Exploring ADAM10 as a Drug Target: Molecular Docking Analysis for Alzheimer's Disease Drug Repurposing

A Dissertation

Submitted in partial fulfilment of the requirement for the degree of

MASTER OF SCIENCE

in

BIOTECHNOLOGY

By:

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June, 2025

ACKNOWLEDGEMENT

At the time of submission of my M.Sc. Dissertation, I express my deepest gratitude to the divine God for providing me with the insight, strength, and perseverance to complete this journey. The successful completion of this project would not have been possible without the support, encouragement, and guidance of many individuals, to whom I am sincerely thankful.

First and foremost, I am profoundly grateful to Delhi Technological University for providing me with the platform and resources to pursue my research work.

I wish to extend my heartfelt thanks to my supervisor, Prof. Pravir Kumar, Department of Biotechnology, for offering me the opportunity to work under his esteemed guidance. His invaluable mentorship, expertise, continuous support, and insightful suggestions have been pivotal to the successful completion of this work.

I am sincerely thankful to Prof. Yasha Hasija, Head of the Department of Biotechnology, for giving me the opportunity to undertake this research project and for her constant encouragement throughout the course of my studies.

I also extend my deepest appreciation to Dr. Meher Sahu, Dr. Neetu Rani, Ms. Shefali Kardam, Mr. Rahul Tripathi, and Ms. Shrutikriti Vashishth for their invaluable guidance, technical advice, and encouragement, which greatly enriched my research experience.

I also wish to acknowledge Mr. Jitender Singh and Mr. C.B. Singh, the technical staff members, for their timely assistance whenever needed. Finally, I am eternally grateful to my family and friends for their unwavering support, patience, and motivation throughout this journey.

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CANDIDATE'S DECLARATION

I, **Ambika Singh**, hereby certify that the work is being presented as the Major Project in the thesis entitled **" Exploring ADAM10 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 **Ambika Singh (23/MSCBIO/59)** has carried out her research work presented in this thesis entitled " **Exploring ADAM10 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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Ambika Singh

ABSTRACT

Aim: AD (Alzheimer's disease) is a chronic and irreversible brain disease identified by cognitive decline, memory loss, and behavioural anomalies. 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, ADAM10 (A Dis-integrin and Metalloprotease 10) plays a pivotal role as an α-secretase, promoting the non-amyloidogenic pathway and preventing the formation of toxic Aβ peptides. Increasing the action of ADAM10 has therefore emerged as a promising therapeutic approach in the management of AD. The present study focused on identifying potential modulators of ADAM10, a critical healing target implicated in AD, through a molecular docking-based drug repurposing approach. Drugs structurally similar with Donepezil, a known enhancer of ADAM10 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 Donepezil, indicating stronger potential interactions with ADAM10's active site. Notably, several drugs exhibited higher binding affinities than Donepezil, suggesting stronger and potentially more effective interactions with ADAM10. Among these, Ziprasidone, Oxatomide, Metergoline, Lasmiditan, and Domperidone emerged as the most promising candidates, demonstrating superior docking scores and favourable interaction profiles. Further, 2D interaction analysis was carried out using BIOVIA Discovery Studio, which visually illustrated key binding interactions such as hydrogen bonds, hydrophobic interactions, and π–π stacking between the ligands and critical amino acid residues of the ADAM10 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 as ADAM10 modulators in AD therapy. These studies provide the scope for future in vitro and in vivo validations to confirm their remedial capabilities.

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LIST OF ABBREVIATIONS

AD	Alzheimer's disease
APP	Amyloids precursor protein
Aβ	Amyloid beta
ADAM10	A Dis-integrin and Metalloproteinase domain-containing protein 10
NFTs	Neurofibrillary Tangles
BBB	Blood brain barrier
PD	Parkinson's disease
PSEN 1 & 2	Presenilin 1 and Presenilin 2
APOE	Apolipoprotein E
CSF	Cerebrospinal Fluid
PKC	Protein Kinase C
BACE-1	Beta-site APP Cleaving Enzyme 1
SAP97	Synapse-Associated Protein 97
CREB	cAMP Response Element-Binding Protein
SH3	Src Homology 3 domain
SRY	Sex-determining Region Y
PAX-2	Paired Box Gene 2
C99	C-terminal fragment
ERK1	Extracellular Signal-Regulated Kinase 1
SOX-2	SRY-Box Transcription Factor 2
GABAB	Gamma-Aminobutyric Acid Type B Receptor
SH SY5Y	Human Neuroblastoma Cell Line SH-SY5Y
LTP	Long term potentiation
LTD	Long term depression
CNS	Central Nervous System

FDA	Food and Drug Administration
AchEI	Acetylcholinesterase Inhibitor
CSV	Comma-Separated Values
PDBQT	Protein Data Bank, Partial Charges, Torsions
ADME	Absorption, Distribution, Metabolism, and Excretion
Log P	Lipophilicity
SMILES	Simplified Molecular Input Line Entry System
PDB	Protein Data Bank
MW	Molecular weight
HBD	Hydrogen bond donors
HBA	Hydrogen bond acceptors
PAINS	Pan-Assay Interference Compounds
SDF	Structure Data File
NCBI	National Center for Biotechnology Information
TREM2	Triggering Receptor Expressed on Myeloid Cells 2
HIF1	Hypoxia-Inducible Factor 1

CHAPTER 1

INTRODUCTION

Up to 24 million people worldwide have been reported to have dementia, and the number is expected to grow every 20 years until 2040, creating a significant disease stress [1]. Deterioration in behaviour, function, and cognition are hallmarks of dementia having significant impact on society. The most popular category of dementia, AD, has been estimated to cost the US government a total of $172 billion in healthcare expenses alone each year [2]. AD has become one of the world's main reasons for death due to global demographic trend towards aging. Despite COVID-19's effects during the last three years, AD remains one of the top 10 causes of death [3]. The patient's memory and cognitive abilities are affected as the disease worsens. Amyloid β having extracellular plaques and intracellular neurofibrillary tangles having tau in neurons are two characteristics of AD [4]. Nevertheless, AD first manifests as temporary forgetfulness, but as the condition worsens, other symptoms as vision and speech impairment and chronic memory failure appear. The usual life expectancy following diagnosis is three to nine years, though the rate of progression might vary [5]. Although researchers are working hard to solve this problem, it is still difficult to find effective remedies [6]. AD is a complex illness, meaning that in addition to hereditary factors, external factors are crucial. Only 2% of AD cases, which are known to begin early and advance more quickly, are inherited [7]. Most of the AD cases are not genetically inherited, and symptoms typically develop later in life, usually around age 65 [8]. About 600 genes have been investigated so far as potential risk factors for AD [9].

Processing of APP is a necessary stage in the biochemical chain of events that causes AD. Alpha or beta-secretase in cells can alternatively mediate the two metabolic pathways that APP, a type I transmembrane protein, could pass through. The Amyloid beta sequence's N-terminus is broken down by beta-secretase to make a cell-associated beta C-terminal fragment, which is further broken down by γ-secretase to produce amyloid beta. Alpha-secretase, on the other hand, cleaves inside the Amyloid beta sequence, eliminating the amyloidogenic component of APP and giving an important soluble extracellular N-terminal fragment (sAPP) in place of it [10]. Despite several attempts to resolve the occurence of AD, no proven treatment has been found till date [11]. More and more data points to the potential benefits of developing treatment plans that specifically address the pathophysiology of the illness. The Aβ production pathway, which involves many enzymes and intracellular components, seems to be one of the best therapeutic prospects for AD. Accordingly,

multiple investigation reveals that blocking processing of amyloidogenic APP and activating the processing of non-amyloidogenic APP pathway may be useful treatments for AD [6] .

It has been demonstrated that some membrane-bound dis-integrin metalloproteinases (ADAMs) can cleave APP at the alpha-cleavage location in a variety of cell systems [12]. ADAM9, ADAM10, and ADAM17, three members of the dis-integrin and metalloprotease family have α-secretase activity. Despite of the fact that all three of the suggested α-secretase candidates assist in APP cleavage, ADAM10 stands out because of its high enzymatic stability under α-secretase cleavage conditions, combined constitutive and regulated activity, and scheduled mRNA expression with the expression of APP in human and mouse cortical neurons. There have been reports of lower sAPP levels and decreased ADAM10 levels in sporadic AD patients [13]. Both plasma and intracellular membranes contain ADAM10 in both its pro-enzymatic and active forms. It has been demonstrated to colocalize with Golgi markers and to be present in membrane vesicles. The proenzyme, on the other hand, has been discovered to reside intracellularly; the plasma membrane includes a significant amount of ADAM 10's enzymatically functional form, which is capable of breaking down APP [14]. The family of widely expressed, transmembrane, secreted proteins known as ADAM has 750 amino acid length and is involved in cell adhesion as well as the proteolytic processing of ectodomains of many receptors on surface of cell and signalling molecules [15]. In non-amyloidogenic route, ADAM10 is the primary secretase that cleaves APP, inhibiting amyloid peptide synthesis, which leads to degeneration of neurons in AD due to its accumulation and aggregation. In addition to APP, the ectodomain of many cell-surface proteins, including cytokines, adhesion molecules and notch, is shed by a membrane-anchored metalloprotease known as ADAM10. ADAM10 produces the neuroprotective APP-derived fragment sAPP by cleaving APP. Since elevated ADAM10 action shields the brain against amyloid buildup in AD, this approach has been indicated to be a successful strategy in managing brain illnesses like AD [16].

There are several approaches to treating AD, including as psychotherapy, behavioural therapies, pharmaceutical and non-pharmacological treatments, and pharmaceutical drugs, which are the primary component of AD therapy. The clinical effectiveness of the most widely prescribed pharmaceutical therapies, donepezil, galantamine, rivastigmine, and memantine, varies from patient to patient [17]. The pathophysiology of AD, which includes acetylcholinesterase, N-methyl-D-aspartate receptor, amyloid plaques, and NFTs, has been the main basis for the development of common AD medications in recent decades [18]. The goals of tacrine, donepezil, rivastigmine, galantamine, and memantine are to alleviate patients' behavioural and cognitive symptoms. Unfortunately, these drugs have serious adverse effects and do not considerably slow the course of AD [3].

The study focused on repurposing FDA-approved drugs having structurally similarity to Donepezil, a known ADAM10 enhancer, by employing molecular docking techniques to evaluate their binding

affinity toward the ADAM10 protein. First, the SwissSimilarity technique was used to identify 284 medications. There was an ADME (Absorption, Distribution, Metabolism, and Excretion) screening to make sure that compounds that were pharmacologically relevant were selected. Blood-brain barrier (BBB) permeability, PAINS (Pan-Assay Interference Compounds), and Lipinski's Rule of Five were among the filters used. After a thorough selection procedure, 42 potential drugs remained, all of which had good pharmacokinetic characteristics and could be used to target the central nervous system. These 42 chemicals' binding affinities for the ADAM10 protein's active site were evaluated by subsequent molecular docking studies. The reference molecule for the comparative analysis was donepezil. Interestingly, 36 drugs showed higher binding affinities than donepezil, indicating more robust and possibly efficient interactions with ADAM10. Considering their excellent docking scores and advantageous interaction profiles, Ziprasidone, Oxatomide, Metergoline, Lasmiditan, and Domperidone came out as the most promising of these. These top candidates developed several stabilizing contacts with important residues in the ADAM10 active site, including π–π stacking, hydrophobic interactions, and hydrogen bonds, according to additional 2D interaction analyses performed with BIOVIA Discovery Studio. Further, a toxicity assessment for the top binding 10 drugs was done using ProTox II server that revealed various toxicity properties such as hepatotoxicity, cytotoxicity, immunogenicity, LD50 and others. This toxicity analysis was crucial in complementing the docking and ADME studies by ensuring that selected drug candidates not only exhibit high binding affinity and good pharmacokinetic properties but also meet the essential safety criteria for further development. The possibility that these medications will function as ADAM10 activity modulators is increased by these interactions and toxicity analysis, which are suggestive of good therapeutic drugs.

