Exploring GABA as a Drug Target: Molecular Docking Analysis for Alzheimer's Disease Drug Repurposing

A Dissertation

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BIOTECHNOLOGY

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I, **Ashish Kumar Sharma** , hereby certify that the work is being presented as the Major Project in the thesis entitled **" Exploring GABA as a Drug Target: Molecular Docking Analysis for Alzheimer's Disease Drug Repurposing"** in partial fulfilment of the requirement for the award of the Degree of Master of Science in Biotechnology and submitted to the Department of Biotechnology, Delhi Technological University, Delhi is an authentic record of my work, carried out during the period from January 2025 to May 2025 under the supervision of **Prof. Pravir Kumar**.

I have not submitted the matter presented in the report for the award of any other degree of this or any other institute/University.

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CERTIFICATE BY THE SUPERVISOR

Certified that **Ashish Kumar Sharma (23/MSCBIO/12)** has carried out his research work presented in this thesis entitled " **Exploring GABA as a Drug Target: Molecular Docking Analysis for Alzheimer's Disease Drug Repurposing**" for the award of Degree of Master of Science in Biotechnology and submitted to the Department of Biotechnology, Delhi Technological University, Delhi under my supervision. This thesis embodies results of original work, and studies are carried out by the student herself and the contents of the thesis do not form the basis for the award of any other degree to the candidate or to anybody else from this or any other University/Institution.

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LIST OF ABBREVIATIONS

AD	Alzheimer's Disease
AB	Amyloid-beta
APOE	Apolipoprotein E
SORL	Sortili-Related Receptor
ABCA	ATP- Binding Cassette Subfamily A
TREM	Triggering Receptor Expressed on Myeloid cell
NGS	Next Generation Sequencing
NFT	Neurofibrillary Tangles
GABA	Gamma Aminobutyric acid
APP	Amyloid Precursor Protein
PSEN	Presenilin
CLU	Clusterin
PICALM	Phosphatidylinositol Binding Clathrin Assembly Protein
PLD	Phospholipase D
UNC	Uncoordinated (gene family from *C.elegans*)
AKAP	A-Kinase Anchoring Protein
ADAM	A Disintegrin and Metalloproteinase
GWAS	Genome Wide Association Study
CSF	Cerebrospinal Fluid
MRI	Magnetic Resonance Imaging
PET	Positron Emission Tomography
GPCR	G-Protein Coupled Receptor
TM	Transmembrane
PV	Parvalbumin
SST	Somatostatin
ERBB	Erythroblastic Leukemia Viral Oncogene Homolog
FDA	Food and Drug Administration
EMA	European Medicines Agency
MHRA	Medicines and Healthcare Products Recovery Agency
GLP	Glucagon Like Peptide

PAINS	Pan – Assay Interference Compounds
SMILES	Simplifies Molecular Input Line Entry System
PDB	Protein Data Bank
GUI	Graphical User Interface
BBB	Blood Brain Barrier
GI	Gastrointestinal
SDF	Structure Data File
NCBI	National Centre for Biotechnology Information
TREM2	Triggering Receptor Expressed on Myeloid Cell
AKT	AKT serine/Threonine Kinase
LD50	Lethal Dose

Exploring GABA A as a Drug Target: Molecular Docking Analysis for Alzheimer's Disease Drug Repurposing

Ashish Kumar Sharma

ABSTRACT

Aim: Alzheimer's disease (AD) is a long-term, irreversible brain condition characterized by behavioral abnormalities, memory loss, and cognitive decline. A major pathological characteristic of AD is abnormal accumulation of Aβ plaques, resulting from the improper breaking of APP. Among the critical enzymes involved in APP processing, Because of its influence on cognitive processes and the balance of excitatory and inhibitory neurotransmission, GABA (γ-aminobutyric acid) is becoming more and more important in Alzheimer's disease (AD).

Since all currently licensed therapeutic medications for AD are cholinergic and glutamatergic system modulators and have only modest effects, it appears that additional pharmacological targets are required to restore the E/I imbalance.

Research suggests that changes in GABAergic signaling may be a therapeutic target for AD since they are linked to the cognitive impairment seen in the disease. The present study focused on identifying potential modulators of GABA receptor in AD, through a molecular docking-based drug repurposing approach. Drugs structurally similar with Clonazepam, a known inducer of GABA identified using computational screening tools. To develop more effective treatment strategies for Alzheimer's disease, the study intends to use molecular docking to find alternative or repurposed therapeutics that might offer better binding affinity and possibly better therapeutic outcomes than donepezil.

Results: Docking simulations revealed that several compounds demonstrated higher binding affinities compared to Clonazepam, indicating stronger potential interactions with Alpha chain of GABA active site. Notably, several drugs exhibited higher binding affinities than Clonazepam, suggesting stronger and potentially more effective interactions with GABA A. Among these, Temazepam, Oxazepam , Prazepam and Nitrazepam emerged as the most promising candidates,

demonstrating superior docking scores and favorable interaction profiles. Further, 2D interaction analysis was carried out using BIOVIA Discovery Studio, which visually illustrated key binding interactions such π–π stacking, hydrophobic interactions, and hydrogen bonding between the ligands and critical amino acid residues of the GABA A protein followed by a toxicity assessment using ProTox II server.

Conclusion: The binding affinity result, interaction profiles and toxicity analysis strongly suggest that certain FDA-approved drugs could serve as promising candidates for repurposing in AD therapy. These studies provide the scope for future in vitro and in vivo validations to confirm their remedial capabilities.

CHAPTER 1

INTRODUCTION

The multifaceted neurodegenerative state named as Alzheimer's disease (AD) is typified by tau tangles along with amyloid-beta (Aβ) plaque buildup, synaptic dysfunction, and cognitive impairment.[1] AD also accounts for two-third of cases reported for the progressive cognitive impairment in older patients aged more than 60.[2] It has also being observed that females are more likely to develop the disease by the ratio of 1.2 to 1.5 as compared to the male, the women with similar amyloid beta concentration are more likely to have a higher tau load.[3] The occurrence the disease is estimated to triple by the year 2050, so it becomes very important to find the therapeutic solution for this problem.[4]

There are many genetic factors which influence the disease , presence of EPOE4 gene is one of them. It has been observed that individual with gene epoe4 is susceptible up to 3 to 4 times to develop AD over time as compared to the individual lacking this gene,[5] although it does not fully account for the heritability of the disease. After this amyloid beta metabolism , immune response metabolism and vascular factors plays a role in AD development.[6] Genes likes SORL1 ABCAT7 and TREM genes are some other genes which has been observed by the several NGS techniques which may also contribute to cause the disease. [7], [8], [9]

These results suggest that these genes are required to be intact which makes them essential in maintaining brain health, although no definite cure for the disease has been found yet this suggest that the presence of beta amyloid or NFT in the brain may be the symptom of the disease and not the causative agents

For proper operation, our brain depends on the delicate balance between excitatory (like glutamate) and inhibitory (like GABA) neurotransmitters. Recent research has highlighted the potential of targeting GABAergic neurotransmission, particularly GABAA and GABAB receptors, as a therapeutic strategy for AD.[10], [11] As studies has found that GABAergic dysfunction can play a major role in memory impairment and disease progression.[10] Also alternation in the GABA receptors may also serves as biomarkers.[12] This approach not only addresses the excitatory/inhibitory (E/I) imbalance but also interacts with other neurotransmitter systems to modulate disease progression.[13]

CHAPTER 2

LITERATURE REVIEW

2.1 Alzheimer's disease

History Background

From its first discovery to its current position as a significant public health issue, Alzheimer's disease (AD) has undergone a complicated progression. When Alois Alzheimer first characterized the illness in 1907, it was thought to be an uncommon type of senile dementia.[14] Over the decades, substantial advances in understanding its pathology, particularly the function of amyloid proteins, have changed AD into a leading cause of dementia worldwide.[15]

Genetic Factors

Strong genetic components have links with AD which include both rare mutation and common genetics variants which contribute to AD risk. Majorly AD is of two types

Early onset AD (Familial <65 years)

This is caused by rare and highly penetrant mutation in the APP , PSEN1 and PSEN2 genes. These genes follow an autosomal dominant inheritance and presence of these mutation almost guarantees that the disease will prevail that is clear grounds are presents.[16] This accounts for less than 10 percent of all AD.[17] The APP gene, which codes for beta APP, is found on chromosome 21 in the 21q11.2-q213 region, which has 32 mutations. which causes increase in Ab production or AB42/AB40 ratio. PSEN1(Presenilin 1) present on chromosome 14 in the region 14q24 which contains 182 mutation this also increase the ratio of AB42/AB40.[18], [19]

Late onset AD (sporadic > 65 years)

The primary cause of it is the gene APOe4, It had been demonstrated to triple or quadruple the risk of AD.,[5] APOE plays a role in metabolism of lipid and repair of tissue. Apart from this it also important in neuronal protection , repair and remodeling through a number of processes which includes antioxidants effects, interaction with estrogen and modulation of synaptodendritic

protein. (narrative review) APOE allele which are found in the human brain is of three types namely e4 , e3 and e2 which are present at 17% , 78% and 7% respectively. The presence of e2 has shown to display protective effect on contrary to which the presence of e4 cause greater risk of AD.[20] This strength of the relationship varies among epidemiological studies. Apart from this there are several genes with moderate and small effects include TREM2 , CLU , PACALM , PLD3 , UNC5C, AKAP9, ADAM10 and many more are identified through genome wide association studies(GWAS) which highlights the polygenic and multifactorial nature of the disease, Apart from this several environmental factors also can influence the disease.[21], [22], [23] It also has been noted that individuals with history of head injury are more susceptible to develop disease although there has been no direct correlation with this information.[24], [25]

