Discovery and Characterization of Small Molecule Inhibitors of Choline Acetyltransferase

Thesis submitted in partial fulfillment for the
Award of Degree of

Doctor of Philosophy

By

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LIST OF ABBREVIATIONS

Abbreviation	Full form
AD	Alzheimer's Disease
WHO	World Health Organization
GBDS	Global Burden of Disease Study
SDI	Socio-Demographic Index
FAD	Familial AD
APP	Amyloid Precursor Protein
PS1	Presenilin 1
PS2	Presenilin 2
SAD	Sporadic AD
GWAS	Genome-Wide Association Studies
Aβ	Amyloid Beta
NFTs	Neuro Fibrillary Tangles
ACh	Acetylcholine
BFCNs	Basic Forebrain Cholinergic Neurons
NGF	Nerve Growth Factor
NAChR	Nicotinic Acetylcholine Receptors
AChE	Acetylcholinesterase
CNS	Central Nervous System
CTFs	α- or β-C Terminal Fragments
C99	C-terminal fragment of APP
AICD	APP Intracellular Domain
PHFs	Paired Helical Filaments
SFs	Straight Filaments
PET	Positron Emission Tomography
MRI	Magnetic Resonance Imaging
CSF	Cerebrospinal Fluid
MCI	Mild Cognitive Impairment
CI	Confidence Interval
FDG	Fluorodeoxyglucose
FDA	Food and Drug Administration
MAO-B	Monoamine Oxidase B
BBB	Blood Brain Barrier
acetyl-CoA	Acetyl-Coenzyme A
VAChT	Vesicular Acetylcholine Transporter
PDB	Protein Data Bank
NVP	Naphthylvinylpyridine
CADD	Computer Aided Drug Design
LBDD	Ligand-Based Drug Design
SBDD	Structure-Based Drug Design
MD	Molecular Dynamics
QSAR	Quantitative–Structure–Activity Relationship

Abbreviation	Full form
ADMET	Absorption, Distribution, Metabolism, Excretion, Toxicity
PK	Pharmacokinetic
AI	Artificial Intelligence
MTDLs	Multi-Target Directed Ligands
PAS	Peripheral Anionic Site
CAS	Catalytic Anionic Site
MIC	Minimum Inhibitory Concentration
PPIs	Proton Pump Inhibitors
BChE	Butyrylcholinesterase
ChAT	Choline Acetyltransferase
α-NETA	2-(α-naphthoyl) ethyltrimethylammonium iodide
RMSD	Root Mean Square Deviation
RMSF	Root Mean Square Fluctuation
RoG	Radius of Gyration
HBN	Hydrogen Bond Number
HBD	Hydrogen Bond Distance
PCA	Principal Component Analysis
FEL	Free-Energy Landscape
SASA	Solvent Accessible Surface Area
RIN	Residue Interaction Network
MM-PBSA	Molecular Mechanics Poisson−Boltzmann Surface Area
ΔG	Binding free energy
XP	Extra Precision
i.v.	Intra Venous
P.O.	Per Oral
HPLC	High Performance Liquid Chromatography
SD	Standard Deviation
HRMS	High-Resolution Mass Spectra
ALS	Amyotrophic Lateral Sclerosis
LBD	Lewy Body Disorder
MM/GBSA	Molecular Mechanics General Born Surface Area
K_m	Michaelis-Menten constant
V_{max}	Maximal Velocity
DMEM	Dulbecco’s Modified Eagle’s medium
FBS	Fetal Bovine Serum
DNN	Deep Neural Network
AI-DD	AI-assisted deep docking
ROC	Receiver Operating Characteristic
PC	Principal component
PDF	Probability density function

PREFACE

Alzheimer's Disease (AD) is one of the most predominant neurodegenerative diseases which accounts for 60 to 80 % cases of dementia, affecting the cerebral cortex and hippocampus of the brain ccharacterized by cognitive decline, memory impairment, and behavioral alterations. A common denominator in such neurodegenerative diseases is the degeneration of the neuronal cholinergic system. Such neurodegenerative disease alone constitutes major challenges and tremendous unmet needs, in term of effective tools for clinical and/or research purposes. This is perhaps one of the reasons why despite the intensive search for the past half century only symptomatic treatments are currently available. Nonetheless, AD is a complex multifactorial disease, making it highly challenging to find a cure.

