"COMPUTATIONAL IDENTIFICATION OF NOVEL NMDA RECEPTOR ANTAGONISTS AS THERAPEUTIC CANDIDATES FOR ALZHEIMER'S DISEASE"

A Dissertation

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BIOTECHNOLOGY

by

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24/MSCBIO/04

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DECLARATION

I, Sonia 24/MSCBIO/04 hereby certify that the work which is being presented in this dissertation entitled as **"Computational Identification of Novel NMDA Receptor Antagonists as Therapeutic Candidates for Alzheimer's Disease"** in partial fulfillment of the requirements for the award of the Degree of Master of Science, submitted in the Department of Biotechnology, Delhi Technological university is an authentic record of my own work carried out during the period from 2025 to 2026 under the supervision of Prof. Pravir Kumar.

The matter presented in this dissertation has not been submitted by me for the award of any other degree of this or any other Institute.

Candidate's signature

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CERTIFICATE BY THE SUPERVISOR

This is to certify that the Dissertation Project entitled as **"Computational Identification of Novel NMDA Receptor Antagonists as Therapeutic Candidates for Alzheimer's Disease'',** submitted by Sonia 24/MSCBIO/04, Department of Biotechnology, Delhi Technological University, Delhi 110042, in partial fulfillment of the requirements for the award of the Degree of Master of Science is a record of the work carried out by student under my supervision. To the best of my knowledge, this work work has not been submitted in part or full for any Degree or Diploma to this University or elsewhere.

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Computational Identification of Novel NMDA Receptor Antagonists as Therapeutic Candidates for Alzheimer's Disease"

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ABSTRACT

NMDA receptors are ionotropic glutamate receptors that are involved in normal synaptic transmission; however, their dysregulation and overactivation are associated with excitotoxic neuronal damage as observed in AD. Therefore, targeting the NMDAR acts as therapeutic strategy to treat AD. This present study is focused on the identification of novel NMDA receptor antagonists, using molecular docking method. Ifenprodil is taken as the reference ligand, a well-known subunit-selective antagonist of the NMDA receptor that binds to GluN2B subunit. A structural similarity search was performed in the PubChem database, by taking Ifenprodil as a query ligand, that lead to the identification of 607 structural analogues, on further sorting, only 73 ligands were selected. The shortlisted ligands were then subjected to molecular docking analysis to evaluate ligand-protein interactions and the docking scores. The ligands exhibiting superior docking scores were further analysed. The ADME analysis was performed to assess the BBB permeability, GI absorption and other pharmacokinetic properties to ensure the CNS suitability. A total of eight compounds that exhibit the favourable docking scores along with acceptable pharmacokinetic properties were identified. These eight compounds may serve as potential candidates to treat AD, but are subjected to wet-lab experimentation.

Keywods: Alzheimer's Disease, NMDA receptor, Ifenprodil, Molecular Docking, BBB, PubChem

Results: Initially, a total of 607 compounds were obtained from PubChem, out of which 73 candidates were selected for the molecular docking stimulation, which gave us the eight compounds having better docking scores than the reference ligand.

Conclusion: The given study proposed eight potential compounds can be taken into consideration for the treatment AD. However, to explore their full potential, molecular dynamics study and wet-lab experimentation are recommended.

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List of Abbreviations

AD	Alzheimer's Disease
NMDA	N-methyl-D-aspartate
BBB	Blood brain barrier
APP	Amyloid Precursor Protein
FAD	Familial Alzheimer's Disease
ADME	Absorption Distribution Metabolism Excretion
EAAT2	Excitatory Amino Acid Transporter 2
ROS	Reactive Oxygen Species
NMDAR	N-methyl-D-aspartate receptor
LTP	Long Term Potentiation
LTD	Long Term Dipression
FOXO	Forkhead box O
SAR	Structure Activity Relationship
GRIN	Glutamate Receptor, Ionotropic, NMDA
Mk	Dizocilpine maleate
CGP	Ciba-Geigy Pharmaceuticals
PCP	Phencyclidine
ATD	Amino Terminal Domain
NDD	Neurodenegrative disease
HTS	High Throughput Screening
PDBQT	Protein Data Bank, Partial atomic charge, Atom type
CID	Compound Identification
SMILES	Simplified Molecular Input Line Entry System
SDF	Structural Data File
CNS	Central Nervous System
QM	Quantum Mechanics
NOS	Nitrogen Oxygen Species
NMR	Nuclear Magnetic Resonance
EM	Electron Microscopy
SBDD	Structure Bases Drug Discovery
LBDD	Ligand Based Drug discovery
vHTS	Virtual High Troughput Screening

SVM	Support Vector Machine
ML	Machine Learning
AI	Artificial Intelligence
GOLD	Genetic Optimization for Ligand Doking
PDB	Protein Data Bank
QSAR	Quantitative Structure Activity Relationship
RMSD	Root Mean Square Deviation
ROC	Receiver Operating Characterstic
AUC	Area Under Curve

1. INTRODUCTION

Alzheimer's disease (AD) is widely known, age-associated progressive neurodegenerative disorder marked by a gradual decline of cognition, impairment in memory, reasoning, behavioural functions, and personality changes [1]. The disease involves a gradual structural and functional decline of neurons within the hippocampus and cerebral cortex, which are the regions of the brain critical for learning and cognitive abilities [2]. The major pathological hallmarks of AD include extracellular aggregation of amyloid-β plaques and intracellular assembly of neurofibrillary tangles consisting of hyperphosphorylated tau protein [3]. Growing evidence further shows that synaptic loss and dysfunction also play a role in cognitive impairment, along with the classical histopathological markers.

The synaptic dysfunction arises due to the calcium overload in neuronal cells, which leads to glutamate-induced cell death in excitotoxic processes [4]. Ca2+ entry into neuronal cells is often mediated by NMDA receptors, which have high calcium permeability [5] [6]. The NMDA receptors are subtypes of the ionotropic glutamate receptors made up of two subunits, GluN2A and GluN2B, and are involved in functions such as learning, synaptic plasticity, memory, and other critical physiological functions [7]. However, their dysregulation or overactivation contributes to neurodegenerative disorders. Studies also demonstrated that the location of NMDA receptors plays a decisive role in determining their effect on neuronal viability. The activation of the synaptic NMDARs has been found to initiate neuroprotective gene expression and support neuronal survival, whereas the activation of extrasynaptic NMDARs leads to pathways that result in neuronal cell death, underlying a possible mechanism of neurodegeneration observed in AD [8] [9]. The overactivation of cellular NMDA receptors or the activation of extrasynaptic NMDA receptors causes sustained calcium influx, and this abnormally elevated Ca^{2+} signalling disrupts synaptic function over time and eventually leads to neuronal cell death. This process is clinically associated with a gradual decline in cognition and memory, along with the emergence of characteristic neuroanatomical abnormalities observed in Alzheimer's disease patients [10] [11].

Therefore, targeting the NMDA receptors offers a therapeutic strategy to treat AD. Their inhibition results in symptomatic relief and protective treatment for the AD patients. Many antagonists have been reported in earlier studies that cause the inhibition of the NMDA receptors, providing neuroprotection and relief from glutamate mediated excitoxicity. Despite their protective effects they also display limitations such as poor BBB permeability, low bioavailability, limited selectivity and off-target effect. The other side effects may include hypertension, hallucinations, headache, agitation, paranoia, cough, back pain, dizziness, vomiting, yawning, shortness of breath, and fatigue, suggesting that, there remains a significant need for NMDA inhibitors that address these limitations, show high efficacy with minimal adverse effects [12].

The computational approach in drug discovery has emerged as a powerful and efficient strategy for screening and refining potential therapeutic agents by using techniques like molecular docking, modelling, pharmacophore generation and leveraging advanced algorithms [13]. Computational drug discovery offers a faster and efficient way to assess the new molecules for their pharmacological profile from large datasets to identify and evaluate lead compounds for drug discovery. Unlike traditional experimental methods, which are often time-intensive and expensive, computational techniques enable the rapid screening and evaluation of vast chemical libraries with improved precision [14] [15].

Leveraging the computational approach of drug discovery, this study is focused on identification of the novel antagonists for the NMDAR inhibition, addressing the AD. By integrating Molecular docking, structure based ligand selection and ADME analysis this study aims contribute in the development of the clinically potential antagonists targeting the Alzheimer's Disease.

2. LITERATURE REVIEW

2.1 Pathophysiology of Alzheimer's Disease

Alzheimer's is a progressive neurodegenerative disease, representing 60-80% of all cases of dementia. It is characterised by the progressive decline in cognition, neurons, impairments in memory, intellect and personality defects [16]. However, in sever cases symptoms like hallucinations, delusions, altered sleep patterns and social withdrawal can be seen. In the early stages, the symptoms of AD remain unexpressed, and changes happen at the molecular level only. The patients transits from mild cognitive impairment to progressing AD within 2 years [17] [18].

Histopathologically, the AD progression is associated with the accumulation of extracellular senile plaques formed by amyloid-beta (Aβ), the presence of intracellular neurofibrillary tangles (NFTs), arise from abnormally phosphorylated tau protein, and progressive synaptic degeneration [19] [20]. The Aβ is formed by the abnormal processing of the APP, causing Aβ overproduction and disruption of its clearance mechanism. During aggregation process the Aβ causes loss of physiological functions, resulting in disease pathology. Soluble Aβ oligomers being neurotoxic, disrupts the cell membrane and Ca2+ balance, triggers oxidative stress, mitocondrial dysfunction and synapse damage, ultimately translating into AD progression [21] [22]. In pathological conditions, the tau undergoes conformational changes and aggregates into oligomers, paired helical filaments, and neurofibrillary tangles within the cell body and dendrities of the neuron. These aggregates disrupt neuronal function, induce cellular toxicity, and promote synaptic failure and cell death. Additionally, tau pathology propagates trans-synaptically, spreading aggregation to neighboring neurons and amplifying neurodegeneration. All these pathological changes primarily affect brain regions involved in cognitive functions, including the neocortex, hippocampus, and other subcortical areas, leading to neurodegeneration [23] [24].

Another mechanism that leads to AD includes glutamate mediated excitotoxicty, through the activation of NMDA receptor, a glutamate ionotropic receptor. The Aβ deposition results in synaptic damage and interferes with various receptors in the central nervous system, thereby disturbing neuronal homeostasis. It causes the activation of the NMDA receptor, which leads to disruption in the calcium homeostasis, reducing the neuron’s buffering capacity and promoting excitotoxic damage [25] [26]. The Aβ causes mislocalisation of astrocytic EAAT2 transporters and impairs the glutamine synthetase activity, which disrupts the glutamate homeostasis. The disruption in glutamate homeostasis causes sustained activation of the NMDA receptor, bringing the synaptic dysfunction [27]. Activation of NMDARs has been shown to enhance amyloid-beta (Aβ) production by favoring APP cleavage through the α-secretase pathway over the β-secretase route [28]. This suggests that even slight disturbances in glutamatergic neurotransmission can contribute to excessive Aβ generation, forming a vicious cycle, leading to disease progression. Tau protein also contributes to glutamate-induced excitotoxicity by undergoing hyperphosphorylation, leading to its mislocalization and ultimately synaptic dysfunction [29].

Cholinergic dysfunction is another hallmark feature of AD, characterized by a significant loss of cholinergic neurons in the basal forebrain (cerebral cortex and hippocampus) and reduced levels of the neurotransmitter acetylcholine [30] [31]. This deficit is strongly associated with impairment in cognition, attention and memory, and forms the basis for currently approved symptomatic treatments such as acetylcholinesterase inhibitors. Neuroinflammation further exacerbates disease progression. Activated microglia and astrocytes respond to Aβ accumulation and neuronal injury by releasing pro-inflammatory cytokines, chemokines, and reactive oxygen species (ROS). While acute inflammation may have protective roles, chronic neuroinflammation contributes to sustained neuronal damage and accelerates disease pathology [32].