2.2 Biomarkers

The combination of biomarkers and imaging methods has greatly improved the diagnosis and tracking of Alzheimer's disease (AD). Cerebrospinal fluid (CSF) proteins and neuroimaging results are examples of biomarkers that are essential for early identification and comprehension of AD disease.[26] Imaging techniques like MRI and PET are crucial for illustrating the disease's anatomical and functional alterations in the brain. The main features of biomarkers and imaging methods in AD are covered in depth in the sections that follow. CSF, or cerebrospinal fluid [27]Biomarkers: Amyloid-beta (Aβ) is an early sign of AD, and other important proteins like as tau (both phosphorylated and total) and Aβ are essential for the diagnosis. Blood-Based Biomarkers: According to recent developments, non-invasive blood tests could offer viable substitutes for early detection.[28]

GABA() has shown potential to be used as a biomarker for AD, which suggest the neurochemical changes attached with the disease. The GABA levels correlate with the burden of beta-amyloid and decline in cognitive levels, which indicates its role in the early detection of AD. In the studies it has been observed that greater level of GABA in gray matter is associated with higher beta amyloid burden,[29] which again indicates its role as early marker for AD. This correlation between them is greatly influenced by the presence or absence of APOE4 allele which is again a known genetic risk factor for AD.[30] The lower level of GABA correlates with damaged cognitive function in AD patients. Disruption in the GABAergic signaling may contribute to the psychological symptoms and behavioral symptoms such as depression and apathy which is

commonly observed in AD.[31] Apart from these changes in the gut microbiota can also affect GABA production influencing both gut integrity and brain health which becomes relevant in AD pathology. Although GABA shows promising results as a biomarker for AD but it may not be used a standalone biomarker as there are many complexities in the neurodegenerative process and pathways which is required to be studied further more.

Table 1: List of biomarkers involved in AD

Biomarker Type	Clinical Relevance & Example	Reference
Amyloid Biomarkers	Detects amyloid plaque burden. E.g. Aβ42 (CSF) Ab PET	[32]
Tau Biomarkers	Indicate tau pathology and neurodegeneration. Total tau(t-tau) phospo-tau(p-tau)(CSF)	[33]
Neurodegeneration	Marker of axonal degeneration e.g. NfL	[34]
Synaptic Biomarkers	Indicates synaptic dysfunction e.g. Neurogranin	[35]
Inflammation Markers	Reflect glial activation and neuroinflammation. E.g. YKL-40 , STREM2	[36], [37]
Imaging Biomarkers	Show structural and functional brain changes. E.g. MRI(hippocampal atrophy), FDG-PET	[38]

Blood Biomarkers	Less invasive markers for early detection Plasma E.g. Aβ42/Aβ40 ratio , p-tau217	[39]

2.3 Imaging Methods

Magnetic Resonance Imaging (MRI): While functional MRI evaluates brain activity, structural MRI can identify hippocampus atrophy. According to [40], these methods are essential for detecting neurodegeneration.

Positron Emission Tomography (PET): PET imaging provides information about the course of the disease by seeing tau accumulation and Aβ plaques.[40]

New Methods: By combining several biomarkers, novel imaging modalities such as radiomics and diffusion tensor imaging improve diagnostic accuracy.[41]

Although these developments in biomarkers and imaging methods offer useful instruments for the early diagnosis of AD, issues with specificity and the invasiveness of some procedures still exist. To enhance diagnostic skills, future studies should concentrate on improving these techniques and investigating novel biomarkers.

GABA

2.4.1 Role of GABA

Numerous neurotransmitters are found in the central nervous system, including GABA, a well-known amino acid neurotransmitter with inhibitory properties.[42] GABAA , GABAB and GABAC are the three major types of GABA receptors. GABAB works through second messenger systems and it trigger metabolic process inside the cell, i.e. it is G-protein coupled receptors,[43] These receptors may be a target for therapeutic drugs because they are believed to be the cause or origin of a number of neuropsychiatric diseases..[43], [44] In addition GABAB receptors expression is linked to the normal memory functioning in aged rats Since the p13k/akt signaling cascade has a major impact on cell development and death in a number of situations, it is well known that it affects neuronal existence. Recent studies have shown that P13K-dependent neutrophil chemotaxis and microtubular rearrangement are triggered when GABAB receptors in neutrophils are activated.[45] For instance, a reduction in the insulin p13k-akt signaling pathway may trigger AD neurodegeneration by lowering O-GlcNAcylation, which in turn increases tau

hyperphosphorylation and aberrant neurodegeneration.[46] This property of GABAB can be used in AD pathology.

2.4.2 Structure of GABA

Different features of GABA A and GABA B receptor structures correspond to their functional roles in neurotransmission. The heterodimeric G-protein-coupled receptors (GPCRs) called GABA B receptors are composed of two subunits. Both GABA B(1) and GABA B(2) In contrast, GABA A chloride ion channel is formed by pentameric ionotropic receptors.

GABAA

GABA A receptor is a complex pentameric structure constitute of five protein subunits symmetrically grouped around a central ion-conducting pore. This receptor has a remarkable subunit diversity with many families, including α1-6, γ1-3,β1-3, θ, δ, ε, π, and ρ1-3.[47] This allows for a wide variety of receptor subtypes, with the most common configuration having two α, two β, and one γ subunit. The four transmembrane domains (M1–M4) that make up each subunit include an extracellular domain that contains the GABA binding location where the α and β subunits meet. and a sizable intracellular loop that connects M3 and M4 and promotes interactions with signaling molecules and cytoskeletal proteins.[47]

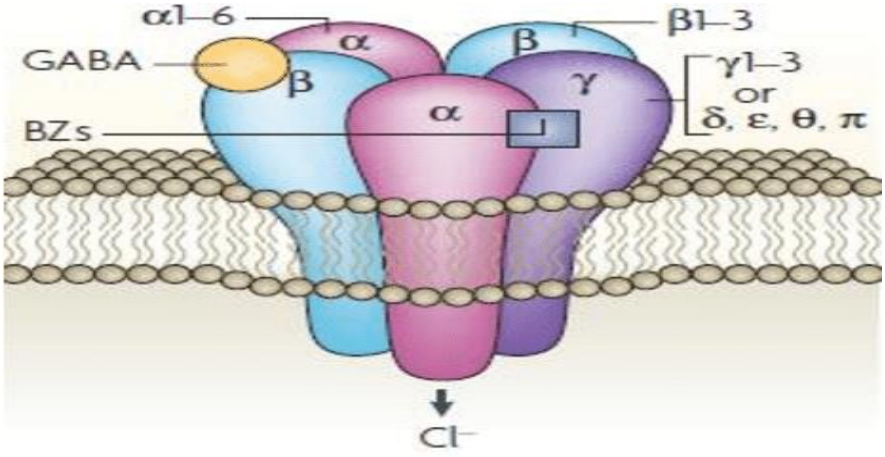

Fig 1. Structure of GABA A Receptor

GABAB

The GABA B receptor differs from other GABA receptors due to its distinct and intricate structure. GABA B receptors are metabotropic G protein-coupled receptors (GPCRs) with a unique heterodimeric architecture, in contrast to GABA A receptors, which have a pentameric ligand-gated ion channel structure.

GABA B(1) and GABA B(2) are the two subunits that make up the GABA B receptor and must combine to generate a functioning receptor. Class C GPCRs are characterized by their seven transmembrane α-helices (TM1–TM7), an internal C-terminal region, and a large external N-terminal domain.[48]The Venus flytrap module found in the extracellular domain of the GABA B(1) subunit acts as an orthosteric binding site for GABA and other agonists, However, despite not binding GABA, the GABA B(2) subunit is necessary for G protein coupling and trafficking.[49]

2.5 GABAergic system

The primary inhibitory neurotransmitter system in the mammalian brain is GABAergic system. which operates as an essential counterbalance to excitatory systems. It helps create brain rhythms necessary for cognitive processes, keeps the brain's excitation/inhibition balance, and stops neuronal overexcitation[50]. GABAergic neurons, GABA transmitters, and GABA receptors are the three primary parts of the GABAergic system. In GABAergic neurons, glutamate decarboxylase changes glutamate into GABA, which is eventually discharged into the synaptic cleft via vesicular exocytosis. [43]Through the plasma membrane, extracellular GABA reuptake can end the inhibitory effects. Unlike projection neurons, which have lengthy axons spanning multiple brain regions, targeting local neurons in the same area of the brain, GABAergic neurons are a type of interneuron that usually has short axons. The bulk of GABAergic neurons are interneurons, despite compelling evidence for the existence of GABAergic projection neurons. In addition to having distinct electrical and chemical characteristics, GABAergic neurons come in a wide range of forms. GABAergic neurons are currently characterized by the calcium-binding and buffering proteins they express, such as somatostatin (SST) and parvalbumin (PV). [51], [52]. All pyramidal neuron subcellular compartments are targeted by GABAergic interneurons, which also modify the firing patterns of pyramidal neurons in various ways. This dynamically controls the pyramidal neurons' spatially separated activity throughout the same or different time periods,

avoiding overexcitation and the development of brain oscillations necessary for memory processing.[42] The two most studied subpopulations of GABAergic neurons in the AD brain are PV and SST neurons, which make up around 70% of all GABAergic neurons.[42] While SST neurons regulate dendrites, PV neurons primarily target the proximal dendrites, soma and axon beginning parts of pyramidal neurons. Ionotropic GABAA and metabotropic GABAB are the two types of GABA receptors. GABAA receptors in mature neurons produce Cl^- influx to hyperpolarize the cell upon binding to GABA. Neurons are protected from neuronal excitotoxicity by this inhibitory current. Usually, GABAA receptors are pentameric proteins made up of two α (1-6) and two β (1-3) subunits, as well as γ (1-3), δ, ε, θ, π, or ρ (1-3) subunits.[53], [54] It is important to note that postsynaptic GABAA receptors generate massive and quick inhibitory postsynaptic currents based on different affinity levels for GABA,[55] while GABAA receptors outside of synaptic areas create modest but persistent inhibitory currents, also referred to as tonic currents.[56]

