Exploring Inflammatory Biomarker, Drug Screening, and Molecular Mechanism for Treating Neurodegenerative Diseases

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DOCTOR OF PHILOSOPHY

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I, Shefali Kardam, 2K22/PHDBT/506 student of Ph.D. Biotechnology, hereby declare that the project synopsis titled "**EXPLORING INFLAMMATORY BIOMARKER, DRUG SCREENING, AND MOLECULAR MECHANISM FOR TREATING NEURODEGENERATIVE DISEASES.**" which is submitted by me to the Department of Biotechnology, Delhi Technological University, Delhi in partial fulfillment of the requirement for the award of the degree of doctorate in philosophy, is original and not copied from any source without paper citation. It is an authentic record of my own work carried out during the period from 12.01.2023 to 31.08.2025 under the supervision of Prof. Pravir Kumar, Department of Biotechnology, Delhi Technological University. The work has not previously formed the basis for the award of any Degree, Diploma, Associateship, Fellowship, or other similar title or recognition.

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DEDICATED

To

My Parents

LIST OF PUBLICATIONS

1. **Kardam, S.,** & Kumar, P. “In Silico Identification of Natural SIRT1 Inhibitors through Molecular Docking, Dynamics Simulation, and MM/PBSA”. Cell Biochemistry and Biophysics (2025). https://doi.org/10.1007/s12013-025-01886-0

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3. **Kardam, S.,** Ambasta, R. K., & Kumar, P. “Overview of pro-inflammatory and pro-survival components in neuroinflammatory signalling and neurodegeneration”. Ageing research reviews vol. 100 (2024): 102465. https://doi.org/10.1016/j.arr.2024.102465

4. Rani, N., Kaushik, A., **Kardam**, S., Kag, S., Raj, V. S., Ambasta, R. K., & Kumar, P. (2024). Reimagining old drugs with new tricks: Mechanisms, strategies and notable success stories in drug repurposing for neurological diseases. Progress in Molecular Biology and Translational Science. https://doi.org/10.1016/BS.PMBTS.2024.03.029

Exploring Inflammatory Biomarker, Drug Screening, and Molecular Mechanism for Treating Neurodegenerative Diseases

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ABSTRACT

Neurodegenerative diseases such as Parkinson's and Huntington's disease arise from a web of pathological events, including protein aggregation, mitochondrial impairment, chronic oxidative stress, and sustained inflammatory activation within the nervous system. Although these mechanisms are increasingly well documented, there are still few therapies that meaningfully alter the disease course. In this thesis, a layered computational workflow was used that combined ligand-based virtual screening with structure-guided docking, molecular dynamics simulations, and MM/PBSA binding-free-energy calculations. Within this framework, candidate regulatory compounds were identified that target Sirtuin-1 (SIRT1) in HD and DJ-1 (PARK7) in PD, and parallel transcriptomic analyses were used to characterize immune-related changes that may drive or modulate neurodegenerative progression. For HD, Selisistat-guided similarity searching across the LOTUS natural-products repository (276,518 compounds; ≥68% Tanimoto) yielded 1,401 structural analogues that were refined through stringent Absorption, Distribution, Metabolism, Excretion, and Toxicity (ADMET), Blood-brain barrier (BBB)-permeability, and drug-likeness filters prior to docking into the high-resolution SIRT1 catalytic domain (PDB 4I5I), prepared using CHARMM force-field optimization and validated via re-docking (RMSD < 2 Å). Subsequent 100-ns all-atom MD simulations (GROMACS/CHARMM36/TIP3P) demonstrated that top candidates, particularly LTS0217483, exhibit exceptional conformational stability, low-fluctuation RMSD/RMSF profiles, persistent H-bonding, and favorable compactness (Rg), while MM/PBSA decomposition revealed pronounced van der Waals and electrostatic contributions, confirming high-affinity NAD^+-competitive

inhibition driven by interactions with catalytic residues His363, Phe414, Val445, and Arg446. Parallel repurposing of FDA-approved anti-inflammatory drugs against the PD-associated mutant DJ-1 (2R1T) employed SwissADME pre-screening followed by CB-Dock and Webina docking, identifying Oxatomide and Levocabastine as high-binding, BBB-permeable modulators that form stable interactions with residues essential for DJ-1's redox-chaperone and glyoxalase functions (e.g., Glu163, Glu170, Arg27, Ala56), thereby potentially mitigating oxidative and inflammatory perturbations characteristic of PD. An overview of the key molecular pathways involved in neuroinflammatory signaling is presented in this thesis to provide a biological context for the computational findings. Toll-like receptors, STAT3, p38 MAPK, and the NLRP3 inflammasome are discussed alongside protective regulators, such as SIRT1, SOCS protein, YY1, and MEF2. It has been shown that disruption of the balance between these pathways contributes to progressive neuronal damage, highlighting potential therapeutic targets. A robust in silico framework for CNS-targeted drug discovery is shown in this multidisciplinary study, and mechanistically validated lead scaffolds are identified for HD (LTS0217483) and PD, as well as a systems-level neuroinflammatory model that informs future translation strategies and precision therapeutic development for NDDs.

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LIST OF ABBREVIATIONS

NDDs	Neurodegenerative Disorders
HD	Huntington's Disease
PD	Parkinson's disease
SIRT1	Sirtuin-1
MD	Molecular Dynamics
CNS	Central Nervous System
MBP	Myelin Basic Protein
SMN	Survival Motor Neuron
AD	Alzheimer's disease
ALS	Amyotrophic Lateral Sclerosis
DAM	Disease-associated microglia
ROS	Reactive oxygen species
GPNMB	Glycoprotein nonmetastatic melanoma protein B
DAMPs	Damage-associated molecular patterns
PAMPs	Pathogen-associated molecular patterns
STAT3	Signal transducer and activator of transcription
ASD	Autism spectrum disorders
FAF1	Fas-associated factor1
TLRs	Toll-like receptors
SOCS	Suppressor of cytokine signaling
MEF2	Myocyte enhancer factor 2
CBDR	Computational-based drug repurposing
GEO	Gene Expression Omnibus
DGE	Differential gene expression
FDR	False discovery rate
UMAP	Uniform Manifold Approximation and Projection

GO	Gene Ontology
BBB	Blood-brain barrier
PME	Particle Mesh Ewald
BDNF	Brain-derived neurotrophic factor
Aβ	Amyloid-β
TDP-43	TAR DNA-binding protein 43

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CHAPTER 1

INTRODUCTION

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Neurodegenerative disorders (NDDs) such as Huntington's disease (HD) and Parkinson's disease (PD) cause irreversible neuronal loss affecting millions worldwide [1, 2]. Despite HD and PD exhibiting distinct symptoms, motor and cognitive decline in HD and tremor associated with dopaminergic loss in PD, their underlying molecular pathways show notable convergence [3]. Mitochondrial dysfunction, violations in proteostasis, excitotoxicity, oxidative stress, and chronic neuroinflammation are some of the key abnormalities common to both PD and AD [4]. In addition, neuroinflammation has been identified as a significant factor influencing PD/AD pathology, whereby cytokines, oxidative radicals, and chemokines produced by activated microglia and astrocytes result in further neuronal damage [5, 6, 7, 8]. In contrast, several endogenous neuroprotective factors (such as MEF2, SIRT1, SOCS family members, and YY1) reduce metabolic and inflammatory mediators (such as TLRs, p38 MAPK, STAT3, NF-κB, and NLRP3 inflammasome), which worsen neurotoxic effects [9, 10, 11]. As seen in **Fig. 1.1,** the presence of both pro-inflammatory and pro-survival signals demonstrates the complexity of NDDs [12, 13].

Due to their significance in maintaining cellular balance and neuronal integrity under stress, two proteins, Sirtuin 1 (SIRT1) and DJ-1 (PARK7), have become appealing therapeutic targets. As a NAD^+-dependent deacetylase, SIRT1 controls a variety of protein substrates involved in transcriptional regulation, mitochondrial bioenergetics, and DNA damage repair [14, 15]. Reduced SIRT1 activity in HD disrupts these defense mechanisms, aggravating transcriptional abnormalities and increasing the death of neurons. Thus, pharmacological SIRT1 regulation suggests a possible approach to enhance cellular flexibility and restore dysregulating signaling networks. Similarly, DJ-1 contributes to neuronal protection by acting as a molecular chaperone, sensing redox imbalances, and safeguarding cells against oxidative stress-induced damage, overall supporting cellular stability and survival. Mutations in the DJ-1 protein can cause two forms of PD: familial and sporadic [16]. Modulating DJ-1 activity may mitigate oxidative stress and autophagic

dysfunction, thereby protecting dopaminergic neurons. Recent advancements in computational drug discovery have enabled new methods for rapidly exploring targets. Tools such as Structure-Based Virtual Screening, Molecular Docking, Molecular Dynamics Simulations, and Pharmacophore will all provide a high degree of detail on how properly designed ligands bind and interact with their targets. Additionally, MM/PBSA free-energy estimation offers insight into the stability of protein-ligand interactions. For this reason, these tools are essential in the early stages of drug discovery, particularly for CNS targets, where BBB permeability, selectivity, and low toxicity are crucial. Moreover, these technologies, Natural Products (plant and animal products), have unique structural properties and are very good at binding to many of the biological targets of interest; therefore, Natural Products are an excellent source for the identification of new SIRT1 inhibitors in HD and for the validation of repurposed SIRT1 inhibitors for HD. Likewise, drug repurposing strategies provide additional advantages by identifying new therapeutic applications for approved drugs with known safety and pharmacokinetic profiles.

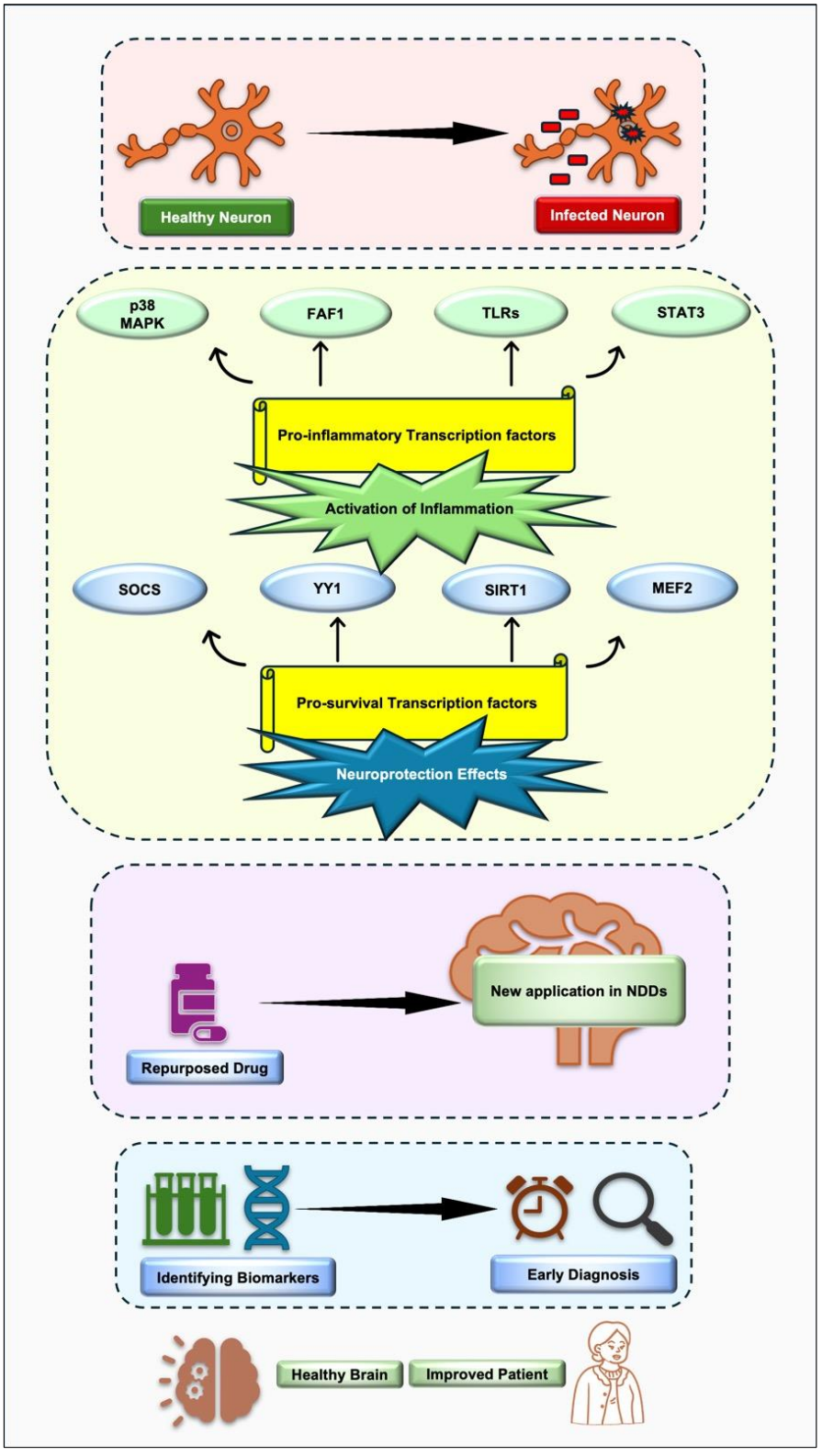


Fig. 1.1 Overview of neuroinflammatory and neuroprotective signaling in NDDs.

Table 1.1 Impacted Brain Regions, Crucial Signs, and Symptoms of Neurodegenerative Disorders

Neurodegenerative Disorders	Impacted Brain Region	Pathological Protein	Key Signs and Symptoms	References
Parkinson's Disease	Substantia nigra	alpha-synuclein	Tremor, bradykinesia, rigid muscles, impaired posture and balance, and speech changes.	[17]
Alzheimer's Disease	Hippocampus	Amyloid-β, tau	Memory loss, poor judgment, loss of spontaneity, and challenges in planning.	[18]
Amyotrophic Lateral Sclerosis	Motor neurons	TAR DNA-binding protein 43 (TDP-43)	Slurred speech, weakness in the legs, feet, or ankles, thinking or behavioral changes, clumsiness.	[19]
Huntington's Disease	Basal ganglia	Huntingtin	Uncontrolled movements like jerking or twitching, ataxia, and dysphagia.	[20]
Multiple Sclerosis	Myelin sheath of neuron	Myelin Basic Protein (MBP)	Numbness, blurry vision, unsteady gait, cognitive problems, and mood disturbances.	[21]
Prion Disease	Cerebral cortex, Thalamus, Cerebellum to Brain stem	Prion Protein (PrP)	Ataxia, disorientation, insomnia, difficulties with thinking and memory.	[22]
Spinal Muscular Atrophy	Motor neurons	A (SMN)	Weak arms and legs, tremors, breathing difficulties, and swallowing problems.	[23]

1.1. AIM AND OBJECTIVES

1.1.1 Aim

The focus of this thesis is to investigate the potential therapeutic benefits of targeting neuroinflammatory signaling, SIRT1, and DJ-1 in major neurodegenerative diseases. The goal of this study is to identify and characterize novel small-molecule modulators of SIRT1 and repurposed FDA-approved anti-inflammatory drugs against mutant DJ-1, with the aim of developing mechanism-based treatment strategies that can slow or halt disease progression.

1.1.2 Objectives

- **Objective I -** To identify specific biomarkers that are associated with NDDs.

- **Objective II -** To identify a systematic approach to screen and select anti-inflammatory drugs for targeting NDDs.

- **Objective III -** To identify potential natural SIRT1 inhibitors for NDDs using a computational approach.

1.2. Summary of the Thesis

The five chapters of this study each examine an aspect of neuroinflammation and pro-survival signaling in the most prevalent NDDs, with emphasis on SIRT1 and DJ-1 as potential therapeutic targets. **Chapter 1** introduces the clinical features and molecular background of NDDs and then summarizes the biological functions of SIRT1 and DJ-1. It also outlines the rationale for the work, including the objectives, expected therapeutic relevance, main limitations, and potential future directions. **Chapter 2** builds on this by reviewing current literature on SIRT1, DJ-1, and neuroinflammatory signaling in NDDs, and highlights mechanistic gaps and unresolved therapeutic issues that motivate a targeted drug-discovery approach. **Chapter 3** then describes the integrated in silico workflow used to identify modulators of SIRT1 and mutant DJ-1, beginning with database screening of natural products and FDA-approved drugs, followed by virtual screening and ADMET/BBB-based filtering. Selected candidates were subjected to molecular

docking and MD simulations, together with binding-free-energy calculations, to evaluate the stability and affinity of the resulting complexes.

