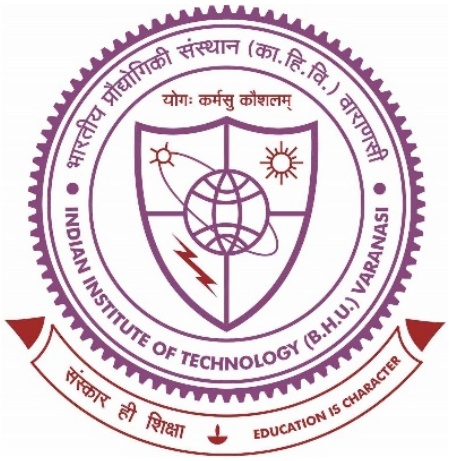
Design, Synthesis, and Biological Evaluation of Quinazoline Derivatives as Multi-targeting Agents in the Treatment of Alzheimer's Disease



Thesis submitted in partial fulfilment for the Award of Degree

DOCTOR OF PHILOSOPHY

By

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Date:

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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
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
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
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
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) has been identified to be a major health issue caused on by the degeneration of neurons and synapses, especially in the hippocampus and neocortex. AD results in anatomical and functional brain damage which causes severe behavioral changes and cognitive dysfunction. According to a 2018 World Health Organization (WHO) assessment, there are 50 million cases of AD globally, and by 2050, this number is expected to triple.

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 leads to the formation of neurofibrillary tangles (NFTs), genetic mutation in apolipoprotein E4 (APOε4), etc. are some of the underlying pathophysiologies linked to the progression of cognitive deficits in the AD condition.

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 of a novel series-I **(AV-1 to AV-21**) containing quinazoline derivatives based on the structure based drug design approach and biologically evaluated them against hAChE, hBChE, hBACE-1, and Aβ aggregation inhibitory potentials. The type

of enzyme inhibition was also estimated using enzyme kinetic study of the most potent compound against hAChE enzyme. The PAMPA-BBB and propidium iodide displacement assay were also carried out to check that the most promising compound was able to permeate the BBB barrier with significant PAS binding, respectively. The neurotoxic liabilities and neuroprotective properties of the compounds were also tested against RA/BDNF differentiated SH-SY5Y neuroblastoma cell lines. 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 of various oxidative biomarkers, and histopathological examination of brain tissue slices to observe any neuronal tissue damage. The molecular expression levels of Aβ, BACE-1, and APP were also estimated using western blotting and IHC analysis. The *in-silico* molecular docking, molecular dynamic simulation studies, and DFT studies were also performed to confirm the ligand-protein complex's stability and overall electronic properties of the active compound. The *in-vivo* BBB permeability and pharmacokinetic analysis of the active compound were also performed.

The second part of the thesis demonstrates the lead optimization based design of quinazoline derivatives (**AK-1** to **AK-14**) using bioisosteric and molecular hybridization approach. The designed series II were synthesized and biologically evaluated for their *in-vitro* inhibitory potential against hAChE, hBChE, hBACE-1, and Aβ aggregation. The neurotoxic liabilities and neuroprotective properties of the compound were also tested against RA/BDNF differentiated SH-SY5Y neuroblastoma cell lines. The A*β*-induced mouse model for AD was studied to evaluate the learning and memory behavior improvements after treatment with the most active compound of the series. *Ex-vivo* studies of hippocampal brain homogenates were performed to investigate the oxidative stress biomarker. The molecular expression levels of

Aβ and BACE-1 were also estimated using IHC analysis. The histopathological examination was performed to observe neuronal appearance in the hippocampal region of the brain. The *Drosophila* eye phenotypic AD model was also performed to check that the active compound possess anti-Aβ aggregation potential. The *in-vivo* BBB permeability and pharmacokinetic analysis of the active compound were also performed.

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

Chapter 1: The pathophysiology, development, and course of AD are briefly discussed in this chapter. It also covers the current treatment strategies for AD, more recent design approaches including computational and molecular hybridization approaches that focuses mostly on to slow the progression of the AD.

Chapter 2: This chapter describes a detailed literature survey on N-benzylpiperidines, benzylpiperzines, quinazoline derivatives, drugs under clinical investigations, and FDA approved drugs against AD.

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.