REPURPOSING OF DRUGS FOR GLUTAMATE RECEPTOR IN HUNTINGTON'S DISEASE: A BIOINFORMATICS EXPEDITION

A Dissertation
Submitted in Partial Fulfilment of the Requirements for the Degree of

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BIOTECHNOLOGY

by

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CANDIDATE'S DECLARATION

I, Surbhi Verma, bearing Roll No. 2K22/MSCBIO/52 hereby certify that the work which is being presented in the thesis entitled **"Repurposing of Drugs for Glutamate Receptor in Huntington's Disease: A Bioinformatics Expedition"** in partial fulfilment of the requirement 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 January 2024 to May 2024 under the supervision of Prof. Pravir Kumar.

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

Signature of Supervisor

Candidate's Signature

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CERTIFICATE BY THE SUPERVISOR

Certified that Surbhi Verma (2K22/MSCBIO/52) has carried out their search work presented in this thesis entitled **"Repurposing of Drugs for Glutamate Receptor in Huntington's Disease: A Bioinformatics Expedition"** for the award of Master of Science from Department of Biotechnology, Delhi Technological University, Delhi under my supervision. The 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 reward of any other degree to the candidate or to anybody else from this or any other University/Institution.

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Repurposing of Drugs for Glutamate Receptor in Huntington's Disease: A Bioinformatics Expedition

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ABSTRACT

Aim: A rare hereditary illness called Huntington's disease (HD) is typified by the build-up of mutant huntingtin protein aggregates. The aggregates cause excitotoxicity, which in turn causes neuronal malfunction and ultimately results in cell death. Glutamate receptors, in particular the NMDA receptors (NMDARs), which are made up of NR1 and NR2 subunits and form a heteromeric complex required for receptor activation, are partially responsible for excitotoxicity in HD. In HD, overactivation of NMDARs causes brain symptoms and neuronal damage. A possible therapeutic strategy involves blocking the NMDAR NR1 and NR2 subunits. Through the use of databases and bioinformatic computational tools, researchers hope to decrease development periods and minimize safety and toxicity issues by repurposing existing drugs to block NMDARs. Identified the 19 antipsychotic medications with the greatest outcomes against the NMDA receptor by using bioinformatic computational tools and approaches. PyRx-Vina was utilized for molecular docking, while Discovery Studio was employed to visualize the interactions between ligands and proteins. When compared to ketamine, an FDA-approved medication utilized as a reference antagonist, we discovered that aripiprazole produced favourable results. By inhibiting NMDAR activity and lowering dopamine production, inhibition of NR1 and NR2 subunits lessens excitotoxic neuronal damage in HD. Repurposing already-approved medications has advantages like accelerated time to market, decreased toxicity, and fewer safety risks.

Result: Identified the 19 antipsychotic medications with the greatest outcomes against the NMDA receptor by using bioinformatic computational tools and approaches. PyRx-Vina was utilized for molecular docking, while Discovery Studio was employed to visualize the interactions between ligands and proteins. When compared to ketamine, an FDA-approved medication utilized as a reference antagonist, we discovered that aripiprazole produced favourable results. By inhibiting NMDAR activity and lowering dopamine production, inhibition of NR1 and NR2 subunits lessens excitotoxic neuronal damage in HD. Repurposing already-approved medications has advantages like accelerated time to market, decreased toxicity, and fewer safety risks. This research indicates that Huntington's illness may be treated therapeutically with aripiprazole due to its inhibition of NMDAR. Molecular docking experiments indicate that apipirazole has a high binding affinity and forms Stable interaction with key sites in the NMDAR. It has

favourable pharmacokinetic properties, such as high solubility, low toxicity, and blood-brain barrier bridging, and a higher binding affinity than ketamine.

Conclusion: Repurposed drug of drug candidates originally known for as Bipolar disorder, Anti-Depression categories for treating for Huntington Disease who have glutamatergic effect by targeting its NMDA Receptor, an glutamate receptor by targeting this receptor can inhibit the excess excitotoxicity in Huntington's disease. Apipirazole has a high binding affinity and forms Stable interaction with key sites in the NMDAR. It has favourable pharmacokinetic properties, such as high solubility, low toxicity, and blood-brain barrier bridging, and a higher binding affinity than ketamine.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

HD	Huntington Disease
HTT	Wild type huntingtin
mHTT	Mutant huntingtin
NMDAR	N-methyl-D-aspartate receptor
LTP	Long Term Potential
ADME	Absorption, Distribution, Metabolism, Excretion
GI	Gastrointestinal
BBB	Blood-Brain Barrier
CAG	Cytosine, Adenine, Guanine

CHAPTER 1

INTRODUCTION

1.1 Overview

The largest medical problem in the world today is neurodegenerative diseases, which impair motor function, cognitive function, psychotic behavior, and other functions. These dysfunctions, such as Huntington's disease, result impacted in patient and their offspring. A well-known example of a disease like Huntington's disease is the intricate interaction of genetic, molecular, and environmental factors that contribute to the pathophysiology of the disease. The (HTT), located on chromosomal number 4, has a point mutation that causes disease, an uncommon autosomal neuropathy illness. Neuronal dysfunction resulting in motor, memory loss, and mutation are the root cause of mental health issues in the polyglutamine sequence of the huntingtin protein. During neuronal misfolding and aggregation, this mutant protein frequently forms inclusion bodies, which are insoluble aggregates.

Within neurons, mHTT aggregation impairs a number of physiological functions, such as internal signalling cascades of events mitochondrial activity, and the degradation of proteins pathways. The eventual loss of cells and malfunctioning of neurons are caused by these disturbances. Glutamate signaling dysfunction is a hallmark of neuronal dysfunction. Excitatory neurotransmitter, glutamate, is essential for synaptic communication and plasticity. Abnormal excessive amount glutamate accumulates in the synaptic cleft of HD patients due to changes in glutamate breakdown, release, and reuptake processes. Glutamate receptors, especially NMDARs, are persistently activated in response to excessive glutamate release and poor glutamate clearance. Synaptic plasticity based on the N-methyld-aspartate receptor (NMDAR) is a promising candidate to mediate hippocampaldependent learning and memory processes. Because of this bidirectional plasticity, it is still unclear how one receptor can drive opposing changes in synaptic weights. The makeup of the NMDAR subunit has been proposed as a potential factor. To be more precise, NMDARs with one subunit composition would induce long-term potentiation (LTP), while NMDARs with a different subunit composition would induce long-term depression (LTD). Regretfully, research on this idea has shown inconsistent results, especially when it comes to LTD [3]. The process of excitotoxicity, In "hich'an excessive calcium influx results in neuronal destruction and an influx of calcium ions into neurons triggers dying cells. Certain studies indicate that NR2B is a selective antagonist that offers improved safety. We extracted the protein structures of NR1 and NR2 using ID5WEJ from the PDB, and we docked drug compounds from the drug bank together. In the drugbank databases, only ariprazole was identified as the ideadrug

with the highest binding affinity to the protein binding site that is involved in the neurotransmitter of Huntington's disease.

CHAPTER 2

LITERATURE REVIEW

2.1 Neurodegenerative Disease

The term "neurodegenerative diseases" refers to a class of illnesses where the nervous system's structure and function gradually deteriorate. Movement problems, cognitive impairment, and other neurological symptoms are frequently brought on by these illnesses. Numerous crippling symptoms are brought on by these illnesses, which can make a person's quality of life considerably poorer over time. The etiology of neurodegenerative disorders are complicated and involves factors related to genes, the environment, and lifestyle.

Incorrect folding of proteins and aggregation, oxidative damage, mitochondrial malfunction, inflammation, and excitotoxicity are some of the pathways that underlie neurodegeneration. The typical symptoms are brought on by a disruption in normal brain function caused by the loss of neurons and synapses. Numerous neurodegenerative disorders are linked to NMDA receptors, which are essential for excitatory synaptic transmission. [1]

Specialized pathogenic features, including aberrant protein processing, the buildup of protein aggregates, neuroinflammation, oxidative stress, and mitochondrial dysfunction are linked to each neurological disorder. Neuronal malfunction and apoptosis are exacerbated by these degenerative alterations. Certain neurodegenerative conditions can be clearly attributed to genetics, but complicated interactions between both environmental and biological factors like its genetics factor may also be involved. Neurodegenerative illnesses can be influenced by aging procedures, decisions about life, environmental pollutants, and abnormalities in the genome.

With worsening symptoms, patients may encounter a decline in general wellbeing, an increase in impairment, and a need on others to perform everyday tasks. The therapy of neurodegenerative illnesses may benefit from pharmaceutical therapies such as antagonists or modulators that target NMDA receptors, particularly particular subunits like NR2B. Research is being done to learn more about the fundamental causes of these illnesses and to create cutting-edge treatment plans that use stem cells, genetic treatments, and personalized therapeutics. Therefore, neurodegenerative disorders essentially constitute a significant health problem that impacts millions of people globally, leaving them severely disabled and with diminished standards of life.

2.2 Huntington Diseases

The most prevalent monoclonal autonomously dominant neurodegenerative disease is called Huntington's disease. George Huntington initially described the illness in 1872, characterizing it as a high penetration hereditary choreiform disorder with behavioral and mental symptoms. The stage in life at which HD manifestations first appear has an inverse correlation with the amount of repeated CAG sequences in the huntingtin gene [10].

The development of neurons in human embryos affected by Huntington's illness can begin at as early as thirteen weeks of gestational period. It is common for neurologists to come with HD as it usually manifests in adulthood. An increased CAG mutation in exon 1 of gene, thereby is a hallmark of Huntington's disease, a hereditary neurological condition. In those who have been impacted, this repeat expands more than usual, resulting in the creation of huntingtin amino acids with unusually lengthy polyglutamine tracts. The MHtt protein is a representation of aberrant CAG repeats with a high glutamate ratio. Widespread glutamate receptors cause aberrant processes that include chronic pain, psychosis, neuronal development, and calcium ion channels. Excitatory neurotransmitters that overexpress NMDA receptors as a result of glutamate overexpression in mutant proteins produce motor impairment and psychotic behaviour.

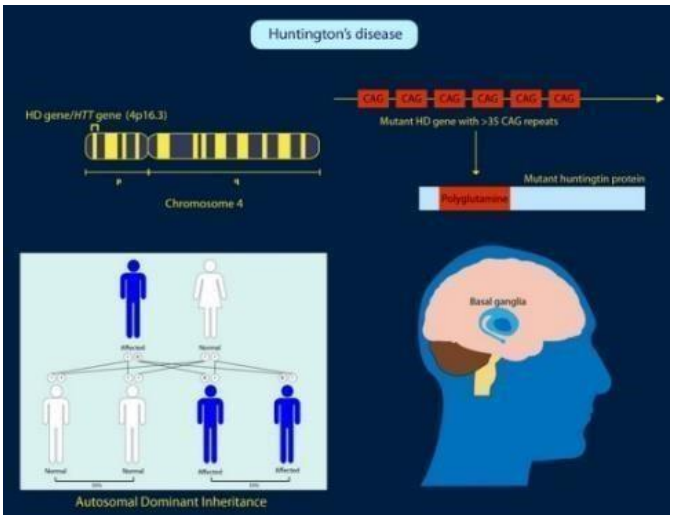

Fig 2.1 : Genetics of Huntington's Diseases (Source: NIH, Huntington Disease)
Research demonstrates aberrant behavior in the developing cortex of the brain, such

as mutant huntingtin incorrect distribution and problems in neuroprogenitor cell polarity, suggesting that HD affects the development of the brain in the embryonic period of development. A minimum of 40 CAG repeats is linked to total the penetration and full clinical symptoms of the illness. People with 36–39 CAG repetitions may have decreased persistence, which could result in older age at diagnosis and varying clinical manifestations of HD. Behavior alterations and moderate cognitive impairment have been identified as linked to intermediate alleles containing 27–35 CAG repeats. In progeny, the proliferation of these intermediary alleles through meiosis may result in greater numbers of CAG repetitions.

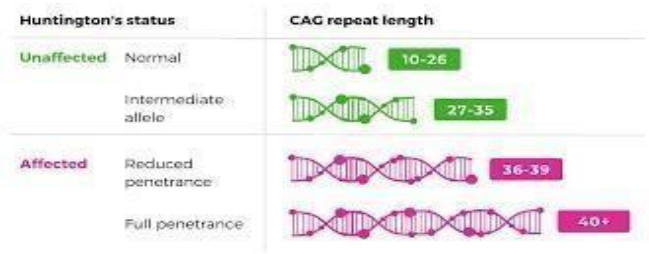

Fig 2.2 : CAG repeat length depicts affected and unaffected Huntington diseases. (image source: Huntington's Disease Association)

These enlarged regions give the protein potentially harmful increases in function, making it more vulnerable to disintegration, which leads to neuronal malfunction and death. According to the findings, the brain's neuroepithelial wall has ventricular zones that extend to the basal and apical surfaces, and the cell nucleus oscillates in response to the progression of the cell cycle. Aberrant protein causes epithelial symmetry to be lost, junctions to be broken, and an early cell cycle that leads to an early neuronal development [12].