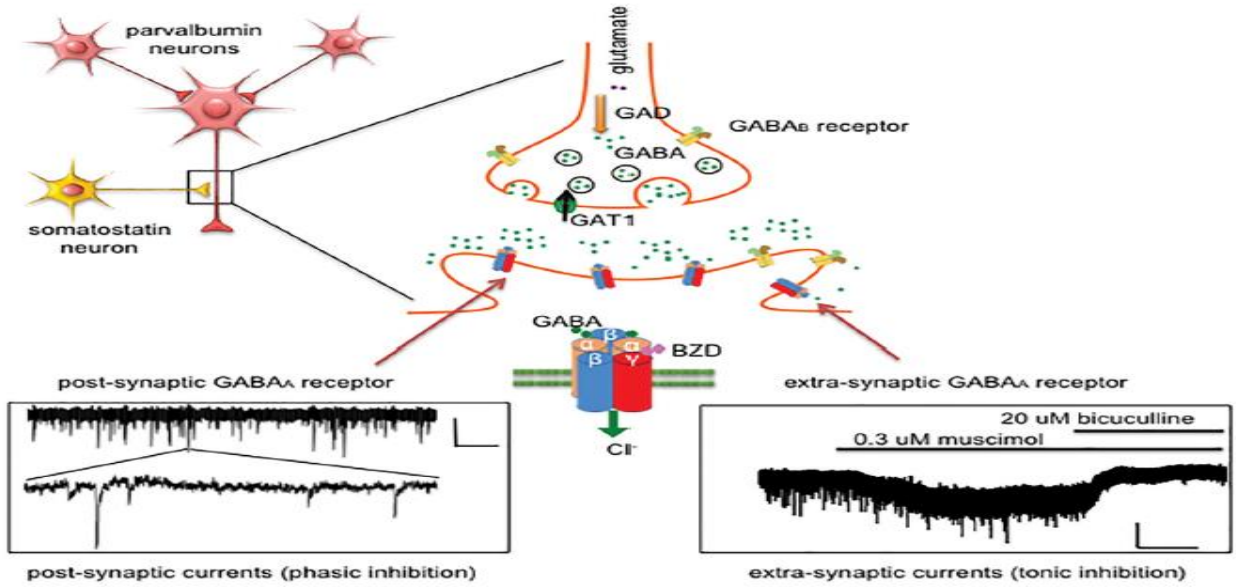

Fig.2 The GABAergic system's main components (GABAergic neurons, GABA transmitters, and GABA receptors.)[10]

2.6 Role of Prominent Genes in AD

2.6.1 Amyloid Beta in GABAergic Dysfunction and Excitation/Inhibition Imbalance in Alzheimer's Disease

Even before AD patients experience cognitive impairment, there is increased Aβ deposition and reducing levels of Aβ42 in cerebrospinal fluid (CSF) and plasma, [57]which can be observed with PET imaging.[40] Aβ oligomers disrupt neuronal networks' and circuits' E/I balance. Numerous research have also shown that Aβ impairs GABAergic functioning, resulting in E/I imbalance and cognitive impairment, using AD mice models.[13] The receptor tyrosine-protein kinase, erbB-4, which is mostly expressed in PV neurons and encoded by the ERBB4 gene, interacts with Aβ.[58] This results in GABAergic neurotoxicity since Aβ-induced memory problems in hAPPJ20 mice[52], [59] are greatly reduced when ERBB4 is specifically deleted in PV neurons.[58], [60]

Maintaining E/I balance and avoiding neuronal hyperactivity and epileptiform discharge depend on parvalbumin-mediated peri somatic inhibition. When levetiracetam, an antiepileptic medication, is administered to hAPP-J20 mice,[59] network hyperexcitability and cognitive impairment are greatly reduced. It has been demonstrated that GABAergic dysfunction caused by Slow-wave activity and spatial memory consolidation deficiencies are caused by Aβ and can be partially addressed by pharmacologically increasing GABAA receptor function. These investigations offer a different explanation for excitotoxicity, the ensuing glutamatergic neuron degeneration, the underlying increased network activity, and the higher seizure prevalence.

Furthermore, because a GABAB receptor antagonist reduces the learning and memory damage caused by Aβ, Aβ also causes hyperactivation of GABAB receptors.[61] E/I balance also depends on GABAB receptors, which are metabotropic G-protein coupled GABA receptors. Activating presynaptic GABAB receptors prevents the release of numerous neurotransmitters. GABA and glutamate release are less likely when released extracellular fragments of amyloid precursor protein (APP) bind agonistically to GABAB receptors.[62]. It is uncertain, meanwhile, how the AD brain's total soluble APP changes.

2.6.2 The S182/PS-1 (Presenilin 1) Gene

Over 25 variants have been described in over 30 families from a range of ethnic origins, including Hispanic, Japanese, Ashkenazi-Jewish, and White. With the exception of one, all of the mutations

are missense mutations, meaning that they produce single aa substitutions as opposed to early cessation and a shortened protein. That indicates that mutations in S182iPS-1 is most probable result in AD via improving the function of Protein as opposed to impairing it. The only exception is a mutation that eliminates the exon 9 splice acceptor site, which codes for a portion of a hydrophilic loop. The reading frame is unchanged despite the absence of exon 9, and the protein should be 29 aa shorter. The final hydrophilic loop contains three alterations, in contrast, the loop connecting the first and second hydrophobic transmembrane domains has two. Only the hydrophilic-hydrophobic junction or the other hydrophobic domains have the remaining 26 alterations.[63] The relative frequency of mutations in the sixth hydrophilic domain/loop, expressed by the successively spliced exon 8, suggests that this region contains a functionally relevant component. Early-onset familial Alzheimer's disease (AD) is the primary clinical characteristic linked to S182/Presenilin-1 (PS-1) mutations.[64] The clinical presentation, age of start, and neuropathological findings of these mutations vary greatly, which can make diagnosis and treatment plans more challenging. There is considerable variation in the mean age of onset for PS-1 mutations; for example, one study found that the A260V mutation had a mean age of 40.3 years, but the A285V mutation had a mean age of 51 years.[65] [66] Additionally, the course of the disease varies; certain mutations cause cognitive function to diminish more quickly than others. The underlying processes of sporadic cases of Alzheimer's disease may differ, indicating that not all cases are caused by genetic mutations in PS-1 or associated genes, even if PS-1 mutations are a key factor in early-onset familial AD.[67]

2.6.3 The STM-2/PS-2 (Presenilin *2)* Gene

Presenilin 2 (PS2), often referred to as the STM-2/PS-2 gene, has important roles in the central nervous system and is linked with familial Alzheimer's disease (FAD).[68]According to research, PS2 is mostly expressed in neurons, especially in areas that are necessary for cognitive processes like the hippocampus and cerebral cortex[69]. FAD is associated with mutations in PS2, which affect the synthesis of amyloid-beta and facilitate the processing of amyloid precursor protein (APP), which is essential to the pathophysiology of Alzheimer's disease.[70] With beginning dates ranging from 40 to 88 years, the penetrance of PS2 mutations varies, suggesting a complicated interplay with other genetic and environmental variables.[71] [72]PS2

plays a role in apoptosis, mitochondrial function, and calcium signaling; mutations impact these mechanisms and exacerbate neurodegeneration.[73] It has been demonstrated that PS2 overexpression in neurons increases apoptotic cell death, underscoring its significance for neuronal survival.[73] On the other hand, sporadic Alzheimer's disease may entail distinct pathways, indicating that not all cases are directly linked to PS2 malfunction, even though PS2 mutations are crucial in familial cases.[71]Developing tailored medicines requires an understanding of these differences.

2.7 DISRUPTED PATHWAYS IN AD

Pathway	**Affected step in Alzheimer's Disease**	**Reference**
Amyloidogenic Pathway	Ab42 peptides accumulate as a result of b-secretase and Y-secretase's aberrant cleavage of amyloid precursor protein (APP).	[74]
Tau Protein Phosphorylation	Hyperphosphorylation of tau protein by kinase (e.g. GSK-3beta) causing microtubule destabilization	[75]
Cholinergic Pathway	Decreased synthesis and release of acetylcholine due to degeneration of basal forebrain cholinergic neurons.	[76]
Neuroinflammatory Pathway	Overactivation of microglia and astrocytes releasing pro-	[77]

	inflammatory cytokines (e.g. IL-1Beta , TNF- alpha)	
Mitochondrial Dysfunction Pathway	Impaired mitochondrial dynamics and increased oxidative stress leading to neuronal damage.	[78], [79]
Insulin Signaling Pathway	Insulin resistance in brain cells, disrupting neuronal survival signaling via the P13K-Akt pathway	[80]
Calcium Homeostasis Pathway	Dysregulation of intracellular Calcium levels impairing synaptic function and promoting apoptosis.	[81], [82]
Ubiquitin Proteasome Pathway	Impaired degradation of misfolded proteins contributing to protein aggregation	[83]
Autophagy Lysosome Pathway	Blocked clearance of damaged organelles and protein aggregate like Ab and tau.	[84]
“Wnt/B-catenin Signaling Pathway”	Cessation of Wnt signaling by Ab leading to decreased neurogenesis	[85]

2.7 DRUG REPURPOSING

Currently, the U.S. Food and Drug Administration has approved seven medications to treat AD: two amyloid β-directed monoclonal antibodies (aducanumab and lecanemab); a glutamate regulator (memantine); three cholinesterase inhibitors (galantamine, rivastigmine, and donepezil); and a combination of a glutamate regulator and cholinesterase inhibitor (donepezil/memantine).