The outcomes of these computational analyses are presented in **Chapter 4**, which details how the shortlisted natural compounds and repurposed drugs interact with the NAD^+-binding pocket of SIRT1 and the catalytic region of DJ-1. The results point to stable, drug-like binding modes and favorable pharmacokinetic profiles, suggesting potential neuroprotective activity. **Chapter 5** discusses these findings in the context of NDD mechanisms, considers the therapeutic implications of the proposed SIRT1 and DJ-1 modulators, and reflects on the constraints of the computational strategy, outlining key experimental steps needed to validate and extend the work.

CHAPTER 2

REVIEW OF LITERATURE

Chapter 2
REVIEW OF LITERATURE

2.1 Neurodegenerative Diseases and Neuroinflammation

Neurodegenerative disorders are long-term conditions characterised by the gradual loss and eventual death of neuronal cells. A range of misfolded proteins, including α-synuclein, amyloid-β, prion protein, and tau, can accumulate in different brain regions, where their distinct physicochemical properties promote abnormal aggregation during disease [24, 25]. These aggregates disrupt cellular homeostasis by driving oxidative stress, interfering with synaptic signaling, and damaging key cellular components, ultimately leading to neuronal dysfunction [26, 27]. As depicted in **Fig. 2.1**, abnormal activation of glial populations, particularly microglia and astrocytes, is another recurring feature across several major NDDs, including AD, PD, HD, and ALS [28, 29]. These disorders are often classified according to the type of aggregated protein involved in their pathology [30]. Increasing evidence highlights that neuroinflammation, driven by reactive glial cells, plays a crucial role in modulating the crosstalk between neurons and glia, profoundly influencing neuronal survival, synaptic plasticity, and disease progression [31, 32]. Increasing evidence highlights that neuroinflammation, driven by reactive glial cells, plays a crucial role in modulating the crosstalk between neurons and glia, profoundly influencing neuronal survival, synaptic plasticity, and disease progression [33].

Neuroinflammation is an umbrella term for inflammation of the nervous system, mainly the central nervous system CNS. Numerous complex disorders, including neuropsychiatric disorders, cancer, and type 2 diabetes, have been interlinked with it. In NDDs, pro-inflammatory transcription factors play a crucial role [34]. The nucleotide-binding oligomerisation domain NOD, leucine-rich repeat LRR, and NLR family pyrin domain-containing protein 3 NLRP3 inflammasome complex is interlinked with pro-inflammatory signaling in PD and AD. Inflammasomes are sophisticated molecular complexes that show significant inflammatory properties

and are strictly regulated at the transcriptional stage. Neuroinflammation biomarkers are widely recognised as a substantial indication of the progression of NDDs [35, 36, 37, 38].

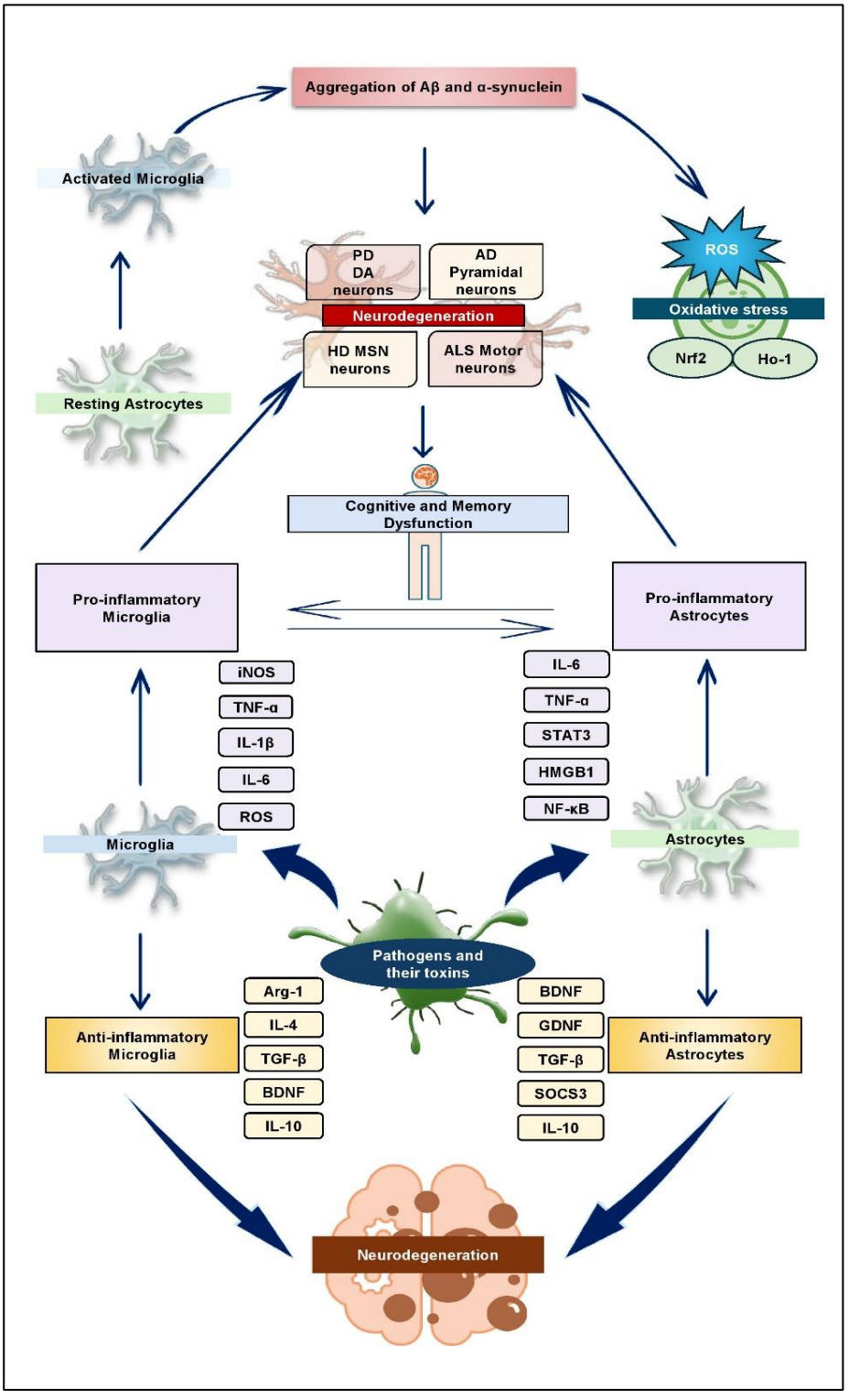


Fig. 2.1. Microglial and Astrocyte Activation in Neurodegeneration. The figure demonstrates the polarization of microglia and astrocytes concerning microbial infection and their involvement in the progression of neurodegeneration. Microglia and astrocytes undergo alterations to their characteristics in response to microbial infection, leading to their activation and polarization. When glial cells become active, they release pro-inflammatory cytokines and neurotoxic substances. These compounds encourage the development of neuroinflammation and disrupt neurons, leading to the development of dementia.

2.2 Implication of Glial Cells and Neuroinflammation in the regulation of NDD homeostasis

2.2.1 Microglia, astrocytes, and DAM key regulators in neuroinflammation

The phases of activation, cytokine release, and resolution are precisely regulated by the neuroinflammatory response, a common mechanism controlling CNS immune surveillance [39]. This complex relationship, which includes interactions between microglia, astrocytes, and disease-associated microglia (DAM), enables accurate progression through various activation states. Understanding the regulatory mechanisms of neuroinflammation is crucial for maintaining CNS homeostasis and preventing abnormal glial reactivity [40].

In response to pathogenic invasion or tissue damage, microglia constantly investigate the environment by generating substances that interact with astrocytes and neurons, resulting in an inflammatory response that is further mediated by the immune system to start tissue repair [41, 42, 43, 44]. Through continuous monitoring and removal of debris from dying neurons or protein aggregation, such as amyloid-β plaques in AD, these cells maintain CNS homeostasis. In order to maintain functional brain circuitry, microglia also eliminate synapses [45, 46].

Activated microglia produce high levels of reactive oxygen species (ROS) and nitric oxide, causing oxidative stress and damaging neuronal components, which is important in the pathogenesis of NDDs [47]. Additionally, microglial activation worsens excitotoxic damage, mainly in ALS, where glutamate overload overstimulates NMDA receptors, triggers calcium overload, and causes neuronal demise. Glycoprotein nonmetastatic melanoma protein B (GPNMB) has a significant impact on NDDs such as PD, ALS, and AD [48].

A particular type of microglia has been identified and titled DAM, as it is associated with NDDs. The transcriptional modification of the microglia was induced by the existence of apolipoprotein E APOE and triggering receptor expressed on myeloid cells 2 TREM2. DAM carries a molecular mechanism that increases the synthesis of genes responsible for inflammatory molecules such as Ccl2 and Itgax, although simultaneously reducing the production of genes

responsible for homeostatic molecules, like Hexb, Cx3cr1, Trem1, and Csf1r [49, 50].

2.2.2 Astrocytic contributions to neuroinflammatory amplification

Pro-inflammatory astrocytes release IL-6, Inos, and TNF-α through the activation of STAT3 and NF-κB. Astrocytes undergo alterations to their characteristics in response to microbial infection, leading to their activation and polarization [51, 52]. When glial cells become active, they release pro-inflammatory cytokines and neurotoxic substances. These compounds promote the development of neuroinflammation and disrupt neuronal function, contributing to the progression of dementia [53].

Neuroinflammation involves the activation of astrocytes. Activated astrocytes produce proinflammatory cytokines (e.g., TNF-α, IL-β, and IL-6) and chemokines that exacerbate neuronal damage and facilitate neurodegenerative processes. Glia also releases nitric oxide and ROS, creating an oxidative stress environment for neuronal injury [54].

2.2.3 Crosstalk between neuroinflammation and neuronal degeneration

Neuroinflammation and neuronal degeneration occur independently. Neuroinflammation is an important hallmark of NDD expansion, as indicated by the activation of immune factors such as microglia and the formation of pro-inflammatory mediators. Neurodegeneration is a key component in the destruction of neuronal cells in NDDs such as AD, PD, and multiple sclerosis [55].

Tau proteins tend to form abnormal structures in the brain, which may result in the development of NDDs. Microglial cells are major components of the immune responses within the CNS. These have a significant role in the progression of NDDs. Inadequate communication between neurons and microglia can cause cellular damage, therefore contributing to the progression of taupathies [56, 57].

2.3. Revival of neuroinflammatory signaling in neurons

2.3.1 Mechanisms specifically involved

2.3.1.1 Glial activation

Activated microglia and astrocytes are key regulators of neuroinflammatory processes within the CNS. Once activated, these glial cells secrete a range of pro-inflammatory cytokines, including TNF-α, IL-β, and IL-6, as well as chemokines that can exacerbate neuronal injury and promote neurodegeneration. Thus, Glial activation serves two roles. In the acute phase, it facilitates debris clearance and host defense. Chronic stimulation, on the other hand, eventually affects neuronal structure and function by releasing neurotoxic mediators [58].

2.3.1.2 Mitochondrial dysfunction

Mitochondrial problems are a recurring feature in many neurodegenerative disorders. When these organelles lose efficiency, they tend to produce higher levels of reactive oxygen species, which progressively injure lipids, proteins, and DNA inside neurons. The resulting oxidative stress can, in turn, trigger microglia and astrocytes, creating a feedback loop in which inflammation and neuronal damage reinforce one another [59]. Disturbed mitochondrial dynamics, for example, excessive fission or impaired fusion, further weaken neuronal resilience and promote degeneration [60].

Several strategies aim to counter this mitochondrial stress. Antioxidants such as coenzyme Q10 and N-acetylcysteine, together with compounds that enhance mitochondrial biogenesis or function, including PGC-1α activators, have been reported to dampen oxidative damage and support neuronal survival [61]. In parallel, mitochondria-targeted molecules like MitoQ have shown promising effects in experimental models by helping to preserve mitochondrial performance and reduce brain injury [62].

2.3.1.3 Protein aggregation

Irregular protein dynamics, such as folding incorrectly and clumping together, play a key role in neurodegenerative diseases. Amyloid-β plaques in AD, tau tangles in AD, and α-synuclein aggregates in PD can activate microglia and cause inflammation. Various protein aggregates demonstrate the complicated interactions between various processes, acting as both a cause and a result of neuroinflammation [63]. Furthermore, protein aggregates can disrupt autophagic and proteasomal degradation pathways, thereby impairing cellular homeostasis [64].

Immunotherapies (such as anti-tau and anti-amyloid antibodies) and small-molecule aggregation inhibitors are two therapeutic treatments that target protein aggregation and improve clearance mechanisms. They help decrease the burden of harmful protein deposits [65]. Furthermore, misfolded proteins can be eliminated by stimulating autophagy with mTOR inhibitors or enhancing proteasomal degradation with proteasome activators [66].

2.3.1.3 Excitotoxicity

Neuronal damage and death result from excitotoxicity, which is brought on by excessive glutamate release and poor glutamate uptake. Glial cells, particularly astrocytes, are responsible for maintaining glutamate homeostasis. Dysfunctional astrocytes in NDDs are unable to eliminate excess glutamate, which leads to an increase in calcium influx, an overactivation of NMDA receptors, and the activation of pathways that cause cell death [67]. In addition to causing oxidative stress and mitochondrial malfunction, chronic excitotoxicity damages neurons [68].

Excitotoxic neuronal damage can be prevented by agents that promote glutamate absorption by astrocytes (e.g., β-lactam antibodies) or modify glutamate receptors (e.g., NMDA receptor antagonists) [69]. Furthermore, therapeutic potential can be achieved by targeting downstream signaling pathways triggered by excitotoxicity, including oxidative stress and calcium signaling [70].

2.4. Factors provoking neuroinflammatory activation in neurons

2.4.1 DAMPs and PAMPs recognition

Damage-associated molecular patterns DAMPs and pathogen-associated molecular patterns PAMPs from damaged brain tissue evoke a reaction from TLRs, beginning activation of supplementary signaling pathways [71, 72].

2.4.2 Oxidative stress signaling cascades

Oxidative stress is a key driver of neuroinflammatory activation in the CNS and results from an imbalance between ROS generation and antioxidant defense mechanisms. Excessive ROS produced due to mitochondrial dysfunction, excitotoxicity, protein misfolding, and impaired redox homeostasis act as secondary messengers that activate multiple intracellular signaling pathways [73] [74, 75]. Prominent among these are the MAPK cascade, including JNK, p38 MAPK, and ERK, which regulate stress-responsive transcription factors such as NF-κB and AP-1, leading to the upregulation of pro-inflammatory cytokines and chemokines [76, 77, 78]. Oxidative stress triggers chromatin remodeling and DNA damage responses, sustaining inflammatory gene expression. It also promotes the release of DAMPs, such as oxidized lipids and mitochondrial DNA, which activate pattern recognition receptors and amplify neuroinflammation, creating a feed-forward loop that drives neuronal dysfunction and neurodegeneration [79, 80].

2.5. Pro-inflammatory transcription factors regulating glial activation

2.5.1 STAT3 signal transducer and activator of transcription 3 in NDDs

A specific class of transcription factors consists of the signal transducer and activator of transcription (STAT)-3. The discovery of a DNA-binding protein was initially reported in 1994. This protein exhibits eminent specificity in its ability to bind to the IL-6-responsive region located within the promoter region of acute-phase genes. These genes are located in hepatocytes that have been stimulated

with IL-6. Moreover, the role of STAT-3 as a protein that interacts with DNA in response to epidermal growth factor was discovered independently [81].

STAT3 has been identified as a crucial factor in NDDs such as schizophrenia, intellectual disabilities, and autism spectrum disorders (ASD). Multiple studies have elucidated the various functions of STAT3 in regulating neuronal activity and synaptic plasticity, ultimately affecting the brain's growth and function.

