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Mechanistic Insight into the Inhibition of Choline Acetyltransferase by Proton Pump Inhibitors

Anurag T. K. Baidya, Bhanuranjan Das, Bharti Devi, Bengt Långström, Hans Ågren, Taher Darreh-Shori, and Rajnish Kumar*

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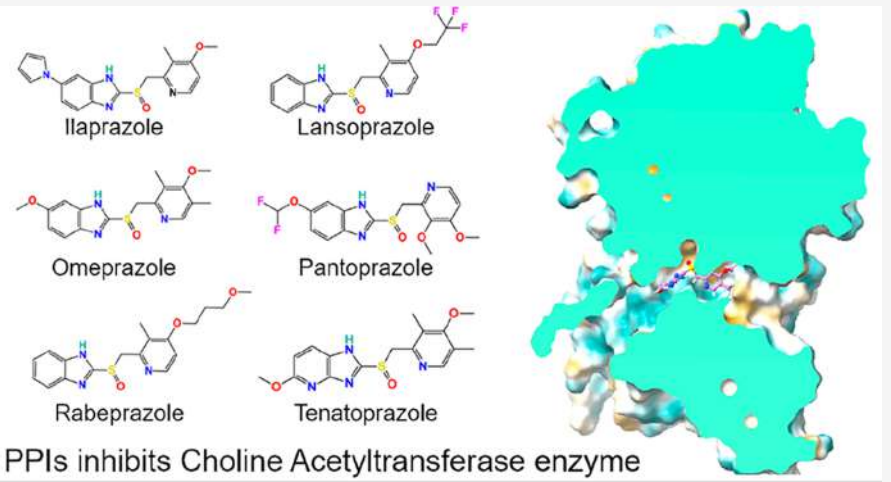

ABSTRACT: Various pharmacoepidemiological investigational studies have indicated that Proton Pump Inhibitors (PPIs) may increase the likelihood of developing Alzheimer's disease (AD) and non-AD related dementias. Previously, we have reported the inhibition of the acetylcholine biosynthesizing enzyme choline acetyltransferase (ChAT) by PPIs, for which omeprazole, lansoprazole, and pantoprazole exhibited IC_{50} values of 0.1, 1.5, and 5.3 μM, respectively. In this study we utilize a battery of computational tools to perceive a mechanistic insight into the molecular interaction of PPIs with the ChAT binding pocket that may further help in designing novel ChAT ligands. Various in-silico tools make it possible for us to elucidate the binding interaction, conformational stability, and dynamics of the protein−ligand complexes within a 200 ns time frame. Further, the binding free energies for the PPI−ChAT complexes were explored. The results suggest that the PPIs exhibit equal or higher binding affinity toward the ChAT catalytic tunnel and are stable throughout the simulated time and that the pyridine ring of the PPIs interacts primarily with the catalytic residue His_{324}. A free energy landscape analysis showed that the folding process was linear, and the residue interaction network analysis can provide 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 Alzheimer's disease is linked to cholinergic dysfunction, our previous and the present findings give clear insight into the PPI interaction with ChAT. The scaffold can be further simplified to develop novel ChAT ligands, which can also be used as ChAT tracer probes for the diagnosis of cholinergic dysfunction and to initiate timely therapeutic interventions to prevent or delay the progression of AD.

KEYWORDS: *proton pump inhibitors, choline-acetyltransferase, Alzheimer's disease, dementia, neurodegenerative disorder, molecular docking, molecular dynamics, MM-PBSA, PET-ligands*

INTRODUCTION

Alzheimer's disease (AD), discovered in 1906,[1] is one of the most common forms of dementia and leads to slow progressive deterioration of cognitive, intellectual, and social functions that can ultimately lead to memory impairment of the patient and gradually to complete loss of independence in their daily living activities.[2] The definitive diagnosis of AD can be accomplished post-mortem, where on examining the brain tissue character-

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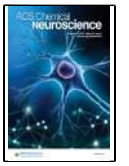

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Discovery and characterization of novel pyridone and furan substituted ligands of choline acetyltransferase

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ABSTRACT

The key to the management of two devastating diseases, namely Alzheimer's Disease (AD) and Amyotrophic Lateral Sclerosis (ALS) lies in an early diagnosis, which is difficult due to its multifactorial nature. However, a common hallmark of AD and ALS is degeneration of cholinergic system. Choline acetyltransferase (ChAT) has been proposed as a potential target for development of cholinergic-specific biomarker. However, lack of selective, potent, brain permeable molecular probes of ChAT hinder development of ChAT biomarkers. In this study, we have successfully utilised structure-based virtual screening approach and identified two ChAT inhibitors from a database of 1.4 million compounds. The compounds were then subjected to rigorous *in vitro* characterization. Compound V6 showed K_i value of 11 μM and IC_{50} value of 21.73 μM, while V15 showed K_i and IC_{50} values of 4.5 and 9.42 μM, respectively for ChAT enzyme. V6 and V15 showed good solubility of 0.21 mg/mL and 0.17 mg/mL respectively and cytotoxicity analysis indicated no toxicity. We also performed a 200 ns molecular dynamics simulation, which revealed the intricate interaction dynamics for V6 and V15 with ChAT binding pocket. Moreover, the Tanimoto similarity analysis indicated the novelty and structural diversity of the hits. In conclusion, these validated hits provide a platform to develop potent, selective, blood-brain barrier permeable small molecules as chemical probes of ChAT or as Positron Emission Tomography tracer for early diagnosis and/or in vivo monitoring of the effect of new therapeutic candidates in spectrum of neurodegenerative disorders, in which cholinergic deficit is one of the hallmarks.