[86], [87]Only small progress has been made so far in creating novel therapies for AD due to high cost and time taking process of drug development methods. Only aducanumab, a novel medication, received contentious approval for treatment in AD between 2003 and 2022. Despite controversy, lecanemab, the most recent approved medication for the treatment of AD, received accelerated FDA approval in January 2023.[88] While lecanemab demonstrated some Although it was successful in halting cognitive decline, it also brought up safety concerns about major side effects such edema and brain bleeding.[89] Since then, lecanemab has obtained complete FDA approval. Approval of a novel medicine is a costly and time-consuming process that can take ten to fifteen years. A different strategy for reducing the amount of time needed to produce a drug is drug repurposing, or repositioning,[90] which is made possible by this drawn-out discovery process. Using medications that regulatory bodies including "the FDA, the European Medicines Agency (EMA), and the Medicines and Healthcare Products Regulatory Agency (MHRA)" have authorized for a new use, and others is known as repurposing. Because a shortened development cycle has such great promise, several pharmaceutical companies are currently embracing drug repurposing to regenerate some of their FDA-approved and previously unsuccessful pipeline compounds as novel treatments for a variety of medical conditions.[90], [91] Utilizing extensive datasets to identify drug-associated patient outcomes that would not have been discovered otherwise is one method of choosing candidates for drug repurposing. Hypothesis-driven repurposing is an alternate approach that identifies possible candidates by integrating data on the disease of interest with the characteristics and targets of currently available medications for other conditions.[92] Also, in vitro models that evaluate the impact of drugs on established target pathways, including amyloid toxicity, can be employed for high-throughput screening. Using disease-associated transcriptional profiles as a tool to find potential treatments is a unique approach. For example, brexpiprazole was effectively repurposed from its approved indications as an adjuvant medicine for the treatment of serious depression in adults and schizophrenia in both adults and children to the approved treatment of agitation in AD-related dementia.[93], [94] Clinical trials are being conducted to evaluate semaglutide, a glucagon-like protein-1 (GLP-1) agonist, for the medication of early AD. It is approved for treatment of diabetes and obesity.[95]

2.9 MOLECULAR DOCKING AS A TOOL FOR DRUG DISCOVERY

Molecular docking is an in-silico technique that use a variety of SF (scoring functions) to determine the protein-ligand complex's suitable binding position and evaluates its strength to determine which position each molecule produces in a rank order.[96] The goal of docking approaches is to effectively place a ligand inside a target protein's binding region. In order to evaluate their binding free energy, this involves balancing and optimizing factors such as hydrophobic, steric, and electrostatic complementarity.[97] Pose prediction, virtual screening, and binding affinity estimate are the three main goals of molecular docking. A trustworthy docking technique should be able to identify the molecular interactions between binding and non-binding sites. the ideal position that each molecule produces to a rank order by determining the protein-ligand complex's strength and suitable binding position. When working with huge chemical libraries, the method must also accurately distinguish binding compounds from non-binding ones and rank the binding compounds among the top compounds in the database.[98] The effectiveness of virtual screening depends on the quantity and precision of structural information available for the target protein and the ligand undergoing docking.[96] To complete this procedure, the molecular orientation of ligand within a receptor is known prior hand and then the complementarity between them is estimated using a scoring function.[99] Molecular docking , MD simulation and ADMET modelling are the three most popular computer modeling technique. these are important in making it simple to identify potential candidates for in vivo and in vitro experiments.[100] There are chance that due to presence of inadequate or inaccurate receptor flexibility modeling the results of docking can be hindered. Physicochemical properties and molecular descriptors of active ligands based virtual screening can be very useful in identifying hits and leads through library.[101] Different search strategies can be applied, classified as systematic or stoichastic while empirical , force field based or knowledge based scoring systems is also used.

When it comes to Alzheimer's disease (AD), where finding effective treatments is still a major challenge, computational methods like docking provide a quick and affordable way to find viable candidates. Docking simulations offer insights into molecular recognition and possible therapeutic efficacy by predicting the ideal binding orientation and affinity of ligands with the active or allosteric regions of target proteins. In this study, FDA-approved medications were screened and

their binding affinity to GABAA and GABA B receptors , The alpha5 subunit of GABA A is activated using different synthetic ligands or Positive allosteric modulators (PAM)while the alpha1 subunit of GABA A is inhibited using PAM [102], [103] this is assessed using molecular docking. The activation or enhancement of Alpha5 od GABA A is thought to be a useful treatment approach for altering the progression of AD by maintaining the balance between excitation and inhibition.[103] GABA A contain Benzodizepine Binding site (present in the interface between alpha- gamma) this site is known to directly activate the receptors apart from this the transmembrane domain(TM2) which forms the ion channel pore and affect the ion conductance and gating of receptor can also be targeted to influence the flow although it is very difficult to target due to subtype selectivity , these sites are selected for molecular docking with several PAM which affect substrate recognition and binding affinity.[104]

Focusing on medicines that have FDA approval was a calculated and sensible approach. Because these compounds have already undergone extensive pharmacological and toxicological testing, the risk, cost, and time associated with early-stage drug development are significantly reduced. Furthermore, because one of the main barriers to drug delivery to the central nervous system (CNS) is the blood-brain barrier (BBB), selecting compounds with known BBB permeability enhanced the likelihood of clinical applicability.We employed a structure-based similarity and function-guided method to repurpose structurally related molecules to Clonazepam), a clinically used AD medication known to balance the AD development at some extend.

The results of the study demonstrate the value of molecular docking as a computational drug repurposing tool, especially for diseases like Alzheimer's where there is an urgent demand for disease-modifying treatments. The promising interactions seen with a few FDA-approved drugs encourage additional in vitro and in vivo studies to validate their efficacy as GABA-targeting therapies.

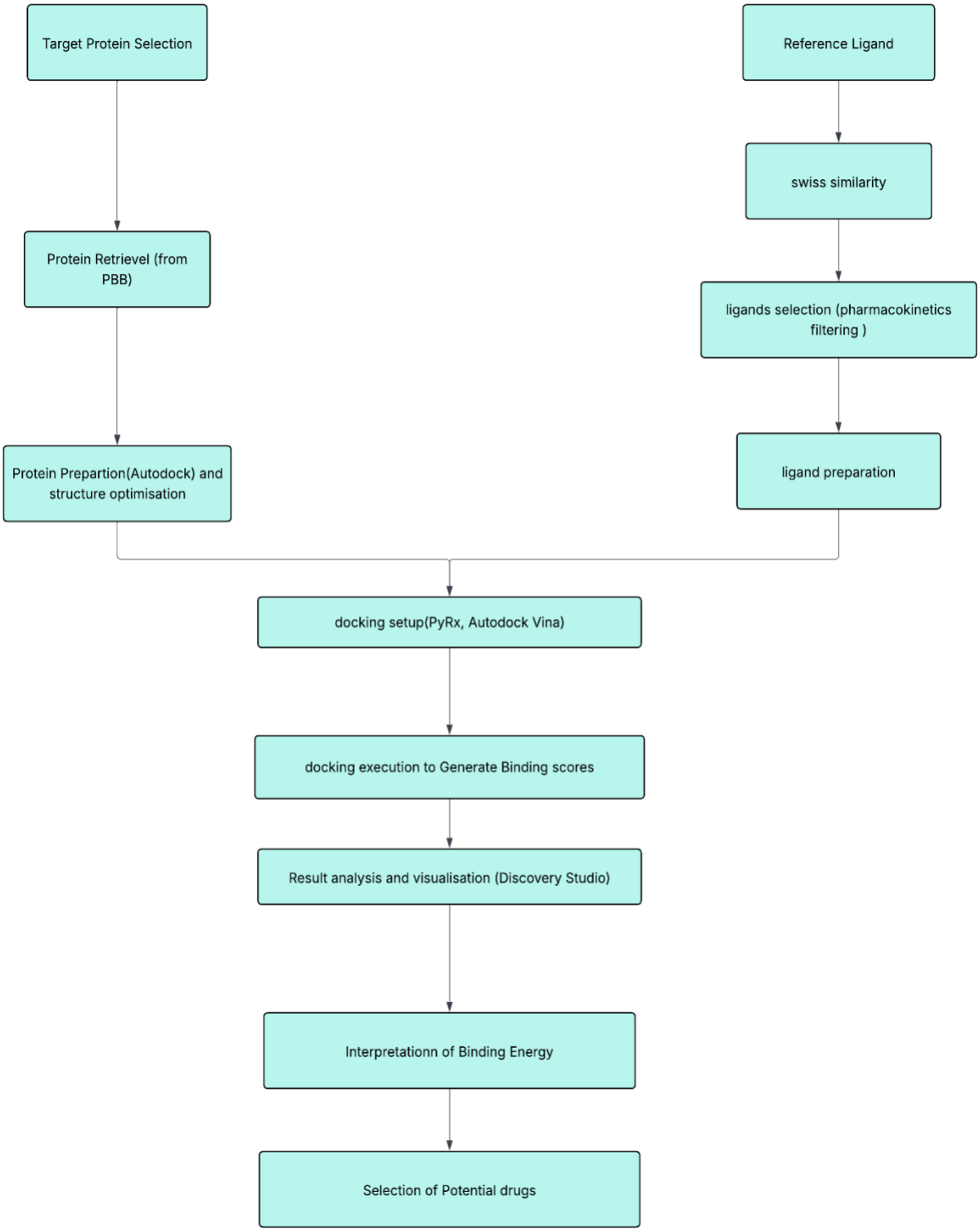

Fig 3 : Molecular docking workflow, key steps involved.

CHAPTER 3

METHODOLOGY

3.1 Collection of data

The protein structure of GABA, the target receptor in molecular docking studies, was retrieved from the Protein Data Bank (PDB). When selecting the compound's structure, the active site's totality and resolution quality were taken into account.