Transcription factors, such as STAT3, play a crucial role in promoting the growth, invasion, and replication of cancer cells [82]. Cholangiocarcinoma is a tremendously metastatic form of cancer characterized by its destructive nature [83]. The cytokine-induced proliferation and migration of cholangiocarcinoma KKU-100 cells are inhibited by MYR, which deteriorates these processes by blocking the STAT3 pathway. Furthermore, MYR suppresses genes linked with downstream metastasis and inflammation, notably MMP-9, cyclooxygenase, intercellular adhesion molecule-1, and inducible nitric oxide synthase [84].

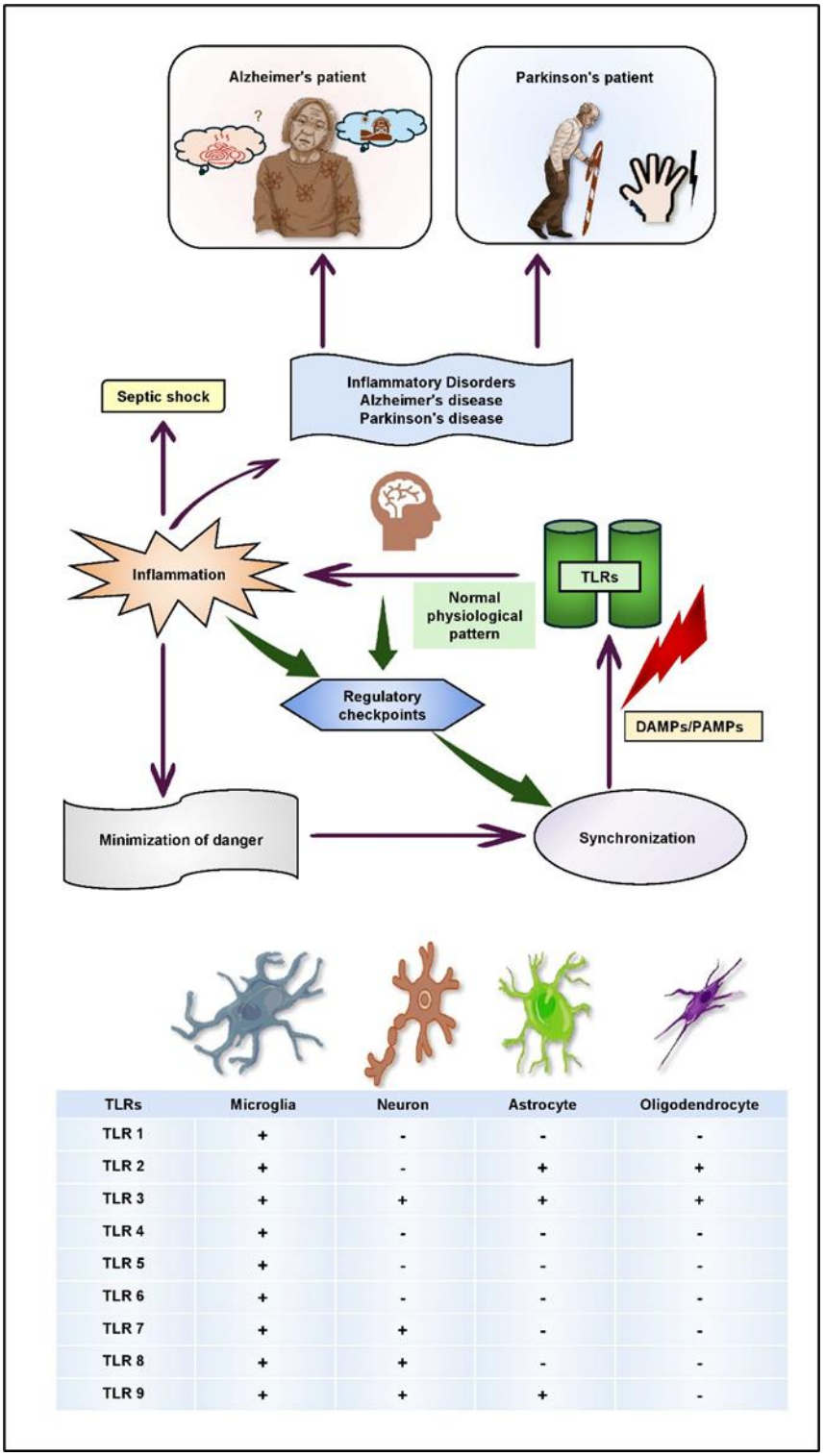
2.5.2 FAF1 Fas-associated factor1 in NDDs

FAF1 is a long non-coding RNA surrounded by the Fas death-inducing signaling complex [85]. It plays a crucial role in multiple biological processes, including programmed cell death, inflammation, cell proliferation, and protein homeostasis [86, 87]. FAF1 is attached to many NDDs, along with PD. It is linked to α-synuclein, a protein associated with PD. Increased expression of FAF1 shows the proliferation of α-synuclein and inhibits its clearance, aggravating PD [88, 89]. FAF1 could have a notable effect on the constant decrease in dopaminergic (DA) release in PD. FAF1 is primarily confirmed as a substrate protein for parkin, functioning as a ubiquitin E3 ligase. When parkin is in deactivated mode, it generates an escalation in the expression of FAF1, resulting in the excessive death of dopaminergic neurons [90, 91]. Postmortem investigation of the brains of individuals with PD reveals a crucial presence of FAF1 expression in the frontal lobe. This expression is powerfully connected to the accumulation of Lewy bodies and the reduction in dopamine transporter expression [92].

2.5.3 TLRs toll-like receptors in NDDs

TLRs are a family of pattern recognition expressed on immune cells, neurons, astrocytes, and microglia in the CNS. They possess an extracellular leucine-rich repeat domain for recognizing pathogen-associated molecular patterns PAMPs and damage-associated molecular patterns DAMPs, and an intracellular IL-1 receptor TIR domain that initiates downstream signaling. In humans, ten TLRs, TLR1-TLR10, have been identified, and their expression is dynamically regulated by infections, cytokines, and environmental cues, shown in **Fig. 2.2.** [93, 94].

In NDDs, DAMPs derived from misfolded proteins and damaged tissue activate TLRs, triggering MyD88- or TRIF-dependent cascades that result in the activation of NF-κB and IRF, leading to the production of pro-inflammatory cytokines and chemokines. In AD, TLR2 and TLR4 participate in both amyloid-β clearance and inflammatory injury, whereas in PD, TLR4-mediated recognition of α-synuclein aggregates promotes microglial activation and dopaminergic neuron loss. Persistent TLR signaling sustains chronic neuroinflammation and contributes to progressive neuronal degeneration [95, 96].



TLRs	Microglia	Neuron	Astrocyte	Oligodendrocyte
TLR 1	+	-	-	-
TLR 2	+	-	+	+
TLR 3	+	+	+	+
TLR 4	+	-	-	-
TLR 5	+	-	-	-
TLR 6	+	-	-	-
TLR 7	+	+	-	-
TLR 8	+	+	-	-
TLR 9	+	+	+	-

Fig. 2.2. TLR-Mediated Immune Balance in the CNS. This figure illustrates the equilibrium between pro-inflammatory and anti-inflammatory responses mediated by TLRs under normal physiological conditions. TLRs detect PAMPs/DAMPs when they penetrate the protective barrier, triggering an inflammatory response. TLRs initiate the activation of inhibitory molecules as a component of the inflammatory reaction, which can also be induced by inflammatory cytokines. The inflammatory response serves to mitigate the threat, whereas negative regulators are in place to limit excessive activation of the immune system to safeguard the host. If there is a lack of proper

stimulation of TLRs, failure to propagate signals, or failure to induce inflammatory mediators, it can lead to the presence of bacteria in the bloodstream. On the other hand, if any component is overactivated, it can lead to the development of inflammatory disorders and septic shock. Additionally, the figure illustrates the behavior of TLR family constituents in CNS cells, signifying their involvement in the immunological response of the CNS.

2.5.4 p38 MAPK mitogen-activated protein kinase in NDDs

MAPKs are serine-threonine kinases that relay extracellular stress signals to nuclear transcriptional responses. Among the classical MAPK pathways, p38 MAPK is primarily activated by inflammatory cytokines, oxidative stress, UV irradiation, and osmotic shock, via upstream MAPK kinases such as MKK3 and MKK6. **Fig. 2.3** demonstrates, once activated, p38 phosphorylates a wide range of substrates, including transcription factors ATF-2, CHOP, and MEF2, as well as NF-κB regulators, thereby modulating inflammation, apoptosis, and synaptic plasticity [97].

Elevated p38 activity has been reported in several NDDs, including AD, dementia with Lewy bodies, and PD, where it is associated with synaptic loss, tau hyperphosphorylation, and α-synuclein pathology. In AD models, p38 inhibition attenuates amyloid-β-induced cognitive deficits and reduces tau pathology. In PD, p38 contributes to the vulnerability of dopaminergic neurons and may influence serotonergic dysfunction. These observations position p38 isoforms as important pro-inflammatory effectors and potential therapeutic targets in NDDs [98].

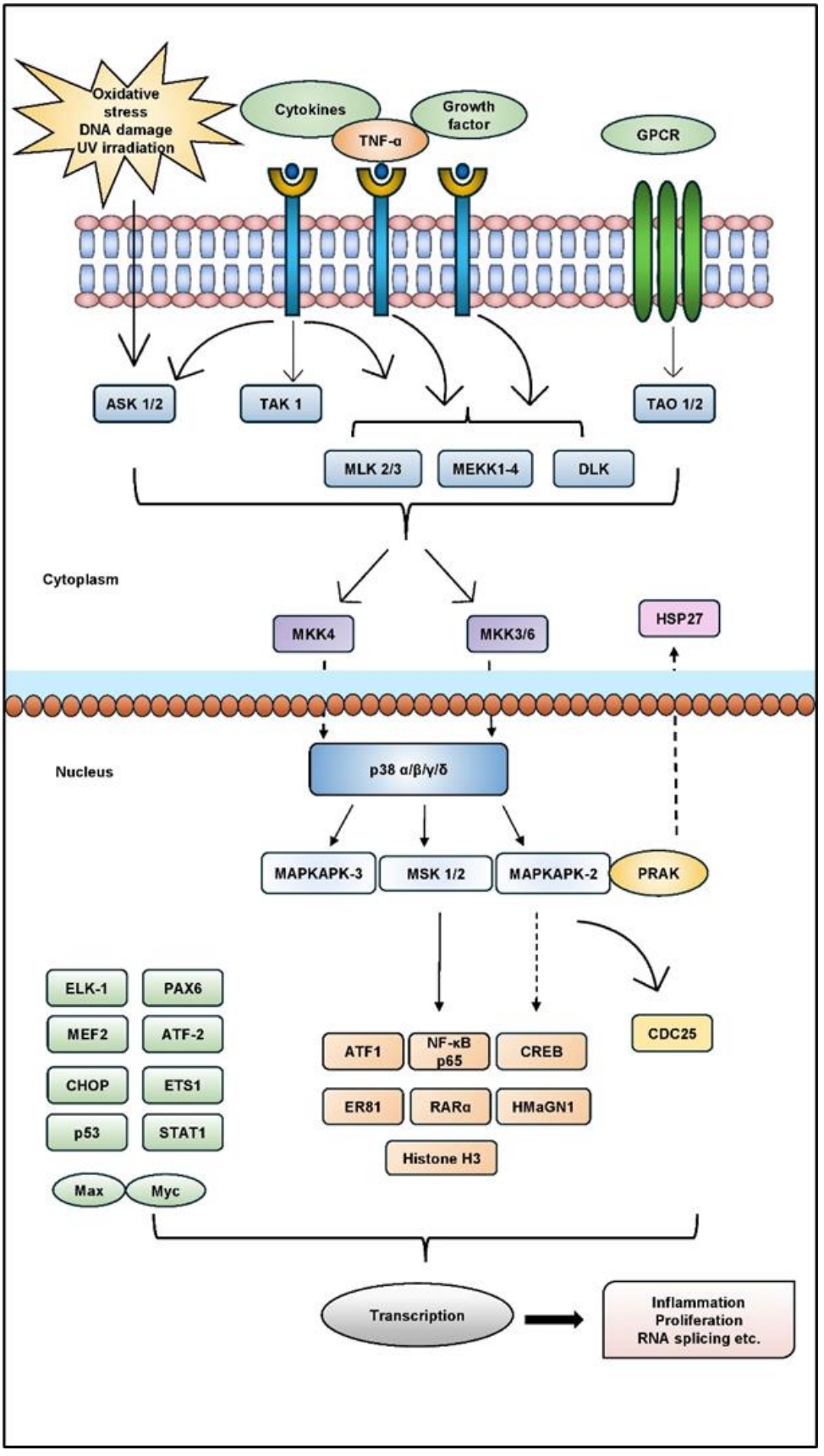


Fig. 2.3. Activation and Signaling of the p38 MAPK Pathway. The p38 MAPK pathway and its activation in both upstream and downstream aspects. The activation of the four members of the

p38 MAPK family (p38α, p38β, p38γ, and p38δ) occurs in response to UV radiation, external stress, or inflammatory cytokines, resembling the JNK pathway. Upon activation, this pathway triggers the synthesis of pro-apoptotic transcription factors involved in inflammation, proliferation, apoptosis, and RNA splicing.

2.6. Pro-survival transcription factors counteracting neuroinflammation

2.6.1 Transcription factor YY1 Yin Yang1 in NDDs

YY1 is a ubiquitously expressed zinc-finger transcription factor originally identified through its binding to immunoglobulin heavy-chain regulatory elements [99]. YY1 can act as both a transcriptional activator and repressor, forming homodimers stabilized in part by RNA binding, and is involved in chromatin remodeling and long-range gene regulation. Conditional knockout studies in mouse models have demonstrated that YY1 is crucial for early cortical development and the survival of neural progenitor cells, as its deletion results in cell cycle arrest and apoptosis in the neuroepithelium [100].

YY1 also exhibits stage-dependent roles in neural crest development, with early loss impairing multiple neural crest-derived lineages, whereas later deletion produces milder phenotypes. These findings suggest that YY1 contributes to neuronal homeostasis and that its perturbation can predispose to neurodevelopmental and neurodegenerative conditions [101, 102].

2.6.2 SOCS suppressor of cytokine signaling pathway in NDDs

The suppressor of cytokine signaling (SOCS) family comprises eight members, SOCS1 to SOCS7 and CIS, that act as classical negative feedback regulators of JAK-STAT signaling [103]. SOCS1 and SOCS3 are particularly important in limiting cytokine-induced inflammatory cascades by binding to JAKs or phosphotyrosine motifs on cytokine receptors via their SH2 domains and targeting them for ubiquitin-mediated degradation through their SOCS box [104].

SOCS3 expression is widely detected in the brain, including hippocampus,

cerebellum, thalamus, and basal ganglia, where it modulates responses to IL-6 family cytokines and other inflammatory mediators. By restraining excessive STAT activation, SOCS proteins help prevent chronic neuroinflammation and insulin-resistance-like signaling in neurons, thereby preserving synaptic and metabolic integrity in NDDs, as shown in **Fig. 2.4.** [105].