Collectively, Alzheimer's disease is a multifactorial disorder involving interconnected pathological processes. The amyloid deposition, tau pathology, excitotoxicity, neuroinflammation, and neurotransmitter deficits forms the common mechanisms that explains AD progression. Among these, NMDA receptor-mediated excitotoxicity serves as a critical link between upstream molecular abnormalities and downstream neuronal degeneration. Therefore, targeting NMDA receptors and modulating glutamatergic signaling represents a promising therapeutic strategy, particularly in combination with approaches aimed at reducing amyloid burden and tau pathology.

2.2 NMDA receptor and Alzheimer's Disease

NMDA receptors are ionotropic glutamate receptors mainly involved in synaptic plasticity and synaptic transmission, which underlie molecular mechanisms of memory formation and learning [33]. Glutamate an excitatory neurotansmitter, released from presynaptic neurons activates NMDA receptors present on the postsynaptic membrane. This activation removes the Mg^{2+} block from NMDARs, allowing the intracelluar Ca^{2+} (acting as secondary messenger) influx into the postsynaptic neuron, which plays a key role in neurotransmission, long-term potentiation (LTP), long-term depression (LTD), and overall synaptic plasticity [34]. In addition to their essential role in synaptic transmission and plasticity, excessive glutamatergic signalling causes overactivation of NMDA receptors (NMDARs) that results in excessive Ca^{2+} influx, which can damage neuronal cells and ultimately lead to neuronal death and synaptic dysfunction, as observed in Alzheimer's disease (AD).

Structurally, the NMDA receptors are heterotetrameric assemblies containing two obligatory GluN1 subunits combined with two modulatory GluN2 subunits [35]. The GluN1 subunits are encoded by the GRIN1 gene and bind to glycine or D-serine, while the GluN2 subunits are encoded by the GRIN2 gene and bind to glutamate. The GluN2A and GluN2B subunits offers 70% sequence similarity yet involved in different physiological and pathological functions. The distribution is also highly specific, while the synaptic NMDARs are often enriched in GluN2A, the extrasynaptic NMDARs frequently contain GluN2B subunits [36] [37]. NMDAR containing GluN2A are typically engaged in signalling cascades that support synaptic plasticity, neuronal survival, and adaptive gene expression, contributing to LTP. Conversely, the GluN2B containing receptor are more strongly associated with mitochondrial impairment, oxidative stress, suppression of pro-survival transcriptional programs and eventually the neuronal death, on their sustained activation [38] [39].

Proper regulation of NMDA receptor (NMDAR) activity is critical for maintaining neuronal survival and function. While the reduced synaptic NMDAR signalling can impair neuronal viability, the excessive glutamatergic stimulation induces excitotoxicity, a process that leads to neuronal injury or death as observed in AD. The prolonged activation of NMDA receptors leads to abnormal intracellular calcium accumulation, that results in mitochondrial dysfunction, ROS production, gradual synaptic loss and neuronal death, causing progressive decline in cognition. Central to Alzheimer's Disease, the Aβ disrupts the glutamate recycling and uptake pathway, leading to sustained NMDAR activation, which in turn causes synaptic dysfunction. Amyloid-β (Aβ) reduces synaptic NMDAR activity, thereby impairing synaptic plasticity [40]. At the same time, it preferentially activates extrasynaptic GluN2B NMDARs, promoting excitotoxic signalling that leads to neuronal damage and cell death, contributing to long-term depression (LTD), which indicates signs of neurodegeneration [41] [42]. In Alzheimer's disease (AD), extrasynaptic NMDA receptor activation suppresses CREB signalling, a master regulator of synaptic plasticity, thereby

impairing long-term potentiation (LTP). Concurrently, it activates FOXO-dependent pathways, contributing to excitotoxic neuronal cell

death. Aβ activates NMDA receptors, causing excessive Ca^{2+} influx that upregulates β-secretase expression, thereby increasing Aβ production and establishing a self-perpetuating pathogenic feedback loop [43].

2.3 Current Pharmacological Landscape of NMDA Receptor Antagonists

The excitotoxicity caused by the NMDA receptor has emerged as the pathological hallmark for neurodegenerative diseases, and several antagonists have been developed targeting the NMDA receptor as therapeutic agents to treat neurodegenerative diseases. Depending on the site of interaction (binding sites) and mechanism of action, these antagonists can be classified into competitive, non-competitive, and allosteric antagonists.

2.3.1 Competitive antagonists of the NMDA receptor

Mechanism of action: These antagonists bind to the Glutamate or Glycine binding site on GluN1 or GluN2 subunits, respectively, and compete with endogenous ligands (neurotransmitter), i.e., glutamate or glycine, thereby blocking the channel opening [44].
Examples of competitive antagonists include compounds like D-CPP/D-CPPene (Midafotel), which binds to the GluN2A subunit; D-AP5/D-AP7 (non-subunit-selective); DCKA, which binds to the GluN1 subunit; Selfotel (GluN2A selective); L689-560 & L701-324 (GluN1 selective); PPDA (GluN2A,C&D subunits selective); NVP-AAMO77 (GluN2A,C&B); SDZ-220-040 (GluN2B selective) and CGP derivatives, which are specific to GluN2A subunits.
Strengths: These functional competitive antagonists can attenuate the glutamate-mediated excitotoxicity and display anti-ischemic, anticonvulsant, antidepressant-like, and anxiolytic-like properties.
Limitations: Many competitive antagonists permeate poorly across the BBB, limiting their CNS exposure. They have also been reported to cause unfavourable side effects like hallucination, agitation, confusion, paranoia, delirium, drowsiness and coma, which leads to their termination in clinical trials [45].

2.3.2 Uncompetitive or Non-Competitive Antagonists of NMDA Receptor

Mechanism of action: These antagonists are also referred to as channel blockers, and occupies the PCP binding region at the channel entrance only when the channel is open. This binding blocks ion flow through the channel, thereby inhibiting calcium influx and suppressing calcium-mediated responses [46].
Examples of uncompetitive or non-competitive antagonists include MK-801, Memantine, Amantadine, PCP, Ketamine and Tiletamine. These compounds act as an open-channel blocker. And out of these compounds, Memantine is approved for the treatment Alzheimer's disease (AD), Amantadine for Parkinson Disease (PD) treatment, Ketamine as an anaesthetic agent and Tiletamine is approved for veterinary use.
Strengths: These compounds have displayed neuroprotective effects in conditions like stroke, cardiac arrest, and neurodegenerative diseases. Also, they possess anti-convulsant, antidepressant properties and anaesthetic effects.
Limitations: Despite having numerous neuroprotective effects, they are reported to induce adverse effects like neuropsychological, psychotomimetic, dopaminergic transmission effects, hallucinations, and schizophrenia-like symptoms, as well.
MK-801, being channel blocker cause the complete inhibition of NMDA receptor, affecting the normal functions also [47].

2.3.3 Allosteric Antagonists of NMDA Receptor

The allosteric antagonists can be categorized as positive and negative antagonists, and both bind to the amino-terminal domain (ATD) of the NMDA receptor channel. The positive antagonists potentiate the Ca2+ responses, while the negative antagonists inhibit the Ca2+-mediated responses.
Mechanism of action: The negative allosteric NMDA antagonists inhibit receptor function by binding to a regulatory site and inducing conformational changes that reduce channel opening and calcium influx [48].
Examples of negative allosteric modulators include Ifenprodil, that selectively GluN2B subunit and interacts at the interface between the GluN1 and GluN2B ATD. Another example is Radiprodil, which also binds to the GluN2B subunit; Ro25-6981 binds to the GluN2B subunit, and DQP-1105 binds to GluN2C & GluN2D [49].
Strengths: The negative allosteric antagonist, when it binds to GluN2B subunits, offers neuroprotective effects by reducing the excitotoxicity. They are found to offer anticonvulsant, antidepressant and neuroprotective effects against glutamate-induced excitotoxicity [50].
Limitations:However, the side effects include impaired cognitive behavioural tasks, vomiting, pyrexia, reduced memory in early life stress mice and motor dysfunction. Allosteric inhibitor like Ifenprodil and radiprodil displays poor BBB permeability and less bioavailability. The other GluN2B anatagonists shows cross-reactivity with another neurotransmitter receptors causing off-target effect [51].

Despite so much development in the NMDA receptor antagonists, there lies a need for the development of the new antagonist that offers maximum therapeutic action with minimal to no negative side effects. NMDA receptors are structurally complex, their hypofunction and hyperfunction both are detrimental for the neurons. Antagonists targeting the negative effects often suppress the positive effects, causing their failure in clinical trails. The competitive antagonists, competing with endogenous neurotransmitter, display strong binding affinity causing complete metamodulation of NMDA receptor to other neurotransmitter. Several antagonist show side-effects like psychotomimetic, dopaminergic transmission, or schizophrenia-like symptoms. Clinically tolerated NMDA receptor antagonists, Amantadine and Memantine offers only symptomatic relief and not without side-effects. For several antagonist selectivity becomes the challenge, causing complete receptor blockade. The GluN2B selective antagonists are less bioavailable and permeate poorly through blood-brain barrier (BBB), limiting their use for neurodegenrative disease treatment. The development of the new antagonists should address these challenges, increasing their chances of successful clinical trails and ultimate their use in treatment of neurodegenrative disease.

2.4 Drug Discovery approaches for neurodegenerative diseases

Drug discovery for neurodegenerative diseases offers significant challenges as the NDDs are multifactorial and complex in nature. There lie multiple mechanisms for their development, like the tau protein aggregation, oxidative damage, mitochondrial dysfunction, neuroinflammation and excitotoxicity. Another major factor that affects the drug development for neurodegenerative diseases is the BBB permeability. The BBB restricts the movement of 98% small molecules entering the CNS, or even if they cross the CNS, the drug concentration will be so small that many drugs become ineffective in treating NDDs. The preclinical period of neurodegenrative diseases is usually very long; it takes a huge amount of time to get translated into symptoms, and during that period, irreversible damage has already taken place, and by that time, the effectiveness of the drugs decreases in later stages. The heterogeneous patient population is another hurdle for drug development; the variability in disease manifestation becomes a challenge in outcome assessment [52] [53].

The drug discovery for neurodegenerative diseases consists of many approaches that have revolutionised the treatment of NDDs.

2.4.1 Traditional approach of drug discovery

Traditional drug discovery approaches primarily include molecular target-based approch and phenotypic screening strategies, which have historically guided drug development.

The molecular target-based approach starts with target identification and validation. The target can be a protein or enzyme responsible for disease manifestation identified through basic research. Various biochemical assays are developed along with high throughput screening (HTS) of chemical libraries to get the hits against target. Usually the top three hits serve as lead compounds, confirmed via biochemical assays, which subsequently undergo chemical optimisation through structure activity relationship (SAR) to enhance properties such as ADME, pharmacokinetics, and pharmacodynamics. After the assessment, the lead compounds undergo preclinical drug development, toxicology studies and clinical trials [54].

In phenotypic screening, characteristics associated with the diseases are utilised for the identification of the lead compounds that ameliorate the disease phenotype through various cell-based assays, without even knowing the underlying molecular mechanism or the target protein. The identified compounds are active against the whole cell, offering potential for the treatment of multiple diseases. Cell-based assays utilise intact cells, giving more physiological relevance. Many drugs have been approved using phenotypic screening approach, and it took a long time to determine their exact mechanism [55].

2.4.2 Computational approach of drug discovery

Computational approaches offer a faster and more efficient strategy for drug discovery, enabling the rapid screening and analysis of numerous compounds for their therapeutic potential and reducing the chances of failure in clinical trials. Compared to traditional methods, these approaches significantly reduce both preclinical development time and associated costs [56].

A) Ligand-based approach

This approach utilises the 3-D structure of the ligand in the absence or unavailability of target protein information. The key features responsible for the biological activity of the reference drug molecules are identified to incorporate into new drug candidates. The phenomenon that molecules with similar structures exhibit similar properties forms the underlying principle of this approach, supporting the discovery of new ligands. Based on strcutcural insights of the reference ligand, a large similarity data gets generated that undergo clustering for initial SAR generation, and based on similarity, potential new molecules are identified. The ligand-based approach comprises pharmacophore modelling and QSAR modelling.