The ligands were identified using a Internet-based virtual screening platform called Swiss Similarity tool (https://www.swisssimilarity.ch/). This program makes it possible to identify structurally related molecules based on known ligands, which aids in ligand-based drug discovery and therapeutic repurposing initiatives. The screening started by obtaining the SMILES notation of Clonazepam, a clinically licensed GABAA enhancer used to treat Alzheimer's disease, from PubChem and verifying it using the Drug Bank database. To ensure that every hit that surfaced had proven pharmacological and safety properties, Swiss Similarity was configured to search just inside the FDA-approved pharmaceutical library.

A CSV file containing 331 medications that showed a high degree of structural similarities to clonazepam was generated when Swiss Similarity was performed. These candidate compounds were next subjected to a blood-brain barrier (BBB) permeability filter using SwissADME and related ADME analysis techniques, as central nervous system (CNS) activity is crucial for Alzheimer's disease therapy. In order to focus on substances that were more likely to have therapeutic effects inside the brain, those that were believed to be non-permeable to the BBB were then removed. Molecular docking studies were conducted against the GABAA active site using the 48 compounds that remained after filtering and had both favorable structural similarity and CNS permeability.

3.2 Target protein preparation

GABAA receptor induction has been chosen as a possible Alzheimer's disease treatment.
The three-dimensional crystal structure of GABAA was retrieved
from the RCSB Protein Data Bank (PDB) (https://www.rcsb.org), a thorough and carefully curate

d repository for experimentally determine
protein structures.Using PDB ID pdb_00005ojm, which represents the extracellular domain of G ABAA (5alpha subunit of the receptor), the exact structure used in this study was identified.
The alpha and gamma subunit, which is the main target for ligand binding and has the active site that propels enzymatic activity, is included in this domain. In order to ensure a clean and physiologically appropriate docking environment, the protein structure was preprocessed using Auto Dock Tools (MGL Tools). Water molecules were removed in this stage to cut down on noise and prevent phony interactions. Because polar hydrogens are not collected during docking and may complicate the interpretation of results, they were also eliminated. Additionally, Gasteiger charges were added and the structure was converted and saved in PDBQT format, which is required as an input file for Auto Dock-based docking simulations. Proper protein preparation is a crucial prerequisite for accurate molecular docking because it ensures that the active site is accurately defined, steric hindrance is minimized, and binding energy calculations are accurate. Any mistakes made at this stage could lead to inaccurate docking results or improper identification of potential lead compounds. Therefore, careful target preparation improves the accuracy, reproducibility, and biological importance of the docking results.

3.3 Selection of ligands

To create effective pharmacological therapies, it is necessary to evaluate the crucial pharmacokinetic parameters known as absorption, distribution, metabolism, and excretion, or ADME. These factors affect not just a compound's drug-likeness but also its bioavailability, toxicity, and therapeutic appropriateness, especially for conditions affecting the central nervous system (CNS), such as Alzheimer's. To ensure the pharmacological significance of selected ligands, this study used SwissADME, a publicly accessible online software developed by the Swiss Institute of Bioinformatics (SIB) (http://www.swissadme.ch/). This platform enables comprehensive profiling of small compounds by using SMILES (Simplified Molecular enter Line Entry System) notation to enter chemical structures.

An initial library of 291 FDA-approved drugs was chosen using a multi-step screening process because of their structural similarity to the well-known GABA A inducer. The first screening step made use of Lipinski's Rule of Five, which evaluates important attributes like MW, log P, HBD, and HBA, all of which are symptomatic of a compound's likelihood to be orally accessible.

Compounds that didn't meet these standards were eliminated. In the next phase, compounds that could result in false-positive results due to assay interference or non-specific biological activity were removed using the PAINS (Pan-Assay Interference Compounds) filter.

Because of the BBB's critical role in CNS drug delivery, blood-brain barrier (BBB) permeability predictions were incorporated into the selection process to ensure that the compounds retained the potential to effectively reach brain tissue. Only substances that were expected to cross the blood-brain barrier were considered promising candidates for further study in the context of Alzheimer's treatment. Additional characteristics such as water solubility, synthetic accessibility, bioavailability score, and gastrointestinal (GI) absorption were evaluated in order to bolster each candidate's pharmacokinetic profile.

Following a comprehensive ADME-based study, 42 drug-like molecules were identified from the original 284 compounds, all of which exhibited encouraging pharmacokinetic characteristics. GABA Molecular docking studies were performed on a subunit to evaluate the binding affinity and interaction patterns of these selected ligands, which were believed to be promising for CNS function. After ADME filtration, only candidates with high potential, CNS permeability, and pharmacological relevance advanced to the final docking phase.

3.4 Ligand preparation

The ligand preparation process was a crucial step in ensuring that each molecule was in a format suitable for molecular docking studies. The first application of Swiss Similarity was to find compounds that shared structural similarities with the reference drug Clonazepam. The matching three-dimensional structures of the compounds were then obtained using the PubChem database (https://pubchem.ncbi.nlm.nih.gov/).The publicly accessible chemical information repository PubChem, which is updated and maintained by the National Centre for Biotechnology Information (NCBI), provides a thorough compilation of compound data, including physicochemical characteristics, biological activities, and structural specifics. The selected compounds were downloaded in the Structure Data File (SDF) format in order to depict 3D chemical structures and associated metadata.

These files were converted into a format compatible with molecular docking tools using Discovery Studio. Discovery Studio is an open-source chemical toolbox that can convert over 110 different

chemical file formats. It also makes molecular modeling activities like identifying molecular descriptors, enhancing geometry, and adding hydrogen atoms easier. All of the ligand structures in this study were translated from SDF to PDB (Protein Data Bank) format using Discovery Studio Biovia in order to guarantee proper alignment and processing during docking simulations. This conversion ensured the preservation of the ligands' spatial arrangement and chemical integrity.

Following format conversion, each ligand was further constructed by adding polar hydrogen atoms, assigning the proper atomic charges, and optimizing shape using Open Babel in PyRx or the ligand preparation tools in the docking suite. This step was crucial to ensure that the molecular docking data appropriately reflected potential interactions with the GABAA target protein. Overall, the ligand preparation process ensured high-quality structural data input, which forms the foundation for reliable and reproducible docking results.

3.5 Molecular docking

To determine their orientation and contact affinity at the protein's active site, FDA-approved ligands selected based on ADME profiling were evaluated for binding potential against the GABA receptor's alpha subunit using molecular docking. PyRx is a well-known open-source application for virtual screening and molecular docking that is mostly used in drug development research. By integrating several powerful tools into a single graphical user interface (GUI), it simplifies and makes computational docking easier, especially for people who have never programmed before.

After eliminating water molecules and non-essential heteroatoms, polar hydrogens got added, and Kollman charges were inserted employing AutoDock tools to create the three-dimensional structure of GABA A (which was taken from the Protein Data Bank). Following energy minimization and maintaining the appropriate torsional flexibility, the ligand structures were created by translating them into PDBQT format in PyRx. The entire GABAA active site region was enclosed by a grid box, which permitted unrestricted investigation of the binding cavity. The grid size measurements were x = 87.1327, y = 82.2439, and z = 115.1061, while the grid center parameters were x =-27.911, y = -150, and z = -40.58 To provide adequate coverage of the catalytic core, the grid spacing was maintained at the default value of 0.375 Å.

Every ligand was docked , and PyRx produced a variety of binding positions for every molecule. The optimum docking pose among them was determined to be the conformation with the lowest

binding energy, or the most negative score. The docking findings were recorded and analyzed using an Excel spreadsheet, with a particular focus on comparing each ligand's binding energy to that of the reference medication.

3.6 Examination of the Protein-Ligand Complex Structure

Following the molecular docking process, a comprehensive structural analysis of the protein-ligand complexes was conducted to ascertain the kind and strength of interactions between ligands and the GABAA receptor. The docking program generated a distinct output file for each ligand that included crucial information such as binding energy scores, interaction distances, and ligand locations inside the active site of the macromolecule. These files served as the foundation for the interaction visualization and validation.

Further analysis and visual understanding of these complexes were conducted using Dassault Systèmes' BIOVIA Discovery Studio, a comprehensive collection of molecular modeling and simulation tools. This platform was chosen due to its powerful visualization capabilities and ability to generate 2D and 3D representations of protein-ligand interactions. In particular, Discovery Studio enabled the creation of 2D interaction diagrams that successfully illustrated significant binding interactions, such as electrostatic forces, hydrophobic contacts, π–π stacking, metal coordination, and hydrogen bonds. These interactions are all particularly significant when considering the mechanism of drug action.

The ligand orientation in the binding pocket and its proximity to crucial active site residues and the catalytic glutamate may be precisely examined thanks to Discovery Studio's 3D visualization features. Validating the docking data and choosing viable lead compounds based on the caliber and specificity of their interactions required these insights. Only the most advantageous binding conformations were taken into consideration for more research thanks to Discovery Studio's interactive display and comprehensive docking output, which greatly improved the interpretability of the docking data.

3.7 Toxicity assessment

A comprehensive toxicity evaluation was carried out to examine the safety profiles of the FDA-approved medication candidates using the ProTox-II (version 3.0) website, a reliable in-silico tool for forecasting a range of toxicological endpoints. One by one, the drug's PubChem and SMILES

names were first entered into the ProTox-II interface. Following submission, each compound was processed by the computer using its machine learning-based models, which integrate molecular fingerprints, chemical similarity, fragment-based descriptors, and toxicophoric detection. ProTox-II then generated a thorough toxicity profile that comprised the estimated LD_{50} value (mg/kg) of each molecule, its toxicity class (based on GHS categorization), and qualitative predictions for cytotoxicity, immunotoxicity, mutagenicity, carcinogenicity, and hepatotoxicity. By evaluating each candidate's relative safety and eliminating medications with potential toxicological problems, our findings aided in the selection of the most promising compounds for further study in AD therapy.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Results of molecular docking

Only 42 of the 284 FDA-approved medications that were first chosen due to their structural resemblance to Clonazepam satisfied the necessary pharmacokinetic requirements, which included permeability of the blood-brain barrier (BBB), the lack of PAINS (Pan-Assay Interference Compounds) alerts, and compliance with Lipinski's Rule of Five. These characteristics suggested that these compounds might have pharmacological effects on the central nervous system. The bulk of these 42 compounds demonstrated substantial binding affinities, according to subsequent molecular docking experiments using the GABA A receptors. Of them, 35 medicines had docking scores better than -7.4 kcal/mol, which is typically seen as a sign of favorable and stable binding.