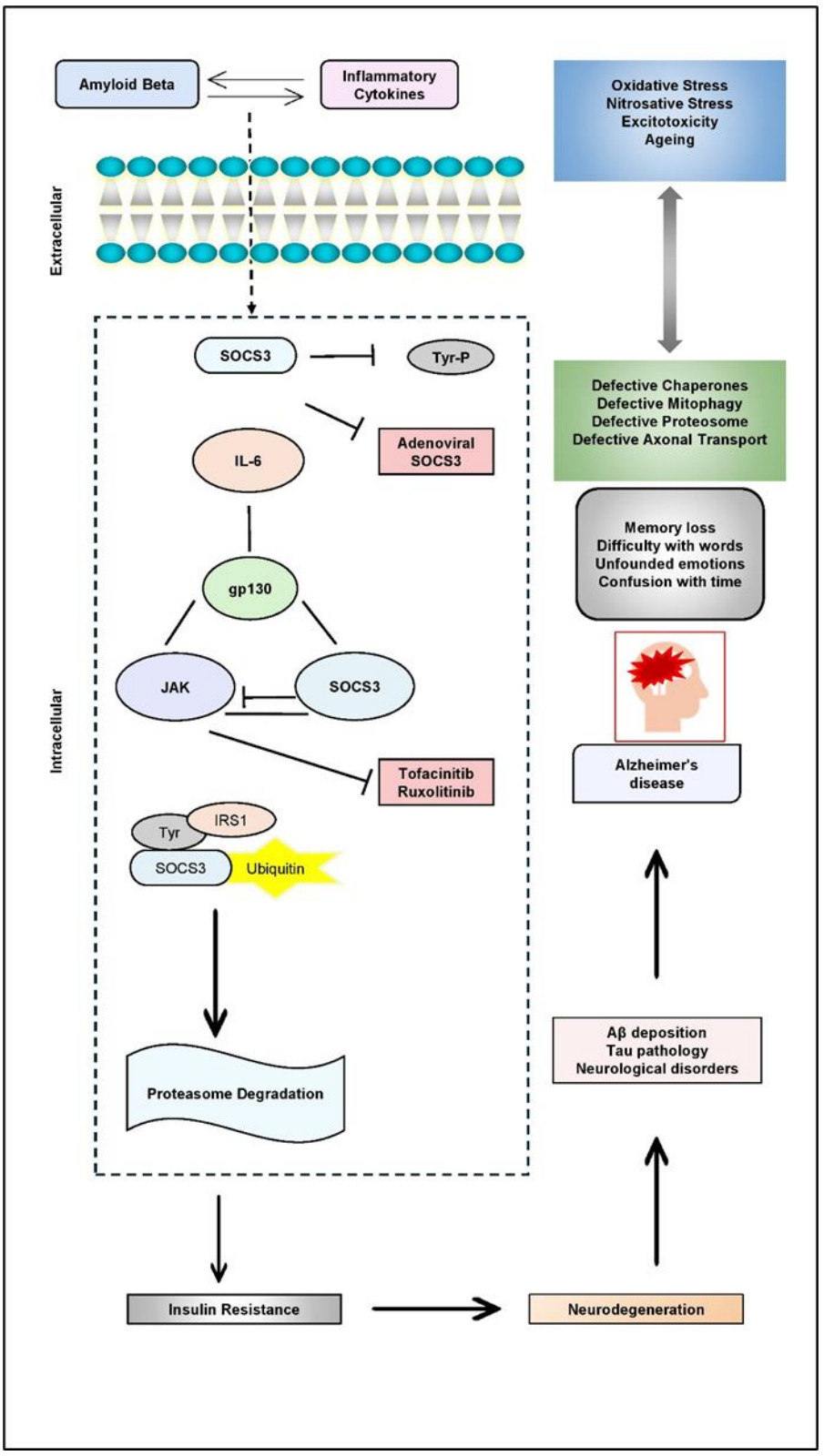


Fig. 2.4. SOCS-Mediated Inhibition of Neuronal Insulin Signaling. Neuronal insulin signaling is regulated by SOCS3. SOCS3 expression is increased subsequent to cytokine activation, resulting in the inhibition of insulin signaling via the tyrosine phosphorylation of IRS1. Furthermore, an increase in serine phosphorylation is observed in IRS1, resulting in the emergence of inflammatory responses and neuronal dysfunction.

2.6.3 MEF2 myocyte enhancer factor 2 in NDDs

MEF2 is a family of MADS-box transcription factors that regulate diverse processes, including cell differentiation, survival, and synaptic remodeling. In neurons, MEF2 isoforms regulate activity-dependent gene expression programs that shape dendritic spine density and synaptic connectivity, thereby linking neuronal activity to structural plasticity [106].

Genetic and functional studies implicate MEF2C in brain development and in the pathophysiology of neuropsychiatric disorders such as ASD, schizophrenia, and epilepsy. MEF2A has been shown to influence neuronal responses to neuromodulators and to regulate mitochondrial function, suggesting that MEF2 integrates metabolic cues with synaptic signaling in the CNS. Disruption of MEF2-dependent transcription may therefore increase susceptibility to both neurodevelopmental and degenerative conditions [107].

2.6.4 SIRT1 silent information regulator in NDDs

SIRT1, a member of the sirtuin family of NAD^+-dependent deacetylases, plays a significant role in defending the nervous system and has been associated with several NDDs, including AD and PD. SIRT1 deacetylates a broad repertoire of substrates, regulating mitochondrial biogenesis via PGC-1α, suppressing apoptosis through p53, maintaining genomic stability, and facilitating DNA repair, as well as exerting antioxidant effects via FOXO transcription factors [108, 109].

Age-related decline in SIRT1 activity has been linked to increased vulnerability to degenerative brain diseases, whereas pharmacological or genetic activation of SIRT1 can enhance synapse formation and support synaptic function. SIRT1 also interacts with pathways involving HEY2, AIRE, miR-132, and PARP-1, and modulates immune responses by affecting T-helper cell subsets, highlighting its pleiotropic role at the intersection of metabolism, inflammation, and neuroprotection [110, 111, 112, 113].

2.7 SIRT1 in specific neurodegenerative diseases

2.7.1 SIRT1 in Alzheimer's disease

In AD, SIRT1 participates in the regulation of tau protein by deacetylating tau, which enhances its ubiquitination and promotes proteasomal degradation, thereby reducing the formation of neurofibrillary tangles. Reduced SIRT1 activity has been linked to increased tau hyperphosphorylation and aggregation, especially under ApoE4-associated pathogenic conditions and disturbances in O-GlcNAc cycling. SIRT1 also modulates amyloid processing and synaptic plasticity-related signaling pathways, which further underscores its neuroprotective contribution in AD models [114, 115, 116, 117].

2.7.2 SIRT1 in Parkinson's disease

In PD, SIRT1 exerts neuroprotective actions that appear to be largely independent of SIRT2. This protective effect is associated with decreased levels of cleaved PARP-1 and NF-κB. As a result, apoptotic signaling and inflammatory gene expression are reduced [118]. SIRT1 regulates mitochondrial function and responses to oxidative stress. This regulation limits dopaminergic neuronal loss caused by environmental toxins and protein aggregation in PD [119].

2.7.3 SIRT1 in Huntington's disease

In HD, SIRT1 has a pivotal function in modulating pathways disrupted by mutant huntingtin. The mutant protein interferes with SIRT1-dependent deacetylation of targets such as TORC1, p53, FOXO3A, and PGC-1α, thereby impairing CREB-DARPP-32-BDNF signaling and mitochondrial biogenesis. This leads to reduced trophic support and increased oxidative and apoptotic stress in vulnerable striatal neurons. Selective modulation of SIRT1 activity is therefore considered a promising strategy to restore transcriptional balance and cellular resilience in HD [120, 121, 122].

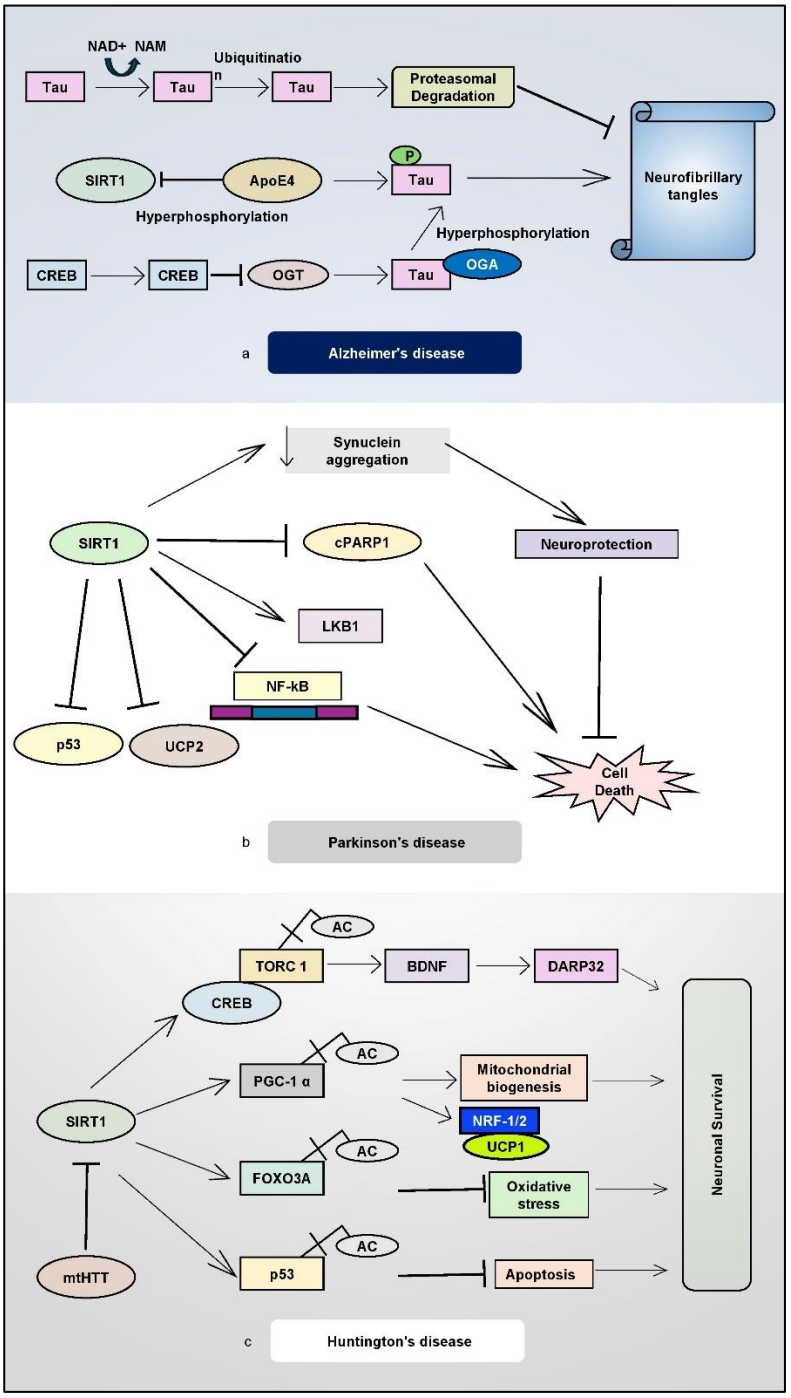


Fig. 2.5. Role of SIRT1 in neurodegenerative diseases. (a) SIRT1's involvement in the regulation of tau protein. It removes acetyl groups from the tau protein, which enhances its ubiquitination and decreases the formation of neurofibrillary tangles. Suppression of SIRT1 results in excessive phosphorylation of tau, which is caused by elevated functioning of ApoE4 and OGTT. (b) The activity of SIRT1 in PD. The effects of SIRT1 and SIRT2 on cell death in PD are independent of each other. SIRT1 suppresses the expression of c-PARP and NF-κB, thereby decreasing the formation of protein aggregates in cells. (c) SIRT1 has a pivotal function in Huntington's disease. The mutant Huntington protein hinders the function of SIRT1, disrupting many pathways. One of these avenues encompasses the suppression of deacetylated TORC1. In

general, when TORC1 is deacetylated, it interacts with CREB and DARPP32, which enhances the production of BDNF in neurons. Nevertheless, mutant Huntington hinders the deacetylation process in various targets, including TORC1, p53, FOXO3A, and PGC-1α, ultimately leading to cell death.

2.8. Targeting neuroinflammatory disorders with drug repositioning

Neuroinflammatory disorders such as AD and PD represent a substantial and growing global health burden. Millions of people are affected across the world. Drug repurposing has emerged as a promising approach for discovering new treatments by assigning new indications to existing drugs. Multiple preclinical and clinical studies indicate that repurposed agents can provide therapeutic benefit in various NDDs [123]. Neuroinflammation is a key factor in the initiation and progression of many neurological diseases. Drugs approved for unrelated issues that have an anti-inflammatory effect make good candidates for therapeutic repurposing. Probenecid stands out as a long-term drug with a documented clinical history. It also has an established safety profile, which makes it a compelling option for further investigation in neuroinflammatory settings. Its potential for repurposing in neuroinflammatory conditions is worth exploring. It is involved in enhancing the removal of uric acid, protecting the nervous system, and reducing inflammation [124]. Drug repositioning is a cost-effective approach that can contribute to new opportunities for treating therapeutic NDDs. Such as kinase inhibitors, developed as cancer treatments, have presented powerful neuroprotective effects in the treatment of NDDs [125]. Novel drug delivery methods have been readily used in preclinical and clinical trials to treat neuroinflammatory disorders by repurposing pharmaceuticals, such as immunomodulators and anti-inflammatory drugs. These statements have demonstrated reduced adverse effects, higher treatment outcomes, and enhanced patient consent in contrast to traditional drug delivery systems [126].

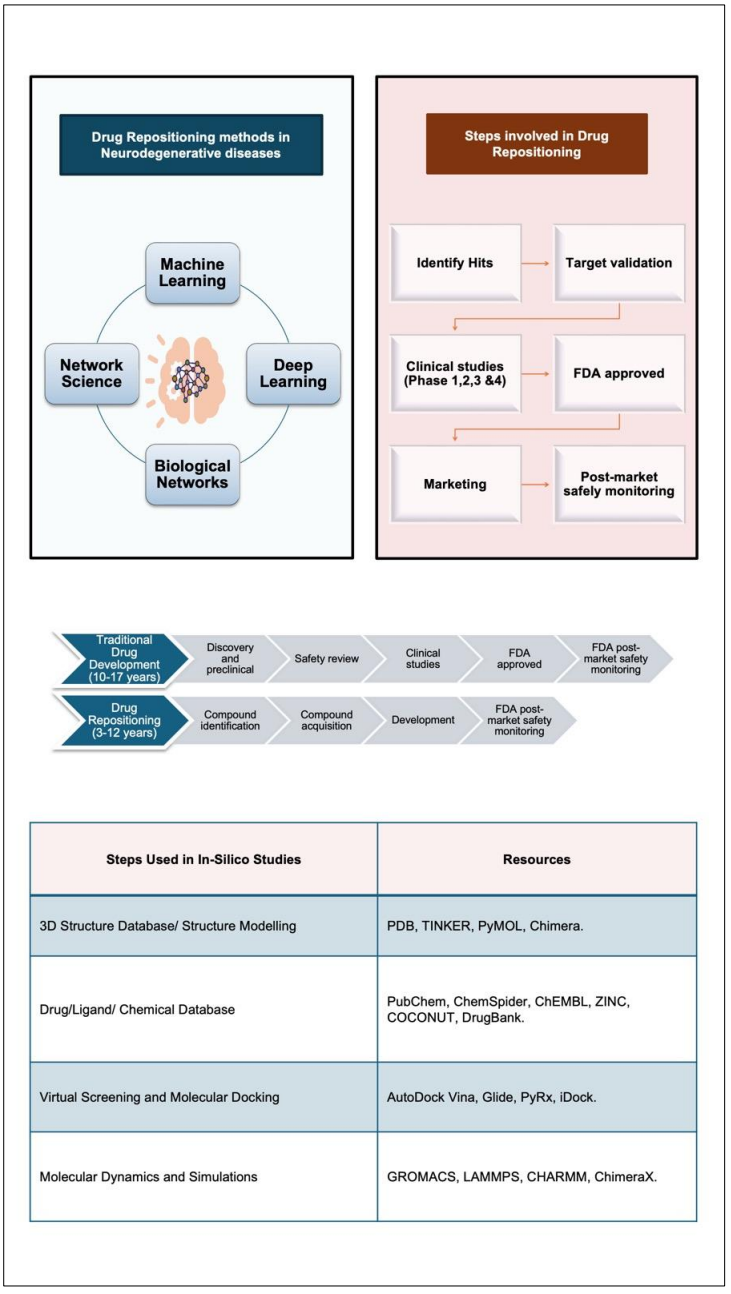
Re-using existing drugs for new indications offers several practical benefits. Because these compounds have already passed preclinical testing and at least some level of clinical evaluation, much of the early safety and pharmacokinetic work does not need to be repeated. This shortens timelines, lowers costs, and

avoids the need to design and characterize entirely new chemical entities from scratch. The established safety records of repurposed drugs in humans also mean that the risk of unexpected toxicity in later-phase trials is generally lower than in a conventional de novo programme. In addition, repurposing helps to overcome one of the main bottlenecks in drug discovery attrition due to safety problems, while at the same time expanding therapeutic options by uncovering new uses for medicines that are already approved for other diseases. This method provides new strategies for diseases that lack effective treatments [127, 128]. **Fig. 2.6** illustrates essential medication repurposing approaches.

