 2: This chapter comprehensively reviews the literature on the relevant research on carbamate and urea hybrids reported in the last two decades for managing AD.

Chapter 3: This chapter outlines the research work's hypothesis, rationale, and detailed plan related to our proposed work.

Chapter 4: This chapter discusses the rationale for synthesizing and evaluating novel diphenylurea-amidine and carbamate-based tryptamine derivatives. The designed molecules were synthesized and subjected to *in-vitro* enzyme inhibition studies. The potent molecules identified from these studies were further investigated for enzyme kinetics, antioxidant activity, metal chelation, Aβ modulation, and neuroprotection. Lead compounds were then selected for *in-vivo* studies in AD animal models to evaluate their effects on working memory and learning.

Chapter 5: This chapter details the general synthetic procedures used for the targeted compounds and their subsequent biological evaluation.

Chapter 6: This chapter provides the final summary and conclusions drawn from the research.