Enzymatic Targets for Drug Discovery Against Alzheimer's Disease

Editors:
Dileep Kumar
Prashant Tiwari

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Edited by

Dileep Kumar
Department of Pharmaceutical Chemistry
Poona College of Pharmacy, Bharti Vidyapeeth
Pune, 411038, India
Department of Entomology and Nematology
UC Davis Comprehensive Cancer Center
University of California Davis
CA 95616, USA

&

Prashant Tiwari
Department of Pharmacology
College of Pharmaceutical Sciences
Dayananda Sagar University
Bengaluru, 560111, Karnataka,
India

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Editors: Dileep Kumar & Prashant Tiwari

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FOREWORD

It is my pleasure to write the foreword for this edited book titled “Enzymatic Targets for Drug Discovery against Alzheimer's Disease” by Dr. Dileep Kumar and his editorial team, which focuses on novel therapeutics and strategies for the treatment and/or management of Alzheimer's disease.

The book summarizes the role of multiple targets and strategies to design and develop novel drug candidates against both established and new drug targets *viz.*, Tau, TREM, and Microglia.

The book highlights the current information in order to unravel the origin, pathogenesis and prevention of AD. The purpose of this book is to capture and discuss improvements in both the diagnosis and potential treatment of AD by established and novel strategies. It brings researchers across the globe having a different scientific interests and expertise under one roof to specifically focus on AD.

The book will benefit not only students but researchers and avid readers around the globe to devour the nuances of AD in a simple yet lucid manner.

I wish all success to the editor Dr. Dileep Kumar and his team for their endeavor to fulfill this assiduous task. I sincerely believe that the book has been prepared with scientific skills and experimental details and will provide useful information to researchers.

Sushil K. Singh
Department of Pharmaceutical Engineering & Technology
IIT(BHU), Varanasi
India

PREFACE

Alzheimer's disease (AD) is a progressive, degenerative, and often fatal neurological disorder. This disorder is clinically attributed to a progressive loss of memory and cognitive functions, eventually culminating in difficulty in carrying out the simplest of tasks. Currently, AD is ranked as the sixth leading cause of death in the United States but is projected to be ranked third, just after cardiovascular diseases and cancer. In 2020, the total payments for healthcare, long-term and hospice care for people aged 65 years and above, amounted to around $305 billion, raising a grave public health concern. Despite epidemiological research statistics directed towards a global Alzheimer's epidemic, the current physician's armamentarium comprises only treatments that provide brief symptomatic relief to the patients.

The prime intent of the book titled, 'Enzymatic Targets for Drug Discovery against Alzheimer's Disease', is to present a comprehensive approach to AD sheerly based on molecular, clinical, and translational research, while addressing the novel targets and recent research in the progression, development, and prevention of AD. However, all the broad multi-disciplinary research in AD would be beyond the purview of the book. Instead, the book would shed light on the various novel mechanistic pathways in the development, diagnosis, and prevention of AD, which would certainly be thoroughly studied and established in the years to come. These chapters address thought-provoking and challenging ideas that could be of great interest to those, both in and out of the AD sector. It is gratifying to receive 15 exceptional contributions that skillfully craft the current book.

Globally, researchers have been relentlessly working to develop treatments and therapeutics to curb the risk and danger inflicted by AD. This has facilitated the unfolding of the byzantine signaling pathways and several contributing factors that form the basis of sporadic AD. The revelation of the latter has engaged multidisciplinary research converging from genetics, pharmacogenomics, proteomics, cell signaling and metabolomics, human nutrition, and physical education, to list a few. The most noteworthy grounds of research include the mechanisms of amyloid plaque depositions, the natural mechanism of deposited amyloid- β plaques (Aβ), and employing anti-Aβ antibodies. However, a deeper understanding of byzantine pathways precedes the development of newer drug molecules and treatment regimens. This would result in the formulation and development of novel drugs for the treatment of AD, and alleviating the symptoms associated with the latter.

This book comprises 15 chapters written by a group of eminent experts in various facets of AD research. These chapters elucidate the recent advances, observations, challenges, and future prospects of various pathways involved in the precipitation of AD. This would further direct the development of drugs that modulate and/or intercept these signaling pathways in a variety of ways.

AD has become the prototype of menacing neurodegenerative diseases. The rising public health concern has elicited the need for remedies at affordable prices, to ensure universal health coverage. In the first chapter, Dorababu underscores recent advances in the design and development of various tacrine-based anti-Alzheimer's. Recent research also reveals that heterocyclic scaffold-based drugs have potent pharmacology. Tacrine was one such quinoline-containing heterocyclic compound, which was an acetylcholinesterase inhibitor. However, it was withdrawn from the commercial markets because of its toxicity profile. To address this, tacrine is currently being chemically modified to produce safer, more effective, and more potent anti-Alzheimer's drugs and to develop their structure-activity relationship maps.

To develop these drugs and ensure their efficient delivery, it is imperative to understand the causal mechanism underlying the pathogenesis. From a clinical point of view, another challenge would be to distinguish and diagnose the type and velocity of AD, to devise the treatment regimen. Chapter 2 authored by Adiga *et al.* aims to explore the epigenetics of AD, which have transpired as key modulators in the pathogenesis and progression of AD. The authors opine that these modalities would prove to be novel and reliable in diagnosing and demarcating different types of AD, directing towards personalized medications.

With the advent of technology in the healthcare sector, there have been several signaling pathways that have been attributed to the progression and development of AD. One such molecule is TMP21, which is imperative in the trafficking of cellular proteins. Dysregulation of this protein has been linked to the production of neurotic plaques, which is the clinical characteristic of AD. The article would aim to explore the various facets that have been correlated to TMP21 dysregulation and the formation of neurofibrillary tangles, synaptic disbalance, and subsequent nerve cell death.

Although Aβ plaques and neurofibrillary tangles (NFT) are potential neuropathological markers, the role of inflammation in the pathogenesis of AD cannot be ignored. Neurodegeneration is caused by abnormal detachment of microtubules (MT) from axon MTs, cellular mislocalization, and tau hyperphosphorylation. Tau's ability to aggregate and form NFTs is thought to be regulated by post-translational modifications, which are thought to be an important regulatory mechanism. Drugs that target tau phosphorylation and aggregation have so far failed to show any therapeutic benefit. The recent finding including deleterious mutations in enzymes involved in the surface modification of MT adds to the relevance of such enzymatic machinery in neurobiology. Chapter 4 authored by Shrivastava *et al.*, presents the therapeutic potential of pharmacologically targeting tubulin-modifying enzymes in the treatment of neurodegenerative diseases, especially AD.

Although several drugs have been studied and researched for the treatment of AD, only a few therapies have been approved by the FDA, especially due to neurotoxicity issues. Memantine is one such drug that resolves the neurotoxicity issue. It acts on glutamate and its receptors to provide a treatment to combat AD. Chapter 5 authored by Kore *et al.* attempts to review the available literature on memantine and other glutamate antagonists used to alleviate symptoms associated with AD.

Irrespective of the causal factor, neuronal cell depletion is often the predominant factor for AD. However, for the treatment of AD, drug delivery remains a major concern, due to poor permeability, which limits the therapeutic utility of drugs. Chapter 6 of this book authored by Geetha *et al*, intends to review the novel approaches reported for therapeutic management, as well as to counteract the conventional therapeutic limitation of AD.

With conventional therapies tossed out, nanotechnology has become the new promising hope for the treatment of AD. Since, the constraints created by the blood-brain barrier surrounding the CNS impede drug delivery to the central nervous system for AD therapy, decreasing therapeutic bioavailability. To overcome these barriers to the effective delivery of medications to the CNS, a wide range of nanoparticle devices are available. The goal of chapter 7 authored by Yadav *et al.* is to describe and highlight recent advances in nanotechnology-based medicines and their implications for the treatment of AD.

However, these nanotechnological interventions must be attributed to a target for the successful therapeutic outcome in the patient. In addition, many targets have been identified for possible therapeutics, and from these targets, numerous drug candidates have been evaluated in clinical trials. Unfortunately, most of these trials failed due to the enigmatic

nature of this disease. Currently, there are 7103 AD targets listed on the Open Targets Platform, where 1240 of them are enzyme-related. The chapter by Ozgen *et al.* aims to explore the various enzymatic targets of AD, which have been claimed to possess disease-modifying effects, according to their clinical significance.

After the administration of the drug, the side effects are often inevitable, due to the intrinsic nature of the drug and/or drug therapy. Furthermore, the growing body of evidence has implied that the regular use of currently available anti-Alzheimer's drugs, which provide symptomatic relief in AD, still causes harmful effects such as mood disorders, sleeplessness, and depression. The importance of sirtuins in cellular biological control and neurological deficits has increased understanding of their novel role, with particular relevance to Alzheimer's disease. The data curated by Bhushan *et al.* in Chapter 9 have thoroughly reviewed the imperative role of sirtuins in the pathogenesis of AD, while simultaneously providing a prospect for drug discovery and delivery.

The structure-activity relationship for various benzothiazolourea derivatives has demonstrated outstanding potential in the alteration of illnesses against the CNS. Chapter 10, by Tiwari *et al.,* aims to curate and compare the statistics acquired from several data sources that demonstrate the potential effect of benzothiazolourea derivatives on the production of a significant lead compound. To date, inhibiting acetylcholine esterase is one of the key Alzheimer's processes discovered. Thus, innovative benzothiazole design and development could have a wide range of applications in the treatment of Alzheimer's disease.

Tau neuronal and glial abnormalities drive the clinical symptoms of Alzheimer's disease (AD) and are often related to human tauopathies. Due to its lack of a stable structure and high flexibility, tau, a microtubule-associated protein, is intrinsically disordered. In the brains of people diagnosed with AD and other tauopathies, intracellular inclusions of fibrillar tau with a sheet structure accumulate. As a result, tau dissociation from microtubules and tau transformation from a disordered to an improperly aggregated state are crucial processes before tau-related disorders manifest. Chapter 11, by Dubey *et al.,* aims to explore the efficacy of some of the most well-developed and/or commercialized molecules, in both pre-clinical and clinical studies for research pertaining to tau-targeted therapies.

The effectiveness of traditional Alzheimer's medications is heavily dependent on physiological factors such as the blood-brain barrier, the blood-cerebrospinal fluid barrier, and drug efflux by P-glycoprotein, all of which limit AD therapies' ability to make it to the central nervous system (CNS). The blood-brain barrier protects the central nervous system, while simultaneously limiting the access of therapeutic compounds to it. Therefore, to overcome the barrier and existing restrictions that CNS drugs face in crossing the BBB, novel drug development approaches have become a necessity. Several nano carrier-based techniques successfully address this goal by improving efficacy and facilitating the continuous release of encapsulated AD medication via targeted drug delivery. The study in chapter 12 by Kumari *et al.* aims to review these nanocarriers, which would mark a milestone in the nanotechnology-based drug delivery options to alleviate symptoms and provide remission from AD.

The function of carbonic anhydrase (CA) and its isoenzymes in Alzheimer's disease (AD) pathology have garnered the interest of researchers all over the world since their discovery in several AD models and the brains of AD patients. The release of the pro-apoptotic factor Cytochrome C (Cyt C) from challenged mitochondria was significantly reduced after treatment with a carbonic anhydrase inhibitor (CAI). As a result, a link was discovered between aging, oxidative stress, mitochondrial malfunction, and AD etiology. Chapter 13 by

Bhatnagar *et al.* aims to review the effects of these CAIs on mitochondrial dysfunction and consequently on AD, which could prove to be a viable strategy for future developments.

Despite decades of study on novel drugs and therapy regimens, AD still has limited treatment options. Although currently available drugs for AD do not slow or stop the progression of the disease, they are used to treat symptoms and provide temporary relief to patients. In recent years, there has been a rise in interest in understanding the mechanism of AD due to the introduction of drugs and other therapy modalities to address an unmet medical need. Somatostatin-evoked Aβ catabolism in the brain is intercepted via the -endosulfan-KATP channel route, according to a growing body of evidence. Chapter 14 by Varghese et *al.* aims to explore Somatostatin-evoked Aβ catabolism in their endeavour for the pursuit of drug design in Alzheimer's disease. The latter can be accomplished by repurposing or repositioning medications that have previously been approved by regulatory bodies and are used to treat different ailments. This study can potentially be a landmark in the treatment of diseases and could revolutionize the way neurodegenerative diseases, especially AD, are perceived and treated. Additionally, the repurposing of the pre-approved drugs would mean no loss of time for the conduction of extensive clinical trials for safety and toxicity profiles. Thus, success in this could potentially be the next treatment regimen for remission from AD. The last chapter 15 by Manjunath *et al.* delves deep into non-invasive, patient-affordable diagnosis methods that are also potential targets to discover new drugs beyond conventional and available drugs against Alzheimer's disease.