Basically at the molecular level, wild-type huntingtin particular, protein is essential for the formation of synapses that stimulate in the brain during development and channels of signaling, transcriptional regulation, neurotrophic support, maintaining the balance of neurotrophin receptors; balancing histone acetylation and deacetylation, which are important processes in regulating the activity of gene expression; keeping track of mitochondrial function and biogenesis, also known maintaining adequate generation of energy and cellbased health; axonal transportation of organelles alongside microtubules, facilitating proper distribution within neurons; signaling pathways within cells, influencing cellular responses in external influences; autophagy, or the removal of destroyed organelles and proteins throughout cells.

Following a mutation in the Huntington's disease (HD) gene, epigenetic modifications resulting to pathophysiology of Huntington disease. Furthermore,

mutant huntingtin impairs its ability to function as a protein scaffold, which has an impact on overall cellular signaling pathways, organelle transport, and synaptic functioning, ultimately resulting in neural dysfunction. The frequency of expression of genes in neurons is impacted by mutant huntingtin protein, which can cause gene transcription factor dysfunction and subsequent signaling cascades. In Huntington's disease, altered gene expression plays a role in neural malfunctioning and neurodegeneration. Proteins that have been improperly folded and aggregates build up as a result of malfunctions in protein degradation which worsens and contributes to neuronal toxic effects. It is also linked to compromised mitochondrial activity, which results in reduced energy, oxidative stress, a condition and destruction of neurons. A mismatch between reactive oxygen molecules and protective antioxidants leads to higher levels of oxidative stress, which in turn causes perturbations in protein transportation mechanisms, organelle malfunctions, and damage to neuron network, which impairs neurotransmission and neural functioning. These neurons' malfunction adds to the damaged cells caused by Huntington's disease.

2.3 Receptors like NMDAR and Their Subtypes

The NMDA receptor could be a significant component of brain work, especially in synaptic transmission, synaptic versatility, learning, and memory. Here is an clarification of the key focuses concerning NMDA receptors within the brain NMDA receptors are included in excitatory neurotransmission, basically within the transmission of glutamate, the brain's essential excitatory neurotransmitter. Synaptic Versatility: NMDA receptors play a basic part in synaptic versatility, which is the capacity of neural connections to fortify or debilitate over time in reaction to neuronal movement. (LTP) and (LTD), two shapes of versatility depend on the actuation of receptors. By encouraging the fortifying of synaptic associations between neurons. The deluge of calcium particles through enacted NMDA receptors starts different intracellular signaling pathways that direct gene NR1, NR2, and NR3 subunits that make up the complex protein structure known as the NMDA receptor each have distinct roles in learning, memory, synaptic transmission, and synaptic plasticity [15] .

The way these subunits interact dictates the NMDA receptor complex's functional characteristics and its involvement in different brain functions. It is possible that the Glumate receptor is a heterotetrameric protein complex composed of distinct subunits, each of which has specific functions. This might be a detailed discussion of the NMDA receptor's structure and the important functions of its subunits. The organization of functional NMDA receptors depends on the NR1 subunit. It is necessary for the collection and trafficking of the receptor to the cell film and is encoded by the GRIN1 quality.. It endows the receptor with calcium porosity, allowing calcium ions to assemble upon activation. The NMDA receptor channel's conductance, energy, and pharmacological characteristics are all influenced by NR2 subunits. The NMDA receptor complex's trafficking and synaptic

localization involve NR2 subunits. Different NR2 subunits have distinct roles in learning forms and synaptic flexibility [5].

For instance, synaptic reinforcement and memory organization are associated with receptors containing NR2A. NR2 subunits support the NMDA's capacity for calcium signaling.
GRIN3A and GRIN3B qualities, independently, encode the NR3 subunits (NR3A, NR3B). In the NMDA receptor complex, they are less prevalent than the NR1 and NR2 subunits [13].

2.3.1 NR1 Subunit

It contains the ligand-binding space that is interatomic with glutamate and glycine, the essential neurotransmitters that enact the receptor. For NMDA receptor complexes to be collected, traded and secured at the postsynaptic layer, NR1 subunits are needed. Channel Arrangement: Within the NMDA receptor complex, it is responsible formation of particle channel pore. It permits the entry of particles, especially calcium, upon actuation. In order to facilitate the transmission of synaptic signals and respond to neurotransmitters, a receptor requires it. The key neurotransmitters that activate the receptor, Glutamate and Glycine, interact with this protein's ligand bind domain. Canonical diheteromeric (d-) NMDARs are formed when two GluN1 subunits, which are present in all NMDARs, are joined with GluN2 subunits of the same kind. When glutamate is removed from Glu2A and Glu2B receptors can all be influenced. Additionally, the GluN1 location can enhance NMDARs containing GluN2C. One gene, which produces eight distinct splice variants through extensive splicing, encodes the NR1 subunit. Four distinct but closely related genes encode the four subtypes that make up the NR2 subunit class, NR2A–NR2D [2] .

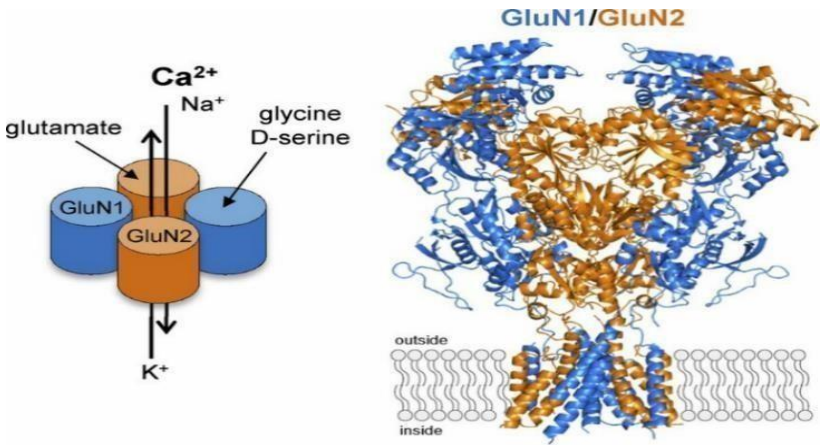

Fig 2.1: GluN1/2 NMDA receptor subunit stoichiometry and subunit organization. (source: The Journal of general physiology. 150.

10.1085/jgp.201812032)

To assemble, transport and anchor to a postsynaptic membrane, the NMDA receptor complex requires NR1 subunits. It allows the transport of ions, in particular calcium, after activation. NMDA receptor calcium permeability, which is required for a number of intracellular signalling pathways and synaptic plasticity. Interactions with the NR1 subunit is interacting with NR2 subunits, such as NR2A and NR2B, to create functional NMDA receptor complexes with unique properties. Where these play role in moulding the functions like Long term potentiation and long term depression, the processes underpinning learning and memory are mediated by NR1 LTPs and Control of NMDA receptor activity. The NR1 subunit is essential to control the function of the NMDA receptor complex in reaction to neurotransmitter binding and membrane depolarization. Abnormal activation of the NMDA receptor, caused by dysregulation or dysfunction of the NR1 subunit, may disrupt neural transmission and synaptic plasticity. The mechanisms underlying NMDA receptor-mediated functions such as memory, learning and synaptic plasticity requires the NR subunit1 [5]. The NR1 regulate in transmitting, transsynaptic plasticity and neuron communication by forming, functioning and regulating a complex of receptors. It is especially relevant to regulate calcium influx, synaptic plasticity and the integration of excitonal signals in the brain.

2.3.2 NR2 Subunits

The NR2 subunits of the NMDA receptor are essential determining functional characteristics the receptor complex. NR2B-containing membrane receptors for NMDA are mostly located. Notably, they are not present in the brainstem. While NR2A and NR2C only show up after birth, with the former primarily found in the frontal lobe and the later being primarily found in cerebellar granule cells, prenatal NMDARs include either NR2B or NR2D.

Structure of NR2:

It produced by four separate genes (GRIN2A, GRIN2B, GRIN2C, GRIN2D) and plays a vital part as a constituent of the NMDA receptor complex. Whereas NR2 subunit is composed of many structural domains forms the ion channel pore. Furthermore, NR2 subunits combine with NR1 subunits to create functional heterotetrameric hence NMDA receptor complexes that possess specific features. It has significant components that enhance the functional variety and specific features of this ionotropic glutamate receptor. NMDARs can engage in intracellular sequences in the development of postsynaptically situated microdomains that interpret Ca2+ signals and initiate the relevant cellular reactions. [9]

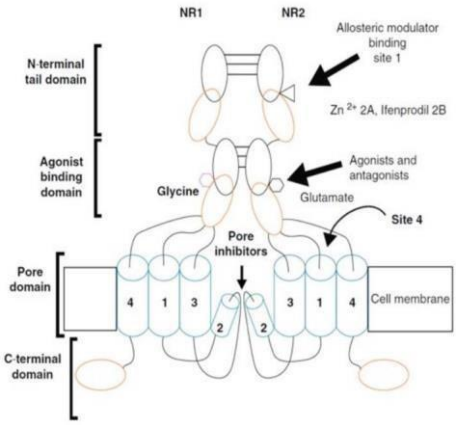

Fig 2.2 : An NMDA-type glutamate receptor's fundamental structure and a few of its pharmacological regulatory sites are shown schematically. (Source:Current pharmaceutical design. 10.2174/1381612811319380003)

Components of NR2:

The NR2 subunits are a necessary group of subunits that, once accompanied with the NR1 subunit, create operational NMDA. The NR2 subunits responsible in enhancing the variety and sensitivity of NMDA receptor activity. The following is an elucidation of he composition and functions of recognized NR2 subunits: NR2A, NR2B, NR2C, and NR2D. [7]

NR2A:

Structure: NR2A subunits have enhanced channel dynamics with regard to other NR2 subunits. They are notable in the forebrain portions of the brain. NR2Acontaining NMDA receptors participate in communication between neurons and plasticity. They serve a role in modulating excitability in neurons and synaptic resilience, facilitating memory and cognition functions.

NR2B:

Properties: Slower channel rates have been demonstrated in this, which are crucial for neuronal plasticity and maturation. Long-term potentiation and longterm depression), two processes underpinning learning and memory formation, are mediated in part by NR2B-containing NMDA receptors.

NR2C:

Structure: Despite being less researched, NR2C subunits differentiate themselves from NR2A and NR2B subunits due to the fact they possess distinctive characteristics.

In particular brain areas or phases of development, NR2C-contain NMDA receptors probably have distinct functions that influence communication and plasticity.

NR2D:

Structure: While the roles of NR2D subunits remain unresolved they differ from other NR2 subunits in several distinctive ways. In particular neural networks or processes related to development, NR2D-containing NMDA receptors could serve distinct functions that affect synaptic communication and memory.

2.4 The NMDA Receptor's Mechanism of Action

Glutamate Binding: Under normal circumstances, NMDAR interacts to glutamate. Glycine binds to the receptor's NR1 subunit while also functioning as a co-agonist.

Ion Channel Opening: The NMDA receptor's ion channel opens when amino acid accept and the membrane depolarizes, enabling calcium and other cations to enter the postsynaptic neuron.

Intracellular Signaling: Gene expression, synaptic plasticity, and neuronal growth are all dependent on the several intracellular signaling pathways that triggered by calcium influx through the active NMDA receptor.

Main Function under Normal Circumstances

Under typical circumstances, the NMDA receptor mediates excitatory transmission between synaptic cells and contributes to synaptic plasticity through the coordinated activation of NR1 and NR2 subunits.

Functions like long-term potentiation (LTP), a biological mechanism behind memory and learning, along with NMDA receptor activation is necessary. NMDA receptors are essential for the growth of neurons, the reinforcement of synapses, and the incorporation of electrical impulses in brain. mHTT , it can interfere with normal pathways of signaling and cellular processes,particularly those including NMDA receptors like NR1 and NR2 subunits, cause of Huntington's disease.

Dysregulation of Calcium:

Calcium dysregulation in neurons can be a result of mutant huntingtin-induced dysfunctional NMDA receptors in HD. High calcium influx via NR1-NR2 subunits and other abnormal NMDA receptors can cause excitotoxicity and injury to neurons in HD.