Cholinergic hypotheses in AD, is linked with the decline in the key cholinergic neurotransmitter, acetylcholine. Choline Acetyltransferase (ChAT) is the enzyme responsible for the biosynthesis of acetylcholine and the maintenance of ChAT expressions is essential for proper neuronal function and overall health of the brain and the body, and disruption in its expression or function can have detrimental effects on both mental abilities and neurotransmitter balance and motor controls. Thereby, it is one of the potential targets for development of biomarkers for monitoring the health of the cholinergic neurons in the central and peripheral nervous system for early-stage diagnosis. In addition, detecting early bio-signature changes of AD should help to halt the progression by suitable therapeutics.

My journey through the corridors of academia has led me to embark on a profound exploration of novel Piperidine based derivatives as potential ChAT inhibitors. The pages that follow document the culmination of years of rigorous research, investigation, and discovery. The work encapsulated herein is divided into four comprehensive studies, each a distinctive facet of the overarching endeavor to unlock the mysteries of AD and advance diagnostic and theranostic interventions.

Study I (**Chapter 3**) Unravels the crucial binding mode mechanism of the proton pump inhibitors to the ChAT binding tunnel, by employing a series of computational tools, namely molecular docking and classical molecular dynamics, followed by the calculation of binding free energies using MMPBSA. Additionally, it was observed that the pyridine ring of the PPI's predominantly interacts with the catalytic residue HIS324. As a major factor for the onset of Alzheimer's disease is linked to cholinergic dysfunction, our present findings give clear insight into the PPI's interaction with ChAT.

In Study II (**Chapter 4**), we embark on to explore the novel piperidine scaffold of the previously identified hit compound B4 from our lab with an objective to simplify the structure, thus, here we synthesized and characterized fifty-two piperidine-based amide derivatives of high purity. These compounds underwent rigorous evaluation for their ability to inhibit ChAT, and also evaluated for its selectivity by screening them against two off-targets AChE and BuChE. Compound A1 emerges as a promising lead, distinguished by its potent and selective inhibition of ChAT in vitro along with good solubility. In vivo pharmacokinetic and brain kinetic studies in rat indicated optimum pharmacokinetic parameters along with good brain permeability. Molecular docking and dynamics simulations reinforce the favorable interaction of A1 with ChAT binding tunnel indicating its interaction with the catalytic residue HIS324.

Study III (**Chapter 5**) We aimed to explore a large chemical space in search for novel scaffolds as potential ChAT inhibitors. Here, we have successfully utilised structure-based virtual screening approach to screen a VITAS-M small molecule library containing ~1.4 million compounds by using a structure-based virtual screen protocol based on MPI-Vina. Identifying 46 top performing hits displaying prominent interaction with the catalytic residue HIS324. The compounds were procured and were then subjected to rigorous in vitro characterization which led to the identification of two novel, selective and potent ChAT inhibitors V6 and V15, having good aqueous solubility and no toxicity. Molecular docking and dynamics simulations revealed

the intricate interaction dynamics for V6 and V15 with ChAT binding pocket. the Tanimoto similarity analysis indicated the novelty and structural diversity of the hits.

Study IV (**Chapter 6**) employs an innovative AI-assisted structure-based virtual screening tool, Deep Docking, to explore an ultra-large library of 1.3 billion compounds from the ZINC database. The virtual screening process, coupled with rigorous filtering and prioritization, which yielded a selection of potential ChAT inhibitors. The top five candidates, CPD1, CPD2, CPD3, CPD4 and CPD5 identified through molecular dynamics simulations and MMPBSA binding free energy calculations, represent promising avenues for further exploration as potential development of diagnostic biomarker in the quest to combat AD.

In conclusion, this thesis signifies a pioneering effort in the pursuit of novel derivatives as selective inhibitors of ChAT. Our synthesis endeavors have yielded compounds of substantial yield and high purity, marked by potent activity against ChAT. In vivo pharmacokinetic studies indicated optimum parameters and good brain permeability. Moreover, traditional as well as an innovative AI-assisted virtual screening initiatives has unearthed a trove of potentially novel ChAT inhibitors, offering a glimpse into the future of AD diagnostics.

This research work represents not only a significant contribution to the field of neurodegenerative disease research but also a testament to the relentless pursuit of knowledge and solutions that drive scientific inquiry. I extend my heartfelt appreciation to my mentors, colleagues, and supporters who have accompanied me on this intellectual journey. May the insights and discoveries contained within these pages inspire further exploration, ignite new avenues of research, and ultimately contribute to the global effort to combat AD and related neurodegenerative conditions.

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