In short, this book shows a window to the future, with the potential remedies and treatment regimen concisely penned down in 15 multidisciplinary yet interconnected chapters. Finally, I extend my gratitude to all authors for their valuable contributions, their perseverance through the peer review process, and their patience with the publication process. The editors and the publishing team appreciate the researchers who contributed to the peer-review, verifying and amending various chapters and the editorial. I would also express my appreciation and gratitude to the authors and publisher of 'Enzymatic Targets for Drug Discovery against Alzheimer's Disease' for their unwavering guidance, unabated efforts, and unrelenting assistance in the development of this book. It is a pleasure to thank Ms. Humaira Hashmi, the Editorial Manager of Publications, and Ms. Asma Ahmed, Senior Manager of Bentham Publication for their valuable assistance during this whole journey.

Dileep Kumar
Department of Pharmaceutical Chemistry
Poona College of Pharmacy, Bharti Vidyapeeth
Pune, 411038, India
Department of Entomology and Nematology
UC Davis Comprehensive Cancer Center
University of California Davis
CA 95616, USA

&

Prashant Tiwari
Department of Pharmacology
College of Pharmaceutical Sciences
Dayananda Sagar University
Bengaluru, 560111, Karnataka
India

List of Contributors

Atukuri Dorababu	SRMPP Government First Grade College, Huvinahadagali – 583219, India
Ayush Kumar	Department of Medicine, Tata Motors Hospital, Telco, Jamshedpur, 831004 India
Achal Mishra	Faculty of Pharmaceutical Sciences, Shri Shankaracharya Group of Institutions, Junwan, Bhiali (CG), India
Ahmet Ozan Ozgen	Department of Medicinal Biotechnology, Akdeniz University, Antalya, Turkey
Abha Deshpande	Poona College of Pharmacy, Bharati Vidyapeeth (Deemed to be) University, Pune, Maharashtra, 411038, India
Arvinder Kaur	Department of Pharmaceutics, KLE College of Pharmacy, Bengaluru, Constituent Unit of KLE Academy of Higher Education and Research, Belgavi, 590001, India
Chandraprabha Sahu	School of Engineering & IT, ARKA JAIN University, Gamharia, Seraikela Kharsawan, Jharkhand-832108, India
Divya Adiga	Department of Cell and Molecular Biology, Manipal School of Life Sciences, Manipal Academy of Higher Education (MAHE), Manipal – 576104, Karnataka, India
Dileep Kumar	Department of Pharmaceutical Chemistry, Poona College of Pharmacy, Bharati Vidyapeeth, Pune, 411038, India Department of Entomology and Nematology, UC Davis Comprehensive Cancer Center, University of California, Davis, CA 95616, USA
Dipanjan Karati	Department of Entomology and Nematology UC Davis Comprehensive Cancer Center University of California Davis One Shields Avenue Davis, CA 95616, USA
Devyani Bhatnagar	Department of Pharmaceutical Chemistry, Poona College of Pharmacy, Bharati Vidyapeeth (Deemed to be University), Erandwane, Pune – 411038, Maharashtra, India
Deepa Bagur Parmesh	Department of Pharmaceutics, KLE College of Pharmacy, Bengaluru, Constituent Unit of KLE Academy of Higher Education and Research, Belgavi, 590001, India
Ekta Shirbhate	Institute of Pharmaceutical Sciences, Guru Ghasidas University, Bilaspur-495 009, (C.G.), India
Gargi Digholkar	Poona College of Pharmacy, Bharati Vidyapeeth (Deemed to be) University, Pune, Maharashtra, 411038, India
Harish Rajak	Institute of Pharmaceutical Sciences, Guru Ghasidas University, Bilaspur-495 009, (C.G.), India
Kavya Manjunath	Department of Pharmacology, KLE College of Pharmacy, Bengaluru, Constituent Unit of KLE Academy of Higher Education and Research, Belgavi, 590001, India

Khushboo Raj	School of Pharmacy, Arka Jain University, Jamshedpur, Jharkhand, 832108 India
Manish Kumar Jeengar	School of Pharmacy, Amrita Vishwa Vidyapeetham, AIMS Health Sciences Campus, AIMS Ponekkara P. O., Kochi, Kerala 682 041, India
Mahesh AR	Faculty of Pharmacy, M.S. Ramaiah University of Applied Sciences, Bengaluru-560054, Karnataka, India
Nadeem G. Khan	Department of Cell and Molecular Biology, Manipal School of Life Sciences, Manipal Academy of Higher Education (MAHE), Manipal – 576104, Karnataka, India
Ozan Emre Eyupoglu	Department of Biochemistry, School of Pharmacy, Istanbul Medipol University, Istanbul, Turkey
Pankaj Kumar Singh	National Institute of Pharmaceutical and Education and Research, Hyderabad, India
Priya Tiwari	Institute of Pharmaceutical Sciences, Guru Ghasidas University, Bilaspur-495 009, (C.G.), India
Rakesh Kore	Institute of Pharmaceutical Sciences, Guru Ghasidas University, Bilaspur-495 009, (C.G.), India
Ravichandran Veerasamy	Faculty of Pharmacy, AIMST University, Semeling, 08100 Bedong, Kedah Darul Aman, Malaysia
Ryan Varghese	Poona College of Pharmacy, Bharati Vidyapeeth (Deemed to be) University, Pune, Maharashtra, 411038, India
S. Sriharikrishnaa	Department of Cell and Molecular Biology, Manipal School of Life Sciences, Manipal Academy of Higher Education (MAHE), Manipal – 576104, Karnataka, India
Sangavi Eswaran	Department of Cell and Molecular Biology, Manipal School of Life Sciences, Manipal Academy of Higher Education (MAHE), Manipal – 576104, Karnataka, India
Shama Prasada Kabekkodu	Department of Cell and Molecular Biology, Manipal School of Life Sciences, Manipal Academy of Higher Education (MAHE), Manipal – 576104, Karnataka, India
Shweta Shrivastava	School of Pharmacy, School of Health and Allied Sciences, ARKA JAIN University, Gamharia, Seraikela Kharsawan, Jharkhand-832108, India
Sonal Dubey	College of Pharmaceutical Sciences, Dayananda Sagar University, Kumaraswamy Layout, Bengaluru 560078, Karnataka, India
Shreya Ladhe	Department of Pharmaceutical Chemistry, Poona College of Pharmacy, Bharati Vidyapeeth (Deemed to be University), Erandwane, Pune – 411038, Maharashtra, India
Shilpa Murthy	Department of Pharmaceutical Chemistry, KLE College of Pharmacy, Bengaluru, Constituent Unit of KLE Academy of Higher Education and Research, Belgavi, 590001, India
Vijay K Patel	Institute of Pharmaceutical Sciences, Guru Ghasidas University, Bilaspur-495 009, (C.G.), India

Yogita Kumari School of Pharmacy, Arka Jain University, Jamshedpur, Jharkhand, 832108 India

CHAPTER 1

Recent Advances In Tacrine-Based Anti-Alzheimer's Drug Design

Atukuri Dorababu[1,*]

[1] *SRMPP Government First Grade College, Huvinahadagali – 583219, India*

Abstract: Alzheimer's has become a common disease in aged people that leads to cognitive impairment and finally results in dementia and death. As the disease has a complicated etiology, it can hardly be prevented and cured. Hence, it turned out to be one of the menacing neurodegenerative diseases. The important concerning factor about Alzheimer's is its unaffordable treatment cost. Also, there are only a few efficient anti-Alzheimer drugs. Now, it is a very urgent need to discover the most efficient and cost-effective anti-Alzheimer's drugs. Nowadays, research reveals drugs based on heterocyclic scaffolds that have attributed to potent pharmacology. Quinoline-contai- ning molecule, tacrine was recommended as an acetylcholinesterase inhibitor. How- ever, its use has been withdrawn because of its toxicity. While research is going on designing derivatives of tacrine. Fortunately, some tacrine derivatives showed the most potent anti-Alzheimer properties. In view of this, here, anti-Alzheimer properties of recently reported tacrine-based Alzheimer's agents are discussed and evaluated. The structure-activity relationship has been helpful in identifying potent molecules in a series of derivatives.

Keywords: Aβ-aggregation inhibitors, Anti-Alzheimer agents, Cholinesterase inhibitors, MAOs, Structure-activity relationship, Tacrine derivatives.

INTRODUCTION

Alzheimer's disease (AD), a neurodegenerative disease is responsible for 60-70% of dementia deaths. AD starts with an early symptom like difficulty in remembering recent events and progresses with problems associated with difficulty in language, loss of motivation, disorientation and behavioral issues [1]. Gradually, AD patients lose bodily functions leading to death. AD is caused by environmental or genetic factors or head injury, clinical depression, and high blood pressure [1].

[*] **Corresponding author Atukuri Dorababu**: SRMPP Government First Grade College, Huvinahadagali – 583219, India; E-mail: dora1687@gmail.com

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Theories such as amyloid hypothesis [2], tau hypothesis [3], cholinergic hypothesis [4] and inflammatory hypothesis [5] have been proposed to determine the cause of AD. Drugs have been designed and discovered based on these hypotheses. However, to date, no Alzheimer's drug could stop the progression of the disease or cure it. These drugs can only slow the rate of progression of the disease. Alongside, there is no well-documented method of prevention of AD [6]. Till now, most promising anti-Alzheimer's medications used for treatment are rivastigmine, galantamine, and donepezil which act as cholinesterase inhibitors [7, 8]. While, memantine is an NMDA receptor antagonist [9]. Aducanumab is a recently FDA-approved anti-Alzheimer's drug that works by reducing amyloid plaques [10]. A continuous effort is being put in by the researchers in bringing up efficient anti-Alzheimer's drugs. Especially, heterocycle-based hybrid molecules have been reported as potent pharmacological agents including anti-Alzheimer's. A quinoline-based drug, tacrine Fig. (**1**) is a centrally-acting acetylcholinesterase inhibitor developed by Adrien Albert.

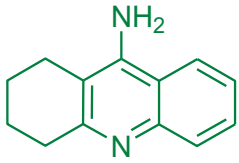

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Fig. (1). Structure of Anti-AD drug tacrine.

However, the use of tacrine has been stopped because of its adverse side effects. While, research is being continued in designing tacrine derivatives as potent anti-Alzheimer agents. In fact, galantamine is a tacrine derivative. This review is aimed at update of recently developed tacrine-based anti-Alzheimer's molecules. Here, structure-activity relationship (SAR) is discussed among the derivatives of a particular family of molecules.

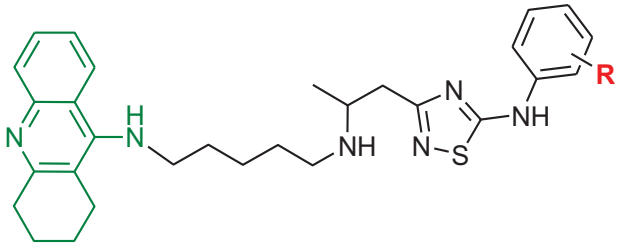
Multi-Target Anti-Alzheimer's Agents

Repeated failure of clinical trials of target-specific drugs suggests the complex multifactorial nature of AD. Most of the anti-AD drugs being designed address a particular signaling pathway by modulation of a single biological target. However, the disease progresses *via* the other signaling pathways resulting in low efficacy of single-target drugs. This fact warrants the discovery of an alternative approach that modulates several biological targets of AD simultaneously [11, 12] which is a challenging job [13]. Here are some tacrine-based multi-target anti-AD agents.

Tacrine-Heterocycle Hybrids

As accumulating evidence suggests 1,2,4-thiadiazole/thiazolidinone derivatives were efficient anti-AD agents [14 - 16], G.F. Makhaeva *et al.* prepared conjugates of tacrine and 1,2,4-thiadiazole and eventually investigated their cholinesterase inhibitory activity [17]. All the evaluated tacrine derivatives showed remarkable cholinesterase inhibitory activity. Especially, propanamine fragment-bearing derivatives **2**-**5** (Table **1**) bestowed the most promising cholinesterase inhibitory activity compared to propanamine analogs. Among them, the strongest AChE activity was noticed for 4-fluorophenyl analog **3**. While, 4-chlorophenyl analog **5** emerged as the most potent BChE inhibitor. However, variation in cholinesterase inhibitory activity was very small with a change in substitution on phenyl ring. Propanamine analogs were inferior to propanamine analogs with respect to CES inhibitory activity. Alongside this, synthesized molecules exhibited good scavenging activity. Particularly, propanamine analogs possessed superior activity (TEAC = 1.28-1.45) to propanamine scaffolds (TEAC = 0.38-0.51). These facts reveal that, compounds **2**-**5** showed moderate scavenging activity and CES activity. Although, they can be developed as potent anti-AD agents.