1. Introduction

Neurodegenerative diseases are a group of pathological conditions negatively affecting the central nervous system (CNS) and peripheral nervous system (PNS), leading to the progressive loss of neuronal structures and its function causing neuronal cell death (DeTure and Dickson, 2019). Alzheimer's Disease (AD) is one of the most predominant neurodegenerative diseases which accounts for 60–80 % cases of dementia, affecting the cerebral cortex and hippocampus of the brain (Andrade-Guerrero et al., 2023). Amyotrophic Lateral Sclerosis (ALS) is another neurodegenerative disorder and a common denominator among these two neurodegenerative diseases is the degeneration of the neuronal cholinergic system, which in case of AD occurs in central cholinergic system, while in ALS the upper and lower motor neurons, and eventually the cholinergic cranial nerves (forming the parasympathetic nervous system) and their interfaces at the neuromuscular sites.

These two neurodegenerative diseases alone constitute 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, both diseases are complex multifactorial diseases, making it highly challenging to find a cure (Melnikova, 2007; Moloney et al., 2014; Theleritis et al., 2019).

Cholinergic hypotheses exist for both diseases. In AD, the decline in the key cholinergic neurotransmitter, acetylcholine, has been observed (Du et al., 2018). In ALS, the "dying-back" hypothesis suggest ALS is a distal axonopathy, where pathological changes occur prior to motor

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AI-Enabled Ultra-large Virtual Screening Identifies Potential Inhibitors of Choline Acetyltransferase for Theranostic Purposes

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Anurag T. K. Baidya, Abhinav Kumar Goswami, Bhanuranjan Das, Taher Darreh-Shori, and Rajnish Kumar*

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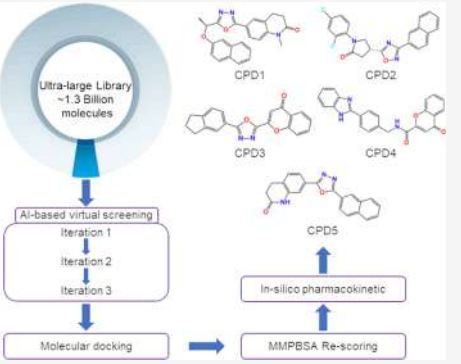
ABSTRACT: Alzheimer's disease (AD) and related dementias are among the primary neurological disorders and call for the urgent need for early-stage diagnosis to gain an upper edge in therapeutic intervention and increase the overall success rate. Choline acetyltransferase (ChAT) is the key acetylcholine (ACh) biosynthesizing enzyme and a legitimate target for the development of biomarkers for early-stage diagnosis and monitoring of therapeutic responses. It is also a theranostic target for tackling colon and lung cancers, where overexpression of non-neuronal ChAT leads to the production of acetylcholine, which acts as an autocrine growth factor for cancer cells. Theranostics is a hybrid of diagnostics and therapeutics that can be used to locate cancer cells using radiotracers and kill them without affecting other healthy tissues. Traditional virtual screening protocols have a lot of limitations; given the current rate of chemical database expansion exceeding billions, much faster screening protocols are required. Deep docking (DD) is one such platform that leverages the power of deep neural network (DNN)-based virtual screening, empowering researchers to dock billions of molecules in a speedy, yet explicit manner. Here, we have screened 1.3 billion compounds library from the ZINC20 database, identifying the best-performing hits. With each iteration run where the first iteration gave ∼116 million hits, the second iteration gave ∼3.7 million hits, and the final third iteration gave 168,447 hits from which further refinement gave us the top 5 compounds as potential ChAT inhibitors. 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 early-stage diagnostics for the onset of AD, for timely therapeutic intervention to halt the further progression of AD.



KEYWORDS: *choline acetyltransferase, Alzheimer's disease, neurodegenerative disorder, deep docking, structure-based virtual screening, PET ligands*

INTRODUCTION

Alzheimer's disease (AD), a neurodegenerative disorder distinguished by memory and cognitive loss, is a major cause of dementia in senile people worldwide.[1,2] The pathophysiological expression of such disorders is often reflected in neurochemical and structural neural networks along with "cholinergic system" dysfunction, eventually leading to failure.[3] Due to the primary association with the cholinergic system, where the key cholinergic neurotransmitter acetylcholine is broken down by the acetylcholinesterase (AChE), which is primarily found at the postsynaptic neuromuscular junctions.

Despite intensive research during the last half of the century, no effective therapeutic interventions are available to completely cure AD; only symptomatic treatment is available.[4,5] Choline acetyltransferase (ChAT) is a globular protein and the key cholinergic enzyme responsible for acetylcholine (ACh) biosynthesis, which plays a key role in the transmission of nerve impulses. ChAT catalyzes the transfer of the acetyl moiety from acetyl-coenzyme A to a choline molecule with the help of the His324 residue.[6−8] ChAT has been known to researchers for a long time; however, many mysteries revolving around the enzyme remain unanswered due to the lack of availability of suitable and selective ligands for ChAT.

One of the major challenges in neurodegenerative disorders like AD is the tremendous unmet clinical need to find newer and more precise diagnostic techniques. Prominent examples are the current positron emission tomography (PET) tracers toward the brain deposits of amyloid-β peptides (Aβ plaques)

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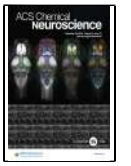

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