Design, Synthesis, and Biological Evaluation of Some Heterocyclic Multifunctional Molecular Hybrids for Alzheimer's disease Therapy

Thesis submitted in partial fulfilment for the Award of Degree

DOCTOR OF PHILOSOPHY

By

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It is certified that the work contained in the thesis titled "**Design, Synthesis, and Biological Evaluation of Some Heterocyclic Multifunctional Molecular Hybrids for Alzheimer's Disease Therapy**" by **Mr. Digambar Kumar Waiker** has been carried out under my supervision and that this work has not been submitted elsewhere for a degree.

It is further certified that the student has fulfilled all the requirements of Comprehensive Examination, Candidacy, and SOTA for the award of Ph.D. Degree.

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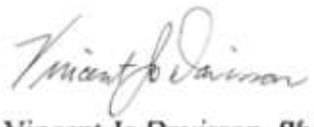

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DECLARATION BY THE CANDIDATE

I, **Digambar Kumar Waiker**, certify that the work embodied in this Ph.D. thesis is my own bonafide work, and was carried out by me under the supervision of **Prof. Sushant Kumar Shrivastava from July 2017 to June 2023** at the **Department of Pharmaceutical Engineering & Technology, Indian Institute of Technology (Banaras Hindu University), Varanasi.** The matter embodied in this Ph.D. thesis has not been submitted for the award of any other degree/diploma.

I declare that I have faithfully acknowledged and given credit to the research workers wherever their works have been cited in my work in this thesis. I further declare that I have not willfully copied any other's work, paragraphs, text, data, results, etc. reported in the journals, books, magazines, reports, dissertations, theses, *etc*., or available at websites and have not included them in this Ph.D. thesis and have not cited as my own work.

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ABBREVIATIONS AND SYMBOLS

ACh	Acetylcholine
AChE	Acetylcholinesterase
AD	Alzheimer's disease
AFM	Atomic force microscopy
AMP	Adenosine monophosphate
AMPK	AMP-activated protein kinase
AP	Anterior – Posterior
ApoE	Apolipoprotein E
Aβ	Amyloid beta
APP	Amyloid precursor protein
ATCI	Acetylthiocholine iodide
ATR	Attenuated total reflectance
BACE-1	Beta amyloid cleaving enzyme 1
BBB	Blood-brain barrier
BChE	Butyrylcholinesterase
BSA	Bovine serum albumin
BTCI	Butyrylthiocholine iodide
CADD	Computer-aided drug design
CaMK	Ca^{2+}/calmodulin dependent kinase
CAS	Catalytic anionic site
CAT	Choline acetyltransferase
$CDCl_3$	Deuterochloroform
CDK-5	Cyclin dependent kinase-5
ChE	Cholinesterase
CNS	Central nervous system
CoA	Coenzyme A
CoMFA	Comparative molecular field analysis
CoMSIA	Comparative molecular similarity indices analysis
COX	Cyclooxygenase
CREB	Cyclic-AMP-response element-binding protein
CST	Conjugated secondary antibody
CYP	Cytochrome P
DMSO	Dimethyl sulfoxide
DPP	Docking-post processing
DTNB	5,5-Dithio-bis-(2-nitrobenzoic acid
DV	Dorsal - Ventral
Dyrk1A	Dual specificity tyrosine- phosphorylation-regulated kinase-1 A
EDGs	Electron donating groups
EDTA	Ethylenediaminetetraacetic acid
ELT	Escape latency time
ERK	Extracellular signal-regulated protein kinases

EtOH	Ethanol
EWGs	Electron withdrawing groups
FDA	Food and drug administration
FRET	Fluorescence resonance energy transfer
FT-IR	Fourier-transform infrared spectroscopy
GSK-3β	Glycogen synthase kinase-3β
hAChE	Human AChE
hBChE	Human BChE
HPLC	High performance liquid chromatography
HTVS	High throughput virtual screening
i.p.	Intraperitoneal
ICV	Intracerebroventricular
JNK	Janus kinase
LBDD	Ligand-based drug design
MAO-B	Monoamine oxidase-B
MAPK	Mitogen activated protein kinase
MARK	Microtubule affinity-regulating kinase
MDA	Malondialdehyde
ML	Medial – Lateral
MM-GBSA	Molecular mechanics generalized Born surface area
mp	Melting point
MTT	3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide
Na-CMC	Sodium carboxymethyl cellulose
NADPH	Reduce form of nicotinamide adenine dinucleotide phosphate
NFI	Normalized fluorescence intensity
NFTs	Neurofibrillary tangles
NMDAR	*N*-Methyl-D-aspartate receptor
NMR	Nuclear magnetic resonance spectroscopy
NOS	Nitric oxide synthase
NOx	NADPH oxidase
NPT	Normality, pressure and temperature
OECD	Organisation for economic co-operation and development
OPLS	Optimized potential for liquid simulations
p.o.	Per oral
PAMPA	Parallel artificial membrane permeation assay
PAS	Peripheral anionic site
PBL	Porcine brain lipid
PBS	Phosphate buffered saline
PBST	Phosphate buffered saline with Tween 20
PDB	Protein data bank
PDE	Phosphodiesterase
PHFs	Paired helical fibrils

PI	Propidium iodide
PKA	Protein kinase-A
PPs	Protein phosphatases
PTPA	Protein tyrosine phosphatase-A
QSAR	Quantitative structure-activity relationship
RIPA	Radioimmunoprecipitation assay
RMSD	Root mean square deviation
RNS	Reactive nitrogen species
ROS	Reactive oxygen species
SBDD	Structure-based drug design
SFKs	Src family non-receptor tyrosine kinases
SOD	Superoxide dismutase
SP	Standard precision
TBARS	Thiobarbituric acid reactive substances
TBST	Tris-buffered saline with Tween 20
TLC	Thin layer chromatography
TPKI	Tau protein kinase-I
TRITC	Tetramethylrhodamine isothiocyanate
UV	Ultraviolet spectroscopy
VSGB	Variable surface generalized Born
WHO	World Health Organization
XO	Xanthine oxidase
XP	Extra precision
XRD	X-ray diffraction