Table 1. Cholinesterase and CES inhibitory activities of tacrine-1,2,4-thiadiazole hybrids.



Compd.	R	Anti-AD Properties		
		AChE (IC_{50}, µM)	BChE (IC_{50}, nM)	CES (IC_{50}, µM)
2	4-CH_3	0.62	10.5	166
3	4-CF_3	0.36	6.3	33.9
4	4-OCH_3	0.49	4.4	20.4
5	4-Cl	0.43	3.8	19.5
Tacrine		0.60	29.5	>100

Literature unveils that pyridine derivatives were good at inhibition of Alzheimer's disease [18, 19]. This fact was considered by K. Czarnecka *et al.* for hybridization

CHAPTER 2

Epigenetics of Alzheimer's Disease: Past, Present and Future

Divya Adiga[1], Sangavi Eswaran[1], S. Sriharikrishnaa[1], Nadeem G. Khan[1], Shama Prasada Kabekkodu[1,*] and **Dileep Kumar[2,3]**

[1] *Department of Cell and Molecular Biology, Manipal School of Life Sciences, Manipal Academy of Higher Education (MAHE), Manipal – 576104, Karnataka, India*

[2] *Department of Pharmaceutical Chemistry, Poona College of Pharmacy, Bharati Vidyapeeth (Deemed to be University), Erandwane, Pune – 411038, Maharashtra, India*

[3] *Department of Entomology and Nematology, UC Davis Comprehensive Cancer Center, University of California, Davis One Shields Avenue, Davis, CA 95616, USA*

Abstract: Alzheimer's disease (AD) exemplifies a looming epidemic lacking effective treatment and manifests with the accumulation of neurofibrillary tangles, amyloid-β plaques, neuroinflammation, behavioral changes, and acute cognitive impairments. It is a complex, multifactorial disorder that arises from the intricate interaction between environment and genetic factors, restrained *via* epigenetic machinery. Though the research progress has improved the understanding of clinical manifestations and disease advancement, the causal mechanism of detrimental consequences remains undefined. Despite the substantial improvement in recent diagnostic modalities, it is challenging to distinguish AD from other forms of dementia. Accurate diagnosis is a major glitch in AD as it banks on the symptoms and clinical criteria. Several studies are underway in exploring novel and reliable biomarkers for AD. In this direction, epigenetic alterations have transpired as key modulators in AD pathogenesis with the impeding inferences for the management of this neurological disorder. The present chapter aims to discuss the significance of epigenetic modifications reported in the pathophysiology of AD such as DNA methylation, hydroxy-methylation, methylation of mtDNA, histone modifications, and noncoding RNAs. Additionally, the chapter also describes the possible therapeutic avenues that target epigenetic modifications in AD.

Keywords: Alzheimer's disease, DNA methylation, Epigenetic therapy, Histone modifications, Non-coding RNAs.

[*] **Corresponding author Shama Prasada Kabekkodu:** Department of Cell and Molecular Biology, Manipal School of Life Sciences, Manipal Academy of Higher Education, Manipal-576104, Karnataka, India
E-mails: shama.prasada@manipal.edu, spbhat81@gmail.com

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INTRODUCTION

Alzheimer's Disease (AD)

Dementia comprises a heterogeneous span of neurodegenerative complications accounting for more than 55 million cases globally [1, 2]. It is one of the leading causes of debility and dependency among older people and is the seventh most common cause of fatality among all the diseases worldwide [3]. Dementia is a comprehensive term encompassing different neurodegenerative disorders such as vascular dementia, dementia with Lewy bodies, and Alzheimer's disease (AD) [4]. AD is a complex, progressive, irreversible, multifactorial, and the most generic form of neurodegenerative dementia, contributing for 60 to 70% of dementia cases [5]. By 2050, AD's prevalence is estimated to cross 131.5 million cases globally [6].

Since its first description in 1906 by Alois Alzheimer [7], research progress has improved the understanding of clinical manifestations and disease advancement, but the causal mechanism of detrimental consequences remains undefined. Short term memory loss is the prominent initial symptom of AD. Other symptoms such as apathy, mood swings, behavioral changes, cognitive disturbances including discrepancies in language, executive, and visuospatial functions emerge with disease progression [8]. With the worsening condition, the patients withdraw themselves from the family as well as from society [9]. The average life expectancy of patients with Alzheimer's is 7 to 10 years, and it may vary from patient to patient [10].

At cellular level, neuroinflammation and accumulation of intracellular NETs (neurofibrillary tangles) and extracellular Aβ (amyloid-β) plaques [11] have been demonstrated in AD. The hyperphosphorylation of MAPT (microtubule associated protein tau) results in the formation of NETs and the extent of accumulation of these NETs in the neocortex is positively associated with dementia severity. Besides NETs, extracellular Aβ plaques are the vital hallmarks of AD's pathogenesis [12]. The insoluble Aβ plaques are formed by the accumulation of amyloidogenic Aβ peptides that are the products of APP (amyloid precursor protein) successive cleavage by β- and γ-secretases [13]. The occurrence and severity of NETs and Aβ plaques in the brain are frequently assessed during the postmortem neurological examination of individuals diagnosed with AD [14]. Other characteristics such as impaired phospholipid, calcium, and cholesterol metabolism are often exhibited early during the disease progression before the deposition of NETs and plaques [15]. The risk of AD development substantially increases after the age of 65 and for people beyond the age of 85, it reaches up to 30% [16].

EOAD (early-onset AD) and LOAD (late-onset AD) are the two different classes of AD [17]. EOAD is comparatively rare and reports less than 10% of total AD cases. It affects individuals below the age of 65 and usually manifests in individuals with the age group of 40 to 50 years [18]. Association of genetic mutations in PSEN-1 or -2 (presenilin-1 or -2), and APP with EOAD results in familial AD, affecting multiple generations within a family [19]. Unlike EOAD, LOAD is often common and affects individuals beyond the age of 65. The etiology of LOAD has not been revealed due to the intricacy of its presentation and pathogenesis [20]. The pathology of LOAD involves the interaction between multiple factors including genetic, biological, and environmental factors, whose complex interactions aggravate the disease progression [21, 22] (Fig. **1**). Studies have identified several genetic risk factors for LOAD. The APOE4 (ε4 isoform of apolipoprotein E) is one of the potent and well-known genetic risk factors for LOAD [23]. DIAD (autosomal dominant inherited AD) has been observed in ≤1% of patients and has been associated with a genetic mutation in PSEN-1, -2, and APP which eventually results in the over accumulation of Aβ [24, 25]. Further, EWAS (epigenome-wide association studies) data analysis have proposed the biological mechanisms involving tau binding proteins, degradation of APP, lipid-related processes, and brain immune function in the pathogenesis of LOAD [26].

Despite the substantial advance in recent diagnostic modalities and research progress, it is challenging to distinguish AD from other forms of dementia. MMSE (mini-mental status examination) [27] and MoCA (Montreal cognitive assessment) [28] tests are often being employed to confirm cognitive difficulties and memory loss. Certain biomarkers such as PET (positron emission tomography) imaging for Aβ and tau deposition in the brain and decreased levels of Aβ in CSF (cerebrospinal fluid) are useful to indicate and evaluate the advancement of AD pathology [29 - 31]. The conclusive AD diagnosis can only be made post-mortem, with the sighting of NETs and Aβ plaques in the brains of AD victims.

Accurate diagnosis is a major glitch in AD as it depends on the symptoms and clinical criteria [32]. Several studies are underway in exploring novel and reliable biomarkers for AD. In this direction, epigenetic alterations have transpired as key modulators in AD pathogenesis with the impeding inferences for the management of this neurological disorder. This chapter discusses the significance of epigenetic modification in the pathophysiology of AD and its potential utility in the clinical management.

CHAPTER 3

TMP21 in Alzheimer's Disease: An Important Target For Effective Treatment Approach

Dipanjan Karati[1] and **Dileep Kumar**[1,2,*]

[1] *Department of Pharmaceutical Chemistry, Poona College of Pharmacy, Bharti Vidyapeeth, Pune, 411038, India*

[2] *Department of Entomology and Nematology, UC Davis Comprehensive Cancer Center, University of California, Davis One Shields Avenue, Davis, CA 95616, USA*

Abstract: Alzheimer's disease (AD) is the most prevalent form of dementia, and it is considered a dynamic cognitive decline. Neurofibrillary tangles and nerve cell injury are important neuropharmacological symptoms for one AD brain. TMP21 is an important molecule in cellular protein trafficking. TMP21, a protein involved in the production of neurotic plaques, appears to be dysregulated in AD. As a result, we want to look into TMP21 dysregulation in Alzheimer's disease, as well as the involvement of TMP21 in neurotic plaque development and the underlying mechanisms. TMP21's significance in the creation of neurofibrillary tangles, synaptic disbalance, and nerve cell death is also explored. It will shed light on the therapeutic potential of regulating TMP21 as a treatment for AD.

Keywords: Amyloid beta protein, Alzheimer's disease, Glycogen synthase kinase-3, Tau phosphorylation, TMP21.

INTRODUCTION

Alzheimer's disease is a neuron-degenerative condition having an indistinct etiology. The cells of the brain are affected by Alzheimer's disease. The various symptoms of the illness include memory loss, cognitive decline, and neurofibrillary tangle formation. It is caused by the aggregation of the beta-amyloid protein and the hyperphosphorylation of the tau protein [1 - 15].

In 2018, the global cost of dementia was projected to be US$1 trillion, and by 2030, it would be US$2 trillion. As a result, it is required to understand the pathogenesis of Alzheimer's disease and establish effective therapies.

* **Corresponding author Dileep Kumar:** Department of Pharmaceutical Chemistry, Poona College of Pharmacy, Bharti Vidyapeeth, Pune, 411038, India; & Department of Entomology and Nematology UC Davis Comprehensive Cancer Center University of California Davis One Shields Avenue Davis, CA 95616, USA; Tel: +916207140054; E-mail: dileep.0@gmail.com

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Transmembrane protein 21KD (TMP21) appears to be a part of the p24 family. TMP21 is a key component of the pathogenesis of AD. It has several important roles in this disorder. It is also dysregulated in AD. This aspect is very significant to draw attention to the link between this protein and the pathogenesis of this disorder [16, 17].

Pathogenesis of Alzheimer's Disease

The production of hyperphosphorylated tau protein and the precipitation of amyloid-beta protein (Aβ) in the form of neuritic plaques are the two key neuropathological hallmarks of AD. β-secretase and γ-secretase help in the sequential cleavage of larger amyloid-β precursor protein to generate Aβ. Despite the elevated intensity of protein produced by the APP (amyloid protein precursor) gene *in vivo*, the development of Aβ by the amyloidogenic process is uncommon in normal conditions. α-secretase, not β-secretase, cleaves the bulk of APP inside the Aβ region in the non-amyloidogenic route, resulting in a secreted version of APP (sAPP) and C-marginal portion C83 [18 - 23].

Structure of the Amyloid Beta Peptide

The Aβ peptides are generated when the bulky APP molecule is cleaved. APP is a membrane protein found in numerous nerves, particularly in neuron synapses, that performs a crucial role in the etiology of AD. A single membrane-spanning domain, a large extracellular glycosylated N-terminus, and a shorter cytoplasmic C-terminus make up the APP protein. It is one of three genes in humans that make up a broader gene family. The APP-related proteins (APLPs) APLP1 and APLP2 are the other two members of the family [24]. Synaptic development and repair [25], anterograde neural transport [26], and iron export [27] have all been linked to APP. It comes in a variety of sizes, ranging from 695 to 770 amino acids in length. The most common form in the brain (APP695) is manufactured mostly by nerve cells and varies from lengthier forms of APP in that its ectodomain lacks a Kunitz-type protease antagonist order [28]. APP695 is mostly found in neurons, while APP751 and APP770, which include the Kunitz-type serine protease inhibitory domain KPI, are mostly found in peripheral cells and platelets [29, 30]. APP is well recognized as the predecessor congener that is cleaved by β-secretases and γ-secretases to generate Aβ [31], a 37 to 49 amino acid residue peptide that is the major component of amyloid plaques identified in Alzheimer's disease patients' brains. People who have sporadic Alzheimer's disease have a higher level of brain Aβ. Aβ is the primary component of parenchymal and vascular amyloid in the brain; it causes cerebrovascular lesions and is neurotoxic [32 - 35]. It's still unclear how Aβ builds up in the central nervous system and causes cell illness. According to several studies, Aβ oligomers cause some

indications of AD by competing with enzymes for the active site on different enzymatic receptors, causing glucose absorption in the CNS to be disrupted [36]. The γ- and β-secretases enzymes, which create Aβ from APP, have received a lot of attention [37 - 40].