A number of licensed drugs already in clinical use have shown promise when tested for new neurological indications because of their anti-inflammatory or neuroprotective actions. Minocycline, an antibiotic, has been reported to protect neurons in preclinical AD and PD models by dampening neuroinflammation and limiting microglial [129, 130, 131]. The widely used antidiabetic agent metformin has been linked to benefits in AD models, where it activates AMP-activated protein kinase (AMPK) and influences mitochondrial function [132]. Riluzole, which was initially approved for ALS due to its ability to regulate glutamate release and decrease excitotoxicity, is now being investigated for other indications across various NDDs [133]. In parallel, the antimalarials chloroquine and hydroxychloroquine are under investigation for their ability to modulate neuroinflammation and autophagy in conditions such as AD and PD [134]. Together, these examples illustrate how repurposing established drugs can open up new therapeutic possibilities for NDDs and highlight why continued work on repositioning strategies remains important.

Existing treatments for neuroinflammatory disorders frequently deliver only limited clinical benefit and are characterized mainly by adverse effects and substantial financial burden. In many cases, currently available treatments ease symptoms but do not adequately address the underlying inflammatory drivers of disease or halt progression. Patients may also experience side effects such as immunosuppression, gastrointestinal problems, and other safety issues that make long-term use difficult. On top of this, the high cost of some therapies limits their

availability, leaving many individuals without realistic access to these treatment options. Drug repurposing strategies may also face challenges related to intellectual property when existing patents remain active for original indications [135]. These problems emphasize the need for novel therapies that effectively treat neuroinflammation [136, 137].



Steps Used in In-Silico Studies	Resources
3D Structure Database/ Structure Modelling	PDB, TINKER, PyMOL, Chimera.
Drug/Ligand/ Chemical Database	PubChem, ChemSpider, ChEMBL, ZINC, COCONUT, DrugBank.
Virtual Screening and Molecular Docking	AutoDock Vina, Glide, PyRx, iDock.
Molecular Dynamics and Simulations	GROMACS, LAMMPS, CHARMM, ChimeraX.

Fig. 2.6. This figure demonstrates various drug repositioning techniques used in the treatment of NDDs.

2.8.1 Overview of clinical trials on neuroinflammation and NDDs

Tables 2.1 and **2.2** provide a concise reference for clinicians, researchers, and others interested in links between neuroinflammation and neurodegenerative disease. They summarize key clinical trials, listing the trial identifier, study title, design, status, phase, and the primary condition being investigated.

Table 2.1. Overview of Therapeutic Drugs, Disorders, Target Cells/Tissues, and Results

Therapeutic Drugs	Targeted Disease	Target cells or Tissues	Observed Results	References
Triptolide	*Alzheimer's disease*	Microglia	Triptolide exhibited anti-inflammatory and antioxidant properties, enhanced spatial learning, and reduced the amount of amyloid beta produced in the brain.	[138]
sRAGE-MSCk	*Alzheimer's disease*	Microglia	M2 microglia were present in more significant numbers. Additionally, sRAGE-MSCs successfully reduced RAGE.	[139]
Sesame oil	*Alzheimer's disease*	Microglia	Reduced levels of acetylcholine esterase and amyloid beta, as well as the expression of TNF-alpha, IL1-beta, and NF-kappa-B, were all enhanced by sesame oil. It also reduced memory impairment and learning.	[140]
SCP2-1	*Alzheimer's disease*	Microglia	By reducing the JNK and NF-kappa-B signaling pathways, the M1 polarisation of microglia was suppressed, and cognition was improved.	[141]

10-hydroxy decanoic acid	*Alzheimer's disease*	Microglia	N9 and BV2 cell lines have lower levels of NO and iNOS. Along with expressing less pro-inflammatory cytokines and more TREM2, these cells were also more expressed than untreated ones.	[141]
DMXBA	*Alzheimer's disease*	Microglia	Decreased accumulated amyloid-beta and enhanced memory and spatial learning. It enhanced microglial amyloid-beta phagocytosis activity and activated nAChR.	[142]
DAPPD	*Alzheimer's disease*	Microglia	Enhanced microglial function, reduced inflammatory cytokines including TNF-alpha and IL6, ApoE, and improved memory through the expression of anti-inflammatory genes like TGFb-beta and IL4, and suppressed NF-kappa-B.	[142]
Astaxanthin	*Alzheimer's disease*	Microglia	The M2 microglia's shift to the pro-inflammatory M1 phenotype was suppressed.	[143]
Cyclosporine	*Parkinson's disease*		Parkinson's disease mouse	[144]

			model with neural repair cyclosporine therapy.	
FK506	*Parkinson's disease*	Various immune cells	FK506 decreased T helper infiltration, cytotoxic T cell infiltration, and the number of macrophages and microglia while enhancing the survival of dopaminergic neurons.	[145]
Ginsenoside Rg1	*Parkinson's disease*	Microglia	Suppression of pro-inflammatory cytokines and elevation of anti-inflammatory cytokines were correlated with the activation of dopaminergic neurons in substantia nigra glial cells. Moreover, midbrain ginsenoside Rg1 reduced Nf-Kappa-B production by microglia and converted M1 microglia to M2 type.	[146]
MCC950	*Parkinson's disease*	Microglia	MCC950 reduced inflammation, alpha-syn accumulation, motor impairments, and nigrostriatal dopamine degradation.	[147]
Pioglitazone	*Parkinson's*	Microglia/	The substantia nigra of 5 mg/kg	[148]

	disease	macrophages	pioglitazone-treated monkeys had fewer macrophage and microglia cells than those in the control group.	
Donepezil	*Multiple sclerosis*	Microglia	Influence of immunomodulation on IL-4 and IFN-gamma	[149]
Ethyl pyruvate	*Multiple sclerosis*	Microglia and astrocytes	Ethyl pyruvate inhibited the infiltration of immune cells into the brain and the quantity of activated microglia and astrocytes.	[150]
PPAR -gamma agonists	*Multiple sclerosis*	Microglia/ astrocytes	They impeded the synthesis of TNF-alpha, IL1, IL6, MCP-1, and nitric oxide.	[151]

Table 2.2. Clinical Trials in Neuroinflammation

Clinical Trial ID	Title	Study Type	Status	Phase	Condition
NCT05040048	Taxonomy of Neurodegenerative Diseases: Observational Study in Alzheimer's Disease and Parkinson's Disease	Observational	Completed	Not Applicable	Parkinson's Disease and Alzheimer's Disease
NCT03457493	The University of Alabama at Birmingham (UAB) Neuroinflammation in Parkinson's Disease-TSPO-Positron Emission Tomography (PET) Sub study	Interventional	Recruiting	Phase 1, Phase 2	Parkinson's Disease

NCT05419453	Microbiome Composition and Function contribute to Cognitive Impairment and Neuroinflammation in Parkinson's Disease	Observational	Recruiting	Not Applicable	Parkinson's Disease
NCT04062526	Evaluation of Neuroinflammation in Parkinson's Disease Using 18F-NOS PET/CT	Interventional	Active, not recruiting	Early Phase 1	Parkinson's Disease
NCT03633513	Parkinson's Disease Inflammatory Biomarker Profiling	Observational	Terminated	Not Applicable	Parkinson's Disease
NCT02319382	Measure of Microglial Activation in the Brain of Parkinson's Disease Patients With PET	Interventional	Unknown status	Not Applicable	Parkinson's Disease
NCT05807581	Clinical, Molecular and Electrophysiological Profiling of Parkinson's Disease: The Role of Non-pharmacological Therapies	Interventional	Recruiting	Not Applicable	Parkinson's Disease
NCT05781711	Clinical Study to Evaluate the Possible Efficacy of Metformin in Patients with Parkinson's Disease	Interventional	Recruiting	Phase 2	Parkinson's Disease
NCT05854524	Exercise Neuroprotection in Parkinson's Disease	Interventional	Not yet recruiting	Not Applicable	Parkinson's Disease
NCT02511015	Hereditary Parkinson's Disease Natural History Protocol	Observational	Completed	Not Applicable	Parkinson's Disease

NCT05815524	Physical Activity in Patients with Parkinson's Disease: a "Disease Modifying" Intervention?	Interventional	Recruiting	Not Applicable	Parkinson's Disease
NCT05395624	Safety, PK, and Biodistribution of 18F-OP-801 in Patients With ALS, AD, MS, PD, and Healthy Volunteers	Interventional	Recruiting	Phase 1, Phase 2	Parkinson's Disease Amyotrophic Lateral Sclerosis Alzheimer's Disease Multiple Sclerosis
NCT04855344	Deep Brain Stimulation Therapy and Intestinal Microbiota	Observational	Unknown status	Not Applicable	Deep Brain Stimulation Gastrointestinal Microbiome
NCT05456451	Effectiveness of Noninvasive Vagus Stimulation for Upper Extremity in Parkinson's Disease	Interventional	Unknown status	Not Applicable	Parkinson's Disease Tremor Upper Extremity Dysfunction
NCT05008094	The Epidemiology of Parkinson's Disease in Croatia and the Influence of Genetic Factors and Microbiota on the Progression and Treatment Outcomes of the Disease (GiOPARK)	Observational	Unknown status	Not Applicable	Parkinson's Disease Genetic Disease Microbiota Neuroinflammatory Response
NCT05962957	Clinical Study to Compare the Possible Safety and Efficacy of Pentoxifylline in Patients with Parkinson's Disease Treated with Conventional Treatment	Interventional	Recruiting	Phase 2	Parkinson's Disease

NCT02377206	Neuroinflammation and Cognitive Decline in Alzheimer's Disease	Interventional	Completed	Early Phase 1	Alzheimer's Disease
NCT04786223	Targeting Neuroinflammation as a Contributing Pathology in Alzheimer's Disease Dementia	Interventional	Enrolling by invitation	Phase 2	Alzheimer's Disease
NCT05077579	Neuroinflammation and Alzheimer's Disease Imaging Biomarkers in Midlife Obesity	Observational	Recruiting	Not Applicable	Alzheimer's Disease
NCT04274998	Evaluation of in Vivo Neuroinflammation in Alzheimer's Disease Using Novel Positron Emission Tomography (PET/CT) Imaging	Interventional	Recruiting	Early Phase 1	Alzheimer's Disease
NCT03548883	Examining Neuroinflammation in Alzheimer's Disease Via Transcriptomic Profiling and Microglia Modeling Using Human Peripheral Blood Mononuclear Cells (ENHANCE)	Observational	Completed	Not Applicable	Alzheimer's Disease
NCT05378659	Neuroinflammation and Alzheimer's Pathology in Post-operative Cognitive Dysfunction: A Pilot Study	Observational	Unknown status	Not Applicable	Alzheimer's Disease

NCT05468073	Therapeutic Evaluation of Low-dose IL-2-based Immunomodulatory Approach in Patients with Early AD	Interventional	Recruiting	Phase 2	Alzheimer's Disease
NCT05114499	Efficacy and Safety of Donepezil and Sodium Oligomannate in Patients with Mild to Moderate Alzheimer's Disease	Observational	Not yet recruiting	Not Applicable	Alzheimer's Disease
NCT03435861	Effect of Neflamapimod (VX-745) on Brain Inflammation Using Positron Emission Tomography (PET) Scan in Alzheimer's Disease (AD) Patients	Interventional	Completed	Phase 2	Alzheimer's Disease
NCT05911178	Impact of Microglial Activation on Synaptic Density in Alzheimer's Disease	Interventional	Recruiting	Not Applicable	Alzheimer's Disease
NCT04517552	Investigation of Inflammation Using [C-11]-CS1P1	Observational	Active, not recruiting	Not Applicable	Alzheimer's Disease
NCT03744312	Imaging Inflammation in Alzheimer's Disease With 11C-ER176	Interventional	Completed	Phase 1 Phase 2	Alzheimer's Disease
NCT05821153	Phase I Trial Using Interleukin-2 (IL-2) to Expand Regulatory T Cells in Patients with Alzheimer's Disease	Interventional	Completed	Phase 1	Alzheimer's Disease

NCT06096090	A Phase II Clinical Trial of Interleukin-2 (IL-2) in Patients with Mild to Moderate Alzheimer's Disease	Interventional	Recruiting	Phase 2	Alzheimer's Disease
NCT04388254	A 12-Month, Open-Label Safety Study of Simufilam Followed by a 6-Month Randomized Withdrawal and 6 Additional Months Open-Label in Mild-to-moderate Alzheimer's Disease Patients	Interventional	Completed	Phase 2	Alzheimer's Disease
NCT05349318	Hyperbaric Oxygen Therapy for Prodromal Alzheimer´s Disease with Cerebrovascular Disease: A Prospective, Randomized, Double Blind Study	Interventional	Recruiting	Not Applicable	Prodromal Alzheimer's Disease

CHAPTER 3

MATERIALS AND METHODS

Chapter 3

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3.1 Research objective 1: To identify specific biomarkers that are associated with NDDs.

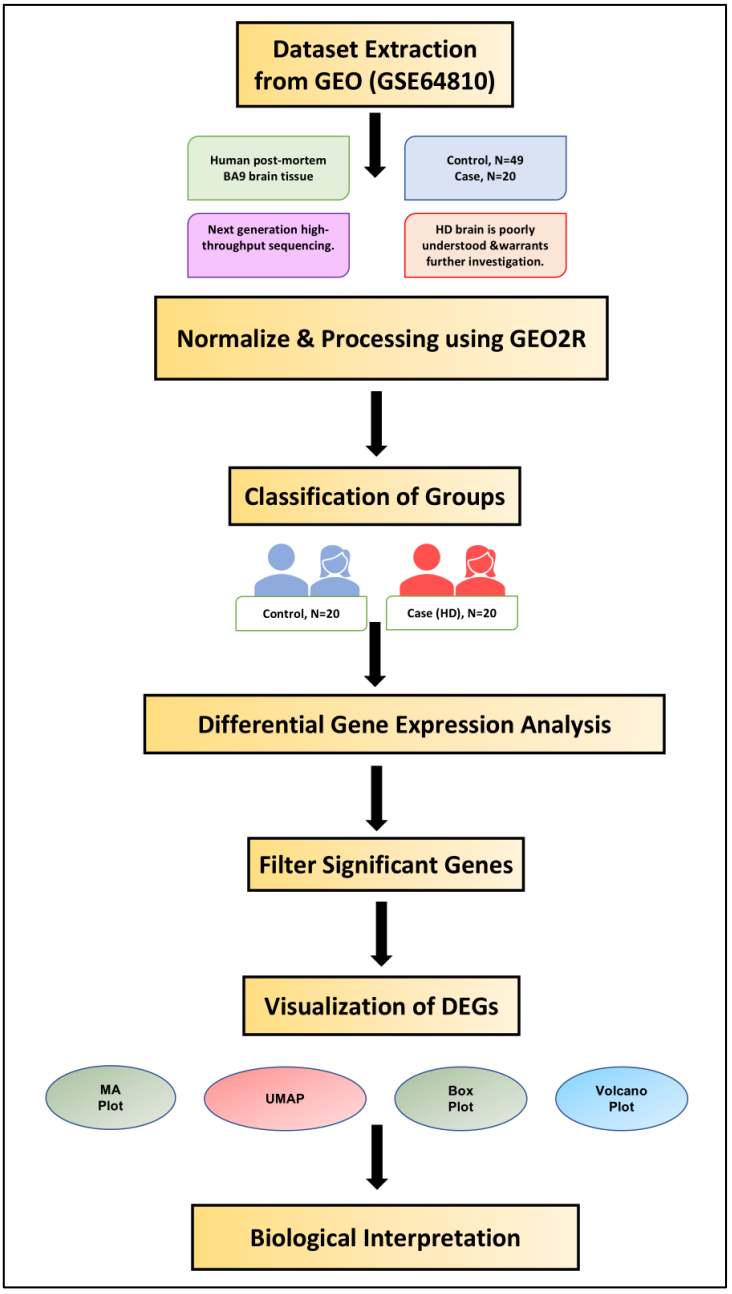


Fig 3.1. Steps involved in the identification of specific biomarkers associated with NDDs.