Apprehensive Dysfunction:

Mutant huntingtin-induced NMDA receptor signaling impairment may impair synaptic plasticity and function, hence impacting neural communication and interaction between neurons. Changes in the NR1-NR2 connections can affect neurotransmitter release and synaptic strength.

Disruption of Glutamate Receptor Function:

Mutant huntingtin protein can have a deleterious effect either directly or indirectly. Different interactions between mutant huntingtin and NMDA receptor subunits may lead to abnormal receptor placement, reduced receptor expression, or impaired receptor functionality.

2.5 Therapeutic Insight of NMDA Receptor

Glutamate responsible for excitatory neurotransmitter NMDARs are essential for synaptic function, it is hardly unexpected that a variety of neuropsychiatric, neurodevelopmental, and neurodegenerative illnesses are linked to aberrant NMDAR signaling. the formation of the channel gate's crossing helical bundle, the cation-selective Na+ and Ca2+ enter cells via their electrochemical gradients. Signal transduction molecules like CamKII can connect to the CTDs, which also contain sites for posttranslational modifications and anchor NMDARs to the intrasynaptic cytoskeletal network. The NMDAR pore's vulnerability to voltage dependent Mg2+ block, which is eased by depolarization, causes channel blockage at resting membrane potentials.

Additionally, GluN2A and GluN2B made up of NMDAR have higher channel conductance levels than GluN2C. Triheteromeric receptors along two distinct GluN2 subunits are also generated. Variations in the splicing location of GluN1 result in variations in the potency of deactivation. Furthermore, different splicing can change the plasticity of synapses. The GluN1 subunit and the distribution of GluN1 splice variants vary by area and stage of development. GluN2A and GluN2B are widely shown in the adult brain, with major neurons in the forebrain expressing them at particularly high levels.

Extrasynaptic glutamate has the ability to activate these receptors, which may be involved in controlling synaptic excitability and membrane potential. preliminary research revealed a higher percentage of the extra synaptic NMDAR pool is made up of GluN2B-containing NMDARs. Normal brain activities, such as neuronal growth depend on NMDAR signalling. NMDARs have physiological functions and pathological implications, making them development treatments for CNS illnesses. NMDAR modulators carry a high risk of side effects in normal physiology. NMDAR modulators may impair cognitive abilities and certain situations when used in humans and in nonhuman preclinical research. The ketamine, an antagonist, as a fast-acting, potent

antidepressant. GluN2B are the archetypal subunit-selective medications. A fresh toolkit has been made available. The networks of neurons and receptor subtypes that support various brain activities and are involved in neurological disorders. This activity-dependent blockage gives functional circuitry selectivity for active receptors. According to estimates, the antidepressant response to ketamine equates to occupying roughly 30% of the entire NMDAR population in the forebrain at the dosage that is typically employed. GluN2B NAMs are notable for two reasons: they display positive interaction as activity-dependence, increased by GluN2B NAMs. The enhancement of synaptic plasticity, learning, and memory that results from GluN2B overexpression in the mouse forebrain. [11].

2.6 NMDAR Role in Synaptic and Learning

The majority of these "synaptic plasticity" at hippocampal excitatory synapses require the activation of a specific kind of glutamate receptor order to be induced. Even though GluN2B has fewer GluN2 subunits than it did during early development, it can still have a significant impact on LTP in the adult hippocampus [6].

NMDARs need to be unblocked by the depolarization of the membrane caused by AMPAR stimulation in order for efficient communication to occur. Glycine and glutamate must also bind to the receptor simultaneously. Typical glutamate neurotransmission can be mediated by AMPARs; at cell membrane resting potentials, external Mg2+ typically blocks the NMDAR channel. NMDARs are made up of two regulatory subunits that have the glutamate binding site and two GluN1 mandatory subunits that are expressed by a single gene and have eight variants that resulted from alternative splicing. The primary regulatory subunits in CNS areas implicated in cognitive processes, such as PFC are NMDAR subunits. In the end, these modifications seem to be reinforced by structural changes [8].

Long-Term Potentiation (LTP): This relates to the hippocampus, a portion of the brain that is closely associated with memory and learning. Deciphering the mechanisms of LTP is essential to understanding the secrets of cognitive health and illness [4].

LTP induction : NMDA receptors (NMDARs), which are specifically activated during the induction of LTP. Glutamate is a neurotransmitter that is released by presynaptic neurons during high-frequency synaptic activity. Concurrently, there is a phenomenon called postsynaptic depolarization, which is brought to the inflow of positively charged ions, mainly sodium (Na+) and potassium (K+). This depolarization releases the magnesium (Mg2+) block from the NMDARs.

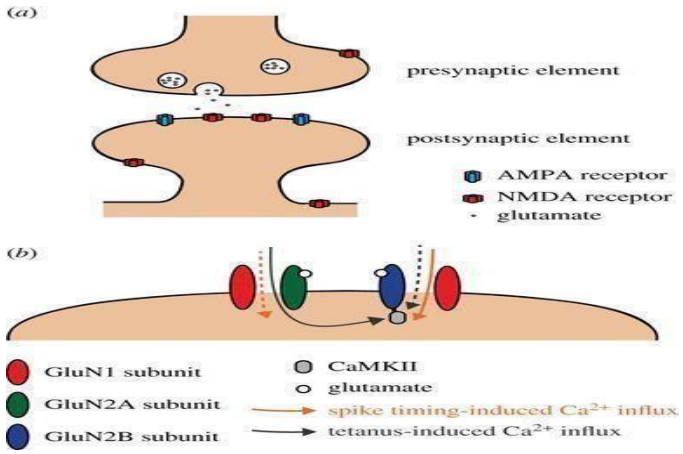

Fig 2.6: Location and subunits of NMDA receptors in synaptic plasticity. (a) Both pre-and postsynaptic NMDAR populations have been identified, receptors are located extrasynaptically, presynaptically, and synaptically in the postsynaptic membrane, where they probably serve a variety of purposes.
(b) Ca2+ influx through GluN2B subunit-containing NMDARs. A greater influx is triggered by tetanic activation via NMDARs (grey arrows) that carry the GluN2A subunit. This Ca2+ influx the GluN2B subunit's C-terminal. Both times, expression, indicating that the GluN2B subunit is present. Though GluN2B has fewer GluN2 subunits than it did during early development, it can still have a significant impact on LTP in the adult hippocampus.

Function AMPA Sense Organs :

Another subtype of glutamate receptors called AMPA receptors is essential for promoting postsynaptic depolarization in response to high-frequency stimulation. Most synapses' rapid excitatory neurotransmission is caused by these receptors.

Influx of Calcium (Ca2+)

NMDARs become permeable to calcium ions (Ca2+) and can enter the postsynaptic neuron after the Mg2+ block is eliminated. A crucial stage in the formation of LTP is the Ca2+ influx via the activated NMDARs.

Practical Importance of Ca2+ Inflow

LTP is expressed as a result of a series of intracellular signaling processes that are set off by the entry of Ca2+ ions into the postsynaptic neuron. As a second messenger, Ca2+ triggers the intiation of phosphatases, and kinases that are responsible for the molecular alterations that underlie synaptic plasticity.

Pathways for Intracellular Signaling

When Ca2+ attaches to calmodulin, conformational changes take place that make CaMKII catalytically active. Numerous synaptic proteins, including as AMPA receptors and other downstream effectors, are phosphorylated by activated CaMKII, which alters their location and function inside the synapse.

Synaptic potentiation during (LTP) is facilitated by phosphorylation i.e. AMPA receptors by CaMKII, which increases their conductance and encourages their trafficking to the postsynaptic membrane.

Activated CaMKII phosphorylates many synaptic proteins, changing their orientation and functionality inside the synapse, such as AMPA receptors and other downstream effectors. The activation of AMPA receptors by CaMKII promotes their transportation to the postsynaptic membrane and raises their conductance, which in turn facilitates synaptic potentiation during long-term potentiation (LTP).

2.7 The Mechanisms of Neuroexcitotoxicity

In many neurological illnesses, overactivation of glutamate receptors, especially NMDA receptors (NMDARs), causes neuronal damage and cell death. This abnormal process is known as neuro excitotoxicity [16].

The cause of this phenomena is a dysregulation of excitatory neurotransmission, in which the neuron's ability to maintain calcium homeostasis is overwhelmed by excessive glutamate release. Apoptosis, which is the final stage of neuronal death, is facilitated by a series of subsequent intracellular events that include calcium overload, oxidative stress, and mitochondrial failure. Comprehending the mechanisms that underlie neuroexcitotoxicity is crucial in order to devise treatment approaches that strive to alleviate neuronal impairment while maintaining brain functionality. The degenerative process known as neuroexcitotoxicity occurs when glutamate receptors are overstimulated to the point of injury and death in neurons. However, excitotoxicity, which is a phenomena linked to a number of acute and chronic neurological illnesses can be caused by dysregulation of glutamate transmission.The mechanisms underlying neurotoxicity are discussed below:-

Overdosage Calcium:

The disruption of calcium homeostasis is central to the pathophysiology of neuroexcitotoxicity. When NMDA receptors are activated by excessive glutamate release, calcium ions can enter the postsynaptic neuron. Calpains, endonucleases, and phospholipases are examples of calcium-dependent enzymes that are activated when intracellular calcium levels are persistently elevated, upsetting cellular homeostasis. These enzymes cleave DNA, membrane phospholipids, and cytoskeletal proteins, which ultimately results in cellular malfunction and death and damages neurons.

Mitochondrial dysfunction and oxidative stress:

Numerous neurodegenerative illnesses are linked to the intertwined processes of oxidative stress and mitochondrial dysfunction. These pathways contribute to the development of neurodegeneration. Gaining knowledge of the complex relationship between mitochondrial malfunction and oxidative stress can help create new therapeutic approaches and provide important insights into the pathogenesis of neurodegenerative disorders.

Oxidative Stress:

ROS, which can harm lipids, proteins, and DNA, are very reactive chemicals like H_2O_2 . Even though reactive oxygen species (ROS) are necessary for physiological functions like immune response and cell signaling, excessive ROS generation can overpower the antioxidant defenses of cells, resulting in oxidative damage and malfunction. Increased intracellular calcium concentrations, which are frequently the consequence of excitotoxicity, can cause neurons to produce ROS. The build-up of oxidative damage accelerates the development of neurodegenerative processes and worsens neuronal injury. Among the main characteristics of oxidative stress-mediated neuronal damage are lipid peroxidation, protein oxidation, and DNA damage, which ultimately result in neuronal malfunction and cell death.

Mitochondrial Dysfunction:

In the processes of cellular energy metabolism, calcium buffering, and apoptotic signaling, mitochondria are essential. A hallmark of neurodegenerative illnesses is mitochondrial dysfunction, which is characterized by reduced ATP synthesis, depolarization. When neurons experience calcium overload, their mitochondrial activity is disrupted, which causes dysregulation of mitochondrial bioenergetics and the start of apoptotic pathways.

The cellular energy supply is compromised by the reduction of mitochondrial ATP synthesis, which affects neuronal function and contributes to synaptic dysfunction. In addition, apoptotic cascades that result in the death of neuronal cells are initiated by the depolarization. In addition to intensifying oxidative stress, mitochondrial failure also increases neuroinflammatory reactions, which exacerbates neuronal damage and neurodegeneration.

2.8 NMDAR Dysregulation in Huntington's Disease

An inherited neurodegenerative disease is a mutation in the HTT gene causes an aberrant extension of polyaminoacid repeats in the coding region of the gene, which is the cause of HD. The huntingtin protein becomes more prone to misfolding and aggregation as a result of the elongated polyglutamine tract that is produced by the enlarged CAG repeat. Mutant huntingtin protein (mHtt) aggregates within neurons impairs cellular activity and adds to neuronal dysfunction and death [17].

The function of NMDAR Inhibition of Growth in HD Pathology

The pathophysiology of Huntington's disease now appears to be significantly influenced by NMDAR dysfunction. Excitotoxic neuronal injury, synaptic dysfunction, and ultimately neurodegeneration in HD are all influenced by dysregulated NMDAR signalling. NMDAR dysregulation in HD pathogenesis is caused by a number of important pathways, including:

Elevated Release of Glutamate

Excessive glutamate release leads to huntingtin protein mutations that interfere with synaptic neurotransmitter release this further discuss below:

Mechanisms of Elevated Release of Glutamate

Disturbance in the Release of Synaptic Neurotransmitters

A mutant huntingtin protein causes dysregulated glutamate release into the synaptic cleft by interfering with synaptic neurotransmitter release pathways. Disruption of presynaptic vesicle trafficking, compromised neurotransmitter release machinery, and changes in synaptic protein expression lead to synaptic dysfunction in HD. Excessive glutamate levels are present in the abnormal release of glutamate, which overrides the regular regulatory systems controlling synaptic transmission.