Non-Amyloidogenic Pathway

APP has three primary isoforms: APP695, APP751, and APP770, all of which are expressed by a single gene on chromosome 21 [41, 42]. The only isoform that lacks an extracellular Kunitz Protease Antagonist field is APP695, which is the predominant form found in the brain. N, O-glycosylation and phosphorylation are all steps in the secretory route [43, 44]. Because of the short half-life of cells on the surface, only a minor percentage of holo-APP is perceived at the superficial portion of cells in comparison to the overall cellular extent [45]. A fraction (14%) of holo-APP is cleaved at the outer membrane of cells by α-secretase between Lys16 and Leu17 within the Aβ domain, preventing amyloid genesis and yielding a dispersible splinter, sAPPa, and a 10 kDa C-terminal piece attached with the membrane. This intra amyloid splitting produces around 92% of C83, according to radiolabeling [46 - 48].

Amyloidogenic Pathway

The process of Aβ biogenesis is referred to as the Amyloidogenic pathway. β-secretase first cleaves APP, yielding soluble β-APP fragments and the C-terminal fragment (C99), which is then further cleaved by γ-secretase, yielding the APP intracellular domain and Aβ.

The β-secretase BACE1 is found on chromosome 11. It is prominently disclosed in the CNS and pancreas [49]. The expression of BACE1 genetic material is firmly controlled at the transcriptional level and is augmented by the imbalanced production of free radicals [50]. In certain circumstances, such as when mRNA expression is minute, the effects of the 5'-untranslated region on translation initiation are minimal [51]. The best-studied BACE1 substrate is APP. The foremost splitting location is between Asp^{597} and Met^{596}, producing C-terminal fragment C99 and secretory APPb [52]. C99 is then cleaved by γ-secretase, resulting in the formation of Aβ.

The human APP Swedish mutation (APPSwe), which changes Lys595-Met596 (KM) to Asn595-Leu596 (NL), causes an increase in the action of β-secretase and deposition of Aβ, leading to afortime-onset dementia [53]. The loss of the YENP endocytosis signal in the cytoplasmic tail of APP, as well as incubating cells in low-potassium media and thereby suppressing endocytosis *via* suppression of clathrin-coat building, indicating that wildtype APP cleavage occurs largely in

CHAPTER 4

Tubulin Modifying Enzymes as Target for the Treatment of Alzheimer's Disease: Old Perspective With A New Angle

Shweta Shrivastava[1,*], Ayush Kumar[2], Manish Kumar Jeengar[3] and **Chandraprabha Sahu[4]**

[1] School of Pharmacy, School of Health and Allied Sciences, ARKA JAIN University, Gamharia, Seraikela Kharsawan, Jharkhand-832108, India

[2] Department of Medicine, Tata Motors Hospital, TELCO, Jamshedpur, India – 831004, India

[3] School of Pharmacy, Amrita Vishwa Vidyapeetham, AIMS Health Sciences Campus, AIMS Ponekkara P. O., Kochi, Kerala682 041, India

[4] School of Engineering & IT, ARKA JAIN University, Gamharia, Seraikela Kharsawan, Jharkhand-832108, India

Abstract: Alzheimer's disease (AD) is a major cause of mental disability in the elderly, accounting for 50-60% of all dementia. While β-amyloid plaques as well as neurofibrillary tangles are neuropathological markers, inflammation plays a critical role in AD development. The aberrant detachment of microtubules (MTs) from axon MTs, cellular mislocalization, and hyperphosphorylation of tau are major factors in neurodegeneration death. Tau's ability to aggregate as well as form NFTs is assumed to be regulated by post-translational changes, which are regarded to be an essential regulatory mechanism. So far, drugs that target tau phosphorylation as well as aggregation have not shown therapeutic impact. It is now clear that tubulin PTMs cause tau dysfunction. High glutamylation and detyrosination levels in the neurons affect MT surface physicochemical characteristics. Further evidence for the relevance of such an enzymatic machinery in neurobiology comes from the recent discovery of harmful mutations in enzymes involved in surface MT modification. In this chapter, we discussed that targeting tubulin-modifying enzymes pharmacologically may be useful in treating neurodegenerative disorders.

Keywords: Alzheimer’s disease, β-amyloid plaques, Microtubules, Microtubules modifying enzymes, Neurofibrillary tangles.

[*] **Corresponding author Shweta Shrivastava:** School of Pharmacy, School of Health and Allied Sciences, ARKA JAIN University, Gamharia, Seraikela Kharsawan, Jharkhand-832108, India; E-mail: shweta.shrivastwa@gmail.com

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INTRODUCTION

Alzheimer's disease (AD) affects 45.0 million individuals globally, making it the most common form of dementia. In AD, aberrant extracellular amyloid plaques and intracellular neurofibrillary tangles develop in the brain [1]. Drugs approved to treat Alzheimer's disease now target the neurochemical systems underlying cognitive and behavioural symptoms, with minimal short-term symptomatic impact [2]. Upstream brain development of Aβ species and plaques occurs 20–30 years before tau spread, neuronal loss, and finally clinical symptoms [3].

Progressive malfunction of neurons and neuronal loss in specific brain areas are the most proximal brain processes related with dementia [4]. While the exact sequence of events triggering the ultimate common route of neurodegeneration remains unknown, the build-up of persistent amyloid is being more recognised as a pathogenetic event [5]. The synthesis of β-amyloid peptides is increased by all known AD mutations [1]. Amyloid precursor protein produces this protein, which forms the core of neurotic plaques. The dementia syndrome is caused by a combination neurotransmitter imbalances and neuronal loss [6]. Oxidative metabolism damage, pruning of dendrites, loss of synapses and cell death are some of the harmful effects [7].

Amyloid plaques and neurofibrillary agglomerates are microscopic features of the disease. Aβ, a 40–42 amino acid peptide that regulates synaptic plasticity and other physiological processes, is the primary component of plaques [8]. Neurofibrillary tangles, which are made up of hyperphosphorylated forms of tau, are associated to the development of Alzheimer's disease as well as cognitive decline [9]. Dysfunctional microglia, reactive astrocytes, and dystrophic neurites are additional AD brain histopathology indicators. Despite the efficacy of cholinesterase inhibitors, an AD carrier continues to lose neuronal tissue even when being treated [10]. Other biochemical mechanisms related with AD pathogenesis for example inhibition of glycogen synthase kinase-3β and β-secretase have also been investigated as therapeutic options [11]. The current book chapter will cover the pathogenesis and treatment of AD, with a focus on novel microtubule modifying enzymes.

PATHOPHYSIOLOGY

Microscopically, amyloid plaques or senile plaques are formed, and hyperphosphorylated Tau protein accumulates, implying neurofibrillary tangles and severe neuronal death [12].

Amyloid and Tau Hypotheses in AD

The mainstream theory for pathophysiology of AD is the amyloid hypothesis. APP gene is located on chromosome 21 [13]. Aβ is secreted from the cell *via* β- and γ-secretase and swiftly eliminated in healthy individuals. However, ageing or pathological circumstances reduce the metabolic capacity to breakdown Aβ, allowing Aβ peptides to accumulate. Aβ-42 production and/or the ratio of Aβ-42 formation are affected by these pathogenic APP mutations located around β-secretase or γ-secretase cleavage sites [14]. β-secretase acts on APP to create sAPP and β C-terminal fragment (CTFβ). γ-Secretase cleaves CTFβ, releasing Aβ and generating AICD. The γ-secretase complex doesn't cut in the same location all the time. As a result, Aβ peptides of 34 to 50 amino acids may be synthesised [15, 16]. Under typical circumstances, majority of Aβ molecules are Aβ40 which make up the majority of Aβ species. Amyloid plaques, on the other hand, may include highly harmful and susceptible to oligomerization and agglomeration Aβ42 and Aβ43 peptide fragments. The AICD controls gene expression and Ca^{2+} signalling, and Aβ participates in a wide range of physiological as well as pathological processes [17]. Aβ 42 increases protofibrils, oligomers or other intermediates lead to the formation of amyloid fibrils, which collect into senile plaques, producing neurotoxicity and tau dysfunction, leading to neuronal death and neurodegeneration [18].

Tau is a microtubule-associated protein that controls tubulin assembly stability [19]. On chromosome 17, the tau gene is found [20]. Specifically, the 3-repeat and 4-repeat tau isoforms are found in the adult brain. Neuronal axons include these isoforms under normal circumstances. Tau is a phosphoprotein linked with microtubules that was found in neurones [21]. Deflation of tau protein causes dissociation from microtubules, resulting in development and accumulation of insoluble neurofibrillary tangles (NFTs) [22]. Some specific tau structural changes, such as hyperphosphorylation due to discrepancies between the activities of kinases (such as GSK3 and CDK5) and phosphatases (such as P1, P2, P5, and P5) in AD, disrupt microtubule assembly and cause tau to aggregate in NFT [23]. Tau is initially hyperphosphorylated followed by tau dimers as well as oligomers are formed. PHF (paired helical filaments) or similar straight filaments are formed by the oligomers. These abnormal inclusions are called neurofibrillary tangles (NFTs) in neuronal cell bodies and threads in dendrites or axons [24]. Tau protein hyperphosphorylation causes neurofibrillary tangles. However, high quantities of Aβ caused modest alterations in mice endogenous Tau, leading to the process of hyperphosphorylation of Tau as indicated in (Fig. **1**). In the last stage of illness aetiology, researchers have established a definite connection with accumulation as well as tau protein aggregation. Although the actual relationship between Aβ and tau protein disease is unknown, GSK-3 is largely assumed to be involved due to

CHAPTER 5

Memantine and Glutamate Antagonists in the Treatment of Alzheimer's Disease: Current Updates

Rakesh Kore[1], Priya Tiwari[1], Vijay K Patel[1], Ekta Shirbhate[1], Ravichandran Veerasamy[2], Achal Mishra[3] and **Harish Rajak[1,*]**

[1] *Institute of Pharmaceutical Sciences, Guru Ghasidas University, Bilaspur-495 009, (C.G.), India*

[2] *Faculty of Pharmacy, AIMST University, Semeling, 08100 Bedong, Kedah Darul Aman, Malaysia*

[3] *Faculty of Pharmaceutical Sciences, Shri Shankaracharya Group of Institutions, Junwani, Bhiali (CG), India*

Abstract: Alzheimer's disease (AD) is the most important cause of dementia and a complex chronic neurodegenerative disease. Many of the currently marketed drugs are used to treat this disease condition, but a major issue with these drugs is their neurotoxicity. Alzheimer's treatment with the FDA approval of memantine resolves the neurotoxicity issue. Memantine acts on glutamate and its receptors in the treatment of AD. Recent studies show that NMDA receptor-acting drugs are doing well in the healing of Alzheimer's patients, because of their selectivity on receptor and neuroprotective activity. The present work is an attempt to collect updated information about memantine and glutamate antagonists used for the treatment of AD.

Keywords: Alzheimer's Disease, Amyloid Beta Precursor Protein (APP), Central Nervous System, Glutamate, Memantine.

INTRODUCTION

Alzheimer's disease (AD) was identified over a century ago but recognized as the major cause of dementia and mortality during the period of 70s of research [2]. It is the most common form of dementia among the elderly across the world. The incidence and prevalence of AD rise with age, and women have a higher incidence and prevalence than men due to their longer life expectancy. AD affects about 1% of people between the ages of 65 and 70 and about 4% of those over the

* **Corresponding author Harish Rajak: I**nstitute of Pharmaceutical Sciences, Guru Ghasidas University, Bilaspur-495 009, (C.G.), India; E-mail: harishdops@yahoo.co.in

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age of 85 [3]. AD is the sixth greatest cause of mortality in the United States (Alzheimer's Association, 2016). According to the World Health Organization, AD is the most important cause of dementia, responsible for 60-70% of cases [4]. The worldwide burden of AD is likely to rise as the population ages, with an estimated 115 million individuals living with dementia by 2050. At present, there is no treatment for AD, and the most feasible treatment objective is to avoid future symptom deterioration [5].