Overall, these results suggest that repurposing these FDA-approved drugs may open up new therapeutic options for Alzheimer's disease treatment, which calls for additional experimental verification.

CHAPTER 2

LITERATURE REVIEW

2.1 Alzheimer's disease

Historical background: The work of Alois Alzheimer initially characterized the neurodegenerative disease that would bear his name more than a century ago, the occurrence of the features he identified, amyloid plaques and NFTs, remains necessary for condition's clinical diagnosis [19]. Alzheimer's disease (AD), the prevalent type of dementia, is neurological disorder that mainly manifests itself by early Cognitive impairment and diminished memory that can ultimately affect speech, behaviour, motor function, and visuospatial orientation [20]. Variant syndromes with early localized atrophy may not always follow this typical presentation; pathological subgroups of AD have been discovered [7]. Considering it is so closely associated with old age, it is believed to be a normal part of aging [21]. Currently, AD does not have any treatments that modify its symptoms [22].

Epidemiology: It has been investigated that worldwide 47 million people suffer from dementia, and as of 2018, the annual price of these illnesses was predicted to exceed $1 trillion [23]. AD is the prevalent form of dementia, making up for 60 to 80% cases, with less than half of dementia cases believed to be pure AD and the balance believed to be mixed dementias [24]. Between 5 and 10% of dementia cases are caused by the other most frequent reasons, which consist vascular dementia, Lewy body dementia, PD with dementia, frontotemporal lobar degeneration, and normal pressure hydrocephalus [25]. The two that tend to coincide with mixed pathology, comprising concomitant AD, are vascular dementia and Lewy body dementia [7]. By the middle century, it is predicted that over 131 million people will get impacted by these physically and financially debilitating illnesses as the population ages. The biggest contributory factor for AD is aging, since every 6.3 years, the number of instances of all dementia cases doubles, rising from 3.9 per 1000 for those aged 60 to 90 to 104.8 per 1000 after age 90 [26]. A rough figure of the rate of incidence is predicted to be 40% for those over 80 and 10% for those over 65. The increasing human and financial costs have led to a need for efficient pre-clinical diagnosis and treatments to halt the progression of the disease before symptoms manifest [7].

Aetiology: The great majority of AD is caused by three genes mutations: PSEN1, APP, and PSEN2 cause a rare familial variant of AD that has less than 0.5% [27]. Symptoms usually appear between the ages of 30 and 50, which occurs at earliest than in sporadic AD [28]. "Typical" late-onset AD is probably brought on by a complicated interaction between environmental and genetic variables.

Approximately 70% of AD risk is currently believed to be attributable to hereditary causes. The three variations of the APOE gene (ε2, ε3, and ε4) are the most significant risk factors for sporadic AD [29]. When compared to non-ε4 carriers, the odds ratio (OR) for AD in ε4 heterozygotes is roughly 3 and in homozygotes, it increases to about 12. More than 20 genetic risk factors have been found through genome-wide association studies involving hundreds of samples, linking endosomal vesicle recycling, cholesterol metabolism, and inflammation [22]. It is now known that a major factor in the pathophysiology of AD is microglial activation in response to amyloid accumulation [30]. When combined to create a polygenic risk score, these relatively common risk genes can nearly double case prediction from chance, even though each one only slightly increases risk [31].

While midlife hypertension and diabetes negatively impact risk, epidemiological evidence indicates that education and physical activity may offer protection against AD. Although obesity has long been thought to increase the incidence of dementia and AD, this has recently come under scrutiny.
Since few epidemiological studies have pathological confirmation of diagnosis, it is still unclear how vascular risk factors may affect AD [32]. Vascular risk factors may "double-hit" with cerebrovascular injury to raise the probability of developing clinical AD, or vascular damage may directly affect the onset of AD pathology [22].

Pathology: NFTs and amyloid plaques are the symptoms of AD pathogenesis. Additionally, Neuropil threads, dystrophic neurites, related astrogliosis, and microglial activation frequently occur with cerebral amyloid angiopathy. One of the results of these degenerative processes is degeneration of neurons, which causes synaptic and neuronal death and macroscopic shrinkage [7]. Mixed pathology, which comprises Lewy bodies and vascular disease, is common, especially in elderly people [33]. Indeed, Lewy body pathology and fAD often coexist, although the precise mechanism is yet unclear [34].

The primary component of amyloid plaques, that are extracellular accumulations, is improperly folded Aβ having 40 or 42 amino acids, which are leftovers of APP processing. Aβ42 is common in plaques than Aβ40 because it is very insoluble and fibrillizes quicker. Deposition of amyloid does not usually progress in an unusual fashion; it typically begins in the iso-cortex and only later spreads to subcortical area. Amyloid plaques affect the entorinal cortex and hippocampus formations less than NFTs do [35].

The main component of neurofibrillary tangles is paired helical filaments formed from hyperphosphorylated tau [36]. Before moving on to the associative iso-cortex, tau pathology usually starts in medial temporal lobe allocortex, which comprise the entorhinal cortex and hippocampus. Visual, motor, and primary sensory domains are typically mostly unaffected [37]. NFT pathology exhibits a stronger correlation with the symptoms and severity of AD [35], while β amyloid pathology

plateaus early in the disease's symptomatic phase. This is because neuronal and synaptic loss usually occurs in tandem with tangle formation [22].

2.2 APP

The pathophysiology of AD is significantly influenced by the APP and its cleavage products, the Aβ peptides which are generated from beta and gamma secretases [38]. α-secretase provides an alternative processing pathway that generates the neurotrophic and neuroprotective cleavage product αAPPs while halting the synthesis of those toxic peptides. The molecular identity of alpha-secretase was only recently determined and ADAM10 is always considered the most physiologically relevant and important enzyme in this class. The extent to which ADAM10 catalytic activity deficiency contributes to the decline in AD pathogenesis in aged adults is unknown [39]. Nonetheless, ADAM10 is proposed to be an attractive target for the prevention and/or therapy of AD [40]. Only 12 of the 21 members of the ADAM family that have been found in the human genome exhibit catalytic activity [41]. The usual zinc-binding motif seen in those enzymatically active ADAMs is expressed by ADAM9, ADAM10, and ADAM17, which also have a similar multidomain structure. Conversely, ADAM10 has been recognized as a physiological α-secretase that is significant using cell culture and mice models [42].

2.3 Structure and synthesis of ADAM10

About 750 amino acids make up the ADAM family of metalloproteinases, which have proteolytic activity and can degrade the ectodomain of many cell-surface receptors [6]. The enzyme ADAM10 is a member of the zinc proteinases family's metazincin subgroup. A prodomain, a catalytical domain with a conserved zinc binding sequence, a cysteine-rich dis-integrin like domain, a transmembrane domain, and a brief cytoplasmic region constitute the conventional multidomain structure of ADAM10 which is a type I integral transmembrane protein [43].

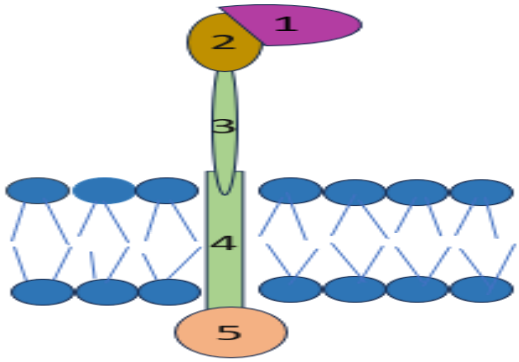

Fig 1: Human ADAM10 domain structure. The five distinct domains that make up ADAM10 include (1) the pro-domain, (2) conserved zinc binding motif, (3) cysteine rich dis-integrin domain, (4) A transmembrane region, (5) A brief cytoplasmic domain.

Although ADAM10 is expressed in a variety of types, the most significant ones include tumour cells, leukocytes, vascular cells, and neurons [44]. The rough endoplasmic reticulum enables the co-translational synthesis of ADAM10, which is then matured and delivered by the Golgi apparatus. The primary alteration in ADAM10 during maturity is the removal of its pro-domain, which maintains ADAM10 in an inactive state by coordinating the zinc ion present in the site of catalysis and inhibiting the proteolytic action of ADAM10 via a cysteine switch mechanism [45]. Through its cleavage at multiple locations, including PC7 in the Golgi apparatus, pro-protein convertase contributes to ADAM10 maturation. The pro-domain appears to have more than just an inhibitory role in ADAM10; it is necessary as an intramolecular chaperon for folding correction. Confocal microscopy has demonstrated this mechanism by identifying a significant amount of ADAM10 in the Golgi apparatus of breast cancer cells [6]. When ADAM10 is transported to the membrane, it undergoes N-glycosylation at four locations in addition to pro-domain removal. An inactive zymogen with a C-terminal and dis-integrin are the other major domains of the ADAM10 structure. The short intracellular C-terminus appears to be essential for ADAM10 protease activity, even though the dis-integrin domain does not appear to be required. This is because it has been shown that ADAM10 cells with an increased expression of cytoplasmic domain deletion mutant of the proteinase have impaired cleavage of epidermal growth factor [46]. Furthermore, various attachment sites have been identified for the cytoplasmic domain of ADAM10 that appear to have function in regulatory activities. These include a binding site for calmodulin and two putative SH3 binding domains rich in proline [47]. While the site of attachment on juxta-membrane is involved in ADAM10 basolateral localization in epithelial cells, the SH3 binding domains drive ADAM10 to the postsynaptic membrane in neurons [48]. Apart from the biosynthesis of ADAM10, other research has examined the method of its translocation to the membrane to alter its physiologic role. At various stages of its maturity, ADAM10 is translocated by many intracellular factors. In this sense, ADAM10 transport from the Golgi outposts to the synapse is primarily regulated by synapse-associated protein-97 (SAP97), which has no bearing on ADAM10 trafficking from the endoplasmic reticulum. Mechanistically, it has been demonstrated that protein kinase C (PKC) mediates the phosphorylation of the SAP97 SRC homology domain 3, that controls SAP97's interaction to ADAM10 along with its migration from the Golgi to the synapse [49]. Since SAP97 is sufficient for ADAM10 to leave the endoplasmic reticulum, additional research has been done to identify numerous of other components that play a role in this process. In this instance, ADAM10 egress from the endoplasmic reticulum was known to be mediated by a subgroup of tetra-spanins made up of eight cysteines in the large extracellular domain (Tspan10, Tspan5, Tspan15, Tspan14, Tspan17, and Tspan33) [6]. Furthermore, it has been found that ADAM10's ineffective surface trafficking and retention in the endoplasmic reticulum are caused by an arginine-rich (723RRR) sequence [50].

The molecular weight of ADAM10 in its mature state is approximately 65 kDa [6]. A membrane-anchored C-terminal fragment weighing about 10 kDa is left behind after ADAM10 ectodomain shedding, and a soluble ADAM10 fragment weighing about 55 kDa is released [51]. This procedure demonstrates that the very significant proteases such as ADAM9, ADAM15 and gamma-secretase, have an impact on this factor itself regardless of ADAM10's protease activity [52]. Presenilin, one of the primary components of γ-secretase, influences ADAM10, causing the intracellular domain to be released. Translocation of this liberated domain to the nucleus is believed to contribute to gene regulation [53].