Pharmacophore modelling: It is used to identify the essential chemical features and their three dimensional spatial arrangements required for a molecule to interact with a biological target. The information derived from structural characteristics of the protein active site, electronic properties, the conformational features of the inhibitors, substrates or metabolites, is utilized to predict the interaction between ligand and the target. The derived information is then subsequently utilised to design the pharmacophore covering all the structure and property of the ligand in 3-D space and stimulate the chemical and spatial properties of binding sites [57] [58].

QSAR modelling: It correlates the chemical structure of compounds with their biological activity using mathematical models. It predicts the biological activity of new untested compounds based on

chemically relevant descriptors like structural count, steric properties, polar surface area, hydrophobicity and molecular properties. The QSAR modelling acts as a replacement for animal testing or supplements the experimental data and prioritises chemicals for such experiments [59].

B) Structure-based approach

This utilises the three-dimensional structure of a biological target protein to design and optimise compounds with high binding affinity and specificity. It relies on detailed knowledge of the target protein's binding site, obtained from techniques like X-ray crystallography, NMR spectroscopy, or cryo-EM. It uses homology modelling, threading or ab initio modelling to predict the 3-D structure. The homology modelling uses the structure of a known protein (acts as template) with >40% sequence similarity to determine the 3-D structure of unknown protein. Structure based drug discovery (SBDD) approach employs techniques like molecular docking or virtual screening to predict ligands that bind to the target's active site [61] [62].

Molecular Docking: This technique analyses the interaction between proteins and small molecules, or protein-protein interaction, to evaluate their binding energies. The interaction between two entities is a function of their shape matching and energy matching, forming underlying principal of molecular docking [63].

Virtual Screening: It is a cost-effective and time-efficient technique for lead compound identification in drug discovery. It enables the rapid screening of large chemical libraries against biological targets and serves as a form of virtual high-throughput screening (vHTS). In contrast to HTS, which involves time-consuming biochemical evaluation of millions of compounds, virtual screening significantly reduces experimental effort, cost, and time. This approach facilitates the efficient identification of biologically active molecules from large datasets [63].

2.4.3 AI & ML in drug discovery

AI has revolutionised drug discovery by accelerating large-scale data analysis efficiently to identify novel therapeutic targets. AI performs datasets analysis for targeting identification and uses pre-trained models to identify the lead compounds. It uses correlations, pattern identification and analysis for predicting the 3-D structure of proteins, protein-protein interactions, and drug activity. AI uses techniques like herurstics, support vector machine (SVM), artificial neural networks, Markov decision and natural language processing for predictions [64].
ML is a branch of AI that utilises datasets for the learning process with progressive accuracy. The ML models, after training, make predictions on similar datasets. ML models are employed for decision-making on pharmaceutical data, QSAR analysis, hit discoveries, and de novo drug design for more accurate results [65].

2.5 Molecular Docking in Drug Discovery

Molecular docking is an SBDD approach that predicts ligand-protein or protein-protein interaction, based on the generated binding mode and affinity between the two. Molecular docking can be employed to carry out many tasks like virtual screening, target fishing, polypharmacology, drug side effects and drug repurposing. Many drugs have been developed using molecular docking, which has revolutionised drug discovery [66].
There are several programs to perform molecular docking that are mainly based on molecular mechanics, using classical physics to represent molecular systems. They rely on force fields, mathematical models that describe how molecules interact, using parameters from experimental data, which are further refined with quantum mechanical methods. These models consider key factors such as energy, bond geometry, molecular flexibility, electrostatic interactions, and the Lennard-Jones potential.

Types of Molecular Docking

a) Rigid Docking: In this type of docking, the structure of the macromolecule does not change or remains fixed, assuming the lock-and-key type of interactions. It produces results fast, is computationally inexpensive, but less accurate. This is employed to study protein-protein and protein-nucleic acid interactions.

b) Semi-flexible docking: In this type of docking, the protein remains fixed, but the ligand is flexible, leading to conformational changes in a certain range, following the induced-fit model. These are used to study protein interactions with small molecules.

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c) Full-flexible docking: In this type of docking, both the protein and the ligand conformations are flexible, allowing the protein re-arrangements, which increases the search space, employing more computing resources, and the process becomes relatively slow [67].

Tools for Molecular Docking

There are various tools, developed by various research institutes, employing different algorithms to carry out docking.

AutoDock and AutoDock Vina: Both the software are open-source and developed by The Scripps Research Institute. AutoDock utilises the Lamarckian Genetic Algorithm, while AutoDock Vina uses the Genetic Algorithm to carry out rigid body-flexible docking. Both are used in conjunction with AutoDock Tools to prepare the molecules [68].

GOLD: It is a commercial software program that uses Genetic Algorithms for docking. It offers flexible docking and multiple scoring functions: GoldScore, ChemScore, ASP and ChemPLP. The evaluation of its accuracy and reliability appeared to give good results [69].

Glide: Developed by Schr¨odinger, it is an exhaustive search-based docking program that is commercial. The ligands are kept flexible during docking. It improves efficiency by restricting the search to a predefined binding region, rather than the entire protein surface and offers efficient virtual screening via SP mode, with XP used for higher-accuracy refinement of top candidates [70].

FlexX: Utilises Incremental Construction search algorithms and is a commercial software program developed by BioSolveIT. It performs rigid body-flexible docking and can be utilised for virtual screening purposes [71].

Swiss Dock: It was developed by the Swiss Institute of Bioinformatics and is a predictive web service for protein-small molecule ligand interactions based on the EADock DSS algorithm.

PyRx: It is an open-source virtual screening tool that integrates AutoDock and AutoDock Vina, enabling the efficient screening of large ligand libraries along with basic preprocessing steps.

Molecular Docking Pipeline :

Molecular docking comprises of various steps:

Protein Preparation: It involves obtaining the target protein's 3-D structure from PDB, and then it is prepared by removing co-crystallised ligands, water molecules, and other heteroatoms with the assignment of hydrogen atoms and appropriate charges. Energy minimisation may also be performed to relieve steric clashes and optimise the geometry of the protein for accurate interaction studies.

Ligand Preparation: The ligands are sketched or retrieved from databases like PubChem, ZINC, DrugBank, etc., and their 3-D structures are downloaded. These ligands are prepared by adding hydrogen atoms, assigning partial charges and optimising geometry through energy minimisation. Rotatable bonds are assigned to permit structural flexibilty during docking. Ligands are then saved in formats compatible with docking software.

Binding site Identification: The binding site or active pocket of the protein is identified using experimental data, literature reports, or computational prediction tools. In many cases, the position of a co-crystallised ligand is used to define the active site, or blind docking may be performed in the absence of any cue about the binding site.

Grid box generation: A grid box is generated around the identified binding site to define the search space for docking, restricting the docking calculations to a specific region, thereby reducing computational time and improving efficiency In the case of blind docking, the entire protein is kept under the grid box.

Docking (pose generation): During docking, the ligand is positioned within the binding site in multiple orientations and conformations (poses), and the algorithm explores different spatial arrangements to evaluate how well the ligand fits within the binding pocket.

Scoring and Ranking: Scoring function is applied to every generated pose to predict ligand-target binding strength, and ranks these poses based on their binding energy, considering various interactions such as H-bonding, hydrophobic interactions, electrostatics, and van der Waals forces.

Post-Docking Analysis: The top-ranked poses of protein-ligand interactions are visualised to identify the key binding residues and interactions involved. Software like Biovia Discovery Studios, UCSF Chimera, Pymol, etc., is used for the visualisation [72].

Scoring Functions:

Scoring functions are mathematical models used in molecular docking to predict and rank ligand–protein binding affinities based on physicochemical interaction energies.

A) Force field (FF) scoring functions: It calculates binding energy through individual interactions like van der Waals, electrostatic, and bonded interactions. It is based on classical molecular mechanics equations and is physically realistic but computationally extensive.

B) Empirical Scoring Functions: It calculates binding affinity as a weighted sum of interaction terms, including van der Waals, electrostatic, hydrogen bonding, desolvation, entropy, and hydrophobic contributions. This is faster and widely used for virtual screening.

C) Knowledge-based scoring functions; These are bases on statistical assessment of experimentally validated protein–ligand complexes and are based on the Potential of Mean Force using the inverse Boltzmann relationship.

D) Consensus scoring functions: Consensus scoring combines multiple scoring functions to evaluate and rank ligand–protein binding more reliably. By integrating different methods, it reduces individual biases and improves the accuracy of docking predictions [73].

Docking Protocol Validation

Molecular docking requires validation to ensure reliability, and this is typically performed by redocking a reference ligand into the target binding site and comparing parameters such as RMSD (root mean square deviation), binding pose, binding affinity, and consistency with previously reported data. Following this, cross-docking may be performed by docking ligands into different conformations of the same protein to test the protocol's robustness against receptor flexibility. Finally, to assess the "enrichment" capability, a virtual screening validation is conducted using a library of known active compounds mixed with "decoys" (inactive molecules); metrics like the Area Under the Curve (AUC) of a Receiver Operating Characteristic (ROC) plot are then used to determine if the protocol can effectively distinguish true binders from non-binders. For complex systems, molecular dynamics simulations are recommended to refine the protein structure, incorporate flexibility, and improve the stability of the docked complex.

Challenges in molecular docking

Molecular docking witnesses several challenges, these are:

Target properties: Many docking programs consider the target protein as a rigid structure, but in real conditions, depending on the extrinsic or intrinsic factors, the structure can fluctuate. Thus, docking programs can give inaccurate results.

Search and Scoring Problems: Docking is complex due to multiple possible ligand–receptor orientations in 3D space. Although algorithms generate and rank these poses using scoring functions, :but scoring functions used in docking are simplified models that may not reliably estimate true binding affinities, often resulting in incorrect ranking of ligands.

Solvent molecules: The solvent molecules often play a crucial role in ligand-receptor interactions, which are ignored while preparing the receptor for docking, generating the false results.

2.6 RESEARCH GAP AND STUDY RATIONALE

The NMDAR dysregulation has been critically linked with the development of AD. Therefore, targeting NMDAR offers a therapeutic target for AD treatment; many antagonists/inhibitors have been developed that target NMDAR, but there remains a significant gap in the development of effective and disease-modifying antagonists for Alzheimer's disease. Several critical limitations restrict their clinical effectiveness. The limitations may include:

Insufficient subunit-selective targeting: NMDA receptors are heteromeric complexes composed of multiple subunits (GluN1, GluN2A–D, GluN3), each with distinct physiological and pathological roles. Current inhibitors largely lack selectivity for specific subunits, leading to off-target effects and reduced therapeutic precision.

Pharmacokinetic challenges: Many inhibitors fail to cross the Blood-Brain Barrier (BBB) effectively due to their hydrophilic nature. Issues such as suboptimal BBB permeability, poor bioavailability, and inadequate receptor binding dynamics can reduce drug efficacy.

Limited efficacy: Memantine, an approved inhibitor (antagonist), has demonstrated benefits predominantly in moderate-to-severe stages of AD, with limited or negligible effects in early-stage disease. Furthermore, patient responses are often variable, indicating heterogeneity in therapeutic outcomes. This restricts the broader applicability of NMDA-targeted therapies.

Translational failures: Many candidates succeed preclinically on animal models, but fail clinically during human trials due to inadequate brain penetration, short duration against chronic excitotoxicity or unexpected toxicity leading to high AD trial failure rates.

Adverse effects and safety concerns: Several antagonists show undesirable adverse effects, such as psychotomimetic, dopaminergic transmission, or schizophrenia-like effects. They interfere with normal NMDA receptor roles in synaptic plasticity, learning, and memory to cause cognitive impairment, dizziness and neuropsychiatric issues.

Poor Selectivity and Disruption of Physiological Function: Many non-selective antagonists fail to specifically block extrasynaptic NMDAR, having implications in disease progression, while sparing synaptic NMDAR having role in pro-survival signalling. Complete blockade of NMDA receptor activity is associated with severe adverse effects, including cognitive impairment and neuropsychiatric symptoms.