Symptoms: The major symptoms of AD include memory loss that causes problems in everyday life, difficulties in solving problems, difficulty in accomplishing routine duties at home, misunderstanding of the passage of time or the site of an occasion, and spatial interactions are hard to understand, when speaking or writing, new challenges with words arise, misplacing items and being unable to retrace your steps. The other symptoms include reduced or faulty judgment, withdrawal from job or social activities is a common symptom of depression, apathy, and despair, as well as changes in mood and demeanor, anxiety, agitation, and sleep disorders are all on the rise [6].

Diagnosis: AD can not be diagnosed with a single, simple test. Currently, the diagnosis of AD is based on the clinical valuation of the patient. The several steps involved in the diagnosis of AD include: (i) obtaining feedback from a family member or other close relatives regarding changes in thinking abilities or behavior; (ii) carrying out cognitive testing as well as physical and neurologic testing; (iii) blood tests and brain imaging are used to confirm the possible reasons of dementia symptoms [7]. Biomarkers, *i.e*., CSF or serum factors, genetic examination, and functional neuroimaging, will likely be used to diagnose AD in the future, and will most likely be added to AD diagnostic criteria [1].

PATHOPHYSIOLOGY OF AD

The pathophysiology of AD comprises both structural and functional aberrations which include a progressive neurodegenerative condition characterized morphologically by brain atrophy and increased cerebral ventricles [3, 8] by the gathering of β-amyloid protein in the form of extracellular senile plaques and the generation of intracellular neurofibrillary tangles in different parts *i.e*., medial temporal lobe, entorhinal cortex, and hippocampus [9]. Trisomy 21, a mutation of the presenilin-1 gene on chromosome 14, and an aberrant allele, ε4, for the lipid-associated protein ApoE on chromosome 19, cause amyloid deposits. The 4 is mostly dependent on the Amyloid precursor protein, same as other prevalent variants (ApoE ε2 and ε3).

Genes Involved in Alzheimer's Disease

Amyloid Beta Precursor Protein (APP) is one of significant protein-coding gene. The ailments related with APP are Cerebral Amyloid Angiopathy, App-Related, and AD. Various cells generate APP, which is processed by the secretory or endosomal-lysosomal routes [3]. Synaptogenesis of static cell-substrate adhesion or neurite outgrowth, synaptic plasticity, and neuronal cell viability appear to be aided by APP [10]. It is also associated with cell mobility modulation [11]. When cortical neurons are subjected to APP monoclonal antibodies, neurite degeneration and apoptosis occur [12]. In both normal and pathological circumstances, apoptosis is significant [13]. It occurs often in the central and peripheral nervous systems during development [14]. However, apoptosis dysregulation could be at the root of a variety of diseases [15]. In both acute and chronic neurodegenerative disorders, an elevated rate of apoptosis may be involved [16]. Studies show that apoptosis may be the final cause of AD Fig. (**1**) [17, 18].

The brain has a significant cholinergic deficiency, though other neurotransmitter systems, such as glutamate and neuropeptide, are also affected. This results in a gradual loss of cognitive function and capacity to perform daily activities, as well as behavioral and psychological abnormalities such as physical aggression, restlessness, inappropriate social behavior, and agitation. The behavioral signs may worsen as the illness progresses [5]. Early AD symptoms are usually caused by malfunction of these structures, which leads to anterograde episodic memory loss, such as repeated queries, missed appointments, missing belongings, and forgotten information in everyday life, which can be seen by the patient and/or family members. Increased dependency and progression toward the akinetic-mute state that characterises end-stage neurologic illness describe the later stages of Alzheimer's disease [19 - 21]. The various genes involved in the pathophysiology of AD have been summarized in Table **1**.

Table 1. Genes involved in pathophysiology of AD.

Genes	Chromosomal Location	Normal Function	Pathology in AD	Ref.
Amyloid precursor protein (APP)	21	Regulating synaptic function	Causing early-onset AD	[36]
Apolipoprotein E4 (ApoE4)	19	Transporting cholesterol	RiskfactorofAD	[37]
Presenilin-1 (PS-1)	14	Processing amyloid precursor protein and forming Aβ	Causing early-onset of AD	[38]

CHAPTER 6

Enzymatic Targets for Drug Discovery Against Alzheimer's Disease

Ahmet Ozan Ozgen[1] and **Ozan Emre Eyupoglu**[2,*]

[1] *Department of Medicinal Biotechnology, Akdeniz University, Antalya, Turkey*

[2] *Department of Biochemistry, School of Pharmacy, Istanbul Medipol University, Istanbul, Turkey*

Abstract: Alzheimer's Disease (AD) is a neurodegenerative disease. The disease itself is progressive and full recovery from it isn't achievable yet. There are several hypotheses asserted (Cholinergic hypothesis, Amyloid hypothesis *etc*.) to explain the mechanisms behind the disease. Also, many targets have been identified for possible therapeutics and from these targets, numerous drug candidates have been evaluated in clinical trials. Unfortunately, most of these trials failed due to the enigmatic nature of this disease. Currently, there are 7103 targets associated with Alzheimer's disease listed in the Open Targets platform where 1240 of them are enzyme-related. In this chapter, enzymatic targets of the AD have been reviewed, and those claimed to have disease-modifying effects were selected and presented according to their clinical significance.

Keywords: Alzheimer's Disease, Amyloid beta-Peptides, Amyloid Precursor Protein Secretases, Angiotensin-Converting Enzyme Inhibitors, Caspases, Cholinesterase Inhibitors, Enzyme Inhibitors, Gingipain Cysteine Endopeptidases, Histone Deacetylases, Insulysin, Metalloproteases, Neurofibrillary Tangles, Protein Kinase Inhibitors, Phosphodiesterase Inhibitors, Synaptic Transmission, Sirtuins, Tau Proteins.

INTRODUCTION

Alzheimer's Disease (AD), which has become a bigger problem with the increasing elderly population, is defined by the Alzheimer's Association as follows [1]: "AD is the most frequent form of dementia, which is a broad term for memory loss and other cognitive impairments that interfere with everyday living. Alzheimer's disease is responsible for about %60~%80 of dementia cases."

* **Corresponding author Ozan Emre Eyupoglu:** Department of Biochemistry, Istanbul Medipol University, School of Pharmacy, Istanbul, Turkey; E-mail: oeeyupoglu@medipol.edu.tr

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AD is a neurodegenerative disease characterized by neurofibrillary tangles formed as a result of accumulation of amyloid-beta (Aβ) peptides and tau phosphorylation in the brain [2]. It is also accompanied by neuronal loss and inflammation in the brain, decreased cognitive functions and dementia. In addition, people with advancing age, family history, female gender, type 2 diabetes, peripheral vascular diseases, atherosclerosis, cardiovascular diseases, head traumas, cerebrovascular diseases and Apolipoprotein E (apoE) ε4 allele gene are at a greater risk of AD [3, 4].

There are many hypotheses suggested, the number of which is still increasing, to reveal the mechanisms behind this disease. The main focus is on cholinergic, amyloid-cascade and hyperphosphorylated tau-cascade hypotheses which are linked in some ways to enzymes such as cholinesterases, secretases, beta-Amyloid degrading enzymes and others [5, 6]. In this section, the enzymes that play a role in the development of AD and the importance of these enzymes in the development of new treatments will be covered.

Enzymes are biocatalysts, and the majority of them are proteins. They are responsible for biochemical processes in living organisms. The molecular weights of enzymes, like structural proteins, range from 12,000 to 1,000,000 Da [7]. Other than the protein structure, certain enzymes require ions or ions known as cofactors such as iron, zinc, magnesium, calcium, manganese, and so on. Coenzymes are complex organic or metalloorganic compounds that are required by some enzymes. Some enzymes require one or more metal ions as well as coenzymes to function [8, 9].

The substrates are the substances with which the enzymes interact. Substrates are converted into products when they interact, and the product and enzyme are then separated as shown in Fig. (**1**) [10]. The three-dimensional structure of the enzymes may change throughout these reactions, but they return to their original shape at the conclusion. Enzymes not only speed up processes, but they also govern several critical metabolic pathways in cells [11].

Enzymes are called by appending the suffix “-ase” to the end of the name of their substrate, but other enzymes are recognized by the names of those who discovered them. However, since the number of identified enzymes has expanded and a more descriptive categorization is necessary, the International Union of Biochemistry and Molecular Biology has created a systematic classification (IUBMB). This taxonomy categorizes enzymes based on the reaction they catalyse as well as their substrate specificities. According to this categorization, all enzymes are assigned an EC number, which is a four-digit number, when they are added to the enzyme catalogue. The first digit denotes membership in one of the six base classes, while

the next two denote subclasses and sub-subclasses. The last digit shows the enzyme's position in the subclass [12, 13]

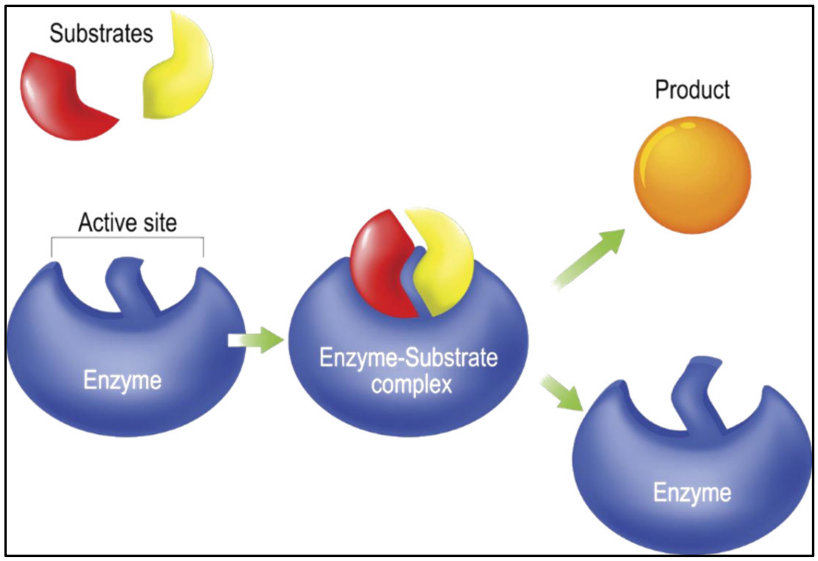

Fig. (1). Representation of enzyme and substrate interaction.

Enzymes with the same reaction specificity can be found in any of the six fundamental classes. Oxidoreductases (EC 1) are enzymes that catalyse the transfer of reducing equivalents from one redox system to another. Other than hydrogen, transferases (EC 2) catalyse the transfer of functional groups from one substrate to another, an example for transferases can be seen in Fig. (**2**) [14]. Coenzymes are required for the majority of oxidoreductases and transferases. Group transfer is carried out by hydrolases (EC 3), although the acceptor is invariably a water molecule. Lyases (EC 4) are enzymes that catalyse processes that involve the removal or creation of a double bond. Isomerases (EC 5) are enzymes that catalyse geometric, optical, or structural isomer interconversion. Ligases (EC 6) catalyse energy-dependent reactions, which invariably include the hydrolysis of nucleoside triphosphates. Translocases (EC 7) catalyse the passage of ions or molecules across membranes as well as their separation inside membranes [12, 14].

There are currently 1240 enzymatic targets associated with AD listed on Open Targets Platform's database [15]. The enzymatic targets for possible therapeutics which are reported, with disease modifying properties, or effects on cognitive functions were selected and presented according to their clinical significance.