3.1.1 Dataset Retrieval and Pre-processing

Gene-expression data for HD were retrieved from the NCBI Gene Expression Omnibus (GEO) using the RNA-seq series GSE64810 (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE64810), which contains profiles from post-mortem human frontal cortex tissue of HD patients and neurologically normal controls. Only human samples were included to maintain direct relevance to disease pathology. The raw count data were first checked for quality and then normalized to minimize technical and batch-related effects. Box-plot inspection after normalization showed comparable expression distributions across samples, indicating improved data consistency for the subsequent differential expression analyses.

3.1.2 Differential Gene Expression Analysis

Differential expression was assessed using a standard RNA-seq workflow comparing HD samples with healthy controls. Raw p-values from the statistical tests were corrected for multiple comparisons with the Benjamini-Hochberg false discovery rate procedure, and genes with an adjusted p-value < 0.05 were retained as significant. To focus on changes of clear biological relevance, an absolute log2 fold-change ($|\log2FC|$) greater than 1 was additionally required. Genes with positive log2FC values were classified as upregulated in HD, whereas those with negative values were considered downregulated. This combination of FDR control and fold-change filtering yielded a final DEG set that was both statistically robust and characterized by pronounced shifts in expression.

3.1.3 Visualization and Sample Clustering

To investigate overall transcriptome differences between HD and control samples, a dimensionality reduction analysis was performed using Uniform Manifold Approximation and Projection (UMAP). This allowed us to see how the samples clustered based on their global gene expression patterns. However, volcano plots were created to show, in a single view, how large the expression changes were (log2FC) and how statistically significant they were (-log10 p-value) for the

DEGs. MA plots were employed to evaluate expression bias across mean expression levels and to validate DEG robustness across the dataset.

3.1.4 Functional Enrichment and Pathway Analysis

Significantly upregulated genes were subjected to functional enrichment analysis to identify overrepresented biological processes and signaling pathways. Gene Ontology (GO) enrichment analysis was performed to explore biological processes associated with the identified DEGs. Pathway enrichment analysis was conducted using curated pathway databases through the KEGG platform (https://www.genome.jp/kegg/pathway.html). Enriched GO terms and pathways with adjusted p-values <0.05 were considered significant and were visualized using dot plots and bar plots to facilitate interpretation.

3.2 Research objective 2: To identify a systematic approach to screen and select anti-inflammatory drugs for targeting NDDs.

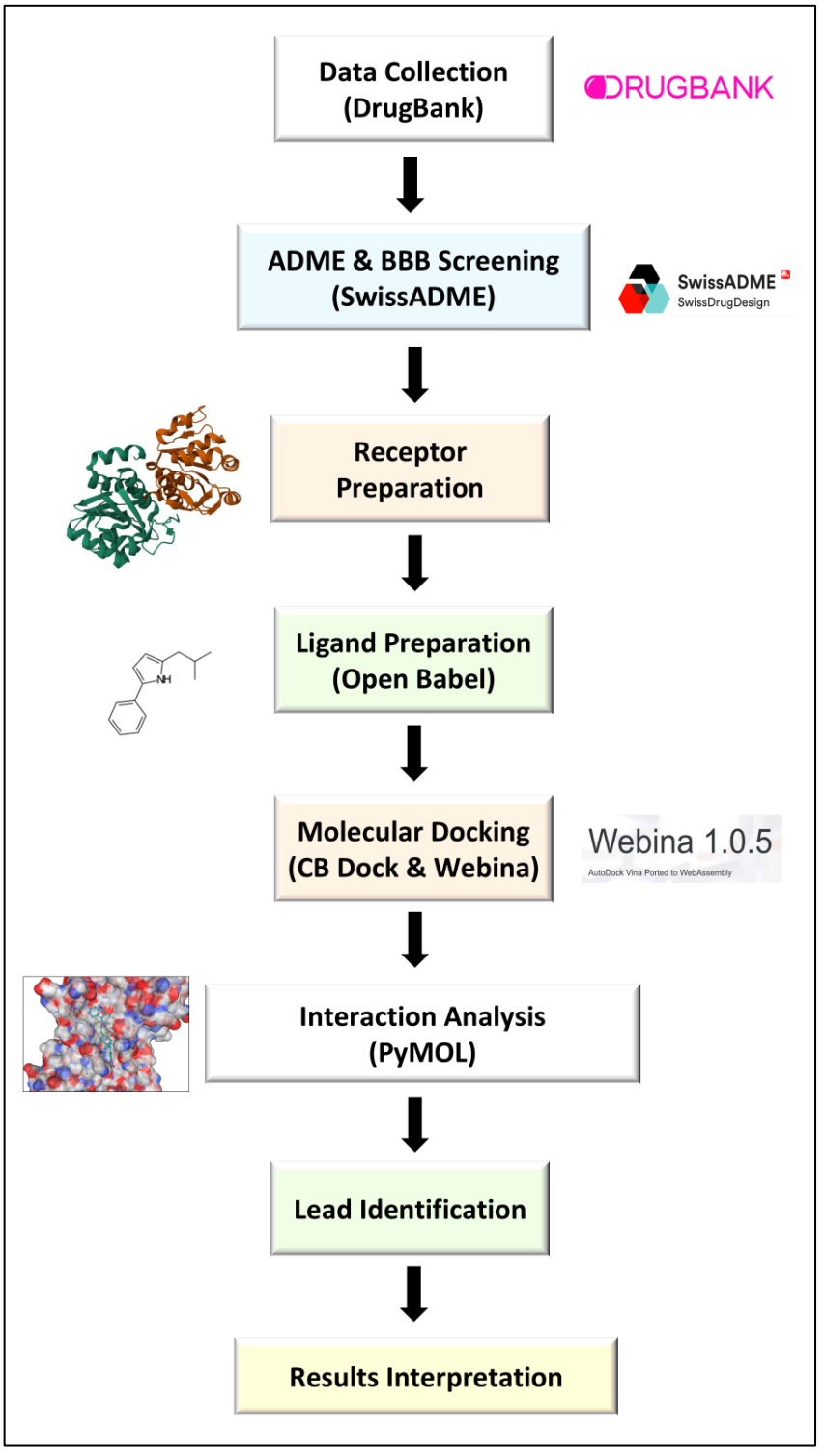


Fig 3.2. Overall steps involved in virtual screening of anti-inflammatory drugs for targeting NDDs.

3.2.1 Data Collection

A list of FDA-approved anti-inflammatory medications was selected from the DrugBank for repurposing as treatments against mutant DJ-1(2R1T). Using the online web-based server SwissADME (absorption, distribution, metabolism, and excretion) tool (http://www.swissadme.ch/) [152, 153, 154], these medications were evaluated to determine their permeability to the blood-brain barrier. The drugs were then downloaded as SDF structures using PubChem (https://pubchem.ncbi.nlm.nih.gov/) [155]. From the PDB database (https://www.rcsb.org/structure/2R1T) [156], the DJ-1 mutant (PDB ID: 2R1T) was obtained.

3.2.2 Receptor and ligand preparation

The DJ-1 structure was prepared for molecular docking using the Autodock Tool. The water molecules and heteroatoms were eliminated from the protein structure, and polar hydrogen and Kollman charges were added. The resulting receptors were saved as a PDBQT file. A docking grid map was created. Additionally, the ligands were transformed from SDF to PDB format, and then, with the help of Open Babel (https://www.cheminfo.org/Chemistry/Cheminformatics/FormatConverter/index.html), these were converted into PBQT format for further use.

3.2.3 Molecular Docking

The proteins and ligands were prepared, and Webina (https://durrantlab.pitt.edu/webina/) and CB-Dock (https://clab.labshare.cn/cb-dock/) were used to perform molecular docking. Entries were evaluated using compound characteristics: RMSD < 1 Armstrong, and free energy of binding < -8.0 kcal/mol. Once the molecular docking is complete, the binding energy, binding mechanism, and interaction between the ligand and protein can be visualized. Several visualization software can be used to graphically examine protein-ligand complexes, such as Chimera, PyMOL, and VMD.

3.2.4 Pharmacokinetic and Blood-Brain Barrier Analysis

In silico pharmacokinetic analysis was performed to evaluate the absorption, distribution, metabolism, and excretion (ADME) properties of the shortlisted compounds. Blood-brain barrier permeability was predicted using the BOILED-Egg model, which estimates gastrointestinal absorption and BBB penetration based on physicochemical parameters.

3.3 Research objective 3: To identify potential natural SIRT1 inhibitors for NDDs using a computational approach.

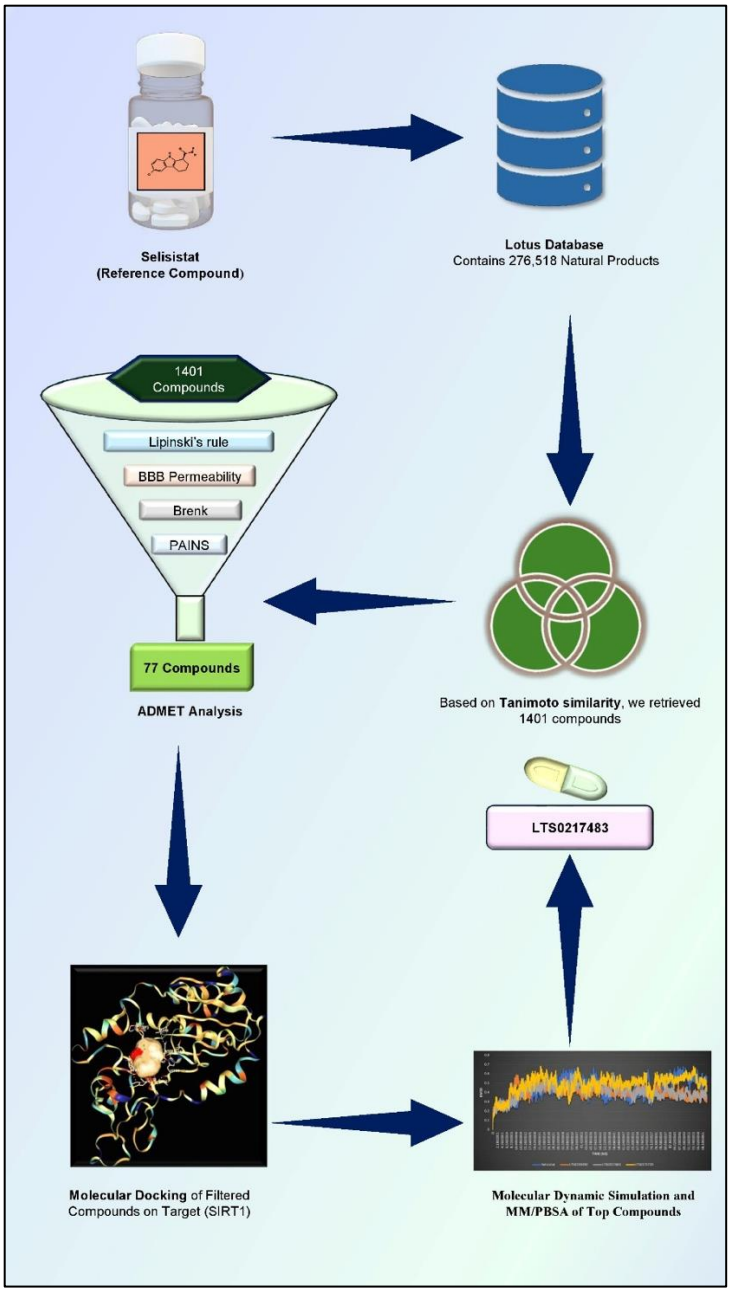


Fig 3.3. Overall steps involved in virtual screening of natural compounds targeting SIRT1.

3.3.1 Structure Retrieval

The X-ray crystal structure of the catalytic domain of human SIRT1 in complex with NAD^+ and the EX-527 analogue (PDB ID: 4I5I; resolution 2.50 Å; R-value 0.202) was obtained from the RCSB Protein Data Bank [156]. This structure was selected based on its fully resolved active site and co-crystallized inhibitor, which facilitated the accurate definition of the docking site. Structural quality was verified by Ramachandran plot analysis (>92.1% of residues in favored regions) in the PROCHECK (https://www.ebi.ac.uk/thornton-srv/software/PROCHECK/) tool and by confirming the absence of missing residues in the binding pocket [157].

Protein preparation was performed in Discovery Studio Visualizer (Dassault Systèmes, version 2021.1.0.20298) (https://discover.3ds.com/discovery-studio-visualizer-download) using the CHARMM force field. Preparation steps included the removal of crystallographic water molecules and non-catalytic heteroatoms, the addition of polar hydrogen atoms, the assignment of protonation states to physiological pH (7.4), and energy minimization to relieve steric clashes [158]. The prepared protein structure was saved in PDB format and converted to PDBQT format using Webina 1.0.5 (https://durrantlab.pitt.edu/webina/) for molecular docking [159].

3.3.2 Ligand Library Preparation

Natural product analogues of Selisistat were retrieved from the LOTUS database (https://lotus.naturalproducts.net/), which contains more than 276,518 curated natural products from diverse taxonomic origins [160]. The LOTUS database was selected for its broad chemical diversity, standardised stereochemical data, and suitability for virtual screening. A Tanimoto similarity threshold of ≥ 68% was applied to retain key pharmacophore features while allowing structural diversity. This threshold is consistent with prior neurotherapeutic screening studies. Values in the 65-70% range effectively balance similarity to the reference ligand with the potential to identify structurally distinct yet biologically relevant analogues.

Ligand structures were downloaded in SDF format. Hydrogen atoms were added,

protonation states were adjusted to pH 7.4, and solvent molecules, counterions, and irrelevant entities were removed. Energy minimisation was performed using the CHARMM force field. The processed ligands were converted from SDF to MOL2 format using Avogadro (https://avogadro.cc/) [161] and subsequently to PDBQT format using Webina (browser-based AutoDock Vina implementation) (https://durrantlab.pitt.edu/webina/) for docking [162].

3.3.3 ADMET Analysis Using SWISSADME

ADMET profiling was performed using SwissADME (http://www.swissadme.ch/), a widely used web-based platform for pharmacokinetic and drug-likeness prediction [152, 153]. Ligand structures retrieved from the LOTUS database were exported in SMILES format and assessed for compliance with Lipinski's Rule of Five (molecular weight ≤500 Da, LogP ≤5, HBA ≤10, HBD ≤5) to ensure oral bioavailability potential. Additional CNS-relevant parameters, including topological polar surface area (TPSA ≤140 Å2), number of rotatable bonds (≤10), BBB permeability, gastrointestinal absorption, and bioavailability score. BBB penetration and CNS drug-likeness were specifically predicted using the BOILED-Egg model [154]. Only compounds meeting both oral bioavailability and BBB permeability criteria were selected for subsequent molecular docking studies. This filtering ensured that the selected ligands had optimal systemic pharmacokinetics and a strong likelihood of CNS activity, which is critical for SIRT1 modulation in the NDD context.

3.3.4 Molecular Docking Screening

Molecular docking studies were performed with Webina (v1.0) (https://durrantlab.pitt.edu/webina/) [162], a browser-based implementation of AutoDock Vina. Webina was selected for its accessibility, platform independence, and reproducibility, as it performs the same scoring function and search algorithm as AutoDock Vina, which has been extensively validated in the literature. While less common for high-throughput screening, Webina ensures a consistent computational environment without local installation, aligning with reproducibility considerations.