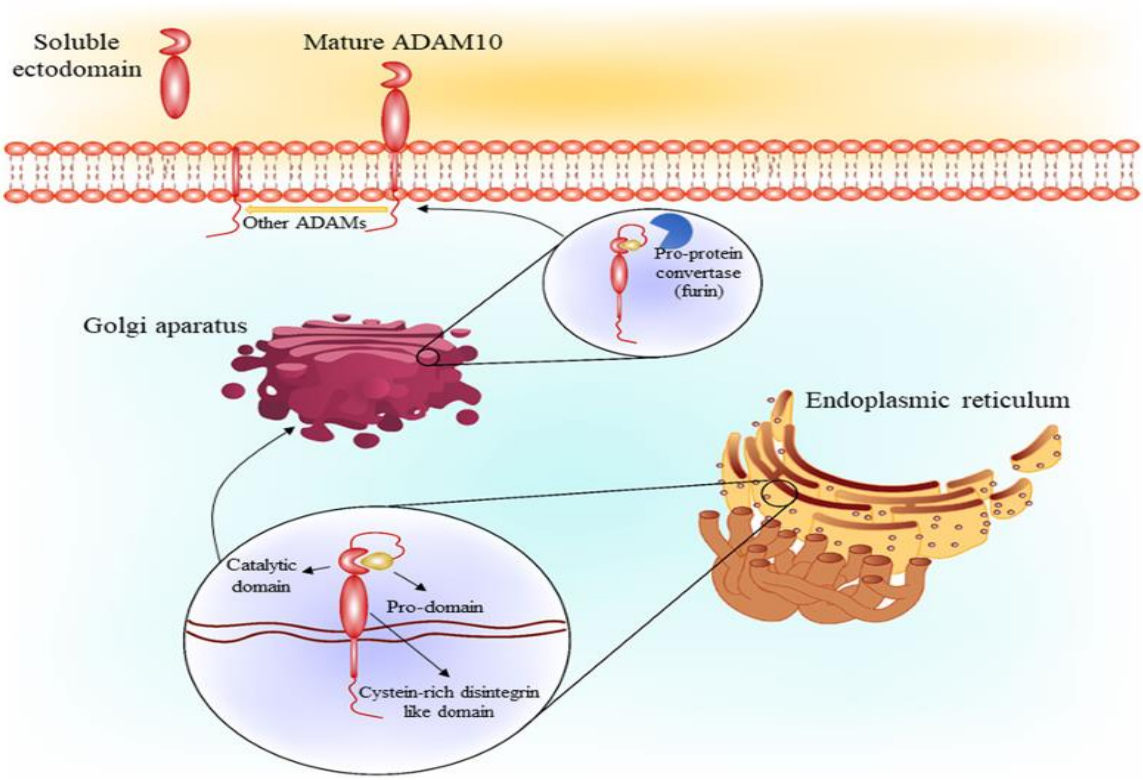

Fig 2: The production and maturation of ADAM10 in cell. The endoplasmic reticulum synthesizes ADAM10, which has pro-domain, catalytic, and cysteine-rich, dis-integrin like domains. Furin and other pro-protein convertases separate the pro-domain during ADAM10 maturation, which involves the golgi apparatus. However, mature ADAM10 can be impacted by other ADAMs to create a soluble ectodomain.

2.4 ADAM10 as an AD biomarker

Human CSF has previously contained ADAM10 in various forms, including an unprocessed form, a large cut solution form, and an immature form that retains protamine [53]. Nonetheless, research on AD patients has demonstrated that alterations are linked to the expression of ADAM10 in their platelets. Although there was initial evidence of a decline in ADAM10, further research shows no discernible correlation between ADAM10 levels and cognitive symptoms in AD patients [54]. As a

result, it has been suggested that these alterations may be brought on by the drugs that the patients are taking. In a study, scientists compared the amount of ADAM10 in AD patient's platelets with those of healthy people who had also reported higher levels of its substrates [6]. While further study is required to support this notion, current evidence points to ADAM10 serving as a potential biomarker for AD diagnosis.

2.5 Role of ADAM10 in AD

As a primary α-secretase enzyme, ADAM10's function in processing the APP is its most well-known activity [55], [56]. Different mammalian cells, particularly neurons, express the type I transmembrane glycoprotein known as APP [57]. Because it functions as an Aβ precursor, APP is well-known. It consists of 28 amino acids from its extracellular area and 12–15 residues from its membrane-spanning portion [58]. Due to its association with other physiological manifestations of AD, such as neuroinflammation and oxidative stress, Aβ buildup as plaques is recognized as a hallmark of the disease, even if the fundamental reason of AD is still not known [59]. This problem has led to a current focus on lowering Aβ production in therapeutic approaches to delay the progression of AD. Addressing the two pathways in APP processing can assist comprehend how ADAM10 contributes to the pathophysiology of AD. The primary β-secretase enzyme, BACE-1, cleaves transmembrane residue of APP, that aids in release of beta-stubs in the amyloidogenesis pathway [60]. Furthermore, APP's soluble N-terminus and a membrane-bound C99 are released upon cleavage by BACE-1. The second phase involves γ-secretase cleaving the C99 fragment, which helps release Aβ into the extracellular region [61]. α-secretase activity starts the non-amyloidogenesis pathway, which is the alternative APP processing pathway. The production and release of soluble APP (sAPP), another APP ectodomain variation referred to as a neuroprotective and neutrophilic factor, is facilitated by the interaction of α-secretase with APP [62]. Furthermore, sAPPα has been identified in many functions, such as modifying basal synaptic transmission, mostly through GABAB receptor subunit 1a [63]. Though more research must be done to support this assertion in practice, conceptually explaining these two paths may be useful in conveying treatment purposes. In this context, it has been demonstrated that inhibiting the Amyloidogenesis pathway by inhibiting BACE-1 and γ-secretase exhibits protective benefits in many AD models [64]. Similarly, Aβ synthesis and accumulation are decreased when the pathway of non-Amyloidogenesis is induced by raising α-secretase expression or activity. One of the primary α-secretases, ADAM10, has got considered as a possible therapy factor to regulate the generation of Aβ [65].

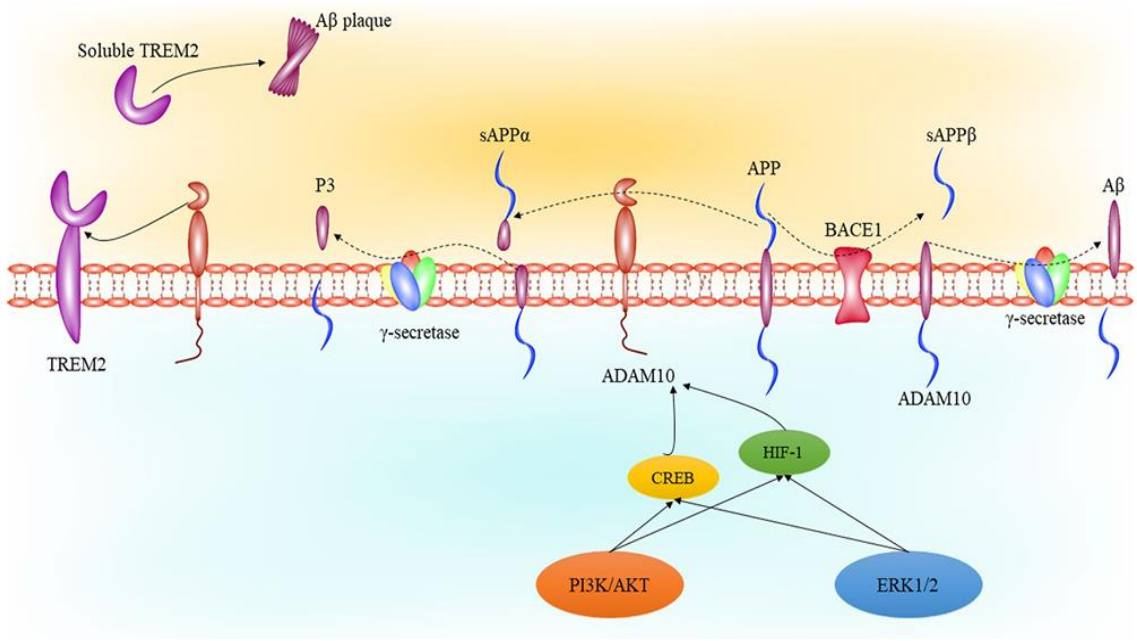

Fig 3: APP processing's amyloidogenic and non-amyloidogenic pathway. BACE1 influences APP in the amyloidogenic pathway, which results in the release of sAPPβ. The action of γ-secretase, another protease implicated in the amyloidogenic process, aids in the production of Aβ. The initial stage in the non-amyloidogenic route involves ADAM10 cleaving APP, which releases sAPPα. The second stage involves the synthesis and release of P3 fragments due to γ-secretase activity. Conversely, TREM2 is cleaved by ADAM10, releasing soluble TREM2, which attaches to Aβ plaque and promotes its removal. The expression of ADAM10 is regulated by the PI3K/AKT and ERK1/2 pathways. CREB and HIF-1 can mediate this impact.

2.6 Regulation of ADAM10 expression and activity by intracellular pathway in AD

ADAM10 expression is controlled at several transcriptional, translation and post-translation stage [66]. However, less research has been done on the link between ADAM10 and various intracellular pathways with altered activity in AD. One of the primary intracellular pathways engaged in controlling various facets in life of cell, such as protein synthesis and cell proliferation, is extracellular signal-regulated protein kinase [67]. Even so, this was proven that this system, that governs the synthesis of Aβ, tau phosphorylation, and neuroinflammation, is disturbed in AD patients [61].

With respect to ERK1/2's impact on ADAM10, it has been shown that ERK1/2 increases CREB activity, that further improves APP processing and sAPPα synthesis [68]. Furthermore, it has been demonstrated that S100A7, a new AD biomarker, promotes the non amyloidogenesis route by activating ERK1/2 and inducing ADAM10 [69]. The second major intracellular pathway with altered activity in AD is the phosphatidylinositol 3 kinase (PI3K)/AKT signalling system [70]. In this context, it has been shown that oestrogen receptor activation enhances ADAM10 activity by promoting non-amyloidogenic processing of APP via PI3K/AKT pathway activation [71]. The precise mechanism of

this regulation in AD is still unknown, despite of the fact that these two pathways will inevitably play a part in controlling ADAM10 activity. Y sex determination region (SRY)-box 2 (SOX-2) and other components involved in the control of ADAM10 expression are, however, observed to be regulated by the PI3K/AKT and ERK1/2 signalling pathways in non-AD animals [72]. While these two pathways will undoubtedly contribute to the control of ADAM10 action, the precise process of how this control works in AD remains unclear. But, in non-AD animals, the PI3K/AKT and ERK1/2 signalling pathways are found to modulate Y sex determination region (SRY)-box 2 (SOX-2) and other elements involved in the regulation of ADAM10 expression [73].

2.7 ADAM10 and synaptic plasticity in AD

Various experiences such as stressful situations, classroom instruction or the use of psychoactive substances alter the activity of particularly, the neuronal circuits in the brain. Synaptic plasticity, the foundation of various learning and memory models is an experience-dependent alteration in the strength of neural connections [74]. Certain activation methods that lead to either LTD or LTP, a defence of the long-term synapses, comprise this form of cellular learning. The theory suggesting a change in the molecular process of synaptic plasticity underpinning the unbalance is generally agreed irrespective of the fact that AD is thought to be linked to the neuron loss in several brain areas [6]. Yet some research has been done to investigate the relationship between synaptic plasticity and other biochemical features of AD.