Reduced Absorption and Elimination of Glutamate

The buildup of extracellular glutamate in HD is caused by defects in glutamate absorption and clearance as well as modified release mechanisms. Excitatory amino acid transporters (EAATs), preserve extracellular glutamate levels. within physiological ranges. Glutamate-mediated excitotoxicity in HD is further exacerbated by diminished glutamate uptake by astrocytes and limited glutamate clearance from the synaptic cleft due to dysfunction. In HD, excessive glutamate release causes NMDARs to become overactivated, which prolongs calcium influx and causes excitotoxic neuronal damage.. On the other hand, aberrant NMDAR activity in HD compromises calcium homeostasis, triggers signaling pathways that support apoptosis and encourages damage and death of neurons.

2.9 Modified NMDAR Expression

HD has a well-established hereditary basis, but little is known about the particular molecular pathways behind neuronal malfunction and death. A key component of HD pathophysiology has been identified as dysregulated NMDAR signaling, with changes in NMDAR expression and subunit makeup being essential to the development of the disease.

Mechanisms of Modified NMDAR Expression :

In the striatum, one of the disease's damaged brain regions, GluN2B subunits of NMDARs in relation to HD pathology. GluN2B-containing NMDARs differ from GluN2A-containing NMDARs in their biophysical characteristics and are primarily found at extrasynaptic locations.

A contributing factor to synaptic dysfunction and neuronal susceptibility in HD is the overexpression of GluN2B subunits, which modifies NMDAR function neuronal excitability. Futhermore, the brains of HD patients express fewer GluN2A subunits of NMDARs in mutant HD brain. It mainly located at synaptic locations and serve a crucial part in recalling processes.

In HD, loss of the GluN2A disturbs regular synaptic function and compromises neuronal communication. It shifted in HD due to an imbalance between the GluN2B and GluN2A subunits, which modifies the subunits' functional characteristics and synaptic location. GluN2B has longer activation times, slower channel kinetics, and greater calcium permeability. The increased calcium influx, excitotoxicity, and neuronal damage caused by the preponderance of GluN2Bcontaining NMDARs at extrasynaptic locations in HD exacerbate the neurodegenerative mechanism. Dysregulated NMDAR signaling and synaptic dysfunction lead to neuronal loss and neurodegeneration [19].

Excitotoxicity, oxidative stress, mitochondrial dysfunction, and apoptotic signaling pathways cause neuronal injury and cell death, especially in vulnerable brain regions, like the cerebral cortex and striatum.

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CHAPTER 3 METHODOLOGY

3.1 Literature Review

Start with scholarly databases that are well-known for providing access to peerreviewed scientific publications. Among the most popular resources for academic articles in a variety of fields are PubMed, Google Scholar, and Web of Science. to find literature reviews that particularly address NMDARs about Huntington's Diseases. A mix of pertinent keywords like "NMDAR", "Huntington's Disease", "review", "neurodegeneration", and associated terms may be used for this. In the designated academic databases, type the prepared search query and carry out the search. Use the sophisticated search options and filters offered by the databases to narrow down the results according to relevance, article type, and publication date. Determine which literature review articles satisfy the inclusion requirements by analyzing the search results. Examine the papers' titles and abstracts to determine how relevant they are to HD NMDARs. Obtain the whole texts of the chosen literature review articles for additional analysis.

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Carefully go over each article to make sure it covers the subject in great detail, cites pertinent main research, and offers important new information on NMDAR dysregulation in HD.To find extra sources that might not have been found in the first database search, look through the reference lists of pertinent articles and review papers in addition to academic databases.Evaluating the selected literature review papers' quality and reliability entails taking into account various variables, including the journal's reputation, the authors' skill, the depth of analysis, and the correctness of the material offered.Maintain a file with the identified literature review papers, including with publication information, citation details, and any pertinent remarks or notes. Having this documentation will make it easier to reference and cite the sources in any further writing or research projects.

Clinical Evaluation

Targeting NMDARs in HD patients may offer therapeutic benefits, according to clinical research. Review articles provide an overview of the results of clinical trials examining neuroprotective medicines, glutamate receptor antagonists, and NMDAR modulators. Reviews of the literature assess clinical studies examining NMDAR modulators in HD patients, including ketamine, dextromethorphan, and memantine. A new era in the development of neuropsychiatric treatments marked by an antidepressant was brought about by the discovery of ketamine as a rapidacting antidepressant. [18]

They evaluate the efficacy, safety, and tolerability of the results and talk about the difficulties in patient recruiting and study design. Review papers address the application of glutamate receptor antagonists, such as amantadine in HD clinical

studies. They provide insights into the possible advantages and restrictions of these drugs by analyzing clinical endpoints, biomarker outcomes, and therapeutic responses.

Therapeutic Approach

The goal of therapeutic strategies that target NMDARs is to lessen HD's neurodegeneration and excitotoxic neuronal damage. Reviews of the literature assess the possible neuroprotective such as ketamine in HD patients.[18] . They highlight difficulties in dose optimization and long-term treatment, and they detail preclinical and clinical data about their efficacy and safety characteristics.[20] Some literature articles go over methods for modifying glutamate signaling pathways in HD to improve excitotoxicity and NMDAR function. They investigate the possible applications of glutamate transport enhancers, AMPA receptor antagonists, and metabotropic glutamate receptor agonists as medicinal therapies.

3.2 Retrieval of Ligand

Preparation and Conversion of The Structure of Ligand:

To guarantee consistency, structures need to be precisely translated into the right file formats and standardized. In order to efficiently manage and manipulate the chemical structures, these procedures require the use of cheminformatic tools. A tool like OPENBABEL are used to convert chemical structures between different file formats using Open Babel, an open-source cheminformatics toolkit. This tool converts chemical structures between PDB, SDF, MOL2, and PDBQT formats. By adding missing hydrogen atoms, adjusting valences, and guaranteeing correct stereochemistry, this technique aids in cleaning and standardizing molecular structures. Finding and extracting ligand molecules from resources such as DrugBank and PubChem is an essential part of the drug development processes.

3.3 Pharmacokinetics of Screened Compound

The Swiss Institute of Bioinformatics created the web-based service SwissADME, which offers pharmacokinetic analysis of medication candidates. With the use of numerous prediction models and computational methods, SwissADME provides insightful information on pharmacokinetic parameters such as excretion, metabolism, distribution, and absorption.

For the purpose of drug discovery and optimization, researchers in academia and the pharmaceutical sector frequently use this user-friendly, easily available platform. Swiss ADME is used to find the best drug candidate that inhibits NMDARc and satisfies the requirements often considered for molecular docking. When these drug compound structures are entered into SMILES, the chemical

formula for that specific drug molecule is displayed. screened or chosen the drug according to criteria like as absorption, solubility, and BBB. Briefly discussed ADME factor

Absorption

One of the basic pharmacokinetic processes that control the entry of medications into the bloodstream following administration is absorption. When it comes to medication research, knowing and anticipating a compound's absorption properties is essential to maximizing both its safety profile and therapeutic efficacy. Through the examination of its physicochemical characteristics, the computational tool SwissADME process offering insights into intestinal absorption and oral bioavailability of medication candidates. A drug's safety, therapeutic efficacy, and pharmacokinetic profile are all greatly influenced by its absorption. The physicochemical qualities of the drug molecule, the route of administration, and the physiological features of the absorption site are some of the elements that contribute to the complexity of absorption. Achieving appropriate systemic exposure and therapeutic benefits while lowering the risk of side effects depends on optimizing absorption. Factors including gastrointestinal transit time, permeability, and solubility are important in influencing a medicine's bioavailability when it comes to oral drug delivery. Specifically, by examining their physicochemical characteristics, SwissADME sheds light on the intestinal absorption and oral bioavailability of medication candidates. These characteristics, such The octanol-water partition coefficient (logP), which is frequently used to quantify lipophilicity, affects a drug's capacity to dissolve in biological membranes and pass through biological barriers.

Distribution

One of the most important pharmacokinetic processes, distribution controls how a drug gets distributed throughout the body and builds up in different tissues and organs. The physicochemical characteristics of the drug, plasma protein binding, tissue perfusion, and the existence of biological barriers like the blood-placental and blood-brain barriers are among the variables that affect distribution. Achieving therapeutic concentrations at the target site while reducing toxicity and off-target effects requires optimizing distribution.

For CNS-targeted therapeutics in particular, SwissADME offers useful methods for assessing drug candidates. It is a unique anatomical composed of inner lining, tight junctions, and transport proteins that regulate the movement of chemicals between the circulation and the brain parenchyma. The BBB's penetration is a

crucial factor in determining a medication's capacity brain and treat neurological conditions including Huntington's disease (HD).

SwissADME uses computer models to forecast BBB penetration chances based on pharmacological candidates' physicochemical characteristics. These characteristics include molecule size, hydrogen bonding ability, lipophilicity, and the existence of substrates for efflux transporters. To direct medication research efforts and improve formulations to increase CNS penetration, this information is extremely helpful. Distribution of NMDAR-targeted treatments for Huntington's disease (HD) is critical to attaining therapeutic success. Progressive neurodegeneration, which largely affects the striatum, is a characteristic of HD. The advancement of HD illness and excitotoxic neuronal injury are both influenced by dysregulated NMDAR signaling.

Metabolism

The term "metabolism" describes how a medicine is biotransformed within the body, mostly in the liver and other metabolic organs. It involves enzymatic processes that change the parent drug's pharmacological activity, toxicity, or excretion characteristics into metabolites. A drug's pharmacokinetic profile is influenced by metabolism. Bioavailability, half-life, and systemic exposure are important factors that determine both therapeutic efficacy and safety. These factors can only be evaluated by having a thorough understanding of a drug's metabolic pathways and kinetics. Researchers can evaluate the metabolic stability of medication candidates and detect potential metabolic liabilities by using SwissADME's useful tools for forecasting their metabolic fate. The platform uses computational models and algorithms to anticipate a compound's vulnerability to enzymatic metabolism by analyzing its chemical structure.Optimizing drug elimination patterns can help reduce the danger of buildup and toxicity in the setting of NMDAR-targeted therapeutics for Huntington's disease (HD), improving therapeutic efficacy and patient safety.

Excretion

Pharmacokinetics' basic mechanism of excretion is essential for eliminating medicines and their metabolites from the body and preserving systemic balance. SwissADME is a computational method that offers useful insights on drug candidate excretion pathways, such as hepatic, biliary, and renal clearance. Maintaining systemic equilibrium and removing medications and their metabolites from the body depend on the fundamental excretion mechanism of pharmacokinetics. A computer technique called SwissADME provides helpful information on drug candidate excretion pathways, including renal, hepatic, and

biliary clearance. Anticipating the routes and rates of drug removal is essential for optimizing dosing regimens, guaranteeing safe and effective treatment outcomes, and understanding clearance kinetics. Adequate CNS exposure is necessary for effective treatment, but systemic exposure and toxicity should be kept to a minimum. Inhibiting hepatic transporters or drug metabolizing enzymes to lower hepatic clearance, extend systemic exposure, and improve treatment efficacy in HD patients.

3.4 Retrieval of Target Protein

Choosing the right targets and creating efficient therapeutic interventions are made possible by the foundation that is laid during the initial data collection stage of the molecular docking process. This process entails compiling and examining data from several scientific sources in order to develop a thorough grasp of the illness mechanism and possible treatment strategies. Few steps to follow while identify an target protein responsible for diseases given below:

(a) Review of the Literature:

Scientific Journals: Peer-reviewed publications offer the most recent information on therapeutic approaches, possible medication targets, and illness causes. These articles frequently include thorough experimental findings and theories that can help with target selection for molecular docking.

Examine Articles: These articles provide an overview of the state of knowledge and point out gaps that can be filled by future studies by summarizing and synthesizing the available research on particular subjects.

Choosing the Target Locations:

Protein Structures: From databases such as PDB identify and retrieve 3D dimensions of target proteins. One important target in HD research is the NMDA receptor (PDB ID 5EWJ). Potential binding sites on the target protein where medication compounds may interact are examined. This entails comprehending the active site, allosteric sites, and any additional pertinent binding areas of the protein.

(b) Therapeutic Target site:

The NMDA receptor plays an important in neuro excitotoxicity, a process that results in neuronal damage and death, in Huntington's disease. By facilitating the entry of calcium (Ca^{2+}) ions into the neuron, these receptors play a crucial role in initiating intracellular signalling cascades that fortify synaptic connections.