CHAPTER 7

Tau Protein: Targets And Development Against Alzheimer's Disease

Sonal Dubey[1,*] and **Mahesh AR**[2]

[1] *College of Pharmaceutical Sciences, Dayananda Sagar University, Kumaraswamy Layout, Bengaluru 560078, Karnataka, India*

[2] *Faculty of Pharmacy, M.S. Ramaiah University of Applied Sciences, Bengaluru-560054, Karnataka, India*

Abstract: The clinical manifestations of Alzheimer's disease (AD) and associated human tauopathies are driven by tau neuronal and glial abnormalities. Tau, a microtubule-associated protein is inherently disordered due to its lack of a stable structure and great flexibility. Intracellular inclusions of fibrillar tau with a sheet shape accumulate in the brains of individuals with AD and other tauopathies. As a result, tau separation from microtubules and tau transition from a disordered state to an inappropriately aggregated state are critical steps before the start of tau-related illnesses. Many studies have demonstrated that this shift is triggered by post-translational changes such as hyperphosphorylation and acetylation. Before the development of tau inclusions, the misfolded tau self-assembles and forms a tau oligomer. Animal and clinical research utilising human samples has shown that tau oligomer development contributes to neuronal death. During tauopathies, tau seeds are released from cells and absorbed into neighbouring cells, resulting in the spread of abnormal tau aggregation. Thus, Tau has become both a physiological and pathological target for AD treatments during the last decade. Evidence reveals many potential techniques for preventing tau-mediated toxicity: (1) direct suppression of pathological tau aggregation; (2) inhibition of tau post-translational changes that occur before pathological tau aggregation; (3) inhibition of tau propagation; and (4) microtubule stabilisation. Aside from traditional low-molecular-weight compounds, newer drug discovery approaches, such as the development of medium-molecular-weight drugs (peptide- or oligonucleotide-based drugs) and high-molecular-weight drugs (antibody-based drugs), provide alternative pathways to preventing the formation of abnormal tau. Suppression of protein kinases or protein-3-O-(*N*-acetyl-beta-D-glucosamin-l)-L-serine/threonine hydrolase, inhibition of tau aggregation, active and passive immunotherapies, and tau silencing using antisense oligonucleotides; in several animal models, have shown the capacity to prevent or minimise tau lesions and treat either cognitive or motor impairment. Immunotherapy, which has already reached the clinical stage of drug development, is the most advanced technique for treating human tauopathies. Tau vaccines or humanised antibodies are designed to target a range of tau

* **Corresponding author Sonal Dubey:** College of Pharmaceutical Sciences, Dayananda Sagar University, Kumaraswamy Layout, Bengaluru 560078, Karnataka, India;
E-mail: drsonaldubey@gmail.com

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species in both intracellular and extracellular environments. Some of them recognise the amino- or carboxy-terminus, while others have proline-rich areas or microtubule-binding domains that they can attach to. In this review, we examine various clinical targets for the treatment of tauopathies as well as the various molecules researched as tau inhibitors that can be used in AD. Furthermore, we explore the efficacy of some of the prominent molecules in clinical studies for tau-targeted therapies research.

Keywords: Alzheimer's Disease, Curcumin, Folic Acid, Immunotherapy, Methylthioninium Chloride, Resveratrol, Tau Protein, Tau Aggregation Inhibitors (TAI), Tau Phosphorylation, Tau Acetylation.

INTRODUCTION

Tau (Tubulin binding protein) was the first microtubule-linked protein to be reported in 1975 by 'Weingarten *et al*.'; which promotes the self-assembly of tubulin microtubules [1, 2]. In neurological science, the Tau research came into focus when anomalous protein deposits were identified in Alzheimer's sufferers' brains. Two proteins were identified in the deposits, one is Aβ, which is an extracellular amyloid plaques' key protein and the second one is Tau, which is the core of intracellular neurofibrillary tangles [3, 4]. Tau research in Alzheimer's has gained momentum with the discovery of tau deposits which are independent of Aβ amyloid-like PiD and PSP; along with frontotemporal dementia caused by Tau mutations (FTDP17) [5].

The clinical manifestations of Alzheimer's disease and associated human tauopathies are driven by tau neuronal and glial abnormalities. Toxic tau entities can transmit illness across the brain by migrating from cell to cell, according to a growing body of research. Tau, both physiological and pathological, has become a popular target for Alzheimer's disease treatments during the last decade. The suppression of protein kinases or 'protein-3-Ortho-(N-acetyl-β-D-glucosaminyl) L-serine/threonine-N-acetylglucosaminyl)-L-serine/threonine-N-acetyl-glucosaminyl hydrolase', tau aggregation inhibition, active and passive immunotherapies, and antisense oligonucleotide tau silencing, in several animal models producing neurofibrillary disease, new tau therapies have shown the ability to prevent or reduce tau injuries, as well as treat cognition or impaired motor.

Immunotherapy that has now made it to the clinical stage of drug development, is the most advanced technique for treating human tauopathies. Humanized antibodies or tau vaccinations are designed to target a variety of tau species in both intracellular and extracellular settings. Some recognise the amino or carboxy terminus, while others contain proline-rich regions or microtubule-binding domains to which they can bind. The major therapy foci in current clinical investigations are Alzheimer's disease, progressive supranuclear palsy, and non-

fluent primary progressive aphasia. Tau therapy is a promising new therapeutic option for a variety of deadly brain illnesses.

Owing to the absence of a strong framework and tremendous adaptability, tau, a microtubule-associated protein, is intrinsically disordered. In the brains of people with Alzheimer's disease and other tauopaffigthies, intracellular inclusions of fibrillar tau in a sheet shape develop. As a result, tau dissociation from the transformation of tau and microtubules from a chaotic to an improperly accumulated state are crucial processes before tau-related disorders manifest. Many studies have shown that post-translational alterations like acetylation and hyper-phosphorylation cause this shift. Before tau inclusions arise, misfolded tau self-assembles and forms a tau oligomer. The conception of tau oligomers contributes to neuronal death, according to animal and clinical research using human samples.

In tauopathies, cells create tau seeds, which are then absorbed by neighbouring cells, causing aberrant tau aggregation to spread. Many possible approaches have been a breakthrough in preventing tau-mediated toxicity (1) direct inhibition of pathological tau aggregation; (2) inhibition of tau post-translational modifications that occur before pathological tau aggregation; (3) tau propagation inhibition; and (4) microtubule stabilisation.

Newer drug discovery initiatives, like the development of 'peptide- or oligonucleotide-based treatments' for medium-molecular-weight pharmaceuticals and antibody-based therapeutics for high-molecular-weight drugs, provide new avenues for suppressing the synthesis of aberrant tau. Recent research found that the initial step in aberrant tau aggregation is the creation of tau droplets *via* liquid-liquid phase separation, as well as data linking tau to dendritic and nuclear activities. In this article, we look at the biology of tau, its targets, and drugs that act on those targets, as well as current clinical trials for tauopathies treatment.

TAU STRUCTURE

Tau protein is a hydrophilic molecule. The polypeptide chain is very flexible and dynamic, with a relatively low secondary structure. Tau's proclivity for aggregation is pushed by two structural factors: charge equalization of the fundamental center portion of Tau by polyanion8; and Tau's proclivity for -structure folding, which makes the structure intrinsically disordered [6, 7]. However, Tau’s ability to form an aggregate in an ordered manner in AD seems to be counterintuitive to its structural properties. Two structural factors which push Tau towards aggregation are- charge compensation of the basic middle part of Tau by polyanion; and Tau’s propensity for β-structure folding [8].

CHAPTER 8

Promising Nano-Carriers-Based Targeted Drug Delivery Approaches for the Effective Treatment of Alzheimer's Disease

Yogita Kumari[1], Khushboo Raj[1] and **Pankaj Kumar Singh[2,*]**

[1] *School of Pharmacy, Arka Jain University, Jamshedpur, Jharkhand, India*

[2] *National Institute of Pharmaceutical and Education and Research, Hyderabad, India*

Abstract: Alzheimer's disease (AD) is an attained disorder of cognitive and behavioral impingement with progressive symptoms over time. It is mostly witnessed in elderly people, and as per the World Health Organization (WHO), it has affected more than 35 million people worldwide, and this figure is presumed to double by the year 2050. The most commonly believed cause of AD is the accumulation of beta-amyloid, which forms extracellular plaques. Presently conventional therapy for treating cognitive impairments in AD relies on a neurotransmitter or enzyme modulation strategy. Conventional approved drugs, such as acetylcholinesterase inhibitors (memantine, tacrine), are widely available for the treatment of mild to moderate AD, but due to their lower bioavailability, poor solubility, and ineffective capability to surpass the blood-brain barrier (BBB), they often fail to produce the desired effect. The potency of conventional AD drugs is highly dependent on various physiological aspects such as BBB; blood-cerebrospinal fluid barrier and drug efflux by P-glycoprotein, which all hampers the capabilities of AD drugs to grasp the central nervous system (CNS). So, in order to conquer the hurdle and these existing limitations faced by CNS drugs to cross the BBB, innovative pathways in drug development have become the need of the hour. Various nanocarriers based approaches profitably meet this demand by improving the efficacy as well as facilitating the sustained release of the entrapped AD drug *via* targeted drug delivery. The blood-brain barrier offers protection to the central nervous system and also limits the entry of therapeutic molecules to the CNS. On the other hand, nanotechnology offers the possibility to deliver small molecules against CNS disorders across BBB due to their enormous properties, such as small surface area, controllable physicochemical properties, higher drug payload, and better drug circulation time. Plenty of nanocarriers and nanoparticle prodrugs have been reported to have inconsequential cytotoxicity in preclinical studies, and these advancements have proclaimed a new juncture for the development of new classes of nano carriers' based potent drug formulations for the treatment of AD. A plethora of nanotechnology-based approaches such as polymers, emulsions, lipo-carriers, solid lipid carriers, carbon nanotubes, and metal-based carriers have been redefined over time, and they have been

* **Corresponding author Pankaj Kumar Singh:** National Institute of Pharmaceutical and Education and Research, Hyderabad, India; E-mail: Pankajksingh3@gmail.com

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successfully focusing on both neuroprotective and neurogenerative techniques for treating AD. Many researchers also reported that nanotechnological-based techniques can improve the early diagnosis of AD and enhance the therapeutic efficacy and bioavailability of drugs.

Keywords: Nano- Carriers, Conventional drugs, β- amyloid, Targeted delivery, blood-brain barrier, drug delivery system, Nanotechnology.

INTRODUCTION

Alzheimer's disease (AD), the most common kind of dementia, is a neurological condition that affects the elderly. The cognitive deterioration associated with AD significantly impacts those living with the disease's social and behavioural effects. Regardless of the societal consequences, AD imposes high financial costs on patients, families, and the society at large. According to the National Institutes of Health (NIH), AD affects around 4.5 million Americans, costing the country $100 billion annually. Even more concerning is the projection that 13.2 million older Americans will develop AD by 2050 if present trends continue and no preventative therapy becomes available. These numbers are aggravated by the lack of contemporary biological markers for AD; as a result, definite diagnoses are established at autopsy, and the effectiveness of novel treatments cannot be prolonged over time. Additionally, therapeutic options for probing the central nervous system (CNS) are constrained by the blood–brain barrier's constrictive tight junctions (BBB) [1]. The current treatment has a number of drawbacks. Currently authorised medications for the treatment of cognitive deficits associated with Alzheimer's disease (AD) are based on the manipulation of neurotransmitters or enzymes. Acetylcholinesterase (AChE) inhibitors have been linked to gastrointestinal side symptoms such as nausea and vomiting, which often result in therapy termination. Tacrine has a short half-life and requires daily doses. Additionally, people who took the dose were obliged to undergo frequent blood tests to check for hepatotoxicity. Memantine may produce dizziness, disorientation, constipation, or vomiting. Frequently, therapy failure arises as a result of unfavourable pharmacological profile of medications. Due to poor hydrophobicity, plasma metabolism, drug instability and toxicity, pharmacotherapy failure arises. By encapsulating pharmaceuticals in nanoplatforms or nanodevices, their pharmacokinetics and pharmacodynamics are enhanced, while their toxicity is minimised. On the one hand, regulated medication delivery into disease areas is a critical component of nanomedicine development [2]. By adding nanotechnology-based medication delivery systems, the efficacy of a therapy may be enhanced [3].

PATHOPHYSIOLOGY OF AD

As the disease progresses, the brain's ability to perform cognitive processes, such as memory and reasoning, suffers from enormous loss of synapses and neuronal death. AD patients have amyloid plaques in their brain parenchyma, as well as aberrant tau protein filaments, which result in neurofibrillary plaques, neuronal loss, and cell activation and irritation in the brain parenchyma, as well as neurofibrillary plaques [4]. First, the amyloidal cascade neuro-degeneration theory, and second, the cholinergic system dysfunction hypothesis, have been postulated for the genesis and pathophysiology of AD. Alzheimer's patients with APP and presenilin mutations had higher levels of deposition. Both metal-mediated neurotoxicity and deposition are related to building-up in the brain. Insoluble amyloid fibres develop in the brain when concentrations are high. Zinc and copper may be incorporated into these fibres, resulting in an even worsening of neuronal toxicity. Alzheimer's disease-causing hydrogen peroxide was formed *in vitro* when copper and A complexed together, according to research. Some metallic elements have also been found as plaques in the brains of Alzheimer's disease sufferers. It is possible to dissolve amyloid plaques in post-mortem tissues of AD patients by using metal chelators. Chelating drugs have also been shown to dissolve amyloid plaques in an animal model of AD [5]. It is caused by a variety of complicated processes. In light of recent breakthroughs in our knowledge of the molecular regulation of these many pathways, we may be able to better diagnose and predict the prognosis of Alzheimer's disease, as well as uncover new molecular targets for the therapy and prevention of this disease [6].