SYMBOLS & UNITS

× g	Relative centrifugal force
°C	Degree Celsius
µL	Microliter
µm	Micrometer
µM	Micromolar
Å	Angstrom
α	Alpha
β	Beta
γ	Gamma
v	Wavenumber
cm	Centimeter
d	Doublet
dd	Doublet of doublets
ddd	Doublet of doublets of doublets
equiv	Equivalent
g	Gram

h	Hour
Hz	Hertz
J-value	Spin-spin coupling constant
K	Kelvin
kg	Kilogram
KHz	Kilohertz
m	Multiplet
mg	Milligram
min	Minute
mL	Milliliter
mM	Millimolar
ng	Nanogram
nm	Nanometer
q	Quartet
RH	Relative humidity
rpm	Rotations per minute
s	Seconds/Singlet
t	Triplet
td	Triplet of doublets
U/mL	Units per milliliter
v/v	Volume by volume
w/v	Weight by volume

PREFACE

Alzheimer's disease (AD) is considered to be an enormous healthcare problem caused by the loss of neurons and synapses, particularly in the neocortex and hippocampus. AD results in remarkable structural and functional damage to the brain, which resulted in severe behavioral alterations and cognitive dysfunction. A recent report 2018 from World Health Organization (WHO) accounts for 50 million cases of AD worldwide and this figure is estimated to be tripled by 2050. There are several underlying pathophysiology associated with the progression of cognitive deficits in AD condition that includes, lower acetylcholine (ACh) levels in the synaptic cleft, amyloid beta (Aβ) aggregation and deposition, *N*-methyl-D-aspartate receptor (NMDAR) activation, oxidative stress in response to neuroinflammation, tau hyperphosphorylation that results in generation of neurofibrillary tangles (NFTs), genetic mutation in apolipoprotein E4 (APOε4), etc.

The current treatment strategy for AD involves the use of some FDA-approved drugs which only provide symptomatic relief to the patient. Certain FDA-approved medications such as AChE inhibitors (donepezil, rivastigmine, and galantamine) and NMDA receptor antagonists (memantine) are being used for the treatment of AD. Recently, Aducanumab and Lecanemab (monoclonal antibodies) have been approved by FDA in an accelerated approval pathway as a disease-modifying therapy for AD, though their use is still controversial in AD progression.

This research work in this thesis was divided into two parts; the first part of the thesis describes the design, synthesis, and biological evaluation of a novel series-I **(SD1-17**) compounds against hAChE, hBChE, hBACE-1, and Aβ aggregation inhibitory potentials. A detailed *in vivo* study of the most active compound of the series was also performed in scopolamine and Aβ-induced behavioral rat models of AD following *ex-vivo* biochemical estimation, and histopathological examination of brain tissue slices to

observe any neuronal tissue damage. The *in silico* molecular docking and Molecular dynamic simulation studies were also performed to confirm the ligand-protein complex's stability.

The second part of the thesis demonstrates the successful design and synthesis of a new class of compounds using in silico e-Pharmacophore hypothesis. A novel series II & III were designed as multitarget-directed ligands to discover new agents for Alzheimer's disease treatment. All the compounds were tested for their *in vitro* inhibitory potential against hAChE, hBChE, hBACE-1, and Aβ aggregation. The neurotoxic liabilities of the compounds were also tested against RA/BDNF differentiated SH-SY5Y neuroblastoma cell lines. Both the scopolamine and A*β*-induced mouse models for AD were studied to evaluate the learning and memory behavior improvements after treatment with the most active compounds of the series. E*x vivo* studies of hippocampal and cortex brain homogenates were performed to investigate the oxidative stress biomarker and pro-inflammatory cytokines (TNF-α, and IL-6 mRNA) levels. The histopathological examination was performed to observe neuronal appearance in the hippocampal and cortex region of the brain. Western blot analysis and immunohistochemical analysis were also performed to investigate the A*β*, APP/A*β*, BACE-1, and tau protein molecular expression levels.

The work has been presented in this dissertation under the following sections:

Chapter 1: This chapter flashes the light on AD, its development & progression, etiology, and various pathophysiology associated with AD. This chapter also deals with the current treatment strategy for AD, a newer designing approach to tackle the disease progression that includes mainly molecular hybridization, and computational methods.

Chapter 2: This chapter describes a detailed literature survey on benzylpiperidines and 1,3,4-oxadiazoles, piperazine, and N-benzylpiperidine derivatives as multitarget directed ligands (AChE, BChE, BACE-1, and Aβ).

Chapter 3: This chapter summarizes the research objectives of the overall study, the rationale for performing different *in vitro* and *in vivo* investigations, and a detailed plan of work that is exemplified in this thesis.

Chapter 4: This chapter describes the experimental procedure used in the synthesis, characterization, protocols for computational studies, and *in vitro* and *in vivo* pharmacological evaluations.

Chapter 5: This chapter covers the overall findings as results and discussion part of the research work.

Chapter 6: Describes the summary and conclusion of the presented work.

Chapter 7: This section includes the references as a source of information to carry out the research work.

Chapter 8: An appendix consisting of the NMR (^{1}H and ^{13}C) and Mass spectra along with HPLC chromatograms of the representative compounds followed by a list of published papers and presentations at international conferences.