Docking was carried out on the designed SIRT1 structure given in the Structural

Retrieval section, as well as the ligand library described in the Ligand Library Preparation section. To validate the docking methodology, Selisistat, the co-crystallized inhibitor, was re-docked into the NAD^+ binding cleft of PDB ID: 4I5I, replicating the crystallographic pose with an RMSD of < 2.0 Å. This confirmed the dependability of the chosen parameters. The docking grid was centered on the geometric center of Selisistat's crystallographic coordinates (x=41.06, y=-25.72, z=22.73) with grid dimensions 10.23×16.60×12.74 Å, fully encompassing the major binding site and adjacent subpockets. Grid spacing was set to 0.375 Å, and the exhaustiveness parameter to 8, following the established small-molecule docking protocol.

Binding affinities (kcal/mol) and interaction profiles, including hydrogen bonds, hydrophobic contacts, and π–π stacking, were obtained for each ligand. Post-docking visualization and interaction mapping were performed in Discovery Studio Visualizer (v2021) to identify key binding residues and compare the interaction pattern with Selisistat. As docking assumes a rigid receptor conformation and may not fully capture protein flexibility or solvent effects, these predictions were subsequently refined using MD simulations and binding free energy calculations.

3.3.5 Molecular Dynamics Simulation

All-atom MD simulations were performed using GROMACS 2023.3 with the CHARMM36 force field and TIP3P water model [163, 164, 165]. Ligand topologies and parameters were generated using the CGenFF (https://www.cgenff.com/) [166, 167] server using MOL2 files obtained from docking. Each protein-ligand complex was placed in a triclinic simulation box with a minimum 10 Å buffer from the protein surface, solvated with explicit TIP3P water molecules, and neutralized with Na^+/Cl^- ions to a 0.15 M ionic strength.

Energy minimization was conducted using the steepest descent algorithm until the maximum force fell below 1000 kJ/mol/nm. Systems were equilibrated in two phases: 100 ps of NVT at 300 K, employing a V-rescale thermostat, followed by 100 ps of NPT at 1 bar using the Parrinello-Rahman barostat. Equilibration

stability was confirmed by monitoring total energy, temperature, pressure, and backbone RMSD. Unrestrained 100 ns production runs were performed with a two fs timestep, Particle Mesh Ewald (PME) electrostatics, a 1.0 nm short-range cutoff for nonbonded interactions, LINCS constraints on bonds involving hydrogen, and periodic boundary conditions.

Backbone RMSD, per-residue RMSF, Rg, and hydrogen bond occupancy were all used in the trajectory analysis to assess protein flexibility and binding mode stability. Hydrogen bonds were calculated using a donor-acceptor distance of ≤ 3.5 Å, and acceptor-donor hydrogen angle ≥ 135°. Rg changes were interpreted with caution to distinguish between conformational shifts and unfolding processes.

3.3.6 MM/PBSA Analysis

To determine the binding energy of protein-ligand complexes, molecular mechanics (MM) energy calculations were carried out using the g_mmpbsa tool (https://rashmikumari.github.io/g_mmpbsa/) as a post-processing step following molecular dynamics simulations in GROMACS [47, 48, 49]. Only the vacuum interaction energy, which comprises van der Waals and electrostatic energy components, was evaluated.

The binding energy (ΔE_{bind}) was calculated using the following equation:

$$\Delta E_{bind} = E_{complex} - (E_{protein} + E_{ligand})$$

Where E denotes the potential energy calculated from the force field, molecular mechanics calculations were performed in the representative trajectory frame extracted from the equilibrated portion of the simulation. Energy decomposition was performed to analyse van der Waals and electrostatic contributions to binding. This MM-based approach enables the calculation of the energy contribution from ligand binding and supports the structural and dynamic findings from the MD simulations.

CHAPTER 4

RESULT AND DISCUSSION

Chapter 4

RESULT AND DISCUSSION

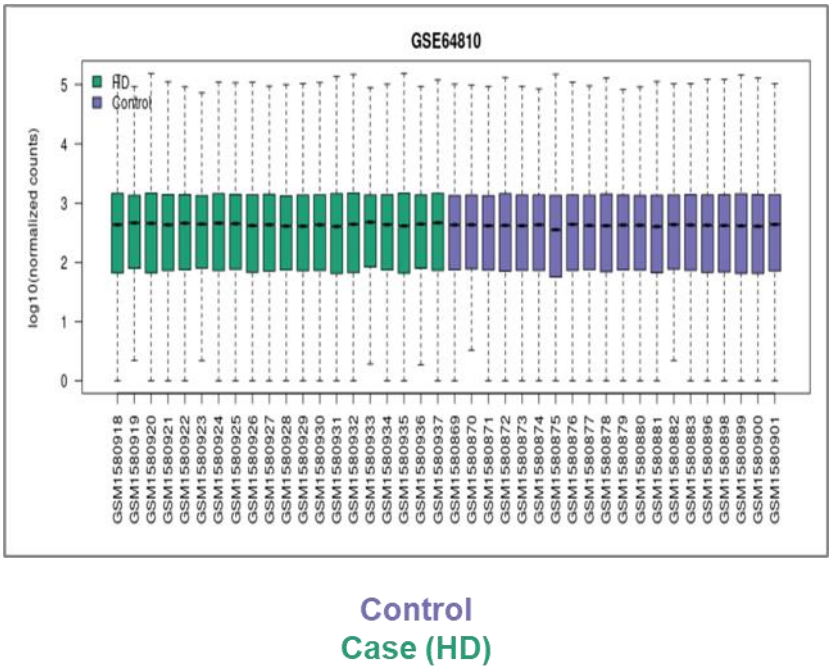
4.1 Research objective 1: To identify specific biomarkers that are associated with NDDs.

4.1.1 Identification of Differentially Expressed Genes

For this analysis, the GSE64810 dataset was used to compare gene expression profiles between HD cases and healthy controls. Differential expression was determined using an adjusted p-value cut-off of < 0.05 together with a log2FC threshold > 1, yielding a set of genes with both statistically and biologically meaningful changes. Within this group, **CXCL1, FOXC2, CLEC2B, OGN**, and **SLC6A12** were consistently upregulated in HD samples, each showing log2FC values above 1 and strong statistical support, indicating marked transcriptional activation in the disease state. Because these genes fall within pathways already implicated in HD pathogenesis, they were selected as priority candidates for follow-up functional experiments and for evaluating their potential as future therapeutic targets.

4.1.2 Quality Control and Sample Separation

Box plots were used to verify whether normalization had been successful, and the expression ranges appeared comparable across all samples, indicating that most technical noise had been removed. Next, UMAP was applied to explore the global structure of the dataset. In **Fig. 4.1** and **4.2**, HD and control cases occupy clearly separated regions of the map, which is consistent with distinct disease-related transcriptional profiles. On this basis, the GSE64810 data were considered suitable for the differential-expression and enrichment analyses carried out in the following.



Control
Case (HD)

Fig. 4.1. Boxplot showing the distribution of log10 normalized gene expression counts across samples in the GSE64810. Green boxes indicate Huntington's disease cases, whereas purple boxes demonstrate control samples. The comparable median values and overall distributions across samples illustrate successful normalization and minimal technical bias between case and control groups.

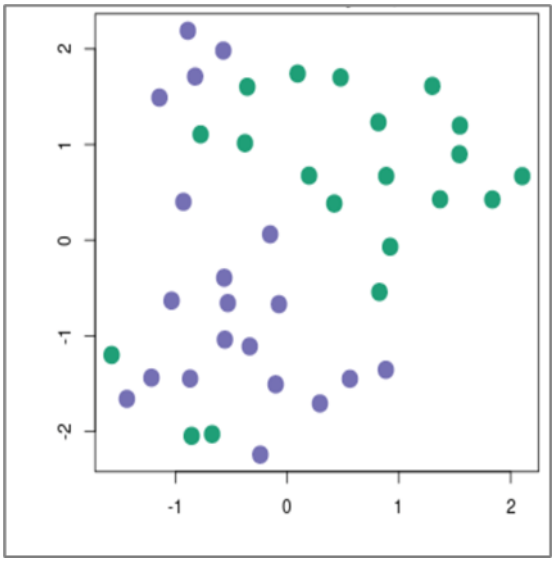


Fig. 4.2. UMAP plot demonstrating clear separation between control and case (HD) samples.

4.1.3 Visualization of Differential Expression

Volcano plots were used to summarize how strongly each gene changed in expression and how statistically reliable those changes were. In **Fig. 4.3**, genes

that were significantly upregulated cluster in the positive log2FC region, while significantly downregulated genes fall mainly on the negative side of the axis. The accompanying MA plot shows that these differentially expressed genes display consistent shifts across a wide span of mean expression values, supporting the robustness of the detected transcriptional changes.

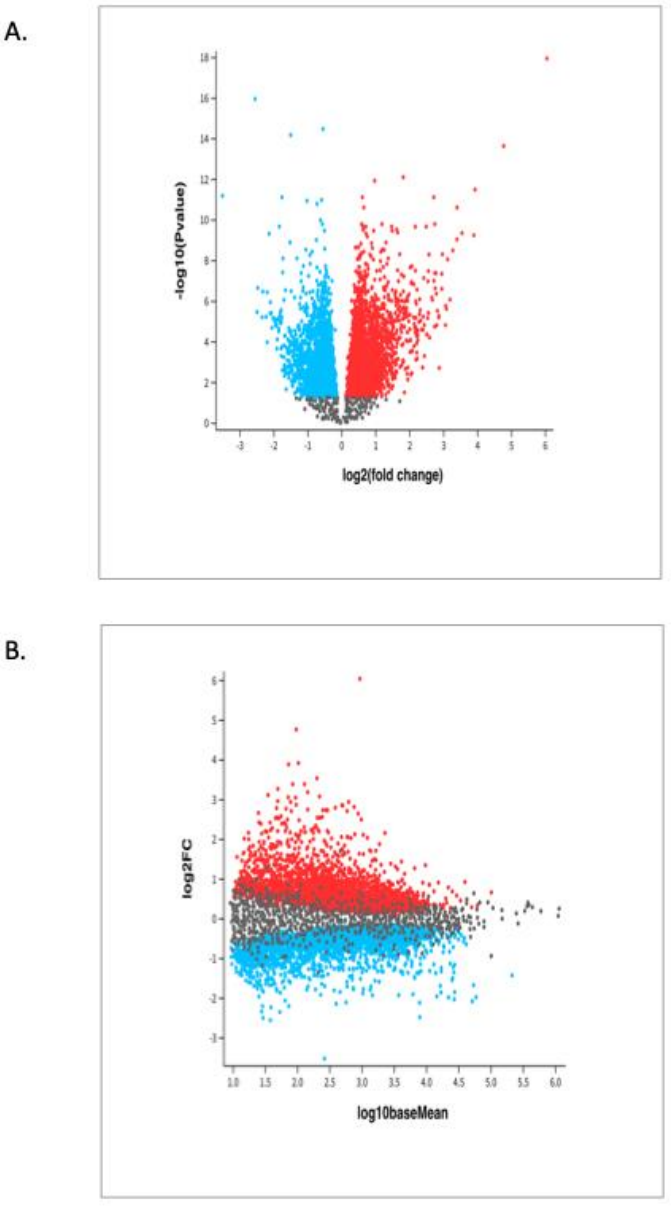


Fig. 4.3. Visualization of differential expression (A) Volcano plot showing log2FC versus -log10 (p-value), where significantly upregulated genes are indicated in red, downregulated genes in blue, and non-significant genes in grey. (B) MA plot depicting log2FC as a function of mean expression, highlighting the strength and distribution of differentially expressed genes across

expression levels, with the same color scheme.

4.1.4 Functional Enrichment and Pathway Analysis

In this study, functional enrichment analysis of the upregulated genes revealed significant overrepresentation of inflammatory and immune-related biological processes. GO enrichment analysis indicated involvement in cytokine-mediated signaling, immune cell activation, and inflammatory responses. Pathway enrichment analysis further identified significant enrichment of **NF-κB signaling, TNF signaling, IL-17 signaling**, and **chemokine signaling** pathways, all of which are well-recognized contributors to neuroinflammatory mechanisms, as shown in **Fig. 4.4**.

The prominent enrichment of these pathways suggests that the identified genes participate in a broader inflammatory signaling network rather than acting as disease-specific causative factors. Among the upregulated genes, CXCL1 emerged as a key inflammatory chemokine associated with multiple pathways, which FOXC2, CLEC2B, OGN, and SLC16A12 were linked to immune regulation, vascular remodeling, extracellular matrix organization, and metabolic stress relevant to HD pathology.

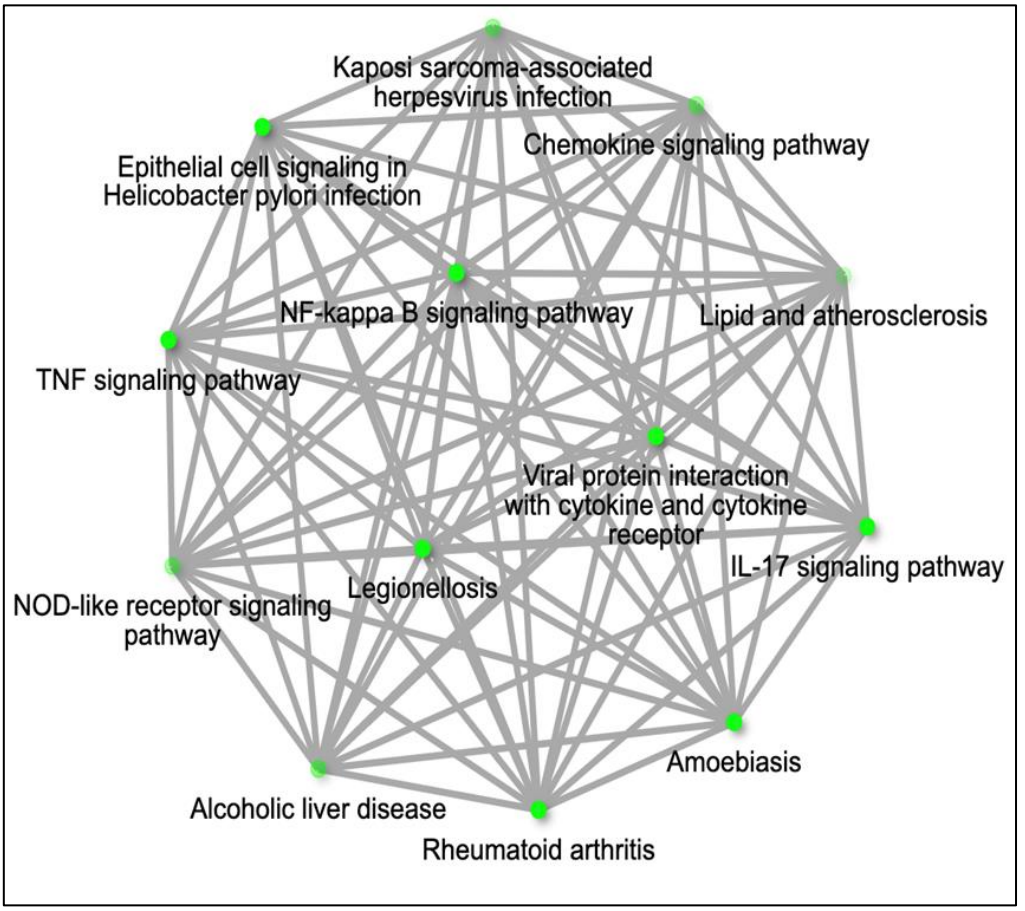


Fig. 4.4. KEGG pathway enrichment and network analysis of upregulated genes.

4.2 Research objective 2: To identify a systematic approach to screen and select anti-inflammatory drugs for targeting NDDs.

4.2.1 Docking-Based Identification of Candidate Compounds

In this study, docking-based virtual screening was performed to identify FDA-approved compounds with strong binding potential toward the DJ-1 protein. Among the screened molecules, **Oxatomide** and **Levocabastine** consistently exhibited the most favorable docking scores across CB-Dock and Webina tools, shown in **Fig. 4.7**. Both compounds occupied the predicted binding cavity of DJ-1, indicating stable and energetically favorable interactions, as shown in **Fig. 4.5**.