Six of these compounds showed remarkably high binding affinities (binding energy < -9.0 kcal/mol), indicating strong and long-lasting interactions inside GABA A's active region. Notably, the reference chemical Clonazepam, which had a binding energy of roughly -7.3 kcal/mol, was outperformed by Nitrazepam (-9.1 kcal/mol) and Temazepam (-9.7 kcal/mol), which had the strongest binding. Furthermore, 19 substances shown moderate-to-significant binding affinities ranging from -7.5 to -8.4 kcal/mol, further supporting their potential as re-purposable GABA A modulators, while two medications showed binding energies precisely at -7.3 kcal/mol, which is equivalent to that of Clonazepam.

The remaining drugs provided important insights into the structural features that can influence GABA A-ligand interactions, even if their binding energies were lower. All of these results point to the potential of certain FDA-approved drugs, such as prazepam, oxyzepam, tamazepam, nitrazepam, and others, as therapeutic options for altering GABA A activity in Alzheimer's disease. Because of their higher or comparable binding affinities to clonazepam, these compounds demand further experimental validation and optimization for the repurposing of GABA A-targeted medications.

TABLE 3. LIST OF DRUGS WITH THEIR ESTIMATED ΔG (KCAL/MOL)

S.no	Drugs	Estimated ΔG (kcal/mol)
1.	Clonazepam (reference drug)	-7.3
2.	Alpha5IA	-7.6
3.	Nitrazepam	-9.0
4.	Flunitrazepam	-7.4
5.	Delorozepam	-9.1
6.	Lormetazepam	-9.7
7.	Lorazepam	-7.5
8.	Nordazepam	-9.1
9.	Diazepam	-7.6
10.	Oxazepam	-8.4
11.	Temazepam	-7.4
12.	Pinazepam	-7.9
13.	Prazepam	-8.6
14.	Fludiazepam	-8.4
15.	Halazepam	-7.1
16.	Clorazepic acid	-7.5
17.	Medazepam	-8.6
18.	Flurazepam	-7.3
19.	Oxazepam acetate	-7.7
20.	Tamazepam acetate	-8.9
21.	Loprazolam	-9.1
22.	Doxefazepam	-8.1

4.2 Visualization of interactions

Following molecular docking of the selected ligands with the GABA A subunit, the two-dimensional (2D) and three-dimensional (3D) binding conformations of the top-performing compounds were analyzed using BIOVIA Discovery Studio. The kind and intensity of the interactions between the ligands and the GABAA active site residues were shown in this picture. Drugs with higher docking scores than the reference molecule Clonazepam, such as Nitrazepam, Nordazepam, Oxazepam, Prazepam, and Loprazepam, were found to have several stabilizing interactions. These included π–π stacking, hydrogen bonding, hydrophobic interactions, and metal coordination with the catalytic zinc ion, which enabled strong and accurate binding inside the active region. The 2D interaction graphs clearly showed important contact residues, which are crucial components of the catalytic site. The 3D visualizations provided additional confirmation of these ligands' correct alignment with the substrate-binding groove, close proximity to the catalytic core, and good fit into the binding pocket. These results provide credence to the compounds' potential as promising inducer for the 5 alpha subunit of GABAA , which calls for more experimental verification

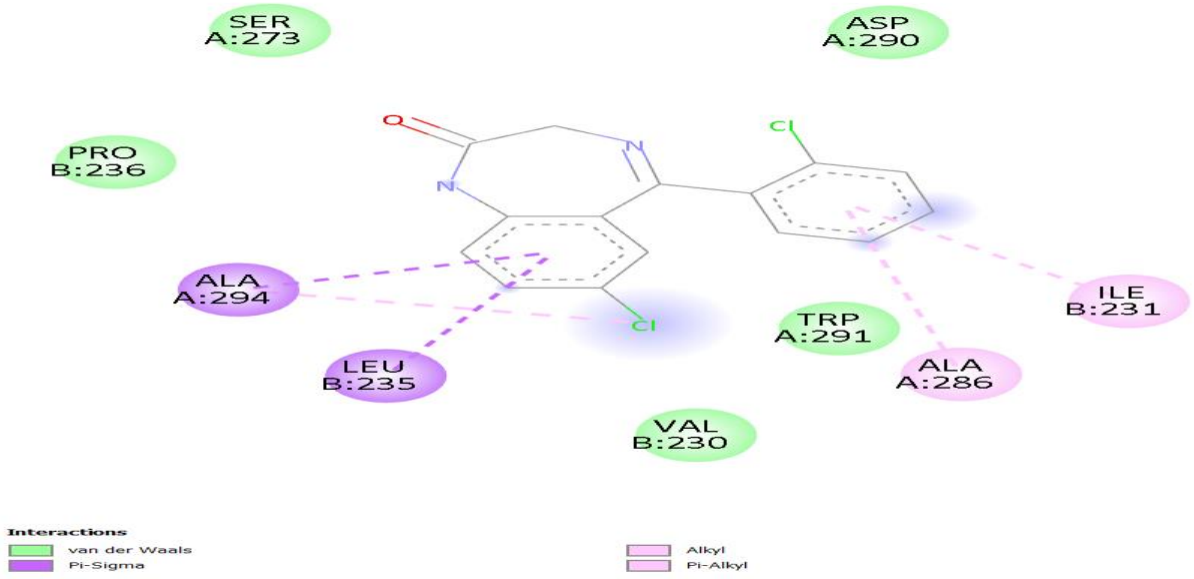

Fig 3. Demonstrates different interactions between Clonazepam (Reference drug) and the GABA A receptor in a two-dimensional graphical representation

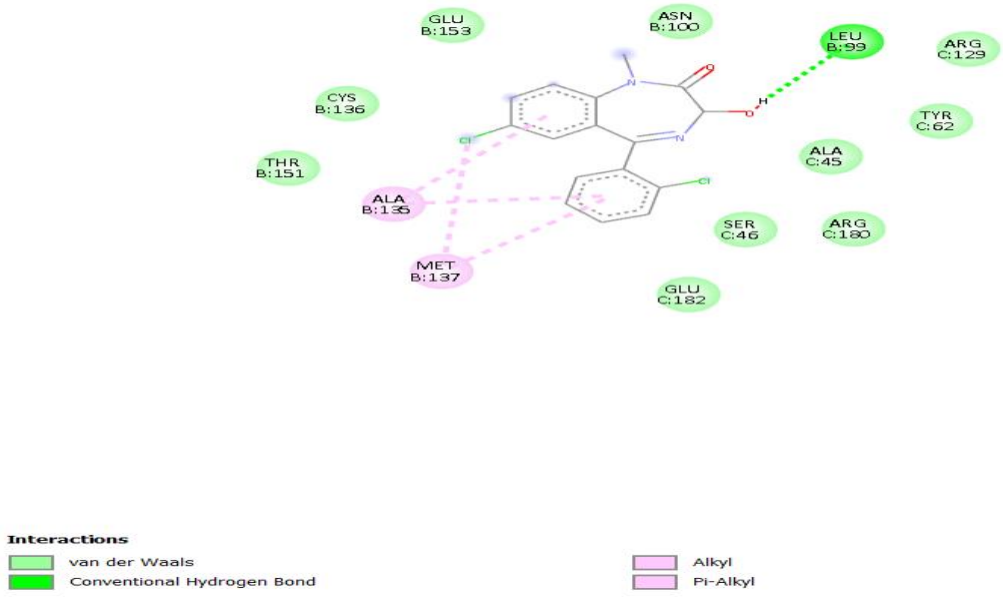

Fig 4. Demonstrates different interactions between Lormetazepam and the GABA A receptor in a two-dimensional graphical representation

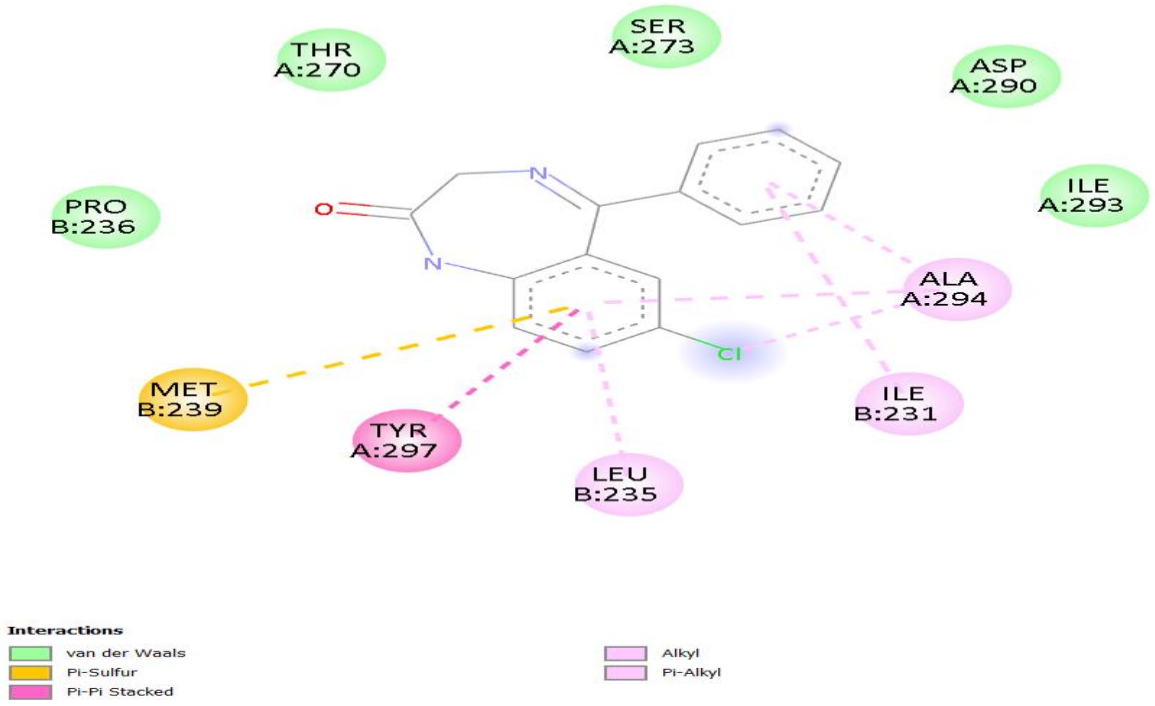

Fig 5. Demonstrates different interactions between Norzazepam and the GABA A receptor in a two-dimensional graphical representation