The above-stated research gaps highlight the urgent need to develop novel NMDA receptor antagonists with improved selectivity, enhanced safety profiles, and superior therapeutic efficacy. In response, the present study is designed to explore and identify potential NMDA receptor antagonists using a computational drug discovery approach where molecular docking is employed to evaluate ligand–receptor interactions and their binding affinities, enabling the prioritization of compounds with superior binding scores. In addition, pharmacokinetic properties, including blood–brain barrier (BBB) permeability and ADME parameters, are assessed to ensure the drug-likeness and therapeutic viability of the selected candidates.

Furthermore, detailed interaction analysis and visualisation are performed to elucidate the molecular basis of ligand binding and receptor modulation. This integrated in-silico framework enables the discovery of candidates exhibiting the superior binding affinity, structural stability, and selective modulation of pathological NMDA receptor activity. Computational drug discovery strategies offer an efficient alternative in terms of cost and time to the conventional experimental screening by facilitating the high-throughput screening of extensive chemical libraries. By reducing the reliance on resource-intensive wet-lab procedures, this approach minimises the risk of late-stage failure in clinical trials and accelerates the identification of promising lead compounds. Therefore, the present study provides a rational and systematic foundation for future experimental validation and the development of more effective therapeutic agents for Alzheimer's disease.

3) METHODOLOGY

3.1. Protein structure selection and preparation:

The crystal structure of NMDA receptor in complex with Ifenprodil, having GluN1-GluN2B subunit (PDB ID: 5EWJ) was sourced from PDB database (https://www.rcsb.org). This structure was selected due to its suitable resolution of 2.77 Å. The structure was inspected and processed using Pymol to remove bound ligand Ifenprodil. Further processing of the protein was carried out using BIOVIA discovery studio 2025 including, elimination of water molecules, addition of polar hydrogen, and then prepared structure was saved for docking stimulation.

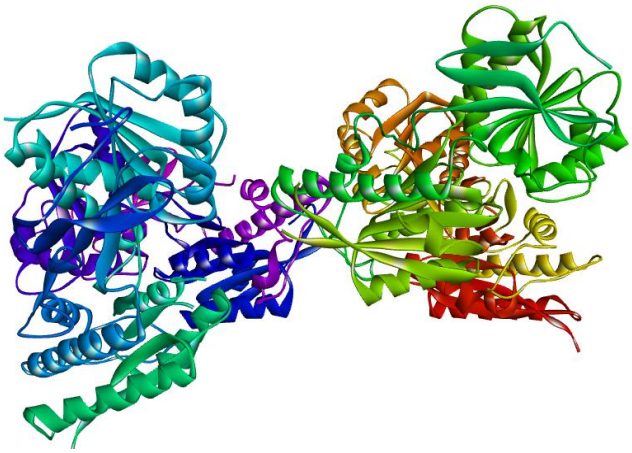


Fig 1: 2-D structure of NMDA receptor

3.2. Ligand Selection and preparation:

Reference ligand Ifenprodil, a well-established inhibitor of NMDA receptor, was chosen as the reference ligand for this study. PubChem database (https://pubchem.ncbi.nlm.nih.gov/) was employed to download the 3-D structure of Ifenprodil with CID:3689, in SDF file format.

Similarity-Based Search: To identify the possible ligands, capable of interacting with the protein, PubChem similarity search was performed using Ifenprodil as query molecule, which yielded a total of 607 compounds with 90 percent structural similarity to the reference ligand. Further refinement was carried out was by applying various filters like molecular mass, heavy atom count, rotatable bonds, polar surface area, which lead to selection of 73 compounds and these were downloaded in SDF format for further processing.

Ligand preparation: All the ligands were prepared for docking using the Open Babel option in PyRx software package where energy minimization steps were done for the ligands. Open Babel is an open source chemoinformatics software package that allows the file conversion into different formats.

3.3. Molecular docking studies

PyRx software package was employed to perform the molecular docking of prepared ligands with the target protein to investigate the interaction between the ligands and protein. A config file was made containing the grid box dimensions and the centroids. A grid box of dimensions 21.27x38.22x25.00 was generated on the protein and centroids center_x= 85.610; center_y= 6.402, center_z= 31.912 were used to carry out the docking studies.

3.4. Interaction Visulisation:

For visualizing the interaction between protein and the ligand, Biovia Discovery Studio Client 2025 was employed. 2-D interactions maps were generated revealing key interactions and residues involved.

3.5. ADME Analysis:

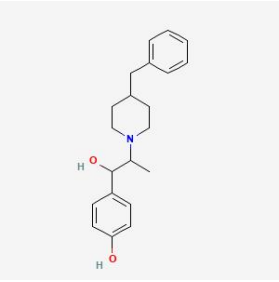
The pharmacokinetic behavior of the top hits was assessed through ADME profiling by using Swiss ADME, to examine their absorption, distribution, metabolism, and excretion characteristics and drug-likeness. Swiss ADME (https://www.swissadme.ch)is an openly assessable web based server, used to carry out the analysis. It was performed to prioritize molecules with favorable bioavailability, BBB permeation, and CNS exposure while eliminating candidates with poor pharmacokinetic profiles.

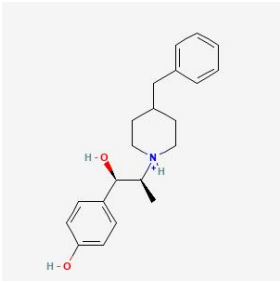
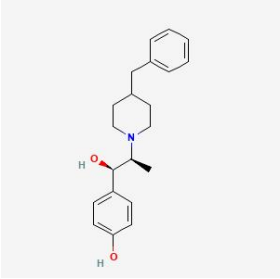
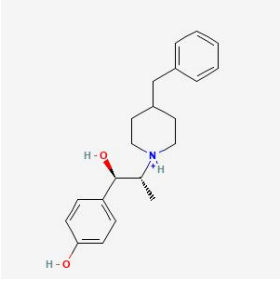
4) . RESULTS AND DISCUSSION

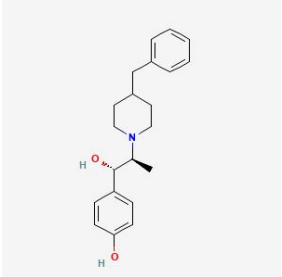
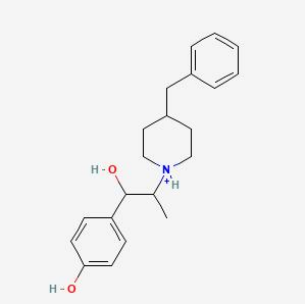
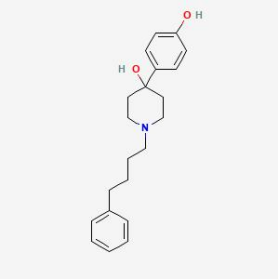
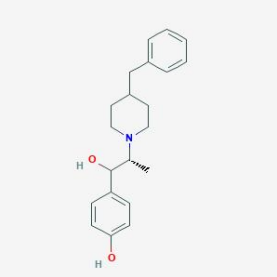
4.1. Shortlisting of Top hits

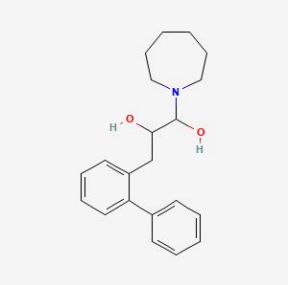
The docking of reference ligand into the active site yielded the binding energy of -10.2 kcal/mol. The molecular docking analysis of selecetd 73 compounds lead to identification of , the top eight hits that were shortlisted, possessing the higher docking scores than the reference molecule Ifenprodil. The binding energy of these top hits, ranges between -11.1 to -10.6 kcal/mol whereas the reference compound possesses the binding energy -10.2 kcal/mol. The compound with CID 25271983 showed the best binding affinity of -11.1 kcal/mol. These compounds exhibited better docking scores suggesting their potential for improved binding affinity towards target protein.

Table I: Tabular representation of compounds, PubChem CID, binding energy, 2-D diagrams and interacting residues

Compound	PubChem CID	Binding energy (kcal/mol)	2-D Diagrams	Interacting Residues
1	3689 (reference)	-10.2		Glu236,Leu135,Phe 176, Arg115,Tyr109, Pro78, Ile111, Ala75, Phe114

2	25271983	-11.1		Arg115, Leu135, Phe176, Ser132, Gln110, pro78, Ile111, Phe114
3	6604117	-10.8		Glu236, Phe176, Leu135, Ala107, Phe114, Pro78, Ile111
4	25271982	-10.8		Gln110, Pro177, Arg115, Leu135, Ser132, Ile133, Ile111, Phe114, Pro78

5	11771731	-10.8		Phe176, Leu135, Ala107, Pro78, Ile111 Phe114
6	74441145	-10.8		Gln110, Pro177, Arg115, Glu236, Leu135, Ser132, Ile133, Ile111, Pro78
7	10592168	-10.6		Pro78, Ile111, Tyr109, Phe114, Glu236, Thr174, Leu135, Phe176, Gln110
8	142065266	-10.6		Ile133,Leu 135 Ser132, Arg115, Tyr109, Ala107, Phe114, Pro78, Ile111,

9	54144945	-10.6		Tyr109, Gln110, Ile133, Ile111, Ser132, Phe176, Leu135

4.2 Visualization of top hits:

The BIOVIA discovery studio 2025 client was utilized to examine and visualize the receptor protein-ligands interactions. The generated 2-D interaction maps reveal the key hydrogen bonds, pi-pi and hydrophobic interactions, and the associated amino acid residues involved in protein-ligand interaction. Leu 135 and Ile 111 were found be consistently interacting residues. Fig 2 is the interaction map of Ifenprodil with the NMDA receptor. Fig 3 represents the interaction of compound 2 with NMDA receptor, fig 4 is the interaction map of compound 3, fig 5 is for compound 4, fig. 6 displays compound 5, fig 7 is the interaction map of compound 6, fig 8 shows interaction of compound 7, fig 9 displays interaction of compound 8 and fig 10 is the interaction map of compound 9 , each interacting with the NMDA receptor.

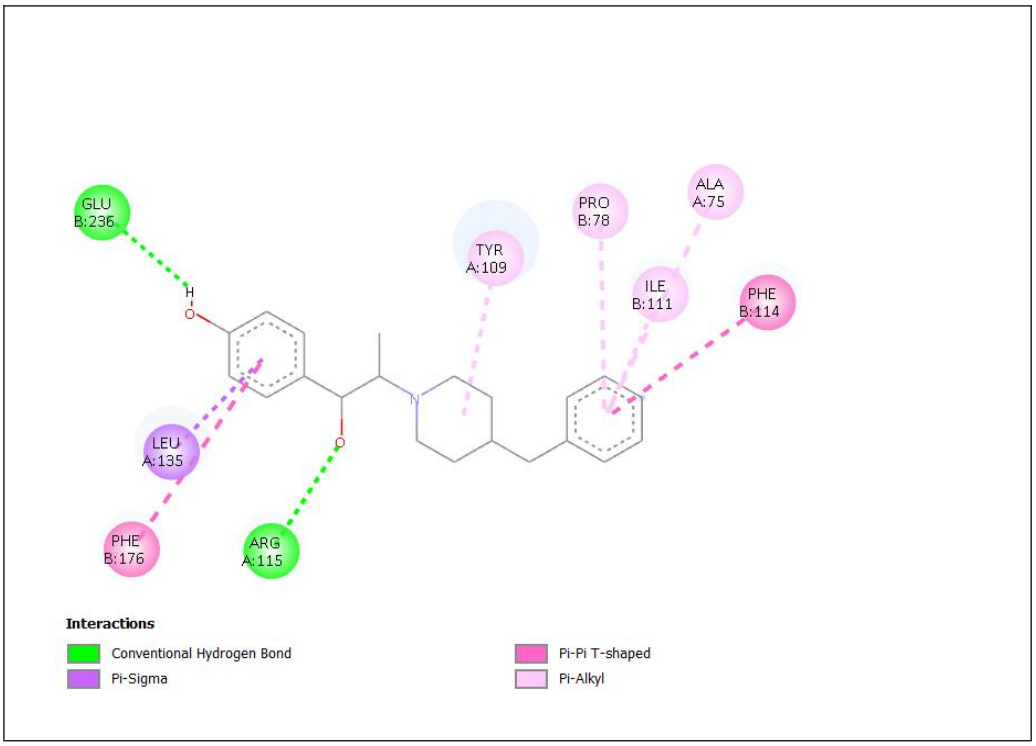


Fig 2: Interaction map of Ifenprodil (reference)