Regarding Aβ, it was noted that oligomers of Aβ modify the molecular processes implicated in LTP, causing LTP to decrease and LTD to rise in slices of the hippocampus [75]. The effect of Aβ on synaptic plasticity may be demonstrated by mice's impaired learning and memory after receiving an injection of Aβ into their brains [76]. Since decreased ADAM10 action in brains of AD can change synaptic plasticity by controlling Aβ accumulation, our findings may provide light on the function of ADAM10 in synaptic plasticity through control of Aβ synthesis. However, studies have shown the connection between ADAM10 activity and processes linked to synaptic plasticity. According to reports, LTD promotes ADAM10's endocytosis, which in turn differentially modulates the synaptic availability and activity of ADAM10. Additionally, it has been demonstrated that in AD mice, stimulation of the PI3K/AKT pathway increases dendritic branch density and synaptic protein expression, which in turn raises ADAM10 levels [77]. According to a mechanistic analysis, modifications in synaptic plasticity may have an impact on intracellular processes, particularly the PI3K/AKT pathway, that is recognized as a controller of ADM10 expression and endocytosis. Given that LTP-induced PI3K/AKT/GSK-3 has been shown to adversely regulate LTD in CA1 pyramidal neurons, this pathway is driven by GSK-3 [78]. Thus, this may be said that LTP activation inhibits LTD and triggers the PI3K/AKT pathway, which in turn causes ADAM10 to be endocytosed by clathrin. However, it has been demonstrated that Aβ oligomers activate caspase-3, which is essential

to the pathogenesis of AD [79]. This cleaves AKT, promotes LTD [80], and may prevent ADAM10 from being endocytosed. Regardless of how synaptic plasticity affects ADAM10 availability, it has been determined that ADAM10 function controls synaptic plasticity primarily through the cleavage of many components, including APP, neuroligin 1, and N-cadherin [81].

2.8 α-secretase ADAM10 upregulation as a potential treatment target for AD

Targeting ADAM10 in AD is based on the theory in which the pathophysiology of the disorder and aging are caused by an imbalance in the action or expression levels of the enzymes that process APP, ADAM10 and BACE-1 [40]. There have been or could be various methods for increasing ADAM10's quantity or catalytic activity. Whether there are adverse effects linked to increased ADAM10 activity in the brain or in peripheral regions is the key question that still to be answered [82]. In terms of appearance, breeding, and everyday handling, ADAM10 mono-transgenic mice with a persistent neural overexpression of ADAM10 to varying degrees were undetectable. This suggests that the homeostasis of the entire body is not significantly impacted by ADAM10 overexpression in the brain [43].

Adult mice with modest ADAM10 overexpression showed only a moderate change in gene expression, according to a recent microarray analysis. In fact, a decrease in inflammation markers was noted, and differentially regulated genes did not outnumber pro-inflammatory or pro-apoptotic proteins. By breaking the extracellular component of this receptor upon binding of the ligand, ADAM10 also contributes to the activation of Notch1 signalling [83]. At postnatal day 15, young ADAM10 transgenic mice displayed a 40% increase in Hes5 gene expression, while mice overexpressing the dominant negative form of the enzyme showed a 50% decrease. However, there were no discernible changes in the quantity of Notch1 target gene Hes5 mRNA in adult mice, suggesting and indicating that as people age, the signalling cascade will weaken. Since ADAM10-based AD therapy is intended for older adults, disruption of this crucial developmental signalling system does not seem to interfere with this strategy [84]. Considering every finding pertaining to elevated ADAM10 levels in vivo, it is said that this method may be a useful substitute for another approaches, like β or γ-secretase inhibition or vaccination in the treatment of AD. Thus, α-secretase activation needs to be moderated and carefully watched [43].

2.9 Molecular docking

In modern drug development, molecular docking has become a potent in silico method, especially in area of drug repurposing, where it makes it possible to anticipate interactions between very small molecules and target proteins quickly and accurately. 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 ADAM10, an α-secretase enzyme that is essential to the non-amyloidogenic pathway of APP processing, was assessed using molecular docking. The activation or enhancement of ADAM10 is thought to be a useful treatment approach for altering the progression of AD by encouraging the cleavage of APP in a way that inhibits the development of harmful β-amyloid plaques. ADAM10's metalloprotease domain contains its active site, which is distinguished by a conserved zinc-binding motif. Three histidine residues, usually His^405, His^409, and His^415 in the human isoform, make up this motif. These residues coordinate a catalytic zinc ion, which is necessary for enzymatic activity. Furthermore, a neighbouring glutamate residue (Glu^406) is essential for triggering the activation of a water molecule needed for the hydrolysis of peptide bonds. The hydrophobic and polar amino acids that make up the surrounding pocket, also known as the S1' specificity pocket, affect substrate recognition and binding affinity.

Acetylcholinesterase inhibitors (AChEIs) are essentially used to treat AD and sole drugs that are currently on the market for treating Alzheimer's disease [10]. Donepezil is a strong and specific AChEI that has been demonstrated to increase secretase activity in vitro by promoting ADAM10 trafficking to the plasma membrane and shifting APP metabolism towards the non-amyloidogenic pathway [16]. Additionally, donepezil treatment of SH SY5Y cells has been shown to promote sAPPα production by inducing ADAM10 activity [85]. Thus, donepezil makes a perfect reference drug for finding structurally comparable substitutes.

It was a strategic and practical move to concentrate on drugs that had FDA approval. These substances have previously undergone thorough toxicological and pharmacological evaluations, which drastically lowers the risk, expense, and time involved in early-stage medication development. Furthermore, choosing chemicals with established blood-brain barrier (BBB) permeability increased the possibility of clinical applicability because the BBB is a significant barrier to central nervous system (CNS) drug delivery. We employed a structure-based similarity and function-guided method to repurpose structurally related molecules to Donepezil, a clinically used AD medication known to promote ADAM10 expression.

The study's findings support the importance of molecular docking as a computational drug repurposing tool, particularly for conditions like Alzheimer's where disease-modifying therapies are desperately needed. The encouraging interactions seen with a few FDA-approved medications support more in vitro and in vivo research to confirm their effectiveness as treatments that target ADAM10.

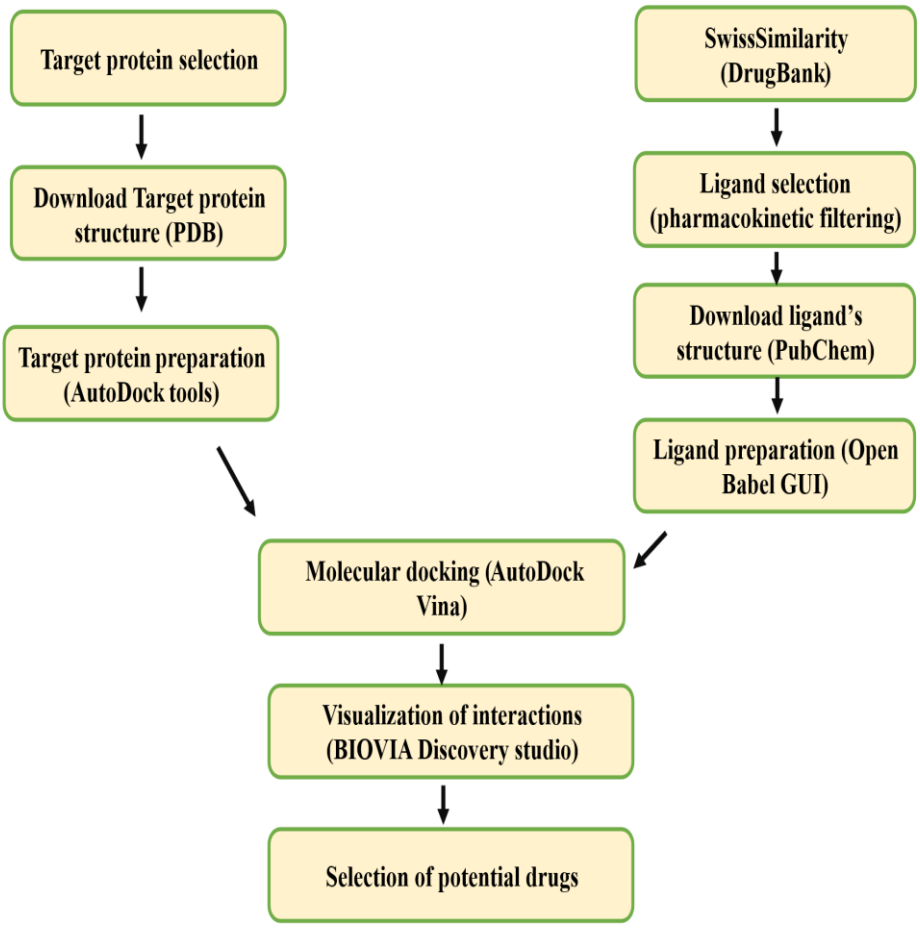

Fig 4: Molecular docking workflow, key steps involved.

CHAPTER 3

METHODOLOGY

3.1 Collection of data

The Protein Data Bank (PDB) provided the protein structure of ADAM10, which was used as the target receptor in molecular docking investigations. The resolution quality and completeness of the active site were taken into consideration when choosing the structure.

The SwissSimilarity tool (https://www.swisssimilarity.ch/), a web-based virtual screening platform, was used to identify the ligands. This tool facilitates ligand-based drug development and therapeutic repurposing efforts by enabling the identification of structurally related molecules based on known ligands. The SMILES notation of donepezil, a clinically approved ADAM10 enhancer used to treat Alzheimer's disease, was obtained from PubChem and verified using the DrugBank database to start the screening. SwissSimilarity was set up to search just inside the FDA-approved medication library, guaranteeing that every hit that came up had pharmacological and safety characteristics that had been established.

When SwissSimilarity was run, it produced a CSV file with 284 drugs that showed a high degree of structural resemblance to donepezil. Since central nervous system (CNS) activity is essential for Alzheimer's disease treatments, these candidate compounds were subsequently put through a blood-brain barrier (BBB) permeability filter utilizing SwissADME and associated ADME analysis tools. At that point, compounds that were thought to be non-permeable to the BBB were eliminated to concentrate on those that were more likely to have therapeutic effects inside the brain. The 42 compounds that were left over after filtering and having both favourable structural similarity and CNS permeability were then used for molecular docking studies against the ADAM10 active site.

3.2 Target protein preparation

Considering ADAM10 potential as a treatment for Alzheimer's disease, the target protein ADAM10—a zinc-dependent metalloprotease involved in the non-amyloidogenic cleavage of APP—was chosen. The RCSB Protein Data Bank (PDB) (https://www.rcsb.org), a comprehensive and well curated resource for experimentally determined protein structures, is where the three-dimensional crystal structure of ADAM10 was obtained. PDB ID 6BDZ, which denotes the extracellular domain of ADAM10 (residues 220–654), was used to identify the precise structure employed in this investigation. This domain includes the metalloprotease region, which is the principal target for ligand

binding and contains the active site that drives enzymatic activity. Although structural data from X-ray crystallography or other experimental techniques is directly included in the raw PDB file, it may also contain extraneous elements like ligands, water molecules, and polar hydrogens that could affect the precision of docking. Therefore, AutoDock Tools (MGL Tools) was used to preprocess the protein structure to guarantee a clean and biologically realistic docking environment. To reduce noise and avoid fake interactions, water molecules were eliminated in this step. Polar hydrogens were also removed because they are not taken during docking and could make results more difficult to understand. The structure was also transformed and saved in PDBQT format, which is the necessary input format for docking simulations based on AutoDock, and Gasteiger charges were added. 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

Absorption, Distribution, Metabolism, and Excretion, or ADME, are important pharmacokinetic factors that must be assessed to produce successful pharmacological treatments. These elements influence a compound's bioavailability, toxicity, and therapeutic appropriateness in addition to its drug-likeness, particularly for disorders that affect the central nervous system (CNS), like Alzheimer's. This work made use of SwissADME, a publicly available online tool designed by the Swiss Institute of Bioinformatics (SIB) (http://www.swissadme.ch/), in order to guarantee the pharmacological relevance of chosen ligands. By employing SMILES (Simplified Molecular enter Line Entry System) notation to enter chemical structures, this platform allows for thorough profiling of tiny compounds.