BLOOD-BRAIN BARRIER

Homeostatic protection of the brain from infections and pollutants is provided by the blood–brain barrier (BBB). Solutes passing across the BBB may be selectively transported into the brain parenchyma because of the BBB's ability to screen for biochemical, physicochemical, and structural characteristics [7]. It was shown in the murine model that the BBB has a biological character, and these findings gave insights into the physiology of the BBB today. Horseradish peroxidase (HRP), an enzymatic tracer, was found in the vascular space and in pinocytotic vacuoles after intravenous injection of HRP into the cerebral cortex [8]. The enzyme was not discovered to be transported *via* pinocytotic vacuoles, and no peroxidase was identified beyond the endothelium of the vasculature, indicating a boundary between the blood and the mind [9].

Mechanism of the Blood-brain Barrier

Angiogenesis, or the growth of new blood vessels from old ones, is the process by which cerebral capillaries are generated [10]. When the ECM and basement

CHAPTER 9

Effects of Carbonic Anhydrase Inhibitors on Mitochondrial Dysfunction and Consequently on Alzheimer's Disease

Devyani Bhatnagar[1], Shreya Ladhe[1] and **Dileep Kumar[1,2,*]**

[1] *Department of Pharmaceutical Chemistry, Poona College of Pharmacy, Bharati Vidyapeeth (Deemed to be University), Erandwane, Pune – 411038, Maharashtra, India*

[2] *Department of Entomology and Nematology, UC Davis Comprehensive Cancer Center, University of California, Davis One Shields Avenue Davis, CA 95616, USA*

Abstract: With the discovery of Carbonic Anhydrase (CA) and its isoenzymes in various Alzheimer's disease (AD) models and the brain of AD patients, the role of CA in AD pathology has become of keen interest among scholars around the world. Several experiments were performed to investigate the same, albeit they didn't provide us with the exact mechanism through which CAs are involved in AD progression, but they gave us an important insight into the beneficial outcomes of CA inhibition. Carbonic Anhydrase Inhibitor (CAI) administration showed a significant reduction in the release of the proapoptotic factor- Cytochrome C (cyt C) from the challenged mitochondria (under oxidative stress). Thus, a link between ageing, oxidative stress, mitochondria dysfunction and pathogenesis of Alzheimer's disease was established. Treatment with CAI indirectly lowers neuronal loss and, thus, cognitive impairment, which are characteristic features of AD. Though, the precise functions of CA in exaggerating or mediating AD still remain hazy, with the support of various scholarships globally, the use of CAII (an isoenzyme of CA) as a potential biomarker for AD can be proposed.

Keywords: Alzheimer's disease, ATZ, Biomarker, CAII, Carbonic anhydrase, Carbonic anhydrase inhibitors, MTZ, Oxidative stress, ROS.

INTRODUCTION

In 1906, a German neuroanatomist, Alois Alzheimer, carried out the autopsy of a dead woman named Auguste D. and found neurofibrillary tangles (NFT) and senile amyloid plaques in her brain's histology [1]. This is how Alzheimer

* **Corresponding author Dileep Kumar:** Department of Pharmaceutical Chemistry, Poona College of Pharmacy, Bharati Vidyapeeth (Deemed to be University), Erandwane, Pune- 411038, Maharashtra, India; & Department of Entomology and Nematology, UC Davis Comprehensive Cancer Center, University of California, Davis One Shields Avenue Davis, CA 95616, USA; E-mail: dileep.0@gmail.com

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disease, one of the most frequent causes of dementia, first came to be known by mankind. On the onset of the disease, the brain incurs neuronal cell death and consequently begins to atrophy [2], leading to its most eminent feature, memory loss and cognition [3]. Although we cannot pinpoint the specific causes leading up to AD, it is believed that aging and the ensuing rise in oxidative processes might be a trigger and thus is regarded as the principal risk factor [4]. Other risk factors deciphered for this neurodegenerative disease so far include- genetic disposition, diabetes, cardiovascular disease, obesity, *etc.* [5 - 8].

Even after billions of dollars being spent globally [9] on various researches trying to determine a cure for Alzheimer's disease (AD) over the past decade, we are still far from our ambitions. Many studies have emphasized the role of oxidative stress in the initiation and enhancement of AD pathology [10]. Oxidative stress theory of aging proposed by K B Beckman *et al.* suggests that the loss of normal physiological functioning with advancing age can be owed to the buildup of oxidative damage caused to the biomolecules by the action of reactive oxygen and nitrogen species [11]. Additionally, mitochondrial dysfunction and its effect on the pathogenesis of AD have been established with various supporting scholarships [12 - 14]. Oxidative stress is mainly mediated by the reactive oxygen species (ROS), whose bulk is generated by the mitochondria [15, 16]. ROS's source also happens to be one of their targets, thus promoting mitochondrial malfunction [17]. Such oxidative activities are also incriminated in brain atrophy, a feature of AD. One of the main pathways by which mitochondrial malfunctioning leads to neuronal cell loss is *via* the pronounced triggering of apoptotic mechanisms by increased mobilization of proapoptotic factors like Cyt c.

To fight off and block this particular pathway, the use of Carbonic Anhydrase Inhibitors (CAI) like Methazolamide, Acetazolamide, *etc.*, in the treatment of AD, was sought. This was done since many studies have confirmed the presence of Carbonic Anhydrase enzyme (CA-II) within amyloid plaques, which suggests the role of CAs in plaque growth [18] and so the curiosity behind this occurrence led to this application of CAIs in AD. After a long spell of failures, the Carbonic Anhydrase (CA) enzyme has emerged as a new target of interest amongst scholars aiming to find a remedy for AD [19]. Other reports like the increased CA-II expression in AD patients [20, 21], the altered catalytic activity of CA during AD [22, 23], *etc.*, have contributed to strengthening the idea of CAs' potential role in neurodegenerative diseases. CAIs are most importantly known to prevent mitochondrial dysfunction [24] and the resulting neuronal death, thereby guarding cognition, amongst their other neuroprotective features. In this review, we also discuss CA-II, an isoenzyme of CA [25] that is acted upon by inhibitors like MTZ & ATZ [26] for the treatment of various diseases, including neuropsychiatric

disorders [27]. Furthermore, we aim to delve deeper into how carbonic anhydrase prevents mitochondrial dysfunction and the empirical evidence backing this finding. Before jumping into the roles of CAIs, we also demonstrate the apoptotic mechanism that leads to neuronal loss in AD patients, owing to oxidative stress. The elevated CA II levels detected in both the central and peripheral systems [28] raise the prospect of CA II being used as a potential biomarker for Alzheimer's disease, as we shall see later. Moreover, we will also review promising preclinical data and the experiments which have been conducted to support this theory. This review cogently discusses, how carbonic anhydrase inhibitors will be helpful in ameliorating the condition of AD and how the possibility of CA's role was determined in AD pathology.

MITOCHONDRIAL DYSFUNCTION AND ALZHEIMER'S DISEASE

Alzheimer's disease (AD), one of the most frequent causes of dementia, has been predominantly reported in the geriatric population. With advancing age, the oxidative processes within the body increase, also known as oxidative stress. Oxidative stress is a result of the disproportion between reactive oxygen species (ROS) production and removal within the cell. When present in excess, ROS can affect the normal functioning of the organelles and biological systems by oxidizing the biomolecules like protein, DNA, RNA, *etc.* [29]. The resulting dysfunction of nucleic acids, proteins, etc. causes cell death of the vulnerable neurons within the brain, which includes neurons in the frontal cortex, entorhinal cortex, amygdala, hippocampus CA1 region, and amygdala in the case of AD [30 - 32]. This ultimately leads to brain atrophy, a typical feature of neurodegenerative diseases. The root cause behind this amplified ROS level may be neurodegeneration (as seen in AD), hypoxia, hyperglycemia, or some other neuronal insult [33 - 35].

Oxidative stress, due to aging and other risk factors, disturb the normal operations of the mitochondria, thus making them generate abnormal amounts of ROS. This high level of ROS, in turn, oxidizes and damages biological molecules, thereby impairing the ability of the mitochondria to carry out metabolic processes like fatty acid oxidation, amino acid metabolism, tricarboxylic acid cycle, *etc.* [36]. Such impaired mitochondria are known to produce more ROS and comparatively lesser energy. Mitochondrial dysfunction is an early and well-recognized feature of AD, and impairment in almost all of its facets has been reported in AD [37].

The majority of the ROS in the cell is produced by the mitochondria. The redox carriers present in it have the ability to transfer a lone electron to the oxygen, thereby producing a superoxide (O_2^-) which is a ROS. Enzymes involved in the tricarboxylic acid cycle, monoamine oxidases, *etc.*, are some sources of ROS

CHAPTER 10

Novel Treatment for Alzheimer's Disease: Tapping the Somatostatin-evoked Aβ Catabolism *via* α-endosulfine-K_{ATP} Channel Pathway

Ryan Varghese[1], Gargi Digholkar[1], Abha Deshpande[1] and **Dileep Kumar[1,2,*]**

[1] *Centre for Advanced Research in Pharmaceutical Sciences, Poona College of Pharmacy, Pune 411038, Maharashtra, India*

[2] *Department of Entomology and Nematology, UC Davis Comprehensive Cancer Center, University of California, Davis One Shields Avenue Davis, CA 95616, USA*

Abstract: Alzheimer's disease (AD) is a debilitating neurological disease that is known to worsen as people age. As a chronic illness, it has a negative impact on the health and financial well-being of patients and their families. Despite decades of research into new medications and therapy regimens, the therapeutic choices for these conditions are still limited. Although currently available medications for AD do not prevent or stop disease progression, they are used to treat symptoms and provide brief comfort to patients. The development of medications and other therapy modalities to address the unmet medical need has sparked a surge of interest in understanding the mechanism of AD in recent years. Growing bodies of evidence direct towards the treatment of AD by intercepting the Somatostatin-evoked Aβ catabolism in the brain, *via* the α-endosulfin--K_{ATP} channel pathway. The latter can be achieved through the repurposing or repositioning of drugs previously approved by the regulatory authorities and indicated in other diseases. With the advent of technology in the healthcare sector, these could be corroborated through various *in-silico* and *in-vitro* techniques. This article aims to explore the various aspects of the byzantine α-endosulfine-K_{ATP} channel pathway while providing information and future prospects for the development of new therapies to combat AD.

Keywords: Alzheimer's Disease, Amyloid β, α-endosulfine, Cell signaling.

[*] **Corresponding author Dileep Kumar:** Centre for Advanced Research in Pharmaceutical Sciences, Poona College of Pharmacy, Pune 411038, Maharashtra, India; & Department of Entomology and Nematology, UC Davis Comprehensive Cancer Center, University of California, Davis One Shields Avenue Davis, CA 95616, USA; Tel: +91 06207140054,
E-mail: dileep.0@gmail.com
[#] Authors contributed equally to the curation of this work

Dileep Kumar & Prashant Tiwari (Eds.)

INTRODUCTION

Alzheimer's Disease (AD) is the most common form of irreversible dementia and accounts for a considerable disease burden on patients, healthcare providers, and society as a whole [1]. AD is characterized by a progressive decrease in cognitive functions, which leaves the individual immobile and reliant on custodial care in the end stages. However, death often occurs about 9 years after diagnosis [2]. However, several factors must be taken into account to determine the velocity of disease progression and the pharmacotherapeutic intervention for the same.

The current gold standard of treatment involves the administration of acetylcholinesterase inhibitors, to improve the cognitive function in patients diagnosed with mild-to-moderate AD. However, the treatment regimen for moderate-to-severe AD includes the utilization of memantine, an NMDA (N-methyl-d-aspartate) antagonist, which aids in enhancing cognitive abilities. Furthermore, the non-cognitive neuropsychiatric symptoms associated with AD often include agitation, mood disorders, mood swings, and sometimes psychosis. These symptoms often necessitate the use of medications, providing symptomatic treatment, as there is no prescribed regimen for the management of the latter [1]. In recent years, non-pharmacotherapeutic interventions have been developed for the management of both cognitive and non-cognitive neuropsychiatric symptoms associated with AD. Although, there have been several articles that discuss various signaling pathways and methods for managing the symptoms associated with AD, this article aims to review the α-endosulfine-K_{ATP} channel pathway, as well as provide the insights into newer avenues for potential research and drug development and/or drug repurposing.