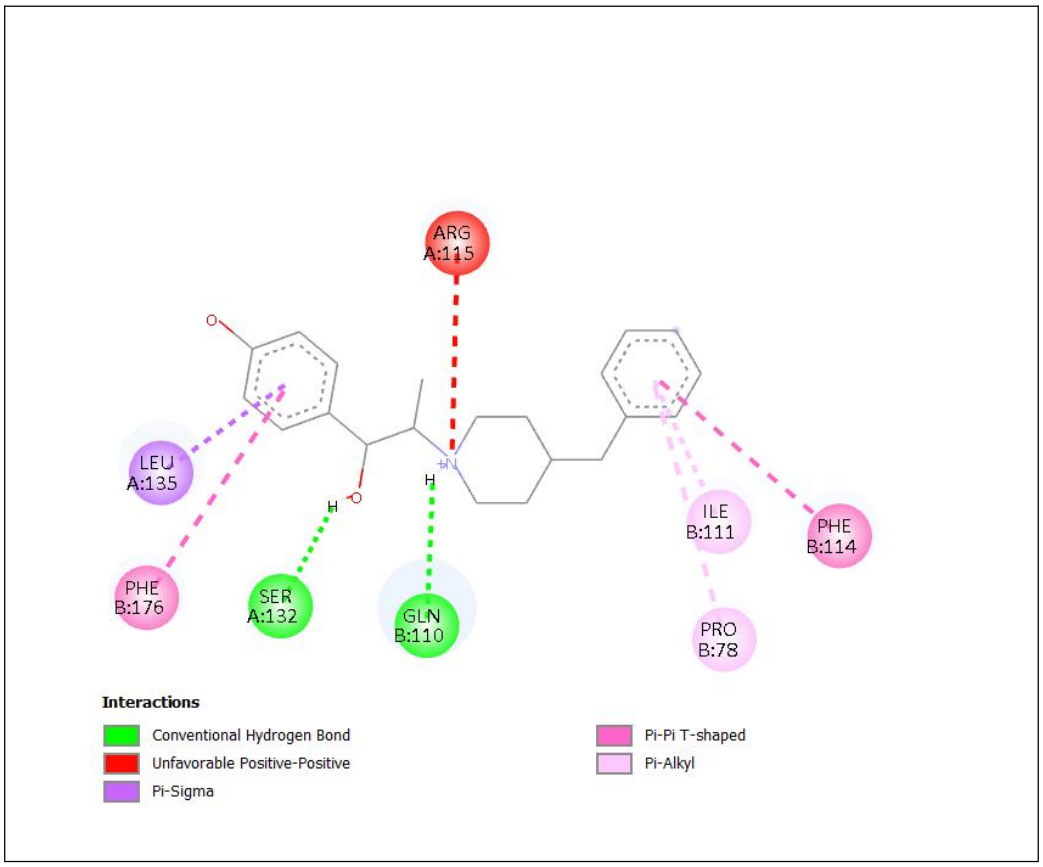


Fig 3: Interaction map of compound 2 (CID 25271983)

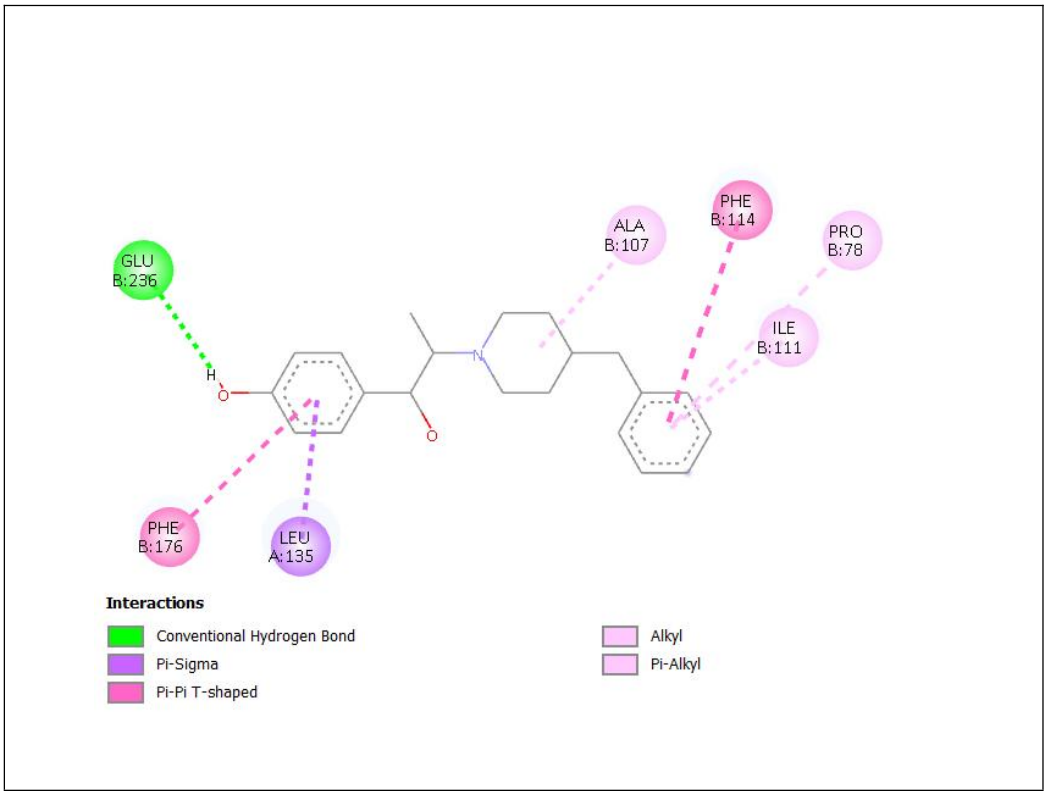


Fig 4 : Interaction map of Compound 3 (CID 6604117)

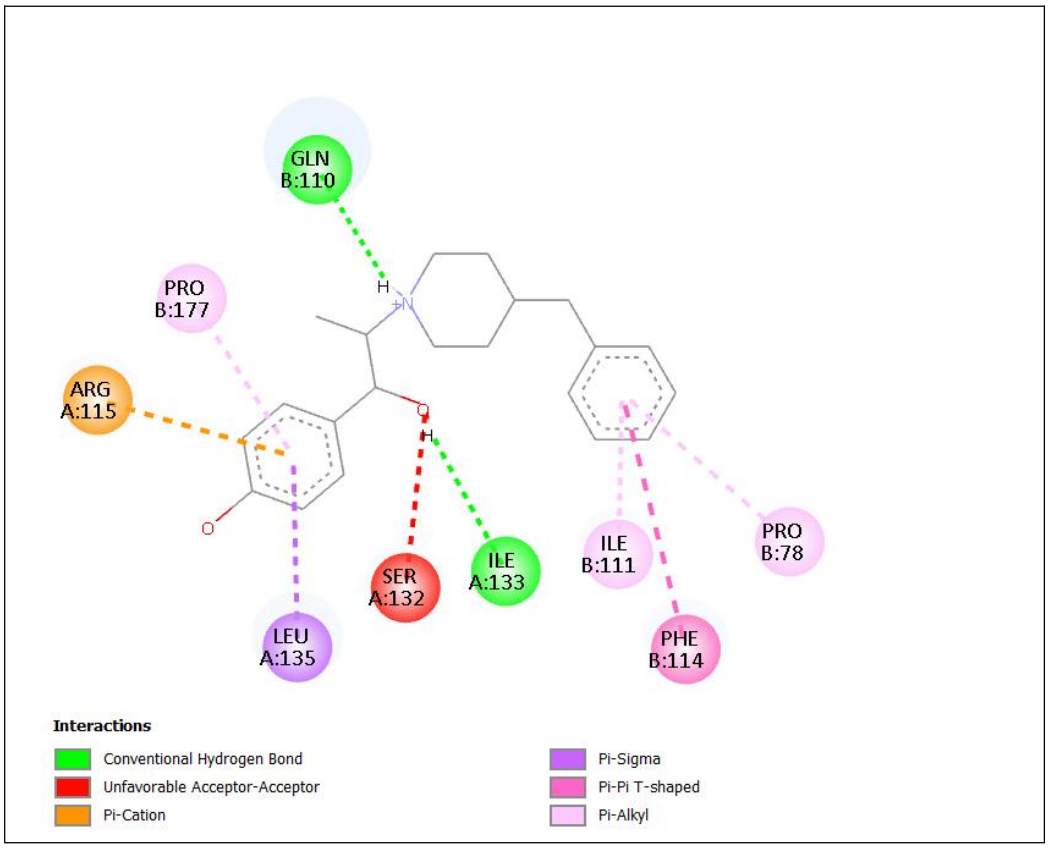


Fig 5 : Interaction map of Compound 4 (CID 25271982)

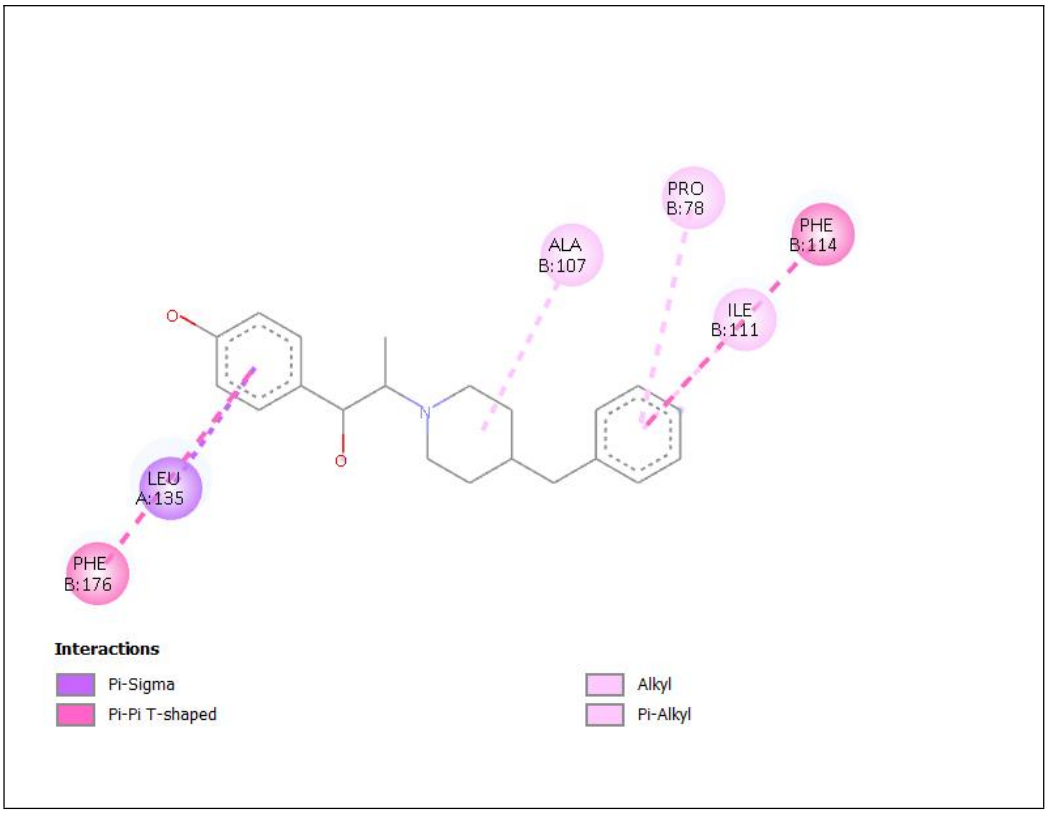


Fig 6 : Interaction map of Compound 5 (CID 11771731)