Both the creation and maintenance of memory depend on the appropriate operation of NMDA receptors. PDB ID 5EWJ designates protein structure selected for molecular docking investigations. The GLU1 (GluN1) and GLU2B (GluN2B) subtypes, which necessary for brain signal transmission, are represented by this structure. PDB is an essential resource for 3D structural data of biological macromolecules. For molecular docking research, it provides precise atomic coordinates of complex assemblies, proteins, and nucleic acids.

The protein of interest is the NMDA receptor subtype, which includes both the GluN1 and GluN2B subunits. Its PDB ID is 5EWJ. Using the PDB Website Navigation: Visit the https://www.rcsb.org website to access the RCSB PDB. Enter the PDB ID (5EWJ) in the search bar. Examine the structure's complete details and summary on the PDB entry page for 5EWJ.

Choose "Download Files" after clicking on it. To download the file, click the "Download Files" tab and choose "PDB format." To utilize the PDB file for molecular docking later on, save it in PDB Format. Every atom in the protein structure has its complete set of atomic coordinates listed in the PDB format. Information on Secondary Structure Specifics about the constituents of the secondary structure, such as loops, beta sheets, and alpha helices. Water molecules and Heteroatoms: The file also contains details on any cofactors, bound ligands, and water molecules, which may be crucial for docking studies, but they might have to be processed or eliminated. Heteroatoms and water molecules are removed in order to prepare for molecular docking. Furthermore, structures is prepared using PyMOL for this Load the PDB file (5EWJ) , downloaded into PyMOL. Herein, to eliminate every water molecule, use the remove solvent command. Carefully Examine the structure visually to determine which heteroatoms are not necessary. To get rid of every heteroatom, use the command remove hetam. Should a portion of the heteroatoms include the binding site or are necessary for the stability of proteins; delete the others and keep the other followed by saving filename PDB command to save the altered structure in PDB format.

3.5 Molecular Docking

3.5.1 Preparation of Protein Structure:

Structural Resolution: The NMDA receptor's 3.D structure, which has a resolution of 2.27 Å, offers precise atomic coordinates necessary for molecular docking. The precise details of the receptor, such as the active site and possible binding pockets, are guaranteed to be well-defined by this high-resolution structure.

3.5.2 Ligand Preparation Database Retrieval

PubChem and DrugBank are two databases that offer extensive information on chemical compounds and their biological properties. These databases are used to obtain ligands. The three-dimensional structure of the ligands is first obtained in 3D SDF format. Docking programs such as PyRx require the PDBQT format since it contains partial charges, torsional degrees of freedom, and atomic coordinates. Open the SDF file in 3D.

3.5.3 Molecular Docking

Software Selection: PyRx is picked because of its robust docking capabilities and user-friendly interface. It incorporates AutoDock Vina, a popular docking program renowned for its precision and effectiveness.

Protein and Ligand Files: PyRx is loaded with the prepared proteins (in PDB format) and ligands (in PDBQT format).

3.5.4 Docking Analysis:

The NMDA receptor is docked to each ligand. Based on the molecular interactions that occur between the ligands and the protein, the software calculates the binding affinities. Values for each ligand-receptor interaction's binding energy are included in the results. Whereas higher the negative binding affinity more strongly it bind each other. Higher negative energy show high binding affinity.

3.5.5 Visualisation of Protein and Ligand Interaction

Analysing protein-ligand interactions and assisting in the identification of chemical and biological materials are made possible by molecular visualization tools like PyMOL, Biovia identification Studio, and Chimera. With the use of these instruments, scientists can see intricate molecular structures, comprehend how they are arranged spatially, and examine the types of interactions that occur ligands and proteins.

Chimera is an effective molecular visualization tool that is frequently utilized in drug discovery and structural biology. It makes it possible for scientists to view and examine macromolecular structures in three dimensions, such as those of proteins and nucleic acids. Some of Chimera's salient features are Protein structure visualization: Chimera can import protein structure files (such as those in PDB format) and show the structures in a variety of ways, including ribbon, surface, and ball-and-stick. Analyzing protein-ligand interactions and assisting in the

identification of chemical and biological materials are made possible by molecular visualization tools like PyMOL, Biovia identification Studio, and Chimera.

With the use of these instruments, scientists can see intricate molecular structures, comprehend how they are arranged spatially, and examine the types of interactions that occur between ligands and protein. Biovia Discovery Studio is a full-featured software suite made for computational chemistry and drug discovery applications including molecular modeling and simulation. It provides cutting-edge resources for researching protein-ligand interactions and logical medication formulation. Researchers can examine and visualize interactions between proteins and ligands in both two and three dimensions using Discovery Studio. Hydrophobic interactions, hydrogen bonding patterns, and binding pockets can all be explored by users. By generating 2D and 3D conformations of protein-ligand complexes, the software aids in the evaluation of the stability and energetics of various binding modalities by researchers Futhermore, Docking scores and binding affinities are used by Discovery Studio to help identify possible therapeutic candidates by virtual screening compound libraries against protein targets

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CHAPTER 4

RESULT AND DISCUSSION

4.1 Retrieval of Protein

NMDA receptor was selected for investigation since it contains the subunits GLU1 (GluN1) and GLU2B (GluN2B). PDB is a repository database for extraction of 3D structure data of biomolecules like protein and nucleic acid etc. for retrieve the protein structure i.e NMDAR subunit, firstly visit to RCSB PDB website (https://www.rcsb.org/) herein, on search engine find ID "5EWJ", download the protein structure with subunit GLU1/GLU2B which play role in synaptic plasticity in PDB format. Once downloaded it further go into removing the unwanted heteroatom like ions, atom and water molecules as these hetroatom will distrup in further docking steps and protein is prepapred. Generally, To guarantee that the protein structure is in the best possible state for accurate simulations, PyMOL protein preparation is a crucial step in computational investigations like molecular docking. Its a powerful tool used to visualize structural biomolecules. Here firstly, PyMol is downloaded on a computer and with input command, the file should be loaded i.e. load 5WEJ.pdb with this protein structure is loaded into its workspace. Herein, visualise the structure carefully by rotating,zooming showing ligands bound with them or sites of water molecules attached on it.

After carefully visualizing water molecules deleted or removed out by writing a command i.e. remove solvent which will remove all water molecular from protein structure. Same as with the heteroatom command should be written as remove hectare which will remove all the atoms from the protein chain and save them.

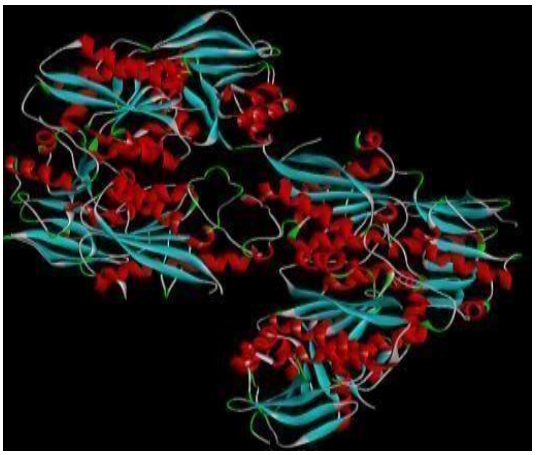

Figure 4.1 5WEJ (PDB ID)

4.2 Ligand Extraction

Firstly FDA approved drug, Ketamine taken as reference drug with respect to other drug. In search for drugs or compound which can be repurposed for disease like huntington particulaary for inhibiting an NMDAR subunits which will bind to their structure and inhibit theor disease progression. Meanwhile method for studying the pharmacological of drugs and retrieved theor structure for futher step p.e docking should be undertaken in some steps like Pubchem database used for protein structre and drugbank for understanding the pharmacology of drugs . Drugbank basically an comprehensive resource of drug library that have details about medications, such as their pharmacological characteristics, targets, indications, and chemical structures (https://go.drugbank.com/). For this study, 50 drugs selected with keywords NMDAR, anti-histamine, and anti-psychotic. Numerous anti-psychotic medications are included in DrugBank, which provides comprehensive details on their pharmacokinetics, modes of action, side effects, and drug interactions.These anti- psychotic drug also referred to as neuroleptics or antipsychotics, or anti-psychotic pharmaceuticals, are mostly used to treat the symptoms of mental illnesses such schizophrenia, bipolar disorder, and psychosis, are employed to treat psychotic disorder symptoms such hallucinations, delusions, and disordered thinking. On account these drug every drug studied carefully espically there pharmacology. Sometimes, Anti-psychotic drugs may occasionally be combined with other medications or psychosocial therapies in order to maximize therapeutic results. After selecting an drug, structure can be dowmloaded in FASTA or SMILES format and save it. With access to chemical and biological data, it is an valuable tool for researchers. It offers details on biological activity, toxicity, safety, and chemical structures and properties. Herein, drug can be searched either using there name or its CID i.e. compound identifier (in numerical form). Furthermore, here will see information of drug related like its 2D OR 3D structure, chemical safety (LCSS Datasheet), their molecular formula and weight, synonyms of same drugs and its description after studying all its pharmacology, the chemical structure of the compound can be downloaded in form of 3D conformer in SDF format.

4.3 Docking Results

A small molecule that is being studied for its ability to bind to a particular target macromolecule—typically a protein—is referred to as a ligand in the context of molecular docking. Predicting the ideal ligand orientation, binding affinity, and interactions inside the target protein's binding site is the aim of docking. The ability of ligands to interact with the target protein is the basis for their identification and selection. These may include medications, substrates, inhibitors, or any other bioactive compounds pertinent to the illness or biological process under investigation. Docking simulations are used to calculate the ligand's binding affinity for the target protein.An connection that is more permanent and advantageous is often indicated by a lower binding energy. Molecular docking

offers a comprehensive understanding of the particular ways in which the ligand and the protein interact. Before docking, the protein and ligand must be prepared for this ligand format, which means they must be converted into a PDB file format. On the other hand, a protein is made by extracting a hydrogen atom, locating the active site, and eliminating ions and water molecules. For protein preparation, a software PyMOL is used for molecular visualization that can also be utilized for activities related to protein production. Before docking, it allows users to optimize protein structures, add hydrogen atoms, and delete water molecules. Whereas Ligand is prepared by OpenBabel, an flexible chemical toolkit that can improve molecular geometries, translate different chemical file formats, and execute molecular structure transformations. After preparation of ligand and protein successfully , molecular docking performed. Software used for molecular docking is PyRx used to docked the protein and ligands. In general, it Predict binding affinity and stability by utilizing scoring systems to assess various ligand poses. Common scoring functions take steric fit, hydrogen bond formation, and binding energy into account. Herein, 50 compound were docked together in which 19 compounds were comes with optimum results with this ought to find out best compound with their highest binding affinity scores. Furthermore Ketamine taken as a reference or controller drug with respect to all other durgs. When Ketamine docked with NMDAR its result with -6.0 Kcal/mol binding affinity and common interacting residue were GLU236, TYR115, ARG115, ASP211, ILE216, LEU209, PRO340, MET207, ILE335.

