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It is certified that the work contained in the thesis titled "**Design, Synthesis, and Biological Evaluation of Multitargeted *N*-Benzylpiperidine Analogs for the Treatment of Alzheimer's Disease**" by **Mr. Piyoosh Sharma** has been carried out under my supervision, and that this work has not been submitted elsewhere for a degree.

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DECLARATION BY THE CANDIDATE

I, **Piyoosh Sharma**, certify that the work embodied in this Ph.D. thesis is my own bonafide work, and carried out by me under the supervision of **Prof. Sushant Kumar Shrivastava from July 2015 to December 2019** 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.

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ABBREVIATIONS & 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
ESI	Electrospray ionization
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
MnSOD	Manganese superoxide dismutase
mp	Melting point
MPO	Myeloperoxidase
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
AUC	Area under curve
cm	Centimeter
C_{max}	Maximal plasma concentration
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
MRT	Mean residence time
N/m	Newton per meter
ng	Nanogram
nm	Nanometer
ppm	Parts per million
q	Quartet
RH	Relative humidity
rpm	Rotations per minute
s	Seconds/Singlet
t	Triplet
$t_{1/2}$	Elimination half-life
td	Triplet of doublets
T_{max}	Time to reach maximum plasma concentration
U/mL	Units per milliliter
v/v	Volume by volume
w/v	Weight by volume

PREFACE

Alzheimer's disease (AD) is an enormous healthcare burden caused by loss of neurons and synapses, particularly in neocortex and hippocampus. AD causes substantial structural and functional damage of the brain, which resulted into 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 pathophysiological mechanisms involved in the progressive cognitive deficits of AD such as, decline in acetylcholine (ACh) levels, amyloid beta (Aβ) aggregation and deposition, activation of *N*-methyl-D-aspartate receptor (NMDAR), oxidative stress, tau hyperphosphorylation and generation of neurofibrillary tangles (NFTs), apolipoprotein E4 (APOε4) gene transcription, cyclic-AMP-response element-binding protein (CREB) signaling pathways, etc.

Current treatment strategies for AD encompass the use of FDA approved medications like cholinesterase (ChE) inhibitors (donepezil, rivastigmine, and galantamine) and N-methyl-D-aspartate (NMDA) receptor antagonist (memantine). However, drug therapy for this ailment is still in its infancy and fails to delay the progression of the disease. The new strategy of multi-targeted inhibitors has been adopted recently, which involves targeting multiple enzymes simultaneously with a single molecule. This strategy was built upon the fact that AD is a multifactorial disorder, linked with multiple targets. Thereby, design and development of multitarget-directed ligands could be successful for mitigating the disease progression rather than providing symptomatic relief only.

This research work is divided into two parts. The first part deals with the designing of Series I ligands based upon computational methods, such as pharmacophore modeling, high-throughput virtual screening (HTVS), docking-post processing (DPP), molecular

mechanics generalized Born surface area (MM-GBSA), molecular docking, and dynamics simulations. The designed series of ligands were evaluated for their multitargeted inhibitory potential against acetylcholinesterase (AChE), butyrylcholinesterase (BChE), beta secretase-1 (BACE-1), and Aβ. Compounds were also assessed for their binding capability with peripheral anionic site (PAS) of AChE by propidium iodide displacement assay and blood-brain barrier (BBB) penetrability was predicted by PAMPA model. The neurotoxic liability of compounds was evaluated against SH-SY5Y neuroblastoma cell lines by MTT assay. The neurobehavioral studies in rats were performed to evaluate the effect of compounds in improving the learning and memory. Moreover, pharmacokinetic study was performed to determine the oral absorption characteristics of lead candidates.

The second part of the thesis work involved designing Series II-V compounds using a molecular hybridization approach on the basis of outcomes from Series I. The compounds were evaluated by several *in vitro* and *in vivo* studies to be established as potential multitargeted ligands for the treatment of AD. Additionally, molecular mechanistic analyses were performed by Western-blot and immunohistochemistry.

To this date, the research work has led to the publication of two research papers and two filed Indian Patents. Suggestions and comments on the part of the readers are always welcome.

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

Chapter 1: The first chapter offers an introductory section which deals with a basic information along with the historical background, pathophysiological mechanisms involved, and current therapeutics available for the treatment of AD. A brief discussion

about novel strategies adopted for the AD, such as multitargeting, computational methods, and molecular hybridization.

Chapter 2: This chapter focused on detailed literature survey on cholinesterase (AChE and BChE), BACE-1 and Aβ inhibitory potential of the compounds bearing *N*-benzylpiperidines and 1,3,4-oxadiazoles.

Chapter 3: This chapter summarizes the research objectives, the overall rationale for carrying out this investigation and plan of work as embodied 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 results and discussion part of the research work.

Chapter 6: This chapter outlines the summary and conclusion.

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, filed patents, and presentations at international conferences.