A multi-step screening procedure was applied to an initial library of 284 FDA-approved medications that were selected based on their structural resemblance to the well-known ADAM10 enhancer donepezil. Lipinski's Rule of Five, which assesses crucial characteristics such MW, log P, and HBD, HBA, all of which are suggestive of a compound's propensity to be orally bioavailable, was used in the initial screening stage. Compounds that did not fit these requirements were removed. The PAINS (Pan-Assay Interference Compounds) filter was used in the following step to eliminate molecules that can cause false-positive results because of assay interference or non-specific biological activity.

Blood-brain barrier (BBB) permeability predictions were included in the selection procedure to guarantee that the compounds maintained the capacity to efficiently reach brain tissue, given the BBB's pivotal position in CNS medication delivery. In the context of Alzheimer's treatment, only compounds that were anticipated to penetrate the blood-brain barrier were deemed promising

candidates for more research. To support each candidate's pharmacokinetic profile, other factors like water solubility, synthetic accessibility, bioavailability score, and gastrointestinal (GI) absorption were also assessed.

After a thorough ADME-based analysis, the original 284 compounds were reduced to a targeted group of 42 drug-like molecules, all of which showed promising pharmacokinetic properties. The ADAM10 protein, a crucial regulator in the non-amyloidogenic pathway of APP processing, was target of molecular docking studies to assess the binding affinity and interaction profiles of these chosen ligands, which were thought to be promising for CNS activity. Only high-potential, CNS-permeable, and pharmacologically relevant candidates made it to the final docking phase following ADME filtration.

3.4 Ligand preparation

To make sure that every molecule was in a format that could be used for molecular docking research, the ligand preparation procedure was an essential step. SwissSimilarity was first used to identify compounds that were structurally similar with reference medication donepezil. The PubChem database (https://pubchem.ncbi.nlm.nih.gov/) was then employed for getting matching 3D structures of those compounds. The National Centre for Biotechnology Information (NCBI) maintains the freely available chemical information repository PubChem, which offers a comprehensive collection of compound data, including physicochemical qualities, biological activities, and structural details. To represent 3D chemical structures and related metadata, the chosen compounds were downloaded in the Structure Data File (SDF) format.

Open Babel was used to transform these files into a format that could be used with molecular docking software. Over 110 distinct chemical file formats can be interconverted using Open Babel, an open-source chemical toolset. Additionally, it facilitates molecular modelling tasks including determining molecular descriptors, improving geometry, and inserting hydrogen atoms. To ensure correct alignment and processing during docking simulations, all ligand structures in this study were converted from SDF to PDB (Protein Data Bank) format using Open Babel. The ligands' chemical integrity and spatial arrangement were guaranteed to be maintained by this conversion.

After format conversion, each ligand was further created using the docking suite's ligand preparation tools or Open Babel by adding polar hydrogen atoms, allocating appropriate atomic charges, and optimizing shape. To guarantee that the molecular docking data accurately represented possible interactions with the ADAM10 target protein, this step was essential. Overall, high-quality structural data input was guaranteed by the ligand preparation method, which serves as the basis for consistent and repeatable docking outcomes.

3.5 Molecular docking

Using molecular docking, the 42 FDA-approved ligands that were chosen based on ADME profiling were tested for binding potential against the ADAM10 protein to estimate their orientation and interaction affinity at the protein's active site. AutoDock Vina 1.1.2 (https://vina.scripps.edu/), a popular open-source docking tool renowned for its speed and accuracy in calculating binding affinities, was used to perform molecular docking. AutoDock Vina predicts the most advantageous binding conformation of a ligand within the designated receptor site using a scoring function based on empirical free energy estimates and a stochastic global optimization method.

After eliminating water molecules and non-essential heteroatoms, polar hydrogens got added, and Kollman charges were inserted employing AutoDock Tools (ADT) to create the three-dimensional structure of ADAM10 (which was taken from the Protein Data Bank). Following energy minimization with Open Babel and maintaining the appropriate torsional flexibility, the ligand structures were created by translating them into PDBQT format. The entire ADAM10 active site region was enclosed by a grid box, which permitted unrestricted investigation of the binding cavity. The grid size measurements were x = 75.26, y = 48, and z = 66.56, while the grid centre parameters were x = 31.68, y = 19.05, and z = 45.96. To provide adequate coverage of the catalytic core, the grid spacing was maintained at the default value of 0.375 Å [65].

Every ligand was docked separately, and AutoDock Vina 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 analysed using an Excel spreadsheet, with a particular focus on comparing each ligand's binding energy to that of the reference medication, Donepezil.

3.6 Examination of the Protein-Ligand Complex Structure

To determine the type and degree of interactions between ligands and the ADAM10 protein, a thorough structural study of the protein-ligand complexes was carried out after the molecular docking procedure. For every ligand, the docking software produced a unique output file that contained important data including binding energy scores, interaction distances, and ligand positions inside the macromolecule's active site. The interaction visualization and validation were based on these files.

Dassault Systèmes' BIOVIA Discovery Studio, a full suite of molecular modelling and simulation tools, was used to further examine and visually understand these complexes. Because of its strong visualization features and capacity to produce both 2D and 3D representations of protein-ligand interactions, this platform was selected. Specifically, Discovery Studio made it possible to create 2D interaction diagrams that effectively depicted important binding interactions like metal coordination,

π–π stacking, hydrophobic contacts, hydrogen bonds, and electrostatic forces—all of which are particularly important in the context of ADAM10's zinc-dependent catalytic mechanism.

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

Using the ProTox-II (version 3.0) webserver, a reputable in-silico platform for predicting a variety of toxicological endpoints, a thorough toxicity assessment was conducted to analyse the safety profiles of the FDA-approved drugs candidates.

First, the drug's SMILES and PubChem name was uploaded to the ProTox-II interface one at a time. The program used its machine learning-based models, which combine molecular fingerprints, chemical similarity, fragment-based descriptors, and toxicophore detection, to process each substance after it was submitted. Each compound's projected LD_{50} value (mg/kg), toxicity class (based on GHS classification), and qualitative predictions for hepatotoxicity, carcinogenicity, immunotoxicity, mutagenicity, and cytotoxicity were all included in the comprehensive toxicity profile that ProTox-II subsequently produced. These results supported the selection of the most promising compounds for additional research in AD therapy by assessing the relative safety of each candidate and weeding out drugs with possible toxicological issues.

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 donepezil 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 ADAM10 protein. Of them, 35 medicines had docking scores better than -7.4 kcal/mol, which is typically seen as a sign of favourable 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 ADAM10's active region. Notably, the reference chemical Donepezil, which had a binding energy of roughly -7.8 kcal/mol, was outperformed by Ziprasidone (-9.9 kcal/mol) and Oxatomide (-9.4 kcal/mol), which had the strongest binding. Furthermore, 21 substances shown moderate-to-significant binding affinities ranging from -8.0 to -8.9 kcal/mol, further supporting their potential as repurposable ADAM10 modulators, while three medications showed binding energies precisely at -7.8 kcal/mol, which is equivalent to that of donepezil.

Despite having lower binding energies, the remaining medications nevertheless offered valuable information about the structural characteristics that can affect ADAM10-ligand interactions. Together, these findings demonstrate the possibility of a number of FDA-approved medications, including Domperidone, Lasmiditan, Oxatomide, Metergoline, Ziprasidone, and others, as therapeutic possibilities for modifying ADAM10 activity in Alzheimer's disease. For ADAM10-targeted medication repurposing, these compounds merit additional experimental validation and optimization due to their greater or equivalent binding affinities to donepezil.

TABLE 1. LIST OF DRUGS WITH THEIR ESTIMATED ΔG (KCAL/MOL)

S.no	Drugs	Estimated ΔG (kcal/mol)
1.	Donepezil (reference drug)	-7.4
2.	Ziprasidone	-9.9

3.	Oxatomide	-9.4
4.	Lasmiditan	-9.3
5.	Domperidone	-9.3
6.	Metergoline	-9.1
7.	Sarizotan	-9
8.	Roluperidone	-8.9
9.	Bifeprunox	-8.8
10.	Darifenacin	-8.8
11.	PF-03635659	-8.7
12.	Lumateperone	-8.7
13.	Pruvanserin	-8.6
14.	Droperidol	-8.5
15.	Benperidol	-8.4
16.	Azaperone	-8.3
17.	GSK-239512	-8.1
18.	Pipamperone	-8
19.	Falnidamol	-8
20.	"(6R)-2-amino-6-[2-(3'-methoxybiphenyl-3-yl)ethyl]-3	-8.3
21.	2R	-8.5
22.	Indoramin	-8.7
23.	{4-[(2R)-pyrrolidin-2-ylmethoxy]phenyl}(4-thiophen-3-ylphenyl)methanone	-8.5
24.	"N-(cyclopropylmethyl)-2'-methyl-5'-(5-methyl-1	-8.6
25.	"5-FLUORO-1-[4-(4-PHENYL-3	-8.1
26.	"N-cyclopropyl-2'	-8.7
27.	Atevirdine	-8.6
28.	"N-{5-[4-(4-METHYLPIPERAZIN-1-YL)PHENYL]-1H-PYRROLO[2	-8.1
29.	ABT-288	-8.0
30.	Emicerfont	-8.5
31.	Niaprazine	-7.8
32.	Butaperazine	-7.8
33.	Niraparib	-7.8

34.	Onalespib	-7.7
35.	Fipexide	-7.6
35.	Sertindole	-7.8
36.	Pirodavir	-7.6

3.2 Visualisation of interactions

After the chosen ligands were molecularly docked with the ADAM10 protein, BIOVIA Discovery Studio was used to examine the two-dimensional (2D) and three-dimensional (3D) binding conformations of the best-performing compounds. The kind and intensity of the interactions between the ligands and the ADAM10 active site residues were clearly shown by this image. Multiple stabilizing interactions were discovered in drugs including Domperidone, Lasmiditan, Oxatomide, Ziprasidone, Metergoline and others that had higher docking scores than the reference molecule Donepezil. Strong and precise binding inside the active pocket was facilitated by these, which included metal coordination with the catalytic zinc ion, π–π stacking, hydrogen bonding, and hydrophobic interactions. Important contact residues, which are essential elements of the catalytic site, were prominently displayed in the 2D interaction diagrams. These ligands' good fit into the binding pocket, near proximity to the catalytic core, and proper alignment with the substrate-binding groove were further validated by the 3D visualizations. These results provide credence to the compounds' potential as promising modulators of ADAM10 activity, which calls for more experimental verification.

