Chapter 2

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Research Envisaged

Neurodegenerative diseases are a collective group of pathological conditions negatively affecting the central and peripheral nervous systems, leading to the progressive loss of neuronal structures and its function causing neuronal cell death. 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. 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.

The cholinergic hypothesis is one of the earliest and most significant theory related to the pathogenesis of the AD. ACh is the key cholinergic neurotransmitter associated with the learning, memory and other cognitive functions. The brains of AD patients exhibit severe neurodegeneration and decline in cholinergic neurons which ultimately leads to the severe deficiency of ACh. This is primarily due to the significant downregulation of the ChAT activity in the AD affected patients. 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.

Recently, Proton pump inhibitors (PPIs) have been reported to inhibit ChAT, suggesting evidences, that Long-term PPI use may increase the risk of developing AD. Given the strong evidence, initially (**Chapter 3**) our research objective was to understand the mechanism of binding of PPIs in the ChAT binding tunnel, we have employed a series of computational tools, namely molecular docking and classical molecular dynamics to gain a mechanistic understanding of the molecular interactions between PPIs and the binding pocket of ChAT. Enabling the elucidation of protein-ligand complexes binding interactions, conformational stability, and dynamic evolution within a time frame of 200 nanoseconds. Further, the binding free energies for the complexes under investigation were calculated using Molecular Mechanics Poisson-Boltzmann Surface Area. The findings indicate that the PPI's have comparable or greater binding affinity to the ChAT catalytic tunnel in comparison to the standard compound α-NETA. Additionally, it was observed that the pyridine ring of the PPI's predominantly interacts with the catalytic residue His324. Moreover, the free energy landscape analysis showed that the folding process was linear, and the residue interaction network analysis provided insight into the roles of various amino acid residues in stabilization of the PPIs in the ChAT binding pocket. As a major factor for the onset of AD is linked to cholinergic dysfunction, our previous and the present findings give clear insight into the PPI's interaction with ChAT.

Simultaneously, we embarked on the synthesis and exploration (**Chapter 4**) of novel piperidine scaffold of the previously identified hit compound B4 from our lab with an objective to simplify the structure to improve its selectivity towards ChAT and also possess better BBB permeability, synthesizing 52 novel derivatives. The compounds were tested in-vitro for their ChAT inhibitory activity and were found to be selective potent inhibitors of ChAT. Which led to the identification of a potent hit compound A-1, which was found to have no toxicity and possess good aqueous solubility. In vivo pharmacokinetic study suggested optimum pharmacokinetic

parameters along with good BBB permeability Moreover, in-silico including molecular docking and molecular dynamics simulation of 200 ns supported the favorable interaction of the compound with the HIS324 amino acid residue and formed a stable complex during the simulation.

These promising findings motivated us to further explore large chemicals database for identifying novel scaffolds as potential ChAT inhibitors. In **Chapter 5**, we explored a 1.4 million compounds library by employing traditional structure based virtual screening methodology. The identified virtual hits were procured and tested in vitro for ChAT inhibitory activity, where two novel hits V6 and V15 emerged to the top displaying selective potent ChAT inhibition, as well as had no cytotoxicity and possessed good aqueous solubility. We also performed a 200 ns molecular dynamics simulation, which 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.

Recognizing the limitations of traditional virtual screening methodology, which can be both time-intensive as well as computational resource intensive, in **Chapter 6** we streamlined our efforts by screening an ultra-large dataset of 1.3 billion compounds using the AI-assisted Deep Docking (DD) structure based virtual screening protocol in search for more novel hits as potential ChAT inhibitors. DD platform leverages the power of DNN-based virtual screening, empowering researchers to dock billions of molecules in a speedy, yet explicit manner. Where a subset of the compound's library is sampled and docked using MPI-Vina and then a QSAR model is built upon the resultant docking scores obtained with the 2D fingerprints of the sampled compounds, which is then used to predict the docking scores of the rest of the compounds in the library. The process is repeated for 'n' number of iterations until the library has been reduced to manageable size. Upon execution of the DD protocol, we finally identified five novel hit molecules as potential inhibitors of ChAT. Furthermore, to understand the

dynamic behavior of the compounds in the ChAT binding tunnel we performed molecular dynamics simulations for 200 ns on the complexes, which suggested that the formed complexes are stable and are compatible with the ChAT binding tunnel. We further extended our analysis to identify the per residue H-bond interactions taking place where it was observed that the compounds were forming H-bond interactions with the catalytic residue HIS324 throughout the simulation, which indicated the compounds can inhibit ChAT. The discovery of novel ChAT inhibitors will enable researchers to develop new probes that can be used as novel theranostic agents against cancer and as an early-stage diagnostics for onset of AD, for timely therapeutic intervention to halt the further progression of AD.