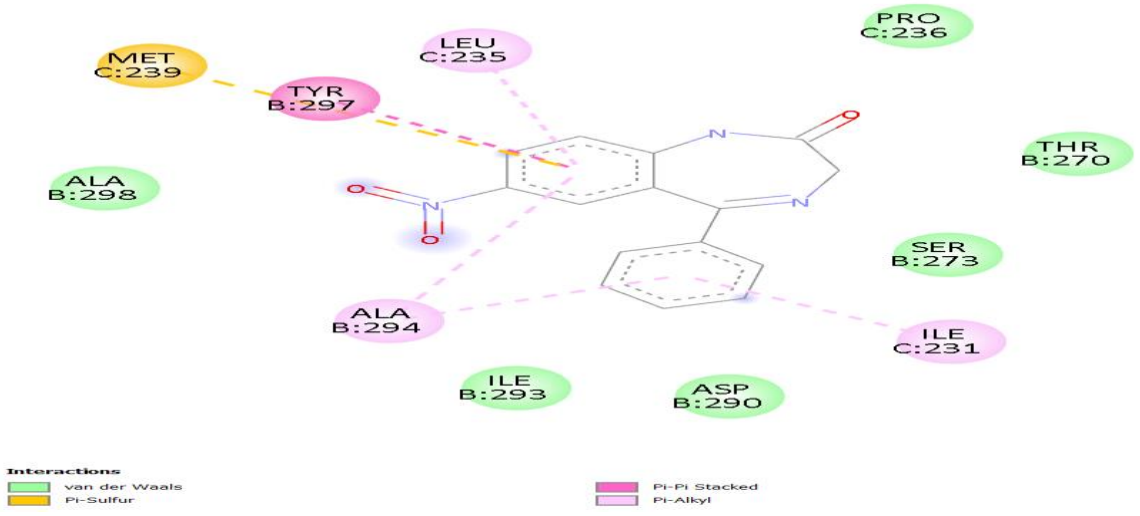

Fig 6. Demonstrates different interactions between Nirazepam and the GABA A receptor in a two-dimensional graphical representation

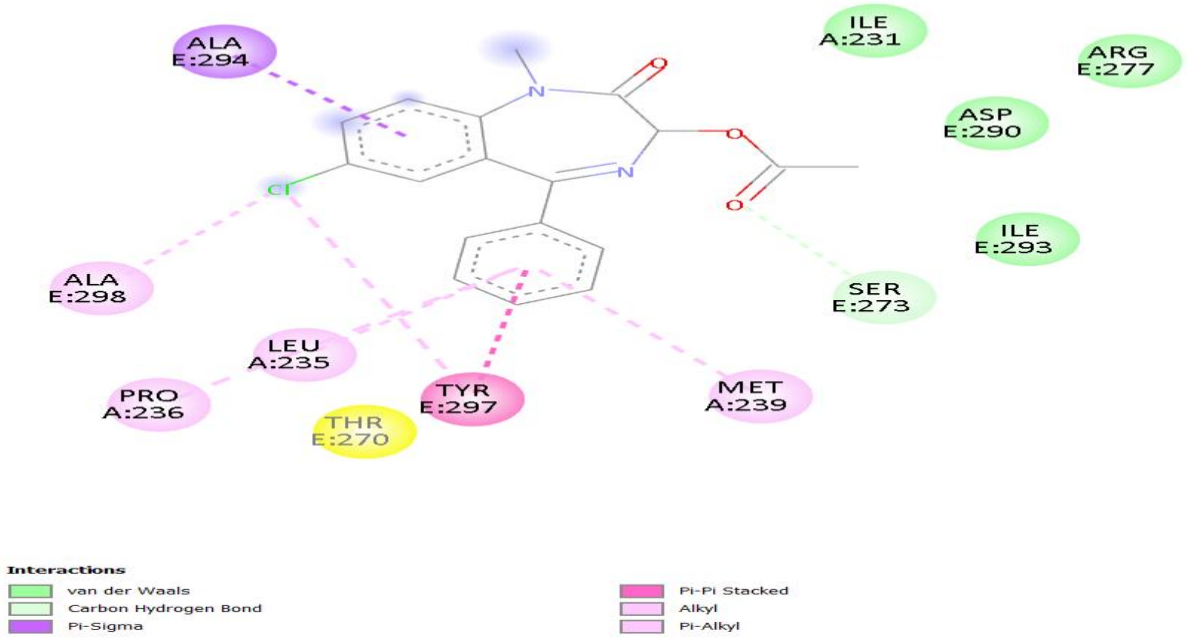

Fig 7. Demonstrates different interactions between Tamazepam and the GABA A receptor in a two-dimensional graphical representation

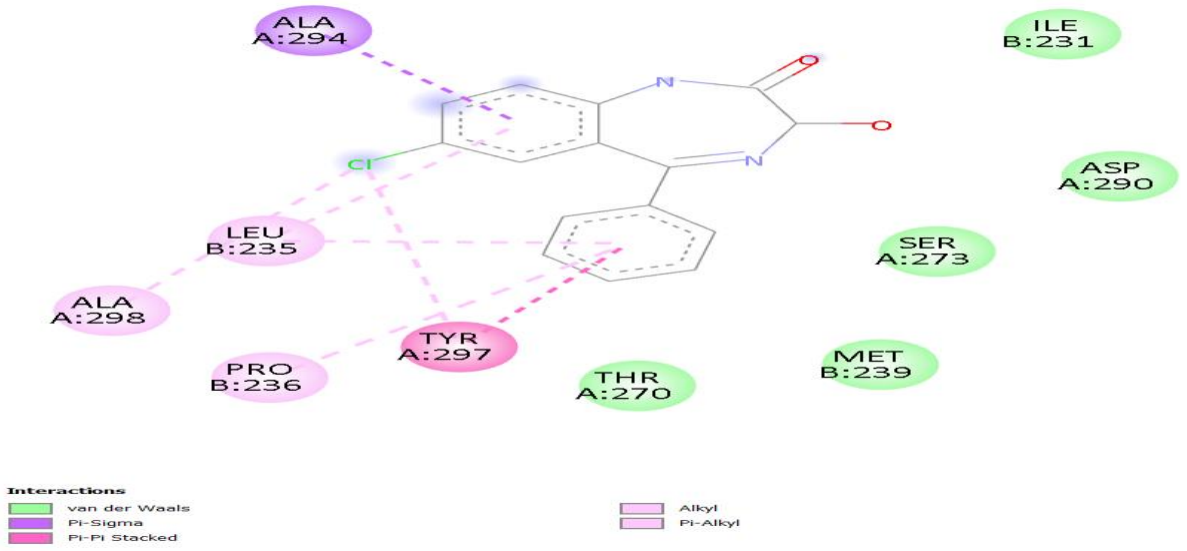

Fig 8. Demonstrates different interactions between Oxazepam and the GABA A receptor in a two-dimensional graphical representation

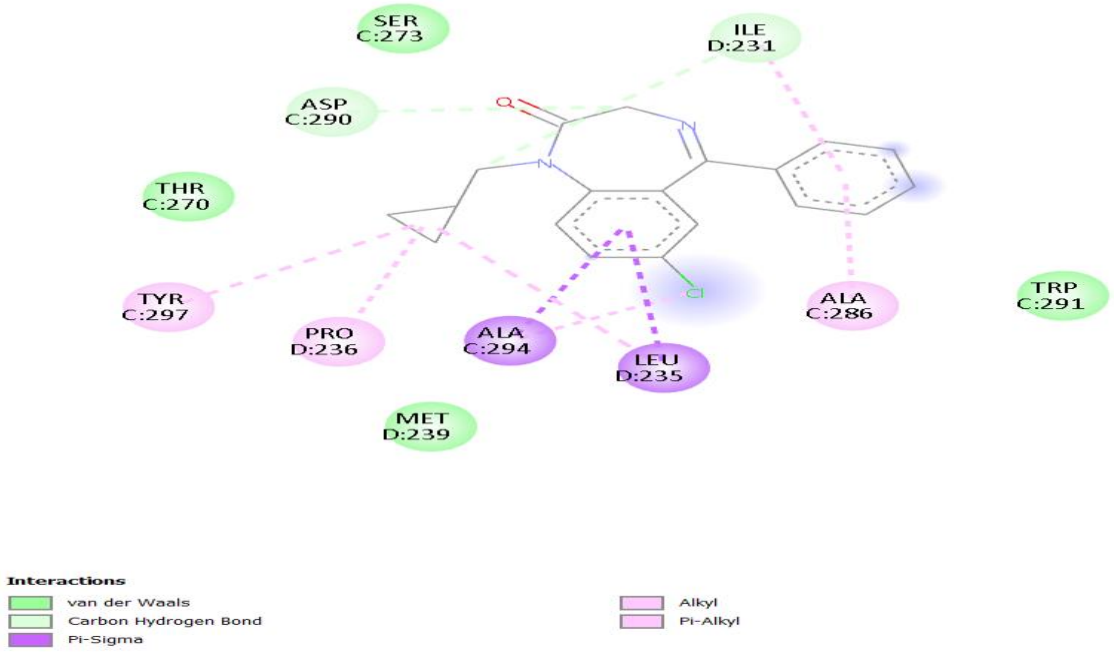

Fig 9. Demonstrates different interactions between Prazepam and the GABA A receptor in a two-dimensional graphical representation