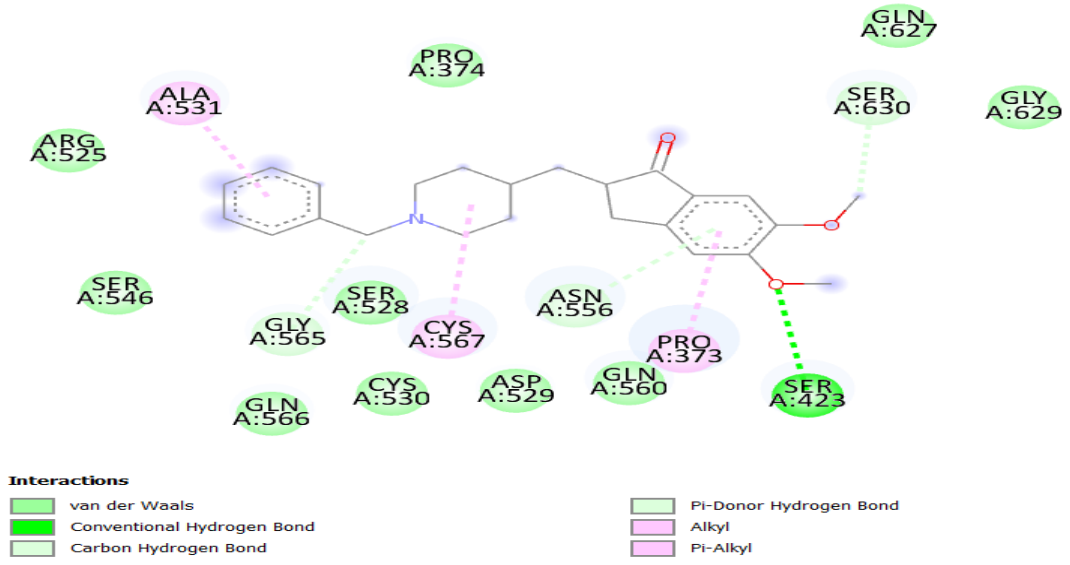

Fig 5. Demonstrates different interactions between Donepezil (Reference drug) and the ADAM10 protein in a two-dimensional graphical representation

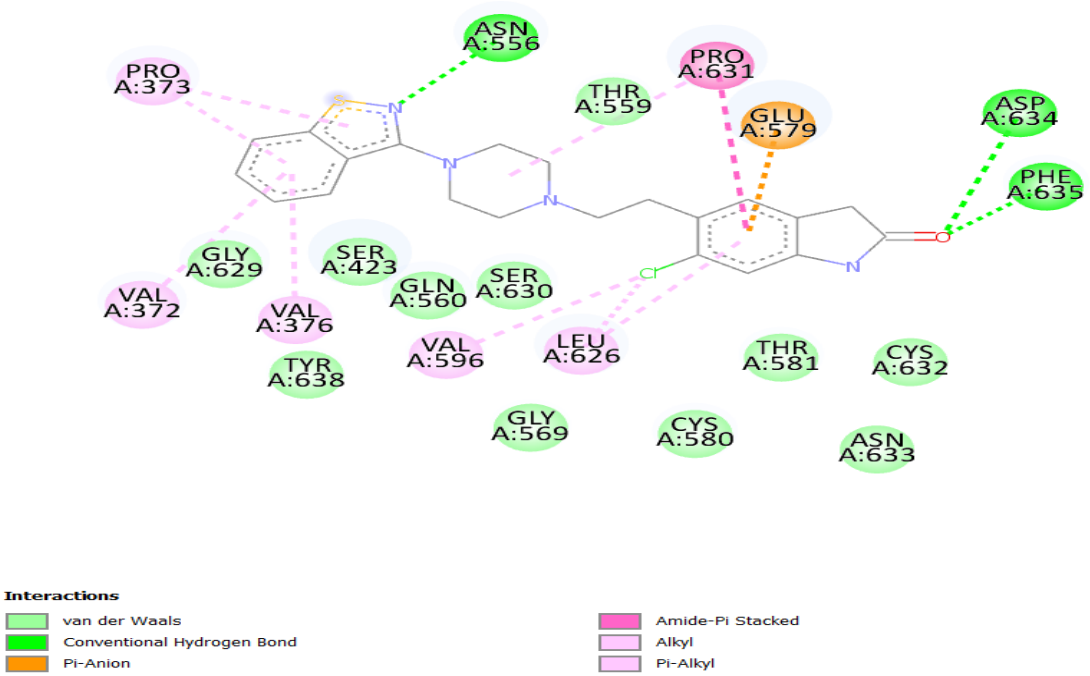

Fig 6. Demonstrates different interactions between Ziprasidone and the ADAM10 protein in a two-dimensional graphical representation.

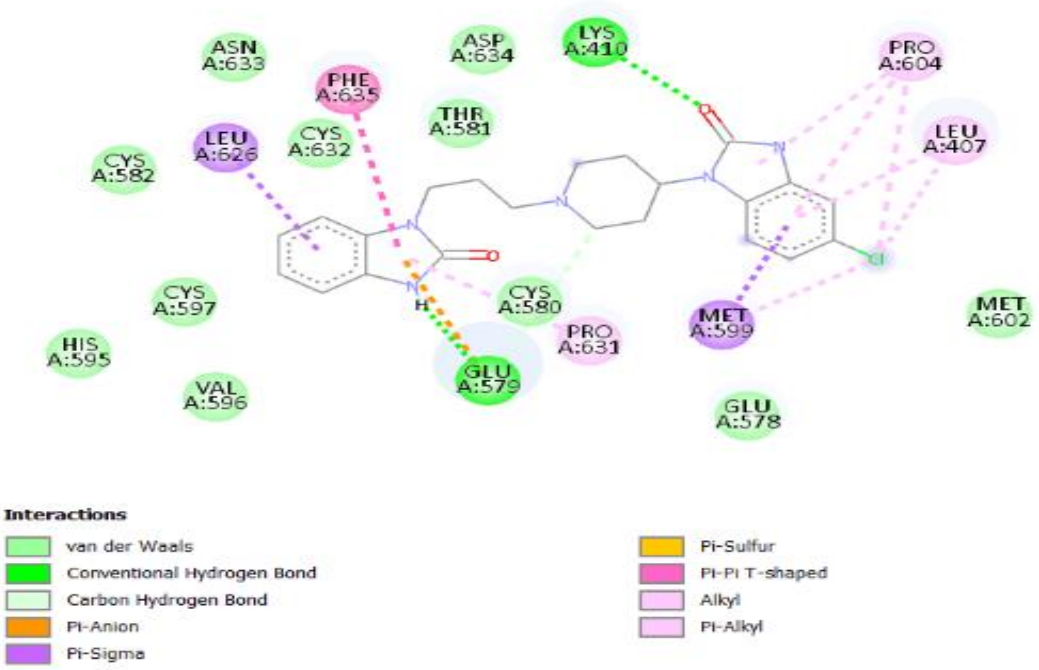

Fig 7. Demonstrates different interactions between Domperidone and the ADAM10 protein in a two-dimensional graphical representation.

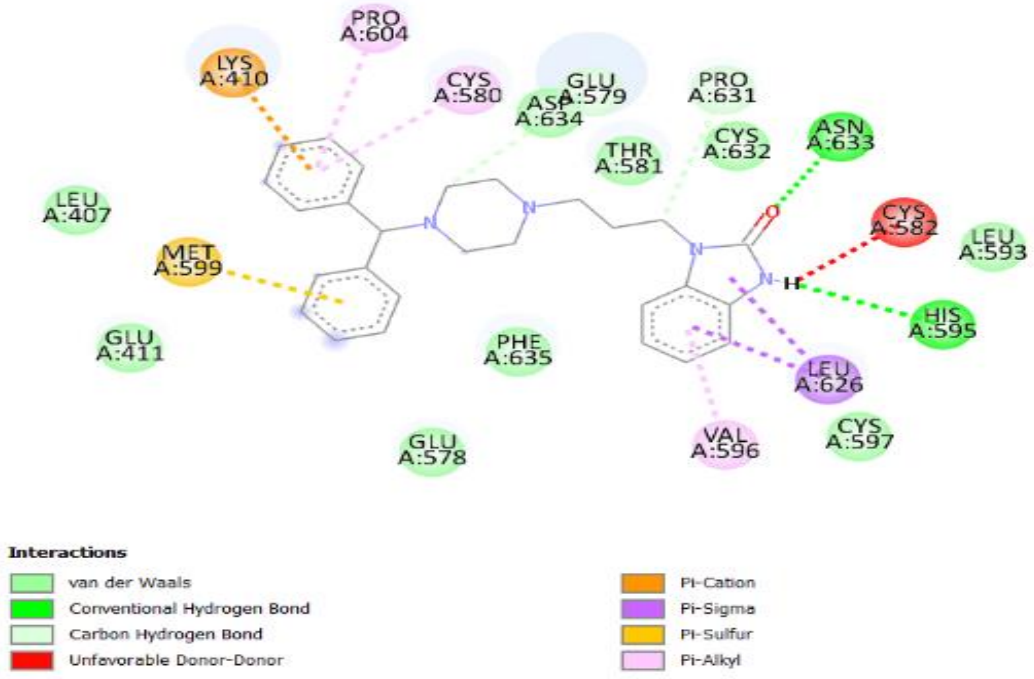

Fig 8. Demonstrates different interactions between Oxatomide and the ADAM10 protein in a two-dimensional graphical representation.

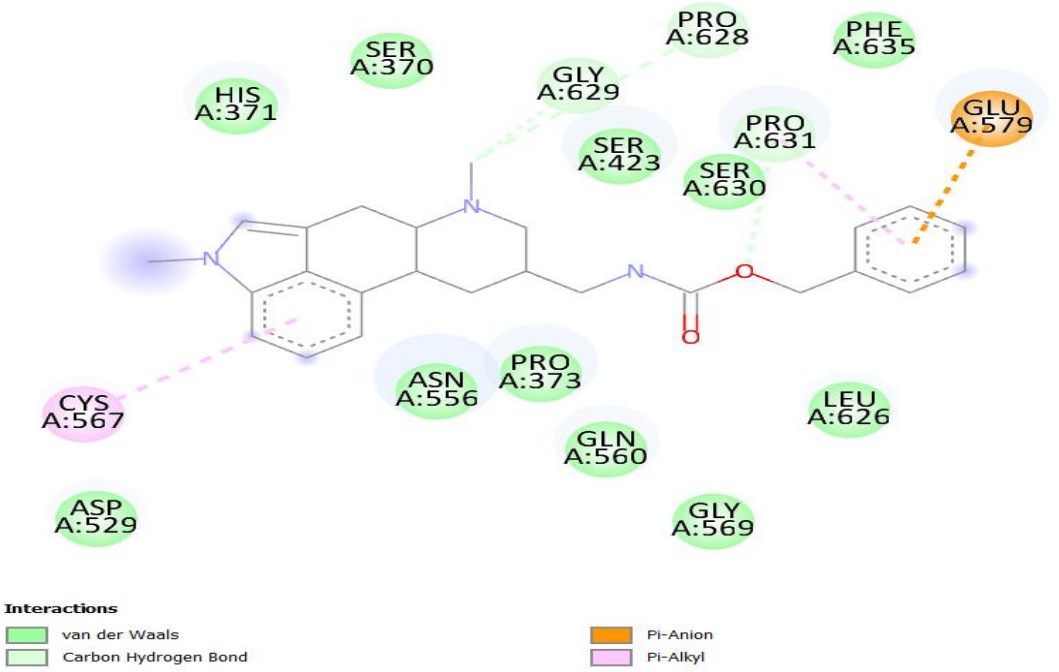

Fig 9. Demonstrates different interactions between Metergoline and the ADAM10 protein in a two-dimensional graphical representation.