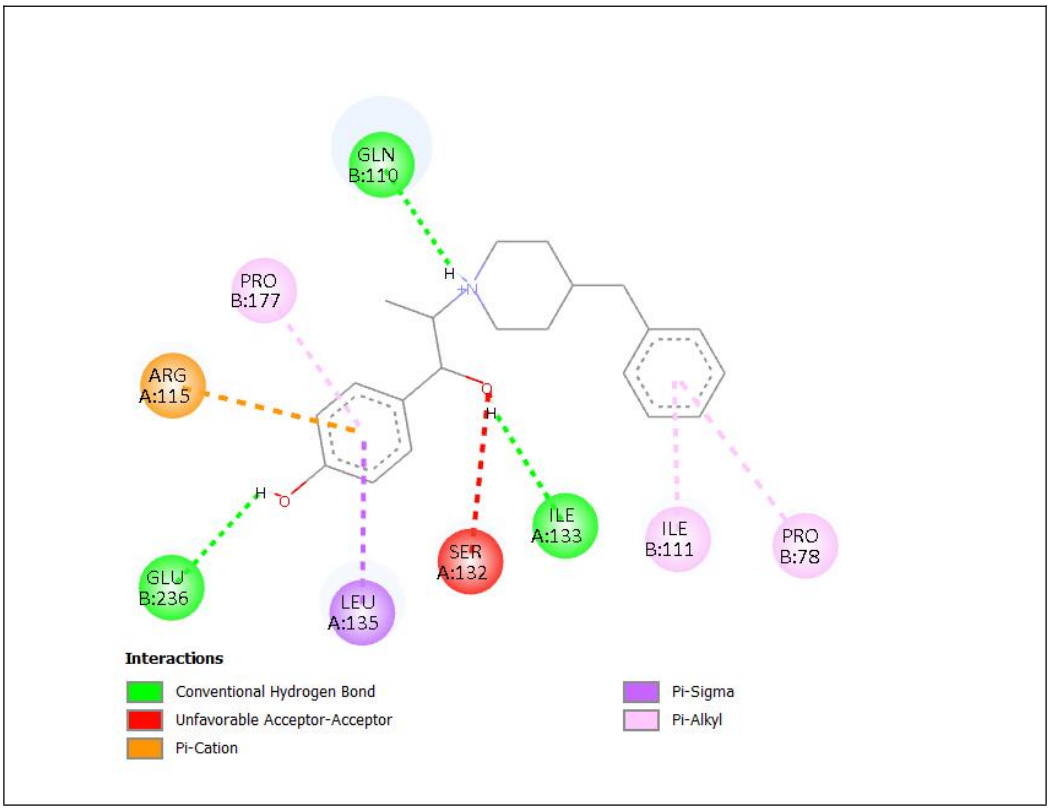


Fig 7 : Interaction map of Compound 6 (CID 74441145)

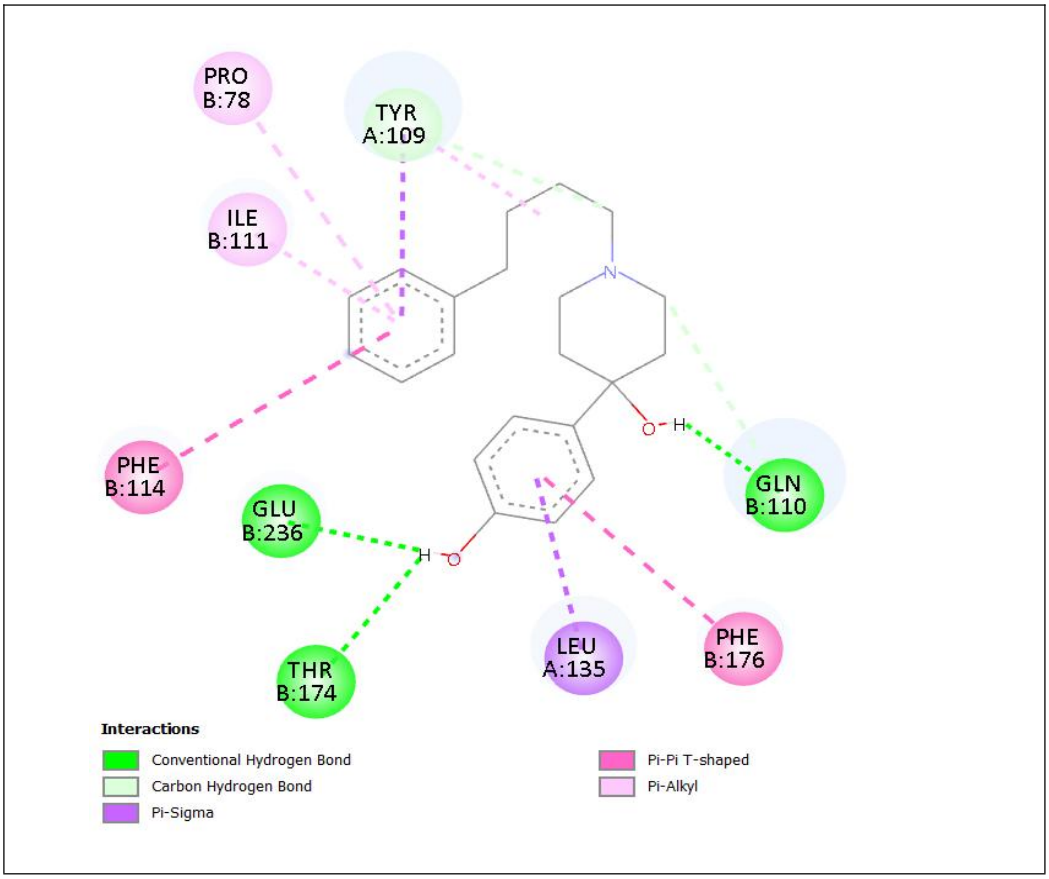


Fig 8: Interaction map of compound 7 (CID 10592168)

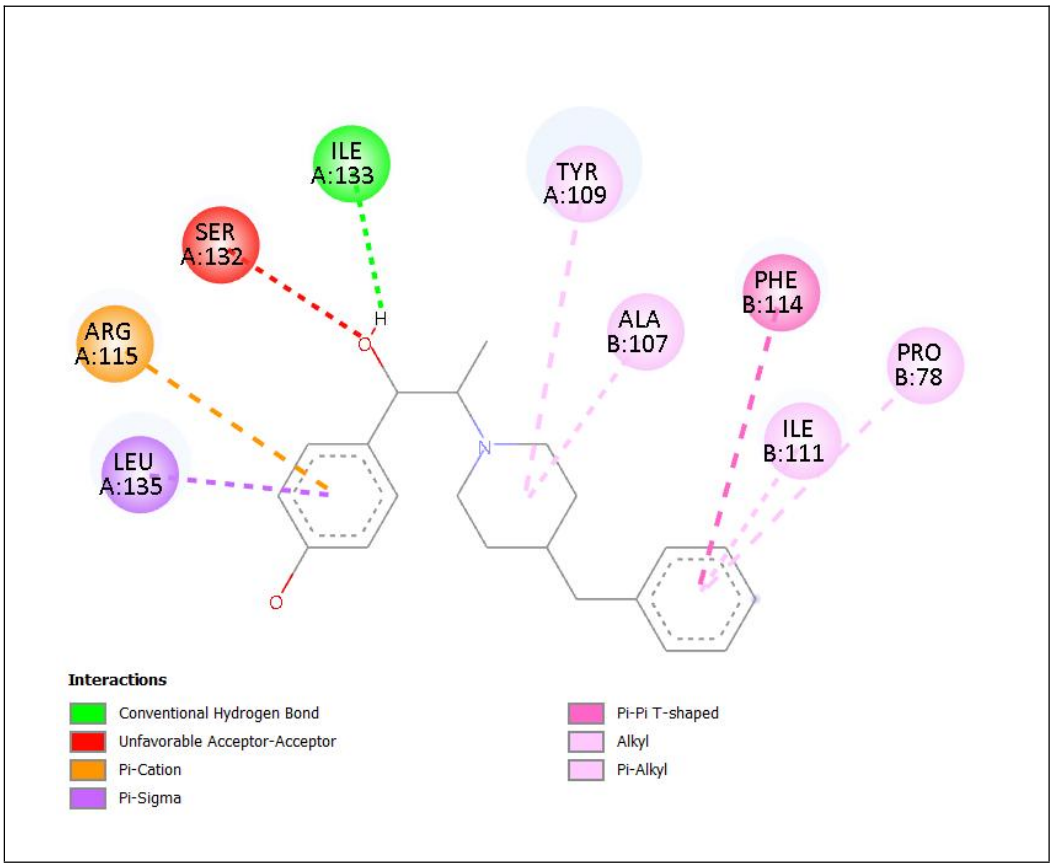


Fig 9 : Interaction map of compound 8 (CID 142065266)

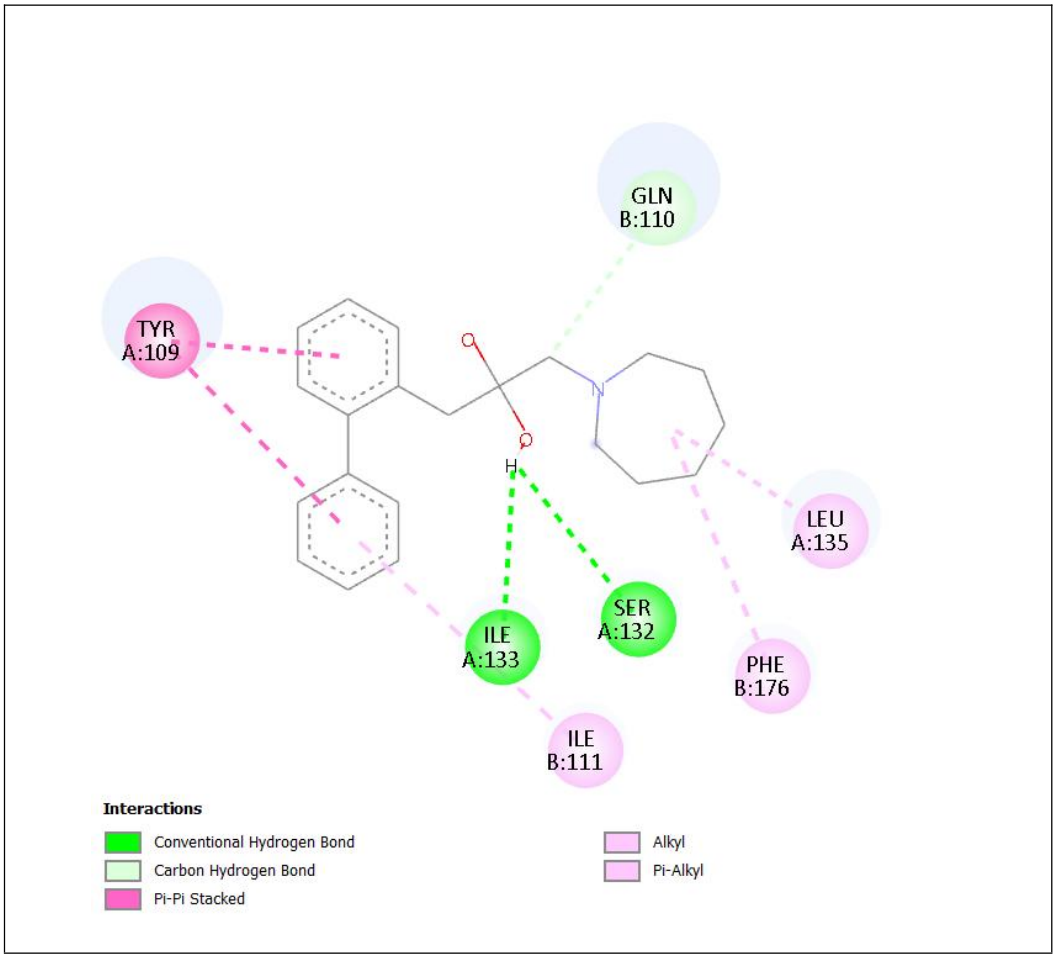


Fig 10 : Interaction map of compound 9 (CID 54144945)

4.3. ADME analysis of shortlisted compounds:

ADME profiling examines the drug-likeness and pharmacokinetic feasibility of the shortlisted compounds. All the shortlisted ligands displayed BBB permeability along with zero violations to Lipinski, making them favourable candidates for neurodegenrative disease drug development. All the shortlisted ligands also possess high GI absorption flavouring their high bioavailability potential. Further, other parameters like consensus logP value, logKp and TPSA were also evaluated that supported the viability of the results (TABLE II).