ETIOPATHOLOGY OF ALZHEIMER'S DISEASE

This progressive neurodegenerative disease derives its name from Alois Alzheimer, who first described the clinical features of the latter about a century ago. He described the clinical characteristics such as amyloid plaques and neurofibrillary tangles that still form the cardinal features of the disease [3]. This impairment is characterized by initial episodic memory loss, and a decline in cognitive function, which ultimately affect speech function, behavior, visuospatial orientation, and motor control, making it the most prevalent form of dementia, accounting for 60 to 80% of cases [4]. Variant syndromes with early focal atrophy may not necessarily manifest in the same way, thus allowing pathological categorization of AD, to ensure a more personalized treatment method [5]. Although several modalities have been developed to evaluate amyloid and tau burden in living patients [6], clinical AD dementia cannot be clearly diagnosed

until a post-mortem examination is thoroughly investigated [6, 7]. AD has been studied to pose a long asymptomatic pre-clinical phase, with even cognitively normal people pathologically developing this condition [7, 8]. Furthermore, it is so closely attributed to aging that both patients and healthcare providers believe it to be an intrinsic part of the natural aging process [9].

In 2018, the global burden of AD was estimated to be approximately 47 million people, with an annual cost of treatment of $1 trillion [7, 10].

With an aging demographic transition, these debilitating and financially catastrophic diseases are projected to become more prevalent, with more than 131 million people being diagnosed with the disease by 2050 [7, 11]. Aging is attributed to be the most conspicuous risk factor for AD, with its prevalence doubling every 6.3 years, from about 3.9 per 1000 for people aged 60 to 90 to over 104.8 per 1000 for people over 90 [7, 11]. Furthermore, people over 65 years of age are predicted to have a 10% prevalence, compared to those over the age of 80, having a 40% prevalence [4]. Due to the increasing personal and financial implications of AD, the need to develop novel drugs or newer treatment modalities has never been greater [7]. The prevalence of various subtypes of AD has been concisely presented in Table **1** [7, 12 - 19].

Table 1. Various subtypes of AD and their prevalence and involved gene.

Sr. No.	Alzheimer's Disease Type	Involved Gene	Chromosome	Prevalence
1.	EOFAD	APP	21	10-15% of total cases of EOFAD
3.	EOFAD	PSEN1	14	30-70% of total EOFAD cases
4.	EOFAD	PSEN2	1	< 5% of the total cases of EOFAD
5.	LOAD	ApoE	19	~ 40% of total cases of LOAD
6.	LOAD	GAB2	11	~ 70% of total cases of LOAD
7.	LOAD	KIBRA	5	40 – 45% of total cases of LOAD
8.	LOAD	PCDH19	X	-
9.	LOAD	SORL1	11	20 – 40% of total cases of LOAD

(EOFAD = early-onset Alzheimer's disease, LOAD = late-onset Alzheimer's disease)

Several researchers have substantiated the etiopathology of AD with the aggregation of Aβ-amyloid (Aβ) within the neocortex [20, 21]. Recent studies have suggested that the Aβ precipitation and subsequent toxicity in individuals progress into AD. Although Aβ could precipitate from a multitude of reasons, its toxicity could potentially be attributed to aberrant interactions with neocortical metal ions, such as copper (Cu), iron (Fe), and zinc (Zn) [20]. An age-related increase in brain Cu and Fe levels could potentially hypermetallate the Aβ

CHAPTER 11

Diagnosis and Potential Strategies to Discover New Drugs for the Treatment of Alzheimer's Disease (AD)

Kavya Manjunath[1,*], Arvinder Kaur[2], Deepa Bagur Parmesh[2] and **Shilpa Murthy[3]**

[1] Department of Pharmacology, KLE College of Pharmacy, Bengaluru, Constituent Unit of KLE Academy of Higher Education and Research, Belgavi, 590001, India

[2] Department of Pharmaceutics, KLE College of Pharmacy, Bengaluru, Constituent Unit of KLE Academy of Higher Education and Research, Belgavi, 590001, India

[3] Department of Pharmaceutical Chemistry, KLE College of Pharmacy, Bengaluru, Constituent Unit of KLE Academy of Higher Education and Research, Belgavi, 590001, India

Abstract: Alzheimer disease (AD) is most common cause of dementia, which is characterized by impaired cognitive and behavioural charateristics. Deposition of Aβ plaques and neurofibrillary tangs (NFTs) are the hallmark of AD. Generally it is a chronic disease where neurodegeneration, and loss of neuronal function arise earlier before it is diagnosed. Early detection of AD is important as it reduces the severity of the disease. In this regard, an effective tools/methods are available including CSF biomarkers, Magnetic Resonance imaging (MRI), Positron emission tomography (PET) but all these methods are painful and often cannot be afforded by the patients.

Therapy of AD includes inhibitors of choline esterases, and antagonists at NMDA receptors. From the studies it is shown that these drugs just offer relief from symptoms rather than alleviating the progression of disease. Multiple pathological processes contribute for AD, like oxidative stress, dysregulation of neurotransmitters, inflammation of neurons, aggregation β-amyloid, phosphorylation of tau protein. It is essential to target multiple causes for an effective outcome in the treatment of AD. Early diagnosis is also crucial as it reduces disease progression thereby cost involved in AD therapy.

This review focuses on non-invasive, patient affordable diagnosis methods and also potential targets to discover new drugs beyond conventional and available drugs.

Keywords: Alzheimer's disease, Diagnosis, Non-invasive, β-amyloid and Tau protein.

[*] **Corresponding author Kavya Manjunath:** Department of Pharmacology, KLE College of Pharmacy, Bengaluru, Constituent Unit of KLE Academy of Higher Education and Research, Belgavi, 590001, India; Tel: +91-6362184250; E-mail - mkavya032@gmail.com

Dileep Kumar & Prashant Tiwari (Eds.)

INTRODUCTION

Alois Alzheimer a German physician described first about Alzheimer's Disease (AD) in 1907 [1]. Dysfunction of Cognitive and non-cognitive features are the characteristic symptoms of AD. Cognitive impairment symptoms includes dementia, impediment of language and dysfunction of execution. Whereas psychiatric, disturbance in behaviour, depression, hallucnations, delusion, agitation are the non-cognitive impairment symptoms [2].

A study from The Global Burden of Diseases, Injuries and Risk Factors reported that around 40-50 million people across the world are living with AD and other forms of dementia. AD accounts for 2.4 million deaths globally and is the fifth major cause of death [3]. It is estimated that approximately 100 million cases of AD by 2050.

The main causes of AD are the loss of neurons and altered synapses due to the deposition of β-amyloid protein, destabilisation of microtubules by hyperphosphorylated tau protein, inefficient cholinergic neurotransmission, and the presence of other diseases like diabetes, hyperinsulnaemia, vascular diseases, neuronal inflammation, altered apolipoprotein gene.

Though it is a major public health problem currently five drugs are available to treat AD and are categorized into two classes. One is inhibitors of cholinesterase (Tacrine, Donepezil, Rivastigmine, Galantamine) and the other one is antagonist at N-methyl-D-aspartate (NMDA) receptor (Memantine). Although new drugs are developed against AD, including small molecules and immunotherapies, but they failed to exhibit different activities between drug and placebo, and moreover, exerted intolerable toxicities [5].

Nearly one hundred compounds have failed during clinical testing of the drug development phase [6]. Monoclonal antibodies are thought to be a promising agents against the amyloid cascade, Ex: Bapineuzumab [7], Solanezumab [8] but were plagued by side effects such as vasogenic edoema. The γ-secretase enzyme inhibitors [9] like Avagacestat [10] and Semagacestat [11] were found to increase the risk of skin rashes, diarrhoea, nausea, and skin cancer.

Symptomatic relivers (neurochemical enhancers) like Encenicline tends to boost cholinergic response at its receptor, while Idalopiridine tends to increase ACh release in the brain. However, these medications demonstrated substantial gastrointestinal toxicity, leading to their withdrawal from phase 3 clinical trials [12].

The development of the primary neuropathological features of AD is influenced by some pathological processes that manifest years before the emergence of amyloid plaques and NFTs. Cellular senescence, oxidative stress, neuroinflammation, decreased neurogenesis, and altered proteostasis are some of these changes [13 - 15]. Many studies have shown how these early changes contribute to the acceleration of cognitive decline, a rise in Aβ load, and tau hyperphosphorylation and neuronal death. The beginning and escalation of the aforementioned degenerative alterations indicative of AD are thought to be mediated by a number of signaling pathways [16–18].

Therefore, we have focused on the PI3K/AKT and Wnt signalling pathways and how their roles are altered in many facets of AD as well as how they interact, particularly in causing apoptosis, angiogenesis, cell division, and viability in the metabolism of the CNS cells.

Rather the symptom remission, disease modifications are the main emphasis of researchers in AD. Since AD is a multifactorial disease it is important to target different aspects. But currently approved drugs fails to act on multiple steps involved in AD.

Diagnosis of AD is equally important to that of therapy because it reduces the risk of disease, cost burden on patients. So it is essential to understand diagnosis and differnet targets for effective outcome in the treatment of AD.

The existence of cognitive impairment, a key component of the dementia profile,may be identified through a combination of the patient's medical history, clinical examination, and an objective cognitive assessment, such as a thorough neuropsychological evaluation or a quick mental assessment [19].

Diagnosis criteria for Alzheimer’s disease are increasingly being updated to include biomarker tests for conditions such as amyloid-protein buildup, neuronal damage, synaptic dysfunction, and neuronal degeneration [20]. Also in identifying stages of AD, CSF biomarkers, and imaging tests are emerging as an essential tools [21].

Hence, the objective of this chapter is to present an overview of potential strategies to discover new drugs in the treatment of AD with respect to important hypotheses, signaling pathways also provide information on diagnosis methods in Alzhiemer’s disease.

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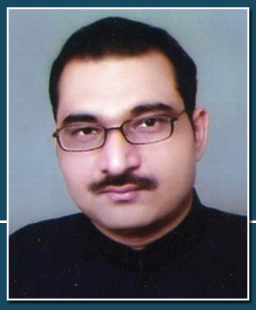

Dileep Kumar

Prof. Dileep Kumar did B. Pharm from Manipal College of Pharmaceutical Sciences, Manipal University, and M. Pharm from S.G.S.I.T.S Indore, Madhya Pradesh. During his research, he published many research and review articles in peer-reviewed journals. He was awarded a junior research fellowship by National Medicinal Plant Board (NMPB) New Delhi, senior research fellowship from University Grants Commission (UGC), teaching assistantship, and institute post-doctoral fellowship from IIT (BHU) Varanasi. At present, he is teaching as an assistant professor at Poona College of Pharmacy, Bharati Vidyapeeth University Pune. He is guest editor of prestigious journals like Current Topics in Medicinal Chemistry, CTMC, Current Drug Target, Combinatorial Chemistry, and Molecules. His current research interests include the design and development of adamantyl analogs as GluN2B selective NMDA receptors antagonist and Amyloid-β protein aggregation inhibitors for the treatment of Alzheimer's disease. At present, he is a visiting professor in the Department of Entomology and Comprehensive Cancer Center at the University of California Davis.

Prashant Tiwari

Prof. Prashant Tiwari is an associate professor in the Department of Pharmacology, College of Pharmaceutical Sciences, Dayananda Sagar University, Bengaluru, India. He received Ph.D. from Siksha O Anusandhan (Deemed to be) University, Bhubaneswar, Odisha, India. He has a rich teaching and research experience of 13 years. He was a regular fellow of the ICMR, New Delhi. He is a lifetime member of multiple professional bodies such as, LASAI, APTI, SPER, ERDA, PESOTS, and IPES. He has several awards in his recognition such as Dr. P. D. Patil National Award for the best thesis in pharmaceutical sciences-2022, Young Scientist Award, Young Talent Award, and Best Teacher Award. He has published 65 papers in peer-reviewed journals, authored 9 books, 6 book chapters, and 07 patents to his credit in the domain of pharmaceutical sciences. His current Google Scholar h-Index is 13; i18-Index No. of citation 6780. He guided 09 M. Pharm and 03 Ph.D. scholars at Dayananda Sagar University, Bengaluru, India. His research areas include neurodegenerative disorders, drug interaction, PK/PD, and metabolic disorders.