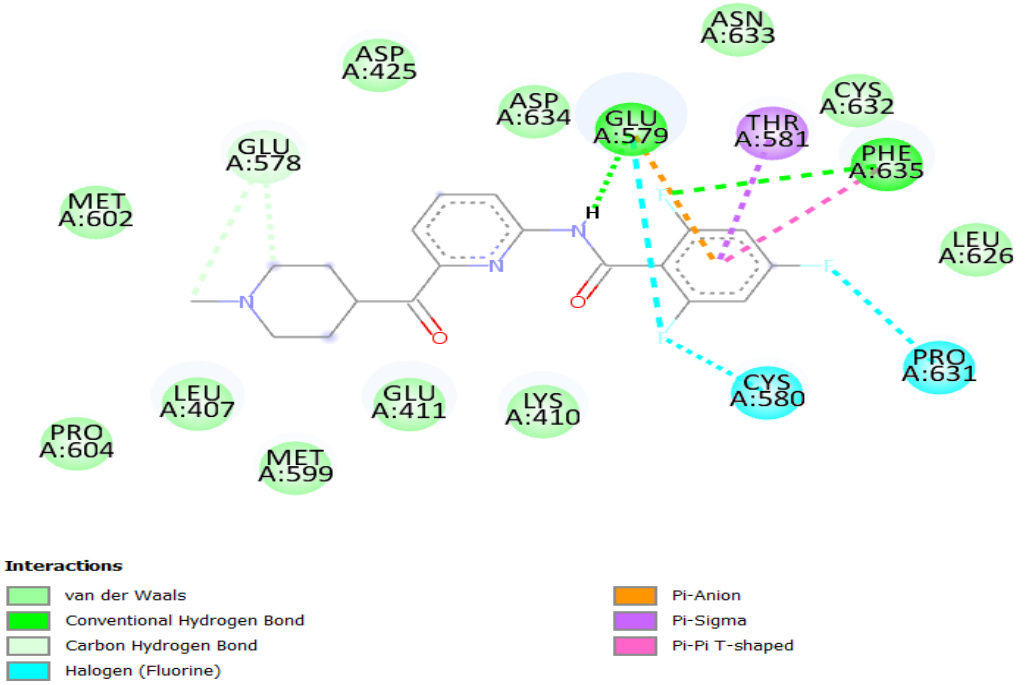

Fig 10. Demonstrates different interactions between Lasmiditan and the ADAM10 protein in a two-dimensional graphical representation.

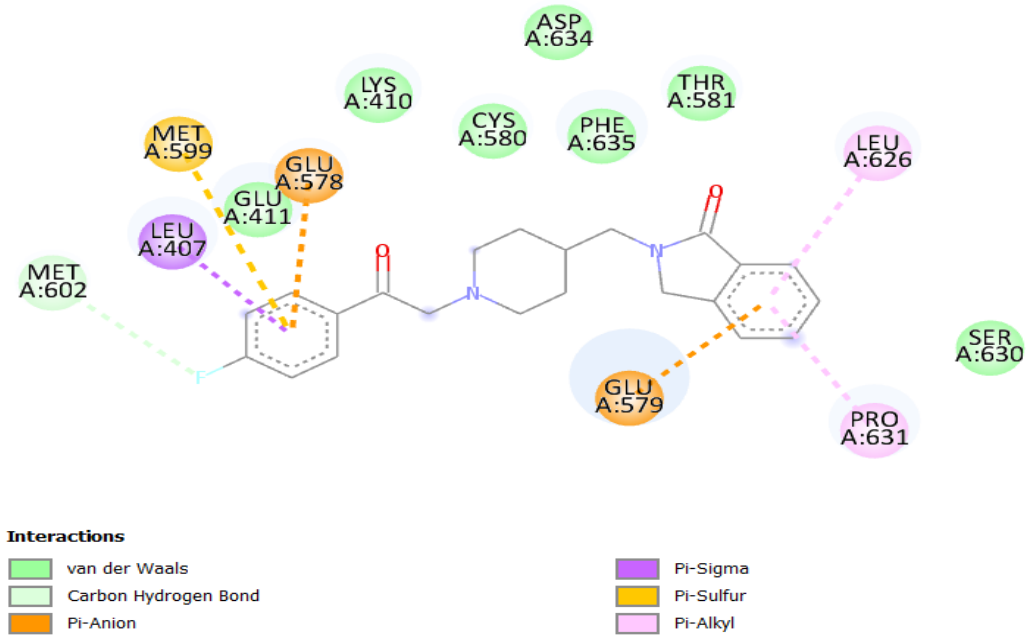

Fig 11. Demonstrates different interactions between Roluperidone and the ADAM10 protein in a two-dimensional graphical representation.

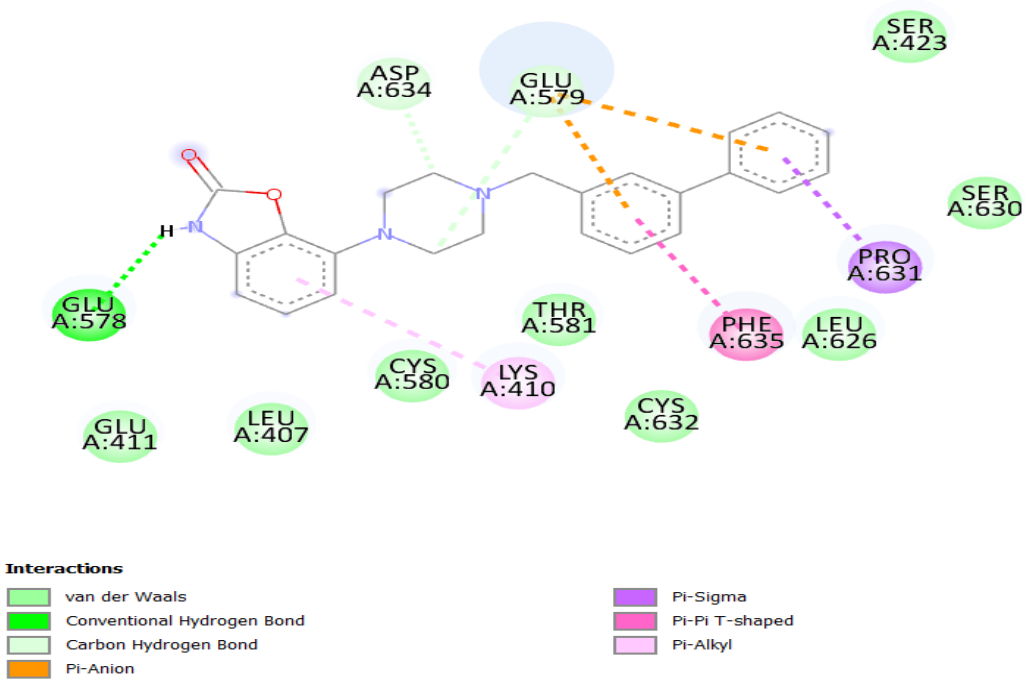

Fig 12. Demonstrates different interactions between Bifeprunox and the ADAM10 protein in a two-dimensional graphical representation.

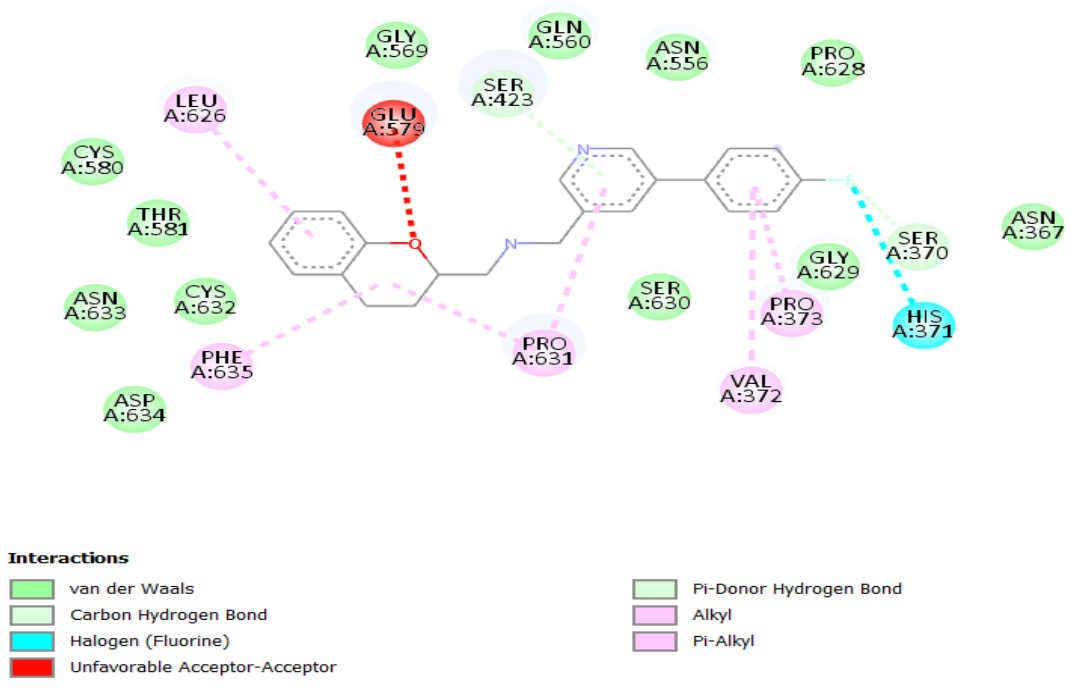

Fig 13. Demonstrates different interactions between Sarizotan and the ADAM10 protein in a two-dimensional graphical representation.

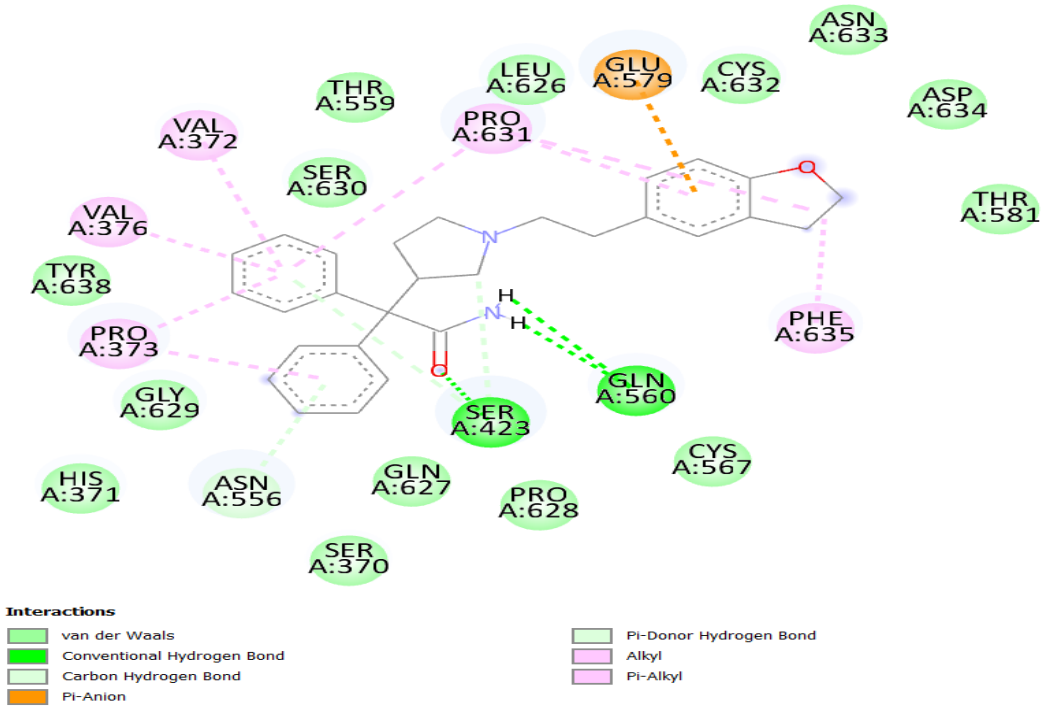

Fig 14. Demonstrates different interactions between Darifenacin and the ADAM10 protein in a two-dimensional graphical representation.