Table II. ADME analysis of top hits

Compound	BBB Permeabiliy	GI absorption	Lipinski violation	TPSA (Å²)	Consensus logP	Log Kp(cm /s)
1	YES	High	0	43.70	3.41	-5.52
2	YES	High	0	44.90	2.38	-5.53
3	YES	High	0	43.70	3.40	-5.52
4	YES	High	0	44.90	2.40	-5.53
5	YES	High	0	43.70	3.39	-5.52
6	YES	High	0	44.70	2.39	-5.53
7	YES	High	0	43.70	3.45	-5.64
8	YES	High	0	43.70	3.41	-5.52
9	YES	High	0	43.70	3.31	-5.51

5) . CONCLUSION

The present study demonstrate the potential and the effectiveness of computational drug discovery approaches for the neurodegenrative diseases, by identifying the eight novel antagonist, targeting the NMDA receptor for the treatment of Alzheimer's diseases. The newly identified eight antagonist compounds display better binding affinities, than the reference ligand Ifenprodil, suggesting better interaction potential to with the target (NMDA). The compound having PubChem CID 25271983 showed the best binding affinity of -11.1 kcal/mol, making it a potential lead compound in drug discovery for Alzheimer's disease. The systematic *in-silico* workflow includes the selection of possible ligands through structure similarity search, molecular docking for identifying the top hits, visualization of interaction involved and finally the ADME analysis for assessing the pharmacokinetic feasibility of shortlisted compounds. All the shortlisted eight compounds offer BBB permeability and no Lipinski violation, suggesting their suitability as potential candidates for the drug development in neurodenerative diseases. The GI absorption was also high for all the eight compounds, offering the better bioavailability. The visualisation of the ligand-protein interactions revealed, Leu135 and Ile 111 as the consistently interacting residues.

This study also highlights the advantages of computational approaches over traditional drug discovery methods, including time efficiency and reduced cost. However, it is essential to recognize the inherent constraints of computational studies. The findings from this study suggests that the identified novel antagonists have the potential to target the NMDA receptor to treat AD. But to validate their actual biological activity, safety, and efficacy, wet-lab experimentation and subsequently in-vivo studies using animal models of Alzheimer's disease is recommended. The future research should employ molecular dynamics simulations for investigating the stability and behaviour of ligand-receptor complexes over time under physiological conditions. In conclusion, this study provides a strong foundation for designing new NMDA receptor antagonists with improved therapeutic profiles. The eight identified compounds represent promising lead candidates that could contribute to the advancement of effective treatments for Alzheimer's disease, addressing current limitations associated with existing NMDA receptor inhibitors, and this work lays the foundation for the future research.

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List of Publications

1. Conference Paper

Title of Paper- "Computational Analysis of Novel Inhibitors of NMDA receptor".

Authors name - Sonia and Prof. Pravir Kumar

Name of the Conference- 2026 IEEE 6th International Conference on Computing, Power, and Communication Technologies (IC2PCT).

Date of Conference- 15th, 16th, and 17th May, Galgotias University , Greater Noida, India

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IEEE IC2PCT 2026: Paper Acceptance Notification and Registration Form | Paper ID- 2446 | 2026 IEEE 6th International Conference on Computing, Power, and Communication Technologies | (MODE-OFFLINE) | Slot -9

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to me, ic2pct

Dear Prof./Dr./Mr./Ms. Sonia Beriwal,

Congratulations!

We are pleased to inform you that your manuscript id-2446 & titled "Computational Analysis of Novel Inhibitors of NMDA Receptor " has been peer-reviewed and accepted for paper presentation, in OFFLINE Mode, at "2026 IEEE 6th International Conference on Computing, Power, and Communication Technologies", which will be held at Galgotias University, Greater Noida, India (203201), from May 15-17, 2026.

IEEE 6th IC2PCT 2026: Presentation Template

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Dear Sonia Beriwal,

Congratulations once again for your work, which is accepted for oral presentation at 2026 IEEE 6th International Conference on Computing, Power, and Communication Technologies.

For presenter details and PPT/PPTX/PDF/ presentation, kindly fill out the Google Form given below by 30/04/2026.

Details of the submitted manuscript are given below:

Track Name: Track 1: Computing

Paper id: 2446

Title: Computational Analysis of Novel Inhibitors of NMDA Receptor

Presentation Mode: As per Registration & Approved by TPC.

Computational Analysis of Novel Inhibitors of NMDA Receptor

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***Abstract*—N-methyl-D-aspartate (NMDA) receptors serves an essential role in excitatory neurotransmission; however, their dysregulation may result in several neurodegenerative and neurological disorders. Alzheimer's is a leading neurodegenerative disorder associated with the glutamate mediated excitotoxicity resulting from the abnormal activation of the NMDA receptor. Therefore, targeting the NMDA receptor overactivation offers a therapeutic strategy for neuroprotection. In this in-silico study, molecular docking was performed to identify the novel inhibitors targeting the NMDA receptor by taking Ifenprodil as the reference ligand, which leads to identification of the eight compounds having higher docking scores than the reference ligand. Further ADME analysis of these compounds supported the BBB permeability and pharmacokinetic feasibility, making them potential candidates for the AD treatment.**

Keywords—NMDA receptor, Alzheimer's disease, Ifenprodil, molecular docking

I. INTRODUCTION

Alzheimer's disease (AD) is a progressive neurodegenerative disorder, linked to aging and is marked by gradual deterioration in memory, logical thinking, behavioral processes, and personality. The disease involves gradual structural and functional decline of neurons within hippocampus and cerebral cortex, which are brain areas critical for learning and cognitive abilities. Extracellular deposition of Aβ plaques and intracellular accumulation of neurofibrillary tangles consisting of abnormally hyperphosphorylated tau protein, are the key pathological hallmarks of AD. Growing evidences further shows that synaptic loss and dysfunction also play a role in cognitive impairment along with the classical histopathological markers [1].

Normal neuronal activity depends on glutamate-mediated excitatory signaling in which NMDA receptors constitute among the most important ionotropic glutamate receptor families. These synaptic receptors contribute to critical processes like neurodevelopment cognitive functions, learning, synaptic plasticity, and memory. However, the activation of the extrasynaptic NMDA receptor or their overactivation leads to synaptic dysfunction and neuronal death. This excitotoxicity is an outcome of their high calcium permeability. Sustained Ca2+ influx through these receptors triggers pathological signaling cascades that leads to synaptic failure and eventual neuronal loss, which is closely associated with the cognitive decline observed in AD [2].

Targeting the NMDA receptor offers a therapeutic strategy to treat AD. In this study an in-silico screening of potential NMDA receptor inhibitors was done, using molecular docking, to identify their novel inhibitors . Ifenprodil is taken as the reference ligand which is an uncompetitive inhibitor of the NMDA receptor that binds to the Glun2B subunit more strongly [3]

II. LITERATURE REVIEW

AD is a well-known neurodegenerative disorder which is chronic and is defined as the steady degeneration of the brain functions, including self-functioning ability, declarative memory, and recall ability. In its early phase, this condition often remains asymptomatic but it gradually advances from early-stage cognitive decline to severe dementia. Neuropathologically, AD can be defined as the extracellular deposition of amyloid-β (Aβ) peptides in hippocampal and cortical regions, accompanied by intracellular aggregation neurofibrillary tangles consisting hyperphosphorylated tau protein [4].

Recent studies indicate that dysregulation of NMDA receptor is a key contributor in AD pathogenesis. Excessive receptor stimulation contributes to excitotoxicity, synaptic dysfunction, and reciprocal interactions with Aβ, and tau further aggravate disease. NMDA receptors are members of ionotropic glutamate receptors family and function as essential mediators of excitatory transmission in CNS. acting as key molecular integrators of synaptic activity which makes them central to processes including learning, memory, and synaptic plasticity. NMDA receptors are heterotetrameric assemblies containing two mandatory GluN1 subunits combined with two modulatory GluN2 subunits. In mature neurons, synaptic NMDARs are often enriched in GluN2A, typically engaged in signalling cascades that support synaptic plasticity, neuronal survival, and adaptive gene expression. Conversely, the extrasynaptic NMDARs frequently contain GluN2B, which is more strongly associated with mitochondrial impairment, oxidative stress, and suppression of pro-survival transcriptional programs, on sustained activation [5].

Central to AD, the Aβ oligomers preferentially accumulate at extrasynaptic GlunN2B containing NMDARs and blocks the astrocytic glutamate uptake through EAAT2 mislocalisation leading to sustained activation of the NMDARs. In parallel, Aβ reduces the activity of the glutamine synthetase activity, resulting in extracellular glutamate elevation and prolonged NMDAR activation. This

hyperactivation of the NMDARs give rise to high calcium permeability, resulting into mitochondrial dysfunction, ROS production, and neuronal death. Tau pathology further intensifies these effects by impairing synaptic NMDAR trafficking and promoting receptor internalization. The extrasynaptic GluN2B NMDAR activation contributes to memory impairments by enhancing long-term depression (LTD) and by suppressing synaptic plasticity by reducing the long-term potentiation (LTP), indicating the signs of neurodegeneration [6][7].The relative activity of synaptic and extrasynaptic NMDA receptor plays decisive role in neuronal fate during Alzheimer's disease. While synaptic NMDA receptor activity supports neuronal plasticity and memory formation, the activation of extrasynaptic NMDA receptors suppresses CREB signalling, disrupts mitochondrial function and promotes neuronal death. Aβ further impairs cognitive function by decreasing CREB phosphorylation and consequently lowering expression of BDNF. The PGC-1α levels decrease weakening the mitochondrial oxidative stress regulation and lowers the neuronal resistance to excitotoxic damage. The Aβ mediated NMDAR activation enhances the β-secretase expression which in turn leads to Aβ overproduction, reinforcing a self-sustaining pathogenic loop [8][9]. Ifenprodil is an uncompetitive antagonist that binds to NMDAR to modulate its activity. The Ifenprodil shows 140x preference towards the GluN2B subunit of NMDAR than the GluN2A and induces the inhibition of GluN2R receptor. This study is focused on determining the novel inhibitors of the NMDA receptors that possess the therapeutic potential to treat AD [3].

III. METHODOLOGY

A. Protein structure selection and preparation

The crystal structure of NMDA receptor in complex with Ifenprodil, having GluN1-GluN2B subunit (PDB ID: 5EWJ) was sourced from PDB database (https://www.rcsb.org). This structure was selected due to its suitable resolution of 2.77 Å. The structure was inspected and processed using Pymol to remove bound ligand Ifenprodil. Further processing of the protein was carried out using BIOVIA discovery studio 2025 including elimination of water molecules, addition of polar hydrogen, and then prepared structure was saved for docking stimulation.

B. Ligand Selection and preparation

Reference ligand: Ifenprodil, a well-established inhibitor of NMDA receptor, was chosen as the reference ligand for this study. PubChem database (https://pubchem.ncbi.nlm.nih.gov/) was employed to download the 3-D structure of Ifenprodil with CID:3689, in SDF file format.

Similarity-Based Search: To identify the possible ligands, capable of interacting with the protein, PubChem similarity search was performed using Ifenprodil as query molecule, which yielded a total of 607 compounds with 90 percent structural similarity to the reference ligand. Further refinement was carried out was by applying various filters like molecular mass, heavy atom count, rotatable bonds, polar surface area, which lead to selection of 73 compounds and these were downloaded in SDF format for further processing.

Ligand preparation: All the ligands were prepared for docking using the Open Babel option in PyRx software package where energy minimization steps were done for the ligands. Open Babel is an open source chemoinformatics software package that allows the file conversion into different formats.