Two hydrogen bond between form with LEU209(2.38 Å) and MET207 (2.91 Å). When all 19 compound docked with NMDAR its showed up that Aripiprazole showed highest binding afiinity (-11.0 Kcal/mol) and Flupentixol showed with 7.3 Kcal/mol with respect to NMDAR compared to Ketamine with this these compound also interacted with NMDARA residues such as ASP210, MET207, PRO340, ASP211, GLU236 which also have common three hydrogen bonds with Aripiprazole and Flupentixol. Aripiprazole and Flupentixol have the highest binding affinity and are hence attractive drugs for NMDAR-targeted therapy. According to the in-depth interactions and hydrogen bond forms, these substances may be useful in regulating NMDAR activity, which may have therapeutic advantages for diseases like Huntington's disease

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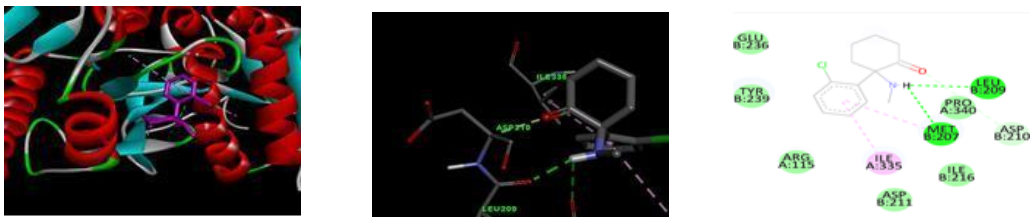
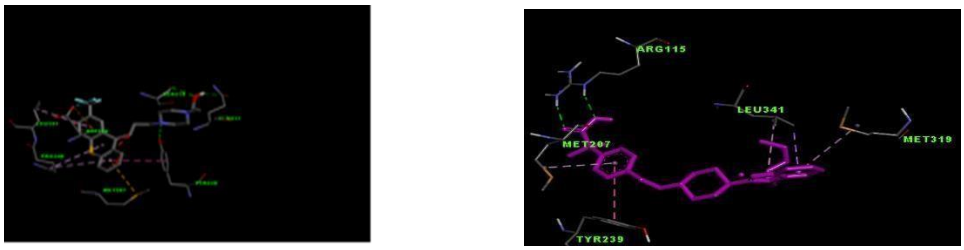
TABLE I TOP DOCKING ANALYSIS OF COMPOUNDS with NMDAR(5EWJ)

Compounds	Docking energies	H-bonds distance	Interaction
Ketamine (Reference drug)	-6.0	LEU209(2.38Å)Met 207(2.91Å)	GLU236, TYR239, ARG115, ASP211, ILE216, ASP210, LEU209, PRO340, MET207, ILE335
Penbutolol	-6.8	Met 207(2.67 Å)	ILE335, ALA107, GLN105, LEU135, LEU209, PRO340, MET207, GLU106, ARG115, TYR239, GLU236, GLU235
Flupentixol	-7.3	GLN 217(2.69 Å)SER214(2.23 Å)TYR239(2.80 Å)	ASP210, GLN217, SER214, GLU242, VAL243, GLY212, ASP211, TYR239, MET207, PRO340, LEU341, LYS337, THR338
Chlorpropamide	-7.4	GLN110(2.83 Å)GLU106(2.81 Å)ARG115(2.31 Å)	ARG115, LEU135, PHE176, GLN110, GLU106, ALA107, PHE113, THR176, MET207, GLU236, SER132 TYR175, LYS131,
Hydrocortisone Acetate	-8.1	ARG115(2.81 Å)ASP211(2.05,2.36 Å)	ARG115, ASP211, ILE335, GLU235, ASP210, LEU209, TYR239, PRO340, LYS337, MET207, GLU236, TYR109
Bilastine	-6.4	THR338(1.93 Å)	ASN321, LEU341, ASP210, ASP211, GLY212, THR323, THR338, LYS3387, MET207, LEU209, TYR239
Aripiprazole	-11	TYR175(2.35 Å)GLU236(1.69 Å)ARG115(2.71 Å)Å)	TYR175, SER132, LYS131, PRO177, PHE176, LEU135, ALA107, GLN110, PHE113, TYR109, ILE111, PHE114, ALA75, PRO78, THR110, ILE82, GLY112, ARG115, GLU106 THR233, GLU236, MET207, THR174
Amlotriptan	-5.9	_	PRO340, ILE335, GLUE235, GLU236, THR233, ARG115, GLU106, THR333, ALA107, PHE113, TYR239, LYS337
Viloxazine	-6.7	Gln110(2.70 Å)	ILE111, LEU135, PHE176, GLN110, GLY112, ALA107, GLU106, ARG115, PHE113, ILE133, TYR109

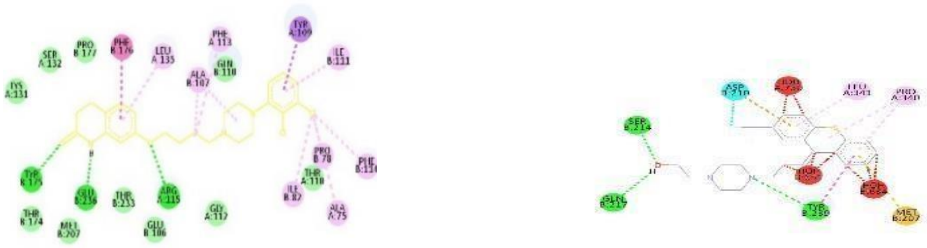


(a) (b) (c)

Figure 4.2: Pattern Interaction of Ketamine with NMDAR (a). The binding site of the active site is visible in the violet structure. (b) The reference drug's binding pattern is depicted in the 3D model. (c) The 2D model illustrates the interaction between amino residues; the alkyl ring and conventional hydrogen bonds are indicated by a dark green color.



(a) (b)



(c) (d)

Figure 4.3: Pattern interaction with Aripiprazole and Flupentixol. (a) displays the aripiprazole 3D structure in conjunction with NMDAR. (b) Flupentixol's 3D structure using NMDAR. (c) depicts the two-dimensional (2D) structure of NMDAR's aripiprazole, with dark green standing in for normal hydrogen bonds, pink for alkyl and dotted purple for Pi-Sigma. (d) A normal hydrogen bond is represented by the color dark green, a halogen (fluorine) by the color blue, and a pi-sulfu

4.4 Pharmacokinetics of Ligands

After selecting and downloading the anti-histamine and anti-psychotic drug compounds from pub chem and Drug bank. These are further analysis by using SWISSADME tool, its an online tool which is freely accessible, forecasts the pharmacokinetic characteristics and drug-likeness of small compounds. web browser to access the SwissADME website at (https://www.swissadme.ch/) , here enter the compound structure convert in the form of smiles format and run which drawn the molecular formula of that particular drug compound. Herein, save and noted the information of drug like ADME kinetic properties i.e. Absorption, Distribution, Metabolism, Excretion.

Larger the molecular size poor the penetration via cell membrane. GI absorption

solubility in digestive fluid directly influence in oral delivery. Distribution provide valuable insights into drug distribution, especially for CNS-targeted therapies, by predicting BBB penetration and helping optimize the pharmacokinetic profiles of drug candidates.. The pharmacokinetic properties and therapeutic potential of drug candidates can be enhanced with the aid of SwissADME's evaluation tools for these pathways and at last Excretion sheds light on the excretion mechanisms of potential drugs.

Predicting the paths and speeds of drug elimination is an essential part of the pharmacokinetic process, which helps the body remove medicines and their metabolites while preserving systemic equilibrium. Pathways including hepatic (liver), biliary, and renal (kidney) clearance are involved in this process. researchers can enhance patient safety and therapeutic effects for CNS-targeted therapies by optimizing medication excretion characteristics. Using the SWISSADME toolbox, 19 pharmacological compounds were tested in this instance to demonstrate the favorable response of an appropriate treatment with regard to neurodegenerative illnesses like Huntington's disease, specifically NMDAR.

TABLE.II Outcome of pharmacokinetics properties of Drug Candidates

parameter		Aripiprazole	Pitoli-sant	Azatadine	Remoxipride
Physicochemical parameter	Formula	C23H34C12N302	C17H30C1NO	C20H33N2	C16H26BrN2O3
	Molecular weight	456.45	2.99.88	302.50	375.3
	Molar refractivity	131.49	12.47	3.24	0.88
	TPSA	52.65	12.47	3.24	58.64
Lipophilicity	LogPo/w(ILOGP)	0.00	0.00	0.00	0.00
	Logpo/w(XLOGP3)	0.99	3.15	2.39	0.96
	Log Po/(WLOGP)	1.46	3.13	2.88	0.65
	LogPo/w(SILICOS-IT)	3.25	4.23	2.45	1.14
Pharmaco kineti c	GI Absorption	High	high	high	HIGH
	(BBB)	yes	yes	yes	NO
	Log Kp(Skin permeation)	-8.38	-5.89	-6.5	-7.91
Wate r. solub le	Solubility	Solubl e	Soluble	Soluble	Soluble

Cont…

Parameter		Compound inhibitors of NMDAR				
		Acrivastine	Viloxazine	Amlotriptan	Bilastine	Erlotinib
Physicochemical parameter	Formula	C1626BrN2O3	C22H23NO3	C15H26F02	C28H48N303	C22H31N3
	Molecular weight	363.56	242.33	257.36	475.71	404.52
	Molar refractivity	119.49	70.69	72.93	155.1	121.94
	TPSA	43.7	55.4	40.46	65.29	49.28
Lipophi-licity	LogPo/w(ILOGP)	0.00	0.00	-8.7	0.00	0.00
	Logpo/w(XLOGP3)	2.07	0.00	4.99	2.33	-1.09
	Log Po/(WLOGP)	3.24	0.51	4.44	3.46	0.54
	LogPo/w(SILICOS-IT)	2.49	0.00	3.68	-5.92	1.36
Pharmac o-kinetic	GI Absorption	high	low	high	high	high
	(BBB)	yes	no	yes	yes	yes
	Log Kp(Skin permeation)	-7.05	0.00	-4.33	-9.54	-9.54
Water. soluble	Solubility	soluble	mild soluble	moderate soluble	high soluble	high soluble

DISCUSSION

When the neurotransmitter glutamate and co-agonist glycine activate the NMDARs, which are ionotropic glutamate receptors, calcium (Ca^{2+}), sodium (Na^{+}), and potassium (K^{+}) ions can pass through the cell membrane. Heterotetrameric complexes known as NMDARs are normally composed of NR1, NR2, and NR3. Every kind of subunit adds distinct functional characteristics to the receptor. The physiological functions and expression patterns of these subunits (A–D) differ. The two most researched, NR2A and NR2B, each contribute differently to excitotoxicity and synaptic plasticity. When present, NR3 subunits lessen the Ca2+ permeability of the receptor and have the ability to alter its activity, which frequently has neuroprotective effects.NR2A-containing NMDARs are mostly expressed in mature synapses and are important in synaptic plasticity and rapid synaptic transmission. They carries a procedure that is necessary for memory and learning.In adult neurons, these subunits are more common in extra-synaptic locations and during development.

Although NR2B-containing linked to neurotoxicity because of their involvement in excitotoxicity, a condition in which an excess of glutamate causes damage to and death of neurons.When these subunits are present in NMDAR complexes, they decrease Ca2+ permeability, which modifies the receptor's receptor's typical ion channel functions.Because of their involvement in glutamate-mediated excitotoxicity, NMDARs play a crucial role in HD.

Excessive Ca2+ influx in HD is caused by dysregulated NMDAR activity, which sets off mitochondrial malfunction, and the activation of apoptotic pathways, all of which contribute to neuronal death.Higher glutamate sensitivity and increased vulnerability to excitotoxic damage in HD have been associated with increased expression and activity of NR2B-containing NMDARs. Because of its established interaction with the NMDAR, ketamine was utilized as a reference medication in this investigation. Using the protein's crystal structure (PDB ID: 5EWJ), docking simulations were run. Ketamine formed two hydrogen bonds with residues LEU209 and MET207, exhibiting a binding energy of -6.0 kcal/mol. Other antipsychotic medication possibilities were compared against these interactions as a baseline. Crucial Results of Docking Studies: -7.3 kcal/mol for the binding energy of fluentixol. Three hydrogen bonds were formed, a sign of stable binding interactions.

The common residues are MET207, GLU236 and ARG115. Showed a steady interaction but was less soluble than aripiprazole in terms of stability. Aripiprazole had the highest binding energy of all the substances studied, at -11.0 kcal/mol,three

hydrogen bonds were formed, each at a distance of 2.32 Å, 1.69 Å, and 2.72 Å where it have robust blood-brain barrier (BBB) crossing capacity, low toxicity, and high solubility.

It can also be effectively absorbed via the gastrointestinal (GI) tract. Common interacting residue demonstrated a steady contact with important residues like MET207, ARG115, and GLU236, among others.s Whereas binding of Cariprazine Energy in kcal/mol is -10.3 formed no hydrogen bonds with NMDAR, which resulted in a weaker interaction than that of aripiprazole compared to aripiprazole, it has a poorer binding and a lower potential for therapeutic use, even if it can pass the blood-brain barrier.

CONCLUSION

This work highlights the potential of aripiprazole as a treatment agent for HD by inhibiting the NMDA receptor (NMDAR). NMDAR are important for neural plasticity and synaptic transmission; nevertheless, overactivation of these subunits causes excitotoxicity, which is a major contributor to HD pathogenesis. Aripiprazole has a strong binding affinity and forms stable interactions with important residues in NMDAR, according to research using molecular docking. Aripiprazole has better binding affinity than ketamine, an FDA-approved NMDAR antagonist, and it also has better pharmacokinetic characteristics, like high solubility, low toxicity, and blood-brain barrier crossing. The results imply that the inhibition of NMDAR activity by aripiprazole may help lessen the excessive calcium influx and consequent cellular death that is specific to HD. Aripiprazole's strong binding affinity and sustained interaction with NMDAR, as predicted by computational models, require extensive preclinical and clinical investigations to validate. Subsequent investigations have to prioritize in vivo analyses to appraise the neuroprotective properties of aripiprazole, succeeded by clinical trials to appraise its safety and effectiveness in people with HD. Aripiprazole's targeting of NMDARs is a viable strategy to lessen excitotoxic neuronal damage in HD, thereby improving patient outcomes and quality of life. This study emphasizes the possibility of repurposing currently available medications with established safety profiles for novel therapeutic uses, which could hasten the creation of efficient cures for difficult-to-treat illnesses like HD. The encouraging computational outcomes offer a solid basis for additional research and the creation of aripiprazole as a cutting-edge HD treatment.