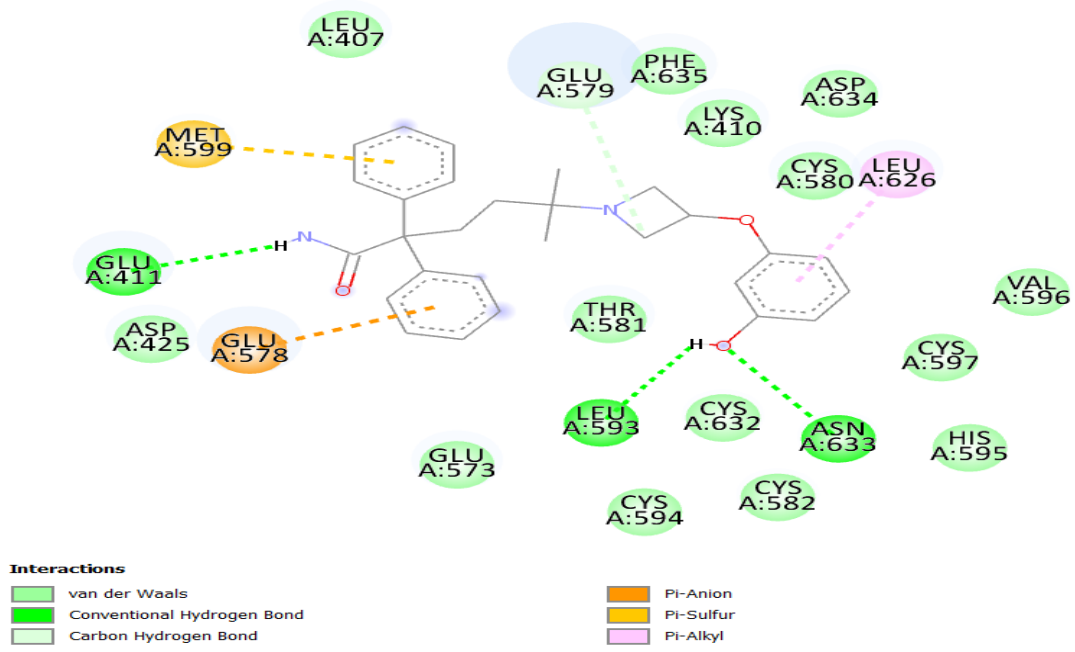

Fig 14. Demonstrates different interactions between PF-03635659 and the ADAM10 protein in a two-dimensional graphical representation

TABLE 2. BINDING ENERGIES OF TOP BINDING DRUGS AND THEIR INTERACTION WITH THE PROTEIN

Drugs	Binding energies (kcal/mol)	Interacting amino acids
Donepezil (Reference drug)	-7.4	SER A:423, PRO A:373, CYS A:567, ALA A:531,
Ziprasidone	-9.9	ASP A:634, PHE A:635, ASN A:556, PRO A:373, VAL A:372, LEU A:626, VAL A:376, VAL A:596
Oxatomide	-9.4	HIS A:595, ASN A:633, LEU A:626, VAL A:596, MET M:599, LYS A:410, PRO A:604, CYS A:604
Lasmiditan	-9.3	GLU A:579, PHE A:635, THR A:581
Domperidone	-9.3	GLU A:579, LYS A:410, PRO A:604, LEU A:407, PHE A:635, LEU A:626, MET A:599
Metergoline	-9.1	GLY A:629, PRO A:628, CYS A:567, SER A:630, PRO A:631, GLU A:579
Sarizotan	-9.0	PRO A:373, VAL A:372, PRO A:631, LEU A:626, PHE A:635, SER A:423, ASP A:634, SER A:370
Roluperidone	-8.9	GLU A:411, MET A:602, PRO A:631, LEU A:626, LEU A:407, MET A:599, GLU A:578
Bifeprunox	-8.8	GLU A:579, LYS A:410, PHE A:635, GLU A:578, ASP A:634, PRO A:631
Darifenacin	-8.8	VAL A:376, PRO A:631, PHE A:635, PRO A:376, PRO A:373, ASN A:556, GLU A:579
PF-03635659	-8.7	MET A:599, GLU A:579, LEU A:593, ASN A:633, LEU A:626, GLU A:411, ASP A:624

4.3 ADMET analysis

The best-performing drug candidates with the highest binding affinities for ADAM10 after the molecular docking experiments were next evaluated for pharmacokinetic appropriateness using ADME (Absorption, Distribution, Metabolism, and Excretion) analysis. The bulk of the 42 nominated compounds showed high gastrointestinal (GI) absorption and good water solubility, indicating considerable potential for development as oral accessible medicines. These medications' bioavailability scores fell within a reasonable range, suggesting that they would probably be effective in the systemic circulation after oral administration. Crucially, the majority of the chosen applicants exhibited no transgressions of Lipinski's Rule of Five, so confirming their adherence to the established standards for drug-likeness. These results lend credence to the idea that the compounds that were found had substantial biological activity against the ADAM10 target together with advantageous pharmacokinetic and safety profiles. The argument for repurposing these FDA-approved medications as possible therapeutic agents for Alzheimer's disease is strengthened by the combination of their significant binding affinity, structural compatibility, and acceptable ADME properties.

TABLE 3. ADME ANALYSIS OF ALL BINDING DRUGS

S.no	Drugs	BBB permeability	Consensus Log P value	GI absorption rate	TPS A value	Lipinski violation
1.	Ziprasidone	Yes	3.5	High	76.1	0
2.	Oxatomide	Yes	3.98	High	44.27	0
3.	Lasmiditan	Yes	2.48	High	62.3	0
4.	Domperidone	Yes	3.38	High	78.2	0
5.	Metergoline	Yes	3.29	High	46.5	0
6.	Sarizotan	Yes	4.2	High	34.15	0
7.	Roluperidone	Yes	3.33	High	40.62	0
8.	Bifeprunox	Yes	3.72	High	52.48	0
9.	Darifenacin	Yes	3.08	High	67.92	0
10.	PF-03635659	Yes	3.96	High	75.79	0
11.	Lumateperone	Yes	3.56	High	26.79	0
12.	Pruvanserin	Yes	3.15	High	63.13	0
13.	Droperidol	Yes	3.56	High	58.1	0
14.	Benperidol	Yes	3.66	High	58.1	0
15.	Azaperone	Yes	3.0	High	36.44	0
16.	GSK-239512	Yes	3.45	High	45.67	0
17.	Pipamperone	Yes	2.55	High	66.64	0
18.	Falnidamol	Yes	3.48	High	78.86	0
19.	"(6R)-2-amino-6-[2-(3'-methoxybiphenyl-3-yl)ethyl]-3	Yes	3.08	High	67.92	0
20.	2R	Yes	4.45	High	62.16	0
21.	Indoramin	Yes	3.43	High	48.13	0
22.	{4-[(2R)-pyrrolidin-2-ylmethoxy]phenyl}(4-thiophen-3-ylphenyl)methanone	Yes	4.35	High	66.57	0
23.	"N-(cyclopropylmethyl)-2'-methyl-5'-(5-methyl-1	Yes	3.97	High	68.02	0
24.	"5-FLUORO-1[4-(4-PHENYL-3	Yes	3.77	High	58.1	0
25.	"N-cyclopropyl-2'	Yes	4.01	High	68.02	0

26.	Atevirdine	Yes	2.5	High	73.49	0
27.	"N-{5-[4-(4-METHYLPIPERAZIN-1-YL)PHENYL]-1H-PYRROLO[2	Yes	2.55	High	77.15	0
28.	ABT-288	Yes	2.97	High	41.37	0
29	Emicerfont	Yes	2.65	High	75.52	0
30.	Niaprazine	Yes	2.62	High	48.47	0
31.	Butaperazine	Yes	3.97	High	52.09	0
32.	Niraparib	Yes	2.29	High	72.94	0
33.	Onalespib	Yes	2.72	High	67.25	0
34.	Fipexide	Yes	2.82	High	51.24	0
35.	Sertindole	Yes	4.0	High	40.51	0
36.	Pirodavir	Yes	3.53	High	64.55	0

4.4 Toxicity assessment using ProTox II (version 3.0) server

The toxicity profile of the top 10 binding drugs was evaluated using the ProTox II server. The server uses various machine learning (ML) methods, which are highly helpful in biological research these days, to forecast the harmful tendencies based on LD50 values. These machine learning algorithms are taught to recognize correlations and trends among various toxicity profiles and chemical structures. Table 3. is a tabulation of the toxicity data where '+' sign in the table denotes an active result while a '-' sign denotes an inactive toxicity outcome.

TABLE 4. TOXICITY ASSESSMENT OF TOP BINDING DRUGS USING THE PROTOX 3.0 SERVER

Drug	LD50 value (mg/kg)	Hepatotoxicity status	Carcinogenicity status	Immunotoxicity status	Mutagenicity status	Cytotoxicity status	Toxicity class predicted
Ziprasidone	715	-	-	+	-	-	Class 4
Oxatomide	1600	-	-	-	-	-	Class 4
Lasmiditan	1600	-	-	-	-	-	Class 4
Domperidone	715	-	-	+	-	-	Class 4
Metergoline	430	-	-	-	+	-	Class 4
Sarizotan	812	-	-	-	-	-	Class 4
Roluperidone	2130	-	-	-	-	-	Class 5
Bifeprunox	1500	-	-	-	-	-	Class 4

Darifenacin	300	-	-	-	-	-	Class 3
PF-03635659	384	-	-	-	-	-	Class 4

Based on factors including LD50 values, hepatotoxicity, carcinogenicity, immunotoxicity, mutagenicity, cytotoxicity and anticipated toxicity class, toxicity prediction was carried out to evaluate the safety profiles of the top binding drugs. The chosen drug's LD50 values varied from 300 mg/kg to 2130 mg/kg, suggesting different levels of acute toxicity. Roluperidone, with an LD50 of 2130 mg/kg, was categorized under Class 5, indicating reduced toxicity, whereas other compounds were classified under Toxicity Class 4, indicating that they are dangerous if swallowed. Darifenacin was classified as having moderate toxicity under Toxicity Class 3, owing to its comparatively lower LD50 of 300 mg/kg.

Remarkably, none of the compounds exhibited cytotoxicity, mutagenicity, carcinogenicity, or hepatotoxicity. However, Metergoline tested positive for mutagenicity, while Ziprasidone and Domperidone were noted for possible immunotoxicity, requiring careful examination in additional research. These results show that even if certain drugs candidates show good ADME profiles and substantial binding affinities, thorough toxicity testing is necessary to guarantee that they are suitable for repurposing in the treatment of AD. Overall, the data indicates that most of the chosen drugs have adequate safety margins; Roluperidone, Lasmiditan, Oxatomide, and Ziprasidone stand out as promising options with low toxicity concerns.

4.5 Selection of potential drugs

Interestingly, 35 drugs showed greater binding affinities than the reference medication, donepezil. Based on their docking scores and important interactions with the catalytic residues inside the ADAM10 active site and toxicity analysis, the most promising candidates were found, particularly the top hits, Roluperidone, Domperidone, Lasmiditan, Oxatomide, Metergoline, and Ziprasidone. These results imply that the drugs on the shortlist have a great deal of promise for use as ADAM10 enhancers in treatment of AD.

CHAPTER 5

CONCLUSION

AD has a complicated aetiology and few available treatments, making it one of the most difficult and complex neurodegenerative diseases. As ADAM10 is essential for the non-amyloidogenic processing of APP, which stops the production of amyloid-beta peptides thar are neurologically toxic, it has become a viable candidate among the different molecular targets. Given that all pharmaceutical treatments for AD have so far failed, finding new and targeted pathways that could form the foundation of innovative medicines is urgent and crucial. Therefore, understanding how ADAM10 is regulated will be crucial to effectively controlling its activity in both healthy and pathological settings.

We used a computational medication repurposing approach to find FDA-approved drugs that share structural similarities with the well-known ADAM10 enhancer donepezil. Using AutoDock Vina for molecular docking and virtual screening, we found many candidates that had a high binding affinity for the ADAM10 active site. Notably, Roluperidone, Oxatomide, Ziprasidone, Lasmiditan, Meterogoline, Domperidone 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 ADAM10 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 ADAM10 activation. Future research should also use molecular dynamics simulations to investigate the stability, conformational behaviour, 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 ADAM10.

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