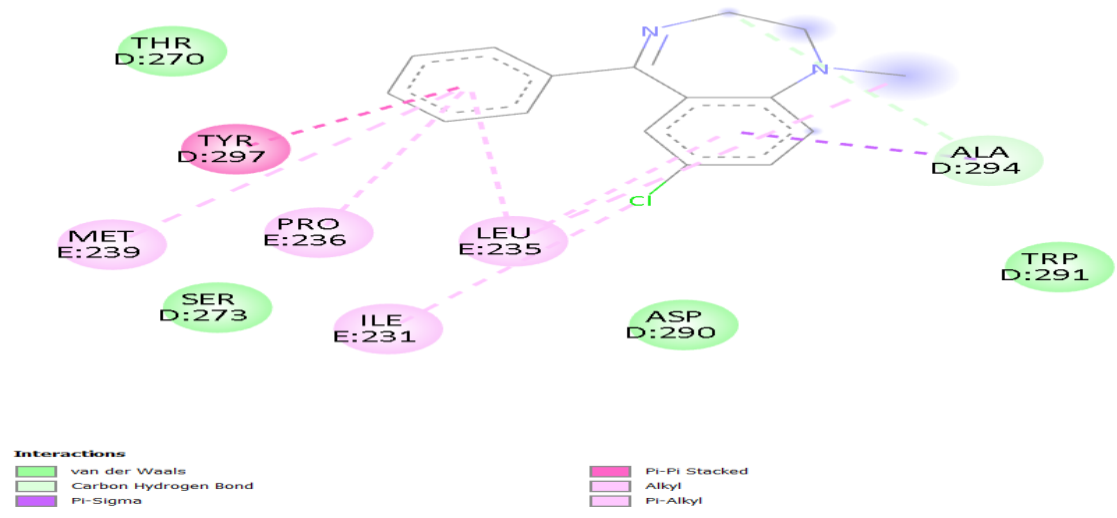

Fig 10. Demonstrates different interactions between Medazepam and the GABA A receptor in a two-dimensional graphical representation

4.3 ADMET analysis

After the molecular docking tests, the top-performing chemical drug candidates with the highest binding affinities for GABA A were evaluated for pharmacokinetic suitability using ADME (Absorption, Distribution, Metabolism, and Excretion) analysis. The majority of the 42 nominated compounds had strong water solubility and high gastrointestinal (GI) absorption, suggesting significant potential for development as oral accessible medications. Following oral administration, these drugs would most likely be efficacious in the systemic circulation because their bioavailability ratings were within a reasonable range. Importantly, most of the selected candidates did not violate Lipinski's Rule of Five, indicating that they met the required criteria for drug-likeness. These findings support the hypothesis that the drugs have favorable pharmacokinetic and safety characteristics in addition to significant biological activity against the GABA A target. The combination of these FDA-approved drugs' strong binding affinity, structural compatibility, and acceptable ADME qualities supports the case for repurposing them as potential therapeutic agents for Alzheimer's disease.

ADME ANALYSIS OF ALL BINDING DRUGS

S.no	Drugs	BBB permeability	Consensus Log P value	GI absorption rate	TPS A value	Lipinski violation
1.	Clanazepam	Yes	1.5	“High”	87.28	0
2.	Flunitrazepam	Yes	1.91	“High”	78.49	0
3.	Delorozepam	Yes	3.22	“High”	41.46	0
4.	Lormetazepam	Yes	2.83	“High”	52.90	0
5.	Lorazepam	Yes	2.68	“High”	61.69	0
6.	Nordazepam	Yes	2.93	“High”	41.46	0
7.	Diazepam	Yes	2.97	“High”	32.07	0
8.	Oxazepam	Yes	2.28	“High”	61.69	0
9.	Temapezam	Yes	2.44	“High”	52.90	0
10.	Pinazepam	Yes	3.24	High	32.67	0

11.	Prazepam	Yes	3.64	High	33.67	0
12.	Fludiazepam	Yes	3.18	High	33.67	0
13.	Halazepam	Yes	3.91	High	32.07	0
14.	Clorazepic acid	Yes	2.32	High	78.76	0
15.	Medazepam	Yes	3.55	High	15.60	0
16.	Flurazepam	Yes	3.82	High	35.91	0
17.	Halazepam	Yes	2.66	High	67.76	0
18.	Clorazepic acid	Yes	2.09	High	97.33	0
19.	Doxefazepam	Yes	2.15	High	79.13	0

4.4 Toxicity assessment using ProTox II (version 3.0) server

The ProTox II server was used to assess the toxicity profile of the top ten binding medications. The server forecasts the hazardous tendencies based on LD50 values using a variety of machine learning (ML) techniques that are quite useful in biological research these days. These machine learning algorithms are trained to identify trends and correlations between different chemical structures and toxicity profiles. A '+' sign in Table 4 indicates an active toxicity outcome, whereas a '-' sign indicates an inactive toxicity outcome. Table 3 is a tabulation of the toxicity data.

Drug	**LD50 value (mg/kg)**	**Hepatotoxicity status**	**Carcinogenicity status**	**Immunotoxicity status**	**Mutagenicity status**	**Cytotoxicity status**	**Toxicity class predicted**
Lorazepam	1790	-	-	+	-	-	Class 4
Diazzepam	48	-	-	-	-	-	Class 4
Oxazepam	1148	-	-	-	-	-	Class 4
Temazepam	1203	-	-	+	-	-	Class 4
Prazepam	2300	-	-	-	+	-	Class 4
Fludiazepam	1502	-	-	-	-	-	Class 4
Halazepam	670	-	-	-	-	-	Class 5

Clorazepic acid	870	-	-	-	-	-	Class 4

To assess the safety profiles of the best binding medications, toxicity prediction was done using LD50 values, hepatotoxicity, carcinogenicity, immunotoxicity, mutagenicity, cytotoxicity, and expected toxicity class. The LD50 values of the selected medication ranged from 350 mg/kg to 2300 mg/kg, indicating varying degrees of acute toxicity. While other chemicals were classified under Toxicity Class 4, which indicates that they are deadly if swallowed, Prazepam, with an LD50 of 2300 mg/kg, was classified under Class 5, which indicates reduced toxicity. Diazepam's very low LD50 of 48 mg/kg led to its classification as having moderate toxicity under Toxicity Class 3.

Interestingly, none of the substances were hepatotoxic, carcinogenic, mutagenic, or cytotoxic. These findings demonstrate that comprehensive toxicity testing is required to ensure that drug candidates are appropriate for repurposing in the treatment of AD, even if they have strong binding affinities and good ADME profiles. Overall, the information shows that the majority of the selected medications have sufficient safety margins; nevertheless, nitrazepam, nordazepam, oxyzepam, prazepam, and loprazepam are particularly noteworthy as prospective choices with minimal toxicity issues.

4.5 Selection of potential drugs

Interestingly, 35 drugs showed greater binding affinities than the reference medication, Clonazepam. Based on their docking scores and important interactions with the catalytic residues inside the GABA A active site and toxicity analysis, the most promising candidates were found, particularly the top hits, , Nitrazepam, nordazepam, oxyzepam, prazepam, and loprazepam. These results imply that the drugs on the shortlist have a great deal of promise for use as GABA A enhancers in treatment of AD.

CHAPTER 5

CONCLUSION

AD is one of the most challenging and complex neurodegenerative illnesses, with a complex etiology and few treatment options. Among the several molecular targets, GABA A induction has emerged as a strong contender since it is necessary for the APP. Finding new and focused pathways that could serve as the basis for novel medications is vital and urgent, especially because all pharmaceutical therapies for AD have failed thus far. To successfully control GABAA's activity in both healthy and pathological conditions, it will be essential to comprehend how it is regulated.

We used a computational medication repurposing approach to find FDA-approved drugs that share structural similarities with the well-known GABAAA enhancer Clonazepam. Using AutoDock Vina for molecular docking and virtual screening, we found many candidates that had a high binding affinity for the GABAA active site. Notably, Nitrazepam, nordazepam, oxyzepam, prazepam, and loprazepam showed best stronger binding affinities, established long-lasting bonds with important catalytic residues in the active site and adequate safety margins in toxicity analysis, suggesting that they may be able to modify GABAA activity. These results demonstrate how useful molecular docking is for quickly and affordably finding novel therapeutic candidates, particularly from among medications that have already received approval, greatly speeding up the drug development process. The docking results are merely the initial stage of drug validation, even though they offer important insights into ligand-receptor interactions. Validating the biological efficacy and neuroprotective potential of these drugs requires experimental research, such as in vivo studies in AD models and in vitro enzymatic assays to evaluate GABA A activation. Future research should also use molecular dynamics simulations to investigate the stability, conformational behavior, and long-term binding properties of these ligand-protein complexes in physiological settings.

All things considered, this study demonstrates the viability and potential of drug repurposing using computational methods as a means of discovering new AD treatments, opening the door for further translational studies that focus on GABA A.

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