C. Molecular docking studies

PyRx software package was employed to perform the molecular docking of prepared ligands with the target protein to investigate the interaction between the ligands and protein. A config file was made containing the grid box dimensions and the centroids. A grid box of dimensions 21.27x38.22x25.00 was generated on the protein and centroids center_x= 85.610; center_y= 6.402, center_z= -31.912 were used to carry out the docking studies.

D. ADME Analysis for BBB permeability

The pharmacokinetic behavior of the top hits was assessed through ADME profiling by using Swiss ADME, to examine their absorption, distribution, metabolism, and excretion characteristics and drug-likeness. Swiss ADME (https://www.swissadme.ch)is an openly assessable web-based server, used to carry out the analysis. It was performed to prioritize molecules with favorable bioavailability, BBB permeation, and CNS exposure while eliminating candidates with poor pharmacokinetic profiles.

IV. RESULTS AND DISCUSSION

The redocking of the reference ligand Ifenprodil into the active site yielded the binding energy of -10.2 kcal/mol with primary pose exhibited an internal RMSD of 0.00Å, with subsequent poses remaining well within the standard threshold of <2.0 Å. The presence of RMSD values around ~2 Å among top poses suggests that the docking protocol can reproduce the experimental binding mode with acceptable accuracy.

A. Docking studies and top hit analysis

After the virtual screening and molecular docking analysis, the top eight hits were shortlisted that possessed the higher docking scores than the reference molecule Ifenprodil. The binding energy of these, ranges between -11.1 to -10.6 kcal/mol whereas the reference compound possesses the binding energy -10.2 kcal/mol. The compound with CID 25271983 showed the best binding affinity of -11.1 kcal/mol. These compounds exhibited better docking scores suggesting their potential for improved binding affinity towards target protein.

B. Visualization of top hits

The BIOVIA discovery studio 2025 client was utilized to examine and visualize the receptor protein-ligands interactions. The 2-D interaction maps were generated to reveal the key hydrogen bonds, pi-pi and hydrophobic interactions, and the associated amino acid residues.

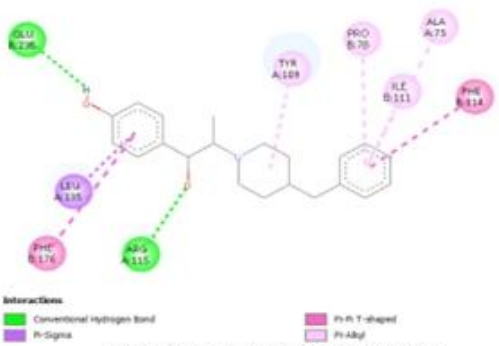


Fig 1: Interaction map of Ifenprodil (reference)

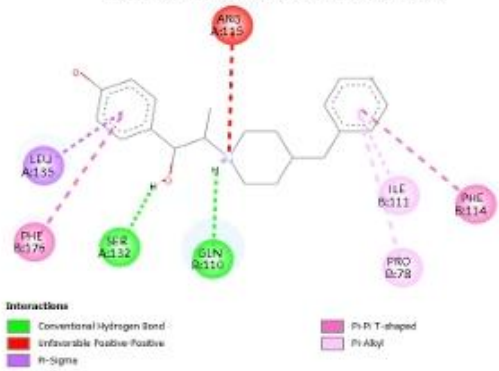


Fig 2: Interaction map of compound 2 (CID 25271983)

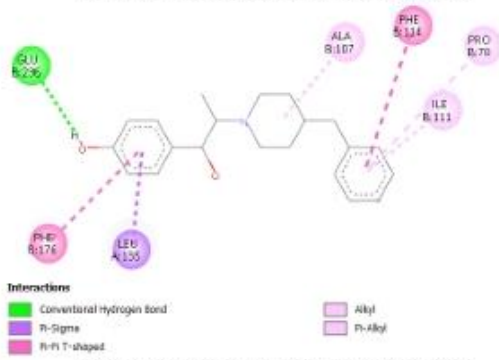


Fig 3: Interaction map of compound 3 (CID 6604117)

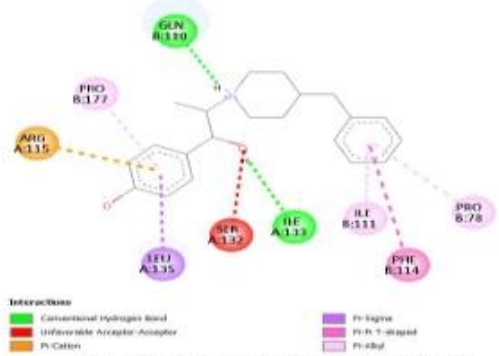


Fig 4: Interaction map of compound 4 (CID 25271982)

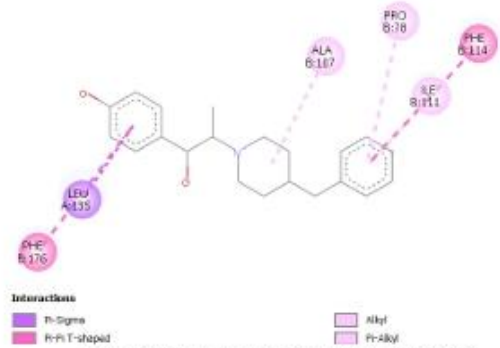


Fig 5: Interaction map of compound 5 (CID 11771731)

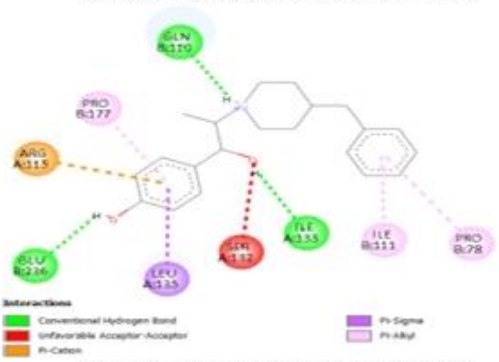


Fig 6: Interaction map of compound 6 (CID 74441145)

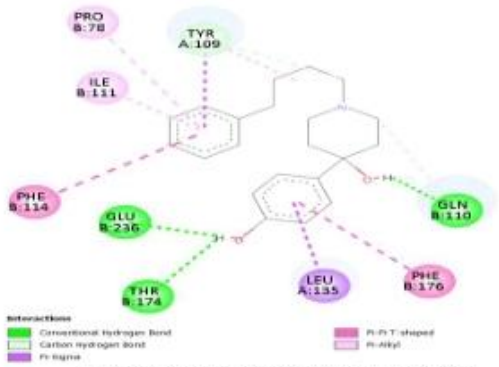


Fig 7: Interaction map of compound 7 (CID 10592168)

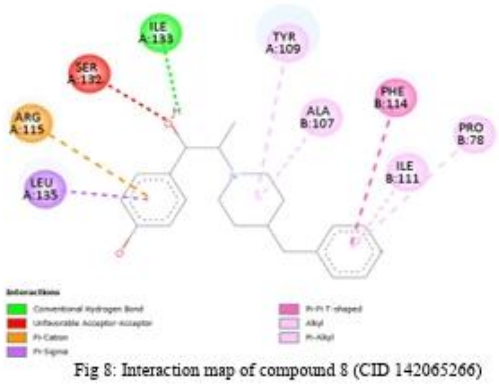


Fig 8: Interaction map of compound 8 (CID 142065266)

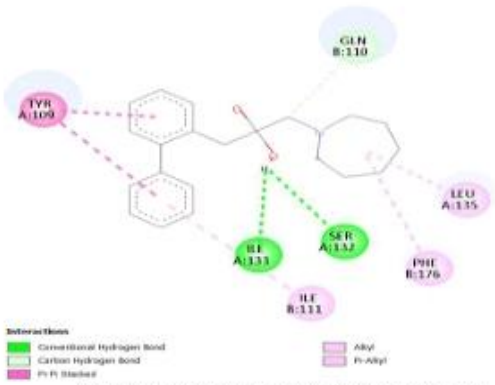
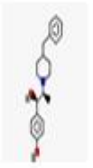
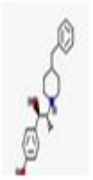
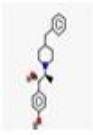
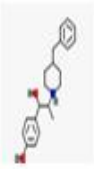
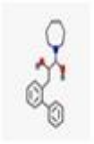


Fig 9: Interaction map of compound 9 (CID 54144945)

TABLE I: TABULAR REPRESENTATION OF PUBCHEM CID, BINDING ENERGY, 2-D STRUCTURES, AND INTERACTING RESIDUES.

Compound	Pubchem CID	Binding energy (kcal/mol)	2D Structure	Interacting residues
1	3689 (reference)	-10.2		Glu236, Leu135, Phe176, Arg115, Tyr109, Pro78, Ile111, Ala75, Phe114
2	25271983	-11.1		Arg115, Leu135, Phe176, Ser132, Gln110, pro78, Ile111, Phe114
3	6604117	-10.9		Glu236, Phe176, Leu135, Ala107, Phe114, Pro78, Ile111
4	25271982	-10.8		Gln110, Pro177, Arg115, Leu135, Ser132, Ile133, Ile111, Phe114, Pro78
5	11771731	-10.8		Phe176, Leu135, Ala107, Pro78, Ile111 Phe114
6	74441145	-10.8		Gln110, Pro177, Arg115, Glu236, Leu135, Ser132, Ile133, Ile111, Pro78
7	10592168	-10.6		Pro78, Ile111, Tyr109, Phe114, Glu236, Thr174, Leu135, Phe176, Gln110
8	142065266	-10.6		Ile133, Ser132, Arg115, Tyr109, Ala107, Phe114, Pro78, Ile111
9	54144945	-10.6		Tyr109, Gln110, Ile133, Ile111, Ser132, Phe176, Leu135

C. ADME analysis of shortlisted compounds

ADME profiling was carried out for the shortlisted compounds to examine their drug-likeness and pharmacokinetic feasibility. All the shortlisted ligands displayed BBB permeability along with zero violations to Lipinski. All the shortlisted ligands possess high GI absorption, zero PAINS and brenk. Further, other parameters like consensus logP value, logKp and TPSA were also evaluated to determine the viability of the results (TABLE II).

TABLE II: ADME ANALYSIS OF THE COMPOUNDS

Compound	BBB Permeability	GI absorption	Lipinski violation	TPSA (Å²)	Consensus logP	Log K_p(cm/s)
1	YES	High	0	43.70	3.41	-5.52
2	YES	High	0	44.90	2.38	-5.53
3	YES	High	0	43.70	3.40	-5.52
4	YES	High	0	44.90	2.40	-5.53
5	YES	High	0	43.70	3.39	-5.52
6	YES	High	0	44.90	2.39	-5.53
7	YES	High	0	43.70	3.45	-5.64
8	YES	High	0	43.70	3.41	-5.52
9	YES	High	0	43.70	3.31	-5.51

In this in-silico study the molecular docking was performed to find out the novel inhibitors for the NMDA receptor. Eight

compounds exhibiting superior binding affinities compared to reference ligand were identified, with binding energies spanning from -11.1 to -10.6 kcal/mol. These compounds were consistently interacted with common residues, notably Leu135 and Ile111. The reference ligand Ifenprodil exhibited binding energy of -10.2 kcal/mol. Improved docking scores indicate, that these compounds can be taken into consideration as the replacement of the reference ligand; however, further experimental analysis is recommended to unfurl the full potential.

V. CONCLUSION

NMDA receptors dysregulation is among the major causes of Alzheimer's disease. This suggests that targeting NMDA receptors offers a therapeutic strategy to treat neurodegenerative disease. In this study, eight compounds demonstrated higher binding energies than the reference inhibitor Ifenprodil. Compound with PubChem CID 25271983 was identified to possess the strongest interaction with the target protein. The ADME analysis of the shortlisted compounds shows the BBB permeability, high GI absorption and zero Lipinski violation strengthening their potential to be used as therapeutic compound.

These results are based on computational predictions; limitations such as rigid receptor model and approximate scoring functions should be considered. The differences between binding affinities should be interpreted with caution due to internal limitations of docking method. To explore their therapeutic potential in AD, molecular dynamics studies and wet lab experimentations also needs to be done further.

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