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Computational Approach for Prediction of NMDA antagonist in Huntingtin's Disease

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***Abstract*- NMDA-R is a type of glutamate receptor that meditates neurotransmission in the central nervous system (CNS) through its three subtypes (GLU1, GLU2, GLU3). In Huntington's disease patients, mutations in the huntingtin gene lead to abnormal CAG repeats that result in polyglutamine aggregation at the end of the N-terminal region of the huntingtin protein. This causes overexpression of glutamate and delocalized NMDA receptors, leading to overexpression of dopamine hormone. Eventually, this leads to chronic and psychiatric disorders. Using computational tools for repurposing the drugs and predicting the anti-psychotic drugs against NMDA receptors. We performed molecular docking through PyRx-vina, where a 3D model structure with PD ID 5EWJ, dock with 50 compounds analyzed inhibition probability scores. Furthermore, Biovia Discovery Studio used for protein-ligand interaction analysis, followed by SWISS ADME analysis for pharmacokinetics, drug properties, and blood-brain barrier permeability Aripiprazole and Flupentixol come with positive outcomes that can inhibit the NMDAR when compared to the reference drug i.e. ketamine which is used as an antagonist for NMDA receptors.**

Keywords- Huntington's disease, NMDA-receptor, molecular docking, disorders, Drug repurposing, drug design, antipsychotic drugs, Ketamine

I. INTRODUCTION

Huntington's disease (HD) is caused due to polygenic, autosomal disease where a wild huntingtin gene translated into mutant huntingtin protein that leads to abnormal polymorphic CAG repeat results in polyglutamine(polyQ) aggregation of mutant huntingtin (Htt) protein [3]. It mainly affects the striatum of GABA which is chiefly an inhibitory neurotransmitter that is generally synthesised by glutamate of GAD in striatum. [13]. Dysfunction of the nervous system, results in neuronal loss and disabilities in speech and communication, etc. MHtt protein is linked to the maturation of neurons and loss of function which directly results in symptoms of disarray. [12]. Normally Ca2+generally plays the role of physiological function as signal transmission under homeostatic regulation activity Ca2+ ion channel, mutation causes an increase number glutamate releases and its subunits NR3 change permeability which leads to Ca2+ ion entering increasingly leads to excitotoxicity due to this, neuronal damage occurs (caudate and putamen region) apoptosis. MHtt protein represents abnormal repeats of CAG, where the number of glutamates is in a high ratio. Glutamate receptors are widespread and result in abnormal mechanisms including chronic, psychosis, neuronal development, and calcium ion channels. Overexpression of glutamate in mutated protein leads to excitatory neurotransmitters that overexpress NMDA receptors in the central nervous system, which will be delocalized and cause motor dysfunction, and psychotic behavior [8].

Dopamine, is a hormone that will lead to agitation, irritability anxiety, hallucination, and delusions like psychotic behavior and jerky movement [6]. NMDA-R (N- methyl-D-aspartate) receptor is associated with 3 subunits: NR1, NR2, and NR3 (rare), where NR2 possesses receptors that usually express predominantly to the stadium and hence have a therapeutic approach to combat their expression as these subunits (NR1, NR2) a heteromeric complex, important for the receptor to become functionally active. ketamine is an FDA-approved drug originally used in aesthesia to patients and an anti-depressant, for inhibition of NMDA secretion—Ketamine is taken as a reference drug for computing existing drugs against NMDR [1].

II. REVIEW OF LITERATURE

NMDA receptors play a role in neuronal flexibility i.e. memory function deals with the ability of the brain to re-wire itself and its effects lead to neuro excitotoxicity [8]. Primarily Excessive release of Glutamate plays a role in excitatory plasma membrane neurotransmitter cause toxicity. Excitotoxicity leads to loss of neurons and cell apoptosis. There have been many mechanisms responsible for neuronal cell death such as calcium channels, oxidative stress, and cascade events downstream of glutamate receptors.[9]. NMDA receptors consist of genes such as GRIN1 and GluN2A-2D) that play a role in protein formation which is further participates in cellular signaling. Defects in different receptors lead to motor dysfunction and psychotic behavior [10]. NMDAR is made up of heteromeric tetramers of two subunits GluN1 and GluN2. Herein, each of the GluN subunits possesses the same structure that is made up of four semi-autonomous domains. The GluN1 is expressed in the central nervous system. Expression of GluN2A and GluN2B differ by

forebrain interneuron meanwhile, GluN2A has comparatively higher expression in the median ganglionic. Whereas Glun2B has higher expression in the central ganglionic region. [11]. Hence GluN2A and GluN2B possess therapeutic properties to inhibit psychotic behavior in huntingtin diseases. Ketamine is an FDA-approved antagonist drug for NMDAR that is used to target symptoms like chronic pain, which is then used as a controller or reference drug concerning other drugs [11]. SwissADME is a web-based server that generates a piece of information related to pharmacokinetics i.e. drug distribution, metabolism, excretion, absorption in the body. Hence swissADME tool for identifying the molecules in drug discovery. Previously available drugs are generally considered safe when repurposing in people and further analysis there new properties which are usually used to treat diseases or conditions with available drugs.

Computational tools like molecular docking are used to docked diseases related to 3D Structured proteins and ligands and the result is noted down carefully. The docking is generally done for available drugs i.e. repurposing of pre-existing drugs particularly those whose treatment is under research or not available. The in silico method includes swissADME for extraction of drug and protein, screening for drugs, molecular docking, and high content screening for protein targets.

III. METHODOLOGY

A. Pharmacokinetics of screened compound analysis

Swiss ADME Online tool is used to predict the ADME (absorption, distribution, metabolism, excretion) properties that play a role in any drug development. Swiss ADME is used to generalize the top candidate for inhibition of NMDARc Drug which meets these properties usually taken for molecular docking. In these structures of drug compounds entered in the forms of SMILES, when run it shows the chemical formula of that particular durg compound. Screened/select out the compound based on the factors like BBB, solubility, and absorption.

B. Retrieval of target protein:

Data was first collected through some preliminary studies like literature and journals which give an insight view of therapeutic approaches for rare diseases hence target sites were selected i.e. NMDA, structural protein (5EWJ) retrieved from Protein Data Bank (RCSB PDB) in PDB format files. 5EWJ, PDB ID that protein which possesses both subtypes of GLU1, GLU2B which is responsible for transmission signal. These further prepare for docking step for this heteroatoms and water molecules removed out from protein. Binding site is further identified using PyMOL, visulising tools.

C. Active site Prediction:

To know about protein compound active site for predicting an active site for ligand . active site is usually predicted for analysis there are many but here FTsite a technique to predict an active site of the protein.

D. Retrieval of ligands

To search for a potential ligand, the drug is extracted from the library of Drug Bank and PubChem database which further extracted in 3D structures of drug compounds in the SDF file format. OpenBabel is open-source file converter software that converts 3D SDF file format into SMILES strings as it is used to convert into SwissADME to convert into the chemical structure of that drug compound.

E. Molecular Docking:

Protein 3D Structure of NMDA, PDB ID: 5EWJ, 5WEJ has a resolution of 2.27 Å that is retrieved from PDB databases designations. Later it is prepared by removing unwanted water molecules by using PYMOL. Ligand retrieved from PubChem and Drug Bank database in 3D SDFs file format which was further converted to PDBQT files by OpenBabelGUI. After ligand and protein are prepared, PyRx software (tool for molecular docking) is conducted for docking protein against all drugs i.e. ligands. By selecting of protein in PDB file format and the ligand in PDBQT format. results of all docks were obtained with their high and low binding energy. Herein, also docked our reference drug i.e. Ketamine as a controller for a better understanding of the biochemistry of protein and ligand interaction.

F. Analyse the protein-ligand complex

Molecular visualization tools like Biovia Discovery Studio, Pymol, and Chimera are used to analyze the protein-ligand complex interaction about the discovery of chemical and biological material. The structure of ligand and protein was visualized by Chimera software whereas these files were converted into pdf format where Discovery Studio was used to visualize the protein and ligand binding and further used to create 2D and 3D confirmations of protein-ligands complex.

IV. RESULT

A. Docking result of protein and ligands

From Google Scholar's literature, for an ideal FDA-approved drug i.e. Ketamine antagonist to NMDA found out as positive controller for NMDA receptors. Hence took this reference drug as a controller/ concerning other retrieved drugs. From the drug bank database, we have selected 50 anti-psychotic, anti-histamine drugs downloaded their structure from the PubChem database in 3D conformer SDF files format, and studied ligand pharmacology.

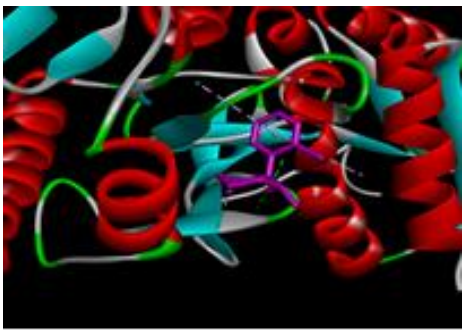
B. Screening of NMDAR inhibitor against compounds

For understanding a physical interaction between drug and ligand, binding affinities were first analyzed with reference

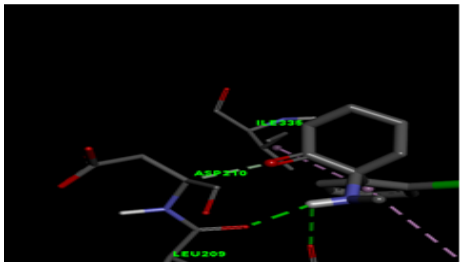
drug ketamine to NMDAR represented in Figure 1(a). where its binding energy is -6.0 Kcal/mol, it showed interacting residues of GLU236, TYR239, ARG115, ASP211, ILEL216, LEU209, PRO340, MET207, and ILE335, where LEU209 and MET207 formed two hydrogen bonds with distances of 2.38 Å and 2.91 Å. From 50 docked compounds from PyRx, we choose the 19 compounds depending upon their best suitable binding energy or docking.

Furthermore, these ligand compounds were visualized with interacting proteins in the Biovia screening studio. screening of the top 24 ligands with protein complex shows interaction between of in virtual physical form. we obtained data in 3d structure and 2d structure complexes. Herein, we observed that compounds aripiprazole, flupentixol, and Caripiprazole had good docking scores that interact with NMDA through important residues. whereas aripiprazole and flupentixol had good docking scores i.e. -11 and -7.3 Kcal/mol. These also show common interacting residues compared to reference drugs which are ASP210, MET207, PRO 340, ASP211, and GLU236. Hydrogen bond formed analysis showed that NMDAR formed 3 bonds with flupentixol and 3 bonds with aripiprazole too respectively. There are 2D and 3D interactions of the reference drug ketamine and with the best two compounds with NMDAR. They are given in **Figure 1** and **Figure 2.**

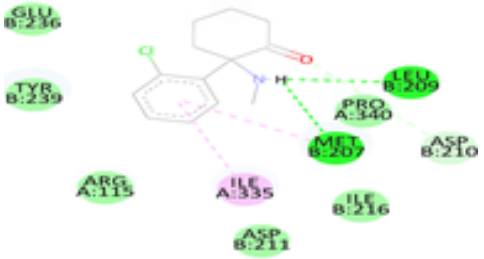
The docking analysis of all 19 docked compounds to their common interacting residues with NMDA receptors is represented in **Table I**. Flupentixol and aripiprazole compounds show common interacting residues with NMDARs regarding amantadine. From the table, we can see ketamine has a -6 Kcal/mol score where aripiprazole gives the highest binding energy i.e. -11 Kcal/mol compared to the reference drug.



(a)



(b)

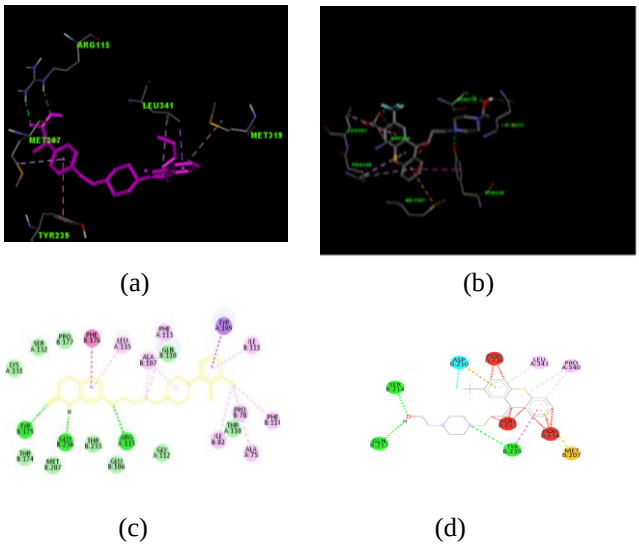


(c)

Fig. 1. (a). Violet structure shows the binding site of the active site. (b) The 3D model shows the binding pattern of reference drug and (c) 2D model shows amino residues interacting where a dark green colour shows conventional hydrogen bonds interacting with the alkyl ring.

TABLE I. DOCKING ANALYSIS OF 19 COMPOUNDS

Compounds	Docking energies	H-bonds with distance	Interaction
Ketamine (Reference drug)	-6.0	LEU209(2.38Å) Met 207(2.91Å)	GLU236, TYR239, ARG115, ASP211, ILE216, ASP210, LEU209, PRO340, MET207, ILE335
penbutolol	-6.8	Met 207(2.67 Å)	ILE335, ALA107, GLN105, LEU135, LEU209, PRO340, MET207, GLU106, ARG115, TYR239, GLU236, GLU235
flupentixol	-7.3	GLN 217(2.69 Å) SER214(2.23 Å) TYR239(2.80 Å)	ASP210, GLN217, SER214, GLU242, VAL243, GLY212, ASP211, TYR239, MET207, PRO340, LEU341, LYS337, THR338
Chlorpropamide	-7.4	GLN110(2.83 Å) GLU106(2.81 Å) ARG115(2.31 Å)	ARG115, LEU135, PHE176, GLN110, GLU106, ALA107, PHE113, GLU236, THR176, MET207, TYR175, LYS131, SER132
Hydrocortisone Acetate	-8.1	ARG115(2.81 Å) ASP211(2.05,2.36 Å)	ARG115, ASP211, ILE335, TYR109, GLU235, ASP210, LEU209, TYR239, PRO340, LYS337, MET207, GLU236
Erlotinib	-6.4	THR338(1.93 Å)	ASN321, LEU341, ASP210, ASP211, GLY212, THR323, THR338, LYS3387, MET207, LEU209, TYR239
Bilastine	-9.4	ARG115(2.36 Å,2.35 Å)	MET319, LEU341, ASN321, ILE322, THR338, PRO340, MET207, GLU235, GLU236, TYR239, ILE335, ARG115, ASP211, ASP210, GLY212
Amlotriptan	-5.9	–	PRO340, ILE335, GLUE235, GLU236, THR233, ARG115, GLU106, THR333, ALA107, PHE113, TYR239, LYS337
Viloxazine	-6.7	Gln110(2.70 Å)	ILE111, LEU135, PHE176, GLN110, GLY112, ALA107, GLU106, ARG115, PHE113, ILE133, TYR109
Acrivastine	-7	ILE133(2.57 Å)	TYR128, THR105, ILE1333, SER132, PRO177, GLN110, MET134, TYR109, ASP136, ASP138, HIS134, ILE127
Remoxipride	-5.6	GLN110(2.74 Å)	GLY112, SER108, GLN110, GLU106, GLU235, ILE335, PHE113, ALA107, ARG115, PHE176, SER132, LEU135, TYR109, PRO177, ILE133
Azatadine	-9.2	-	THR233, GLU236, GLU106, TYR175, MET207, GLN110, PRO177, SER132, ILE133, GLY112, LEU135, PHE113, ALA107, ARG115
Pitolisant	-5.3	ASP211(2.35 Å)	ARG115, ILE335, LEU209, LYS337, THR338, PRO340, ASP211, GLU235, GLU236, TYR239, ASP210
Fosaprepitant	-8.6	THR323(2.74 Å) ASN321(2.03 Å)	GLY212, ASP211, ASP210, SAN321, THR323, THR338, LYS337, LEU341, PRO340, ILE335, LEU209, MET207, ILE216, TYR239
Desipramine	-6.7	-	ILE133, PHE113, PHE114, ALA107, ARG115, LEU135, GLN110, PRO78, THR110, ILE82, ILE111, TYR109
Protokylol	-8.1	GLN110(2.40 Å) GLU236(2.16 Å)	GLU235, ILE335, ARG115, ALA107, GLN110, GLU106, LEU135, PRO177, PHE176, SER132, LYS131, TYR175, GLU236, MET207, PHE113, THR233
Aripiprazole	-11	TYR175(2.35 Å) GLU236(1.69 Å) ARG115(2.71 Å)	TYR175, SER132, LYS131, PRO177, PHE176, LEU135, ALA107, GLN110, PHE113, TYR109, ILE111, PHE114, ALA75, PRO78, THR110, ILE82, GLY112, ARG115, GLU106, THR233, GLU236, MET207, THR174
Cariprazine	-10.3	-	TYR109, ILE111, GLN110, PHE176, ARG115, PRO177, SER132, GLU236, MET207, SER208, TYR175, ASP206, THR174, THR233, LEU135, PHE113, ALA107, ILE82, PHE114, THR110, ALA75, PRO78
Zafirlukast	-9.4	TYR239(2.54 Å) ASP211(2.89 Å) ARG115(2.23 Å)	SER214, GLN217, GLU242, VAL243, TYR239, PRO340, GLU235, ILE335, GLU236, ARG115, MET207, ASP211, ASP210, LYS337, LEU309, LYS337, ILE216



(a) (b)

(c) (d)

Fig 2. Pattern interaction; (a) shown the 3D structure of aripiprazole with NMDAR. (b) 3D structure of flupentixol with NMDAR. (c) shown 2D structure of aripiprazole of NMDAR where dark green colour represents conventional hydrogen bonds, pink colour represents alkyl and dotted purple colour represents the Pi-Sigma. (d) dark green colour represents a conventional hydrogen bond, Blue colour represents halogen (Fluorine) and orange colour represents the Pi-Sulfur.

C. Pharmacokinetic properties of screened compounds

Swiss ADME is commonly used to analyze the kinetics of drug compounds, generally, it screened their physical, chemical, and pharmacokinetics parameters factor that affect drug design. The factor responsible for physicochemical properties includes their number of rotational bonds, various ADMET descriptors, and topological polar surface area (TPSA), where all 19 compounds screened including reference drugs. When compared to reference drug i.e. ketamine analysis where it is very soluble and with positive gastrointestinal absorption. Flupentixol, Bilastine, azatadine, and aripiprazole come with good output results when these were screened against ketamine, furthermore, aripiprazole comes with very high solubility and Gastrointestinal absorption. Water solubility, toxicity, pharmacokinetics kinetics properties like Gastrointestinal absorption and Blood-brain barrier (BBB), octanal and rest other that found to be suitable and hence considered as important factors in the case for designing a drug for neurodegenerative disease more likely Huntington's diseases. After screening all 19 drugs, the best of the five drugs were selected for penetrating the blood-brain barrier (BBB) and it showed positive responses which are given in **Table II.** Herein, concluded that flupentixol, Bilastine, azatadine, and aripiprazole which a screened compounds not show any undesired effects such as mutagenicity, toxicity, tumorigenicity, reproductive effect, or hepatotoxicity. Hence, with this analysis, it has been concluded that out of compound four, aripiprazole comes out with a highly water soluble compound and can cross (BBB) easily and with high Gastrointestinal absorption. Blastine, azatadine,flupentixol and ketorolac can also cross the blood-brain barrier but ketorolac and azatadine show moderate solubility in water whereas Bilastine and flupentixol are soluble in water.

Table II. SWISS ADME ANALYSIS OF TOP-5 NMDAR INHIBITORS

Parameter		Topmost compound inhibitors of NMDAR				
		Flupentixol	Bilastine	Ketorolac	Azatadine	Aripiprazole
Physico-chemical parameter	Formula	C15H26FO2	C28H48N303	C15H22NO3	C20H33N2	C23H34C12N302
	Molecular weight	257.36	475.71	264.34	302.50	456.45
	Molar refractivity	72.93	155.11	80.29	104.88	131.49
	TPSA	40.46	65.29	69.21	3.24	52.65
Lipophilicity	Log Po/w (ILOGP)	-8.7	0.00	0.00	0.00	0.00
	Log po/w (XLOGP3)	4.99	2.33	0.48	2.39	0.99
	Log Po/w (WLOGP)	4.44	3.46	1.78	2.88	1.46
	Log Po/w (SILICOS-IT)	2.16	-5.92	0.62	2.45	3.25
Pharmaco-kinetics	GI Absorption	Yes	Yes	Yes	Yes	Yes
	BBB Permeation	Yes	YES	Yes	Yes	Yes
	Log Kp (Skin permeation)	-5.47	-7.55	-7.57	-6.45	-8.38
Water solubility	Solubility	Moder-ate Soluble	Moder-ate soluble	Soluble	Solubl e	Very soluble

V. DISCUSSION

NMDAR is the receptor of glutamine of subunit of NMDA-R1(NR1), R2(N2A), these are membrane-bound, non-selective cation channel, high calcium permeability. Where NR2B- has the sensitive functional role of magnesium and can increase their sensitivity to glutamate. These subunit receptors release excitatory neurotransmitters and are thus delocalized as these macromolecules are soluble and result in cytoskeleton dysfunction where NR3 subunits interfere with a Ca2+ ion channel permeability and neuronal death in HD. Hence NMDA has now become a therapeutic site where NR2A has pharmacological properties.

Ketamine, as controller against retrieved anti-psychotic drugs compound. ketamine docked with extracted protein 5EWJ shows -6.0 binding energy with NMDA receptor with the formation of 2 hydrogen bonds, likewise compound Flupentixol shows the highest docking hits scores and forms three hydrogen bonds form stable binding interaction, analysed by online tool Biovia discovery studio.

Candidates possess some common residues like ARG115, GLU236, and MET207. However, Ketamine not only shows low binding affinity to NMDA but also is not very soluble as compared to aripiprazole which is very soluble and has no toxicity. aripiprazole shows the highest hit scores with a -11.0 Kcal/mol docking score resulting in stable interaction with NMDA forming when visualizing their hybrid virtual interaction, we found these complexes come up with good interaction due to stable three hydrogen bonds with distance- 2.32 Å, 1.69 Å, 2.71 Å.

Furthermore, it also shows higher stability and solubility that can easily penetrate the BBB (blood-brain-barrier in CNS which will inhibit NMDA secretion. Although Cariprazine shows good binding energy with a score of -10.3 and can also penetrate inside BBB but does not form any hydrogen bond with NMDA and is concluded as having weak interaction and stability with the target protein. As seen in **Table I.** which showcases the highest binding affinity shown by Aripiprazole followed by Cariprazine, also some common amino interaction residues – GLU236, ARG115, MET207, when compared to ketamine as a controller meanwhile where aripiprazole shows low toxicity, and high solubility, no mutagenicity and can cross BBB and absorbed by GI tract. NMARs, more deeply to NR1, NR2B subtype now a hallmark and attractive therapeutic approach to overcome psychotic behavior by Huntington patients where they continuously release high amounts of dopamine which neuro excitotoxicity causes neuronal cell death due to high percentage of glutamine- NMDA receptor and hence our work showcase computational bioinformatics tools to repurposing a drug from pre-existing anti-psychotic or anti-depressive drugs and repurposed to overcome secretion of NMDA in HD patient specifically.

Altogether by virtual screening-based studies from protein extraction to drug analysis. Aripiprazole and Flupentixol showed the highest docking energies for NMDAR and flupentixol showed low energy binding.

VI. CONCLUSION

NMDARs subtypes NR1 AND NR2B have now become a hallmark for treatment for Huntington's disease. Herein, by virtual screening based on bioinformatics tools and techniques, we analyzed that can bind to NMDAR. Bilastine, azatadine, aripiprazole, Cariprazine, ketorolac, and flupentixol show good binding energies to NMDARs better than ketamine. Aripiprazole, flupentixol and zafirlukast formed stable three hydrogen bonds with NMDAR. whereas amantadine which is taken as a reference drug shows two hydrogen bonds with NMDAR and pharmacokinetics shows positive outcomes aripiprazole shows the highest binding energy and also crosses BBB and is highly absorption in the gastrointestinal. Furthermore, more study is required to carry out *in-vivo* and *in-vitro* analyses where can transfer these inhibitor compounds into clinical trial drugs. Hence this study, anticipated that aripiprazole could be an ideal antagonist to NMDARs based on the highest binding energy, water solubility, and BBB permeability as compared to ketamine.

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