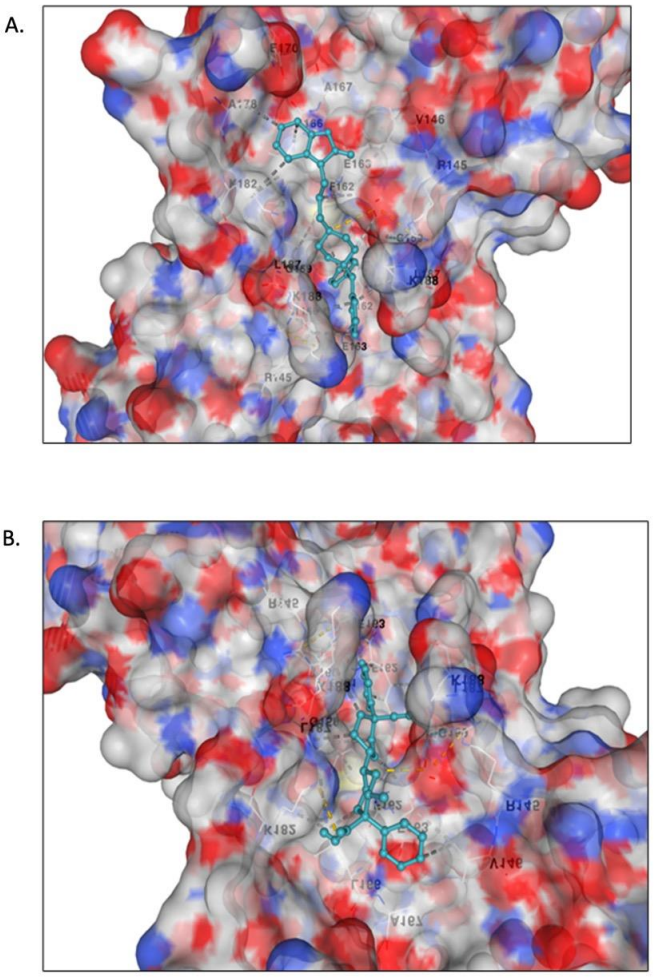


Fig. 4.5. Binding poses of (A) Oxatomide and (B) Levocabastine within the DJ-1 active site.

4.2.2 Protein-Ligand Interaction Analysis

We further analyzed the binding interactions of Oxatomide and Levocabastine within the DJ-1 binding pocket to understand their interaction profiles. Both ligands formed multiple stabilizing interactions, including hydrogen bonds and hydrophobic interactions with residues lining the binding cavity. These interactions suggest favorable ligand accommodation and structural compatibility with functionally relevant regions of DJ-1. Such interaction stability is essential for potential modulation of DJ-1-associated pathways in PD.

4.2.3 Pharmacokinetic and BBB Permeability

In this study, in silico pharmacokinetic evaluation was conducted to assess the drug-likeness and CNS suitability of the shortlisted compounds. Both Oxatomide and Levocabastine demonstrated acceptable ADME profiles. BBB permeability analysis using the BOILED-Egg model predicted efficient GI absorption and the ability of both compounds to cross the BBB, indicated in **Fig. 4.6. and Table 4.1**. These properties are particularly important for neurological applications, as effective CNS penetration is required for therapeutic relevance in PD.

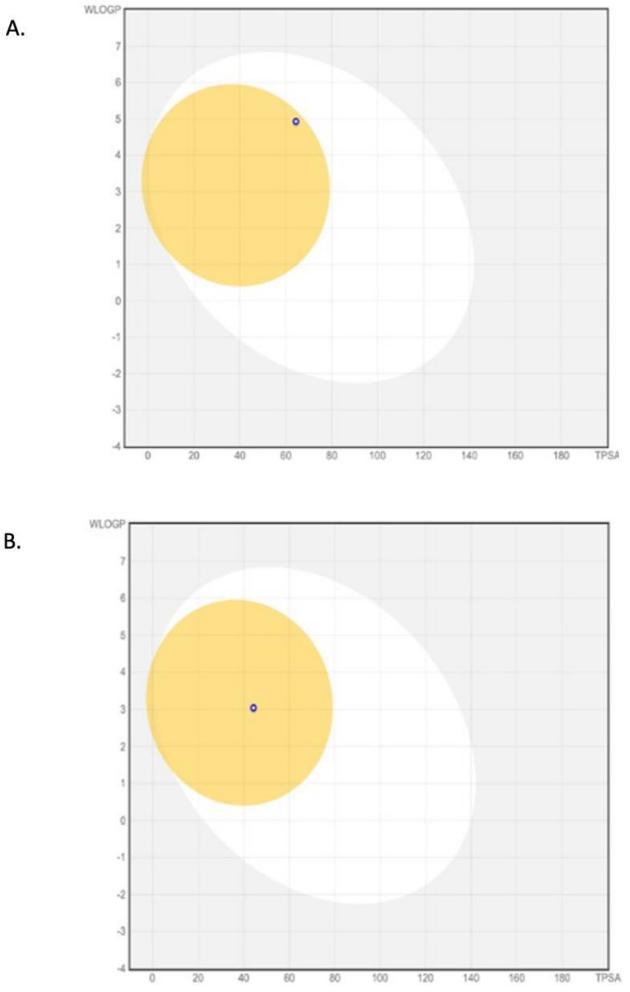


Fig. 4.6. BOILED-Egg plot showing predicted ADME properties of (A) **Oxatomide** and (B) **Levocabastine**, illustrating their GI absorption (white region) and BBB permeability (yellow region).

Table 4.1. Tabular Representation of Docking Score of Drugs

Drug Name	Compound CID	Webina Docking Score	CB Dock Docking Score	Blood-Brain Barrier Permeability
Oxatomide	**4615**	**-8.673**	**-8.5**	**Yes**
Loteprednol	9865442	-8.556	-8.5	No
Fexofenadine	3348	-9.409	-8.6	No
Levocabastine	**54385**	**-8.332**	**-8.9**	**Yes**
Ritanserin	5074	-9.648	-8.7	No
Prednisone	5865	-9.107	-8.9	No

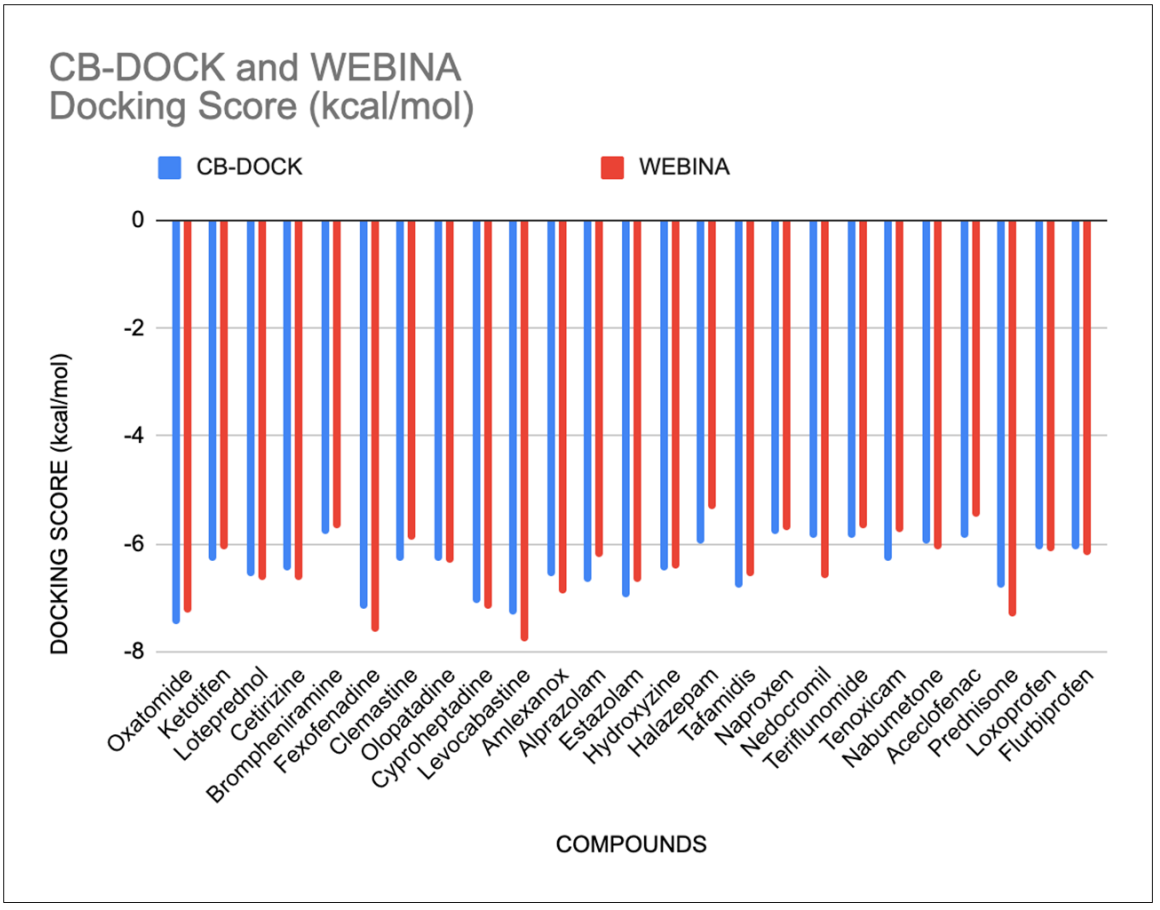


Fig. 4.7. Comparison of molecular docking scores obtained using CB-Dock and Webina

4.3 Research objective 3: To identify potential natural SIRT1 inhibitors for NDDs using a computational approach.

4.3.1 Structural Analysis of SIRT1 Binding Site and Key Residues Relevant to Ligand Interaction

The catalytic domain of human SIRT1 (PDB ID: 4I5I, 2.50 Å resolution) encompasses residues 241-516 and exhibits the canonical sirtuin fold required for NAD^+-dependent deacetylase activity (**Fig. 4.8.)** [156]. It consists of a Rossmann-fold NAD^+-binding domain (residues 241-370) and a $Zn2^+$-binding domain (residues 371-516) [168]. The $Zn2^+$-binding domain stabilizes the catalytic framework through a tetrahedral zinc ion coordinated by Cys371, Cys374, Cys395, and Cys398, while His363 and Asp292 play critical catalytic and stabilization roles.

The active sites form a hydrophobic tunnel lined by Phe414, Leu418, and Val445, with Glu416 and Arg 446 contributing polar interactions. Inhibitors such as Selisistat target the nicotinamide pocket, forming hydrogen bonds with Gly415 and Arg446, and hydrophobic contacts with Phe414, Leu418, and Val445 [168]. These residues have been previously characterized in crystallographic and mutagenesis studies; however, our analysis verified their spatial arrangement in the selected crystal structure and validated their relevance as the functional docking site for our screening approach.

By focusing docking and MD simulations on a functionally validated SIRT1 pocket, the study established a consistent framework for comparing natural inhibitors with existing reference compounds. The main innovation is in demonstrating how conserved active-site residues accommodate new chemotypes, revealing alternative binding arrangements and potential inhibition mechanisms that are directly relevant for HD therapy.

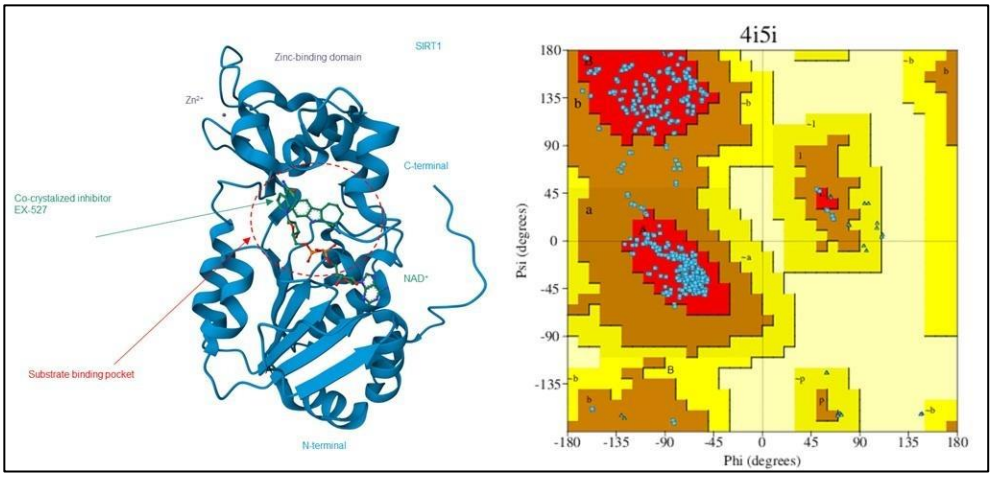


Fig. 4.8. Structural Features and Stereochemical Validation of SIRT1 (PDB ID: 4I5I) (A) Crystal structure of SIRT1 (PDB ID: 4I5I) showing the zinc-binding domain, N- and C-terminal regions, NAD^+, and the co-crystalized inhibitor EX-527 located near the substrate binding pocket. (B) Ramachandran plot of the SIRT1 structure (PDB ID: 4I5I) displaying the distribution of ϕ and Ψ backbone torsion angles, with residues clustered in favored, allowed, and disallowed regions.

4.3.2 Database Mining

Selisistat (EX-527), a strong and specific inhibitor of SIRT1, was selected as the reference drug because of its proven mechanism of action, which involves blocking the enzyme's nicotinamide pocket and preventing NAD^+-dependent deacetylation activity. Essential hydrogen bonds and hydrophobic interactions involving residues His363, Phe414, Leu418, and Arg446 allow Selisistat to interact with the catalytic domain of SIRT1. These interactions keep SIRT1 in an inactive conformation and successfully block substrate binding. By regulating SIRT1 activity and upregulating the expression of neuroprotective factors like BDNF, this particular inhibition has shown therapeutic promise in HD [169, 170]. A similar search was conducted in the LOTUS database using the Tanimoto coefficient, a popular method in molecular comparison, to find structurally related natural compounds [160, 171]. A 68% similarity threshold was used in the Selisistat SMILES notation, which was submitted for structure-based screening to select closely related compounds and filter out less relevant matches [172]. This threshold was chosen to ensure pharmacologically relevant matches during virtual screening by affecting a balance between sensitivity and specificity [173]. Since they were less likely to mimic Selisistat's binding interactions, compounds with a similarity score below this cutoff were disqualified. A total of 1401 natural

compounds that matched the similarity criteria were found, and their .sdf files were retrieved for further computational [169]. This strategy supported the development of HD treatments by enabling the identification of novel SIRT1 inhibitors among natural compounds.

4.3.3 ADMET and Drug-Likeness Evaluation of Selected Natural Compounds

We performed a thorough in silico ADMET profiling of 1401 selected natural compounds that were obtained from the LOTUS database for this study. The SwissADME profiler predicted drug-likeness, toxicities, and pharmacokinetic properties. All shortlisted molecules met Lipinski's Rule of Five, with molecular weight, lipophilicity, hydrogen-bond counts, and rotatable bonds falling within ranges associated with good oral bioavailability. Additional Veber and Egan filtering confirmed that topological polar surface area (TPSA $\leq$ 56.33 Å^2) and the number of rotatable bonds ($\leq$ 6) were compatible with efficient intestinal uptake. Within this set, LTS0217483 stood out as the strongest lead, combining the highest docking score **(Table 4.2)** with a consensus LogP of 4.4, high predicted gastrointestinal absorption, and blood-brain barrier penetration, suggesting suitability for CNS indications. SwissADME also indicated that LTS0217483 can inhibit several CYP450 isoforms (CYP1A2, CYP2C19, CYP2C9, CYP3A4), pointing to a broad metabolic profile, while the absence of P-glycoprotein substrate status reduces the risk of efflux-mediated loss of exposure. At the same time, one Muegge rule violation and two PAINS alerts linked to its quinone motif highlight potential assay-interference liabilities that would need to be addressed during optimization.

LTS0173725 and LTS0193492 also emerged as attractive candidates. Both compounds were highly soluble, carried no PAINS alerts, and showed at most one Brenk alert, while LTS0173725 had the lowest synthetic accessibility score, implying that it should be relatively straightforward to prepare. LTS0193492 combined favorable lipophilicity with predicted BBB permeability, further supporting its relevance for NDD therapy. Radar plots summarizing the physicochemical and ADME profiles of Selisistat and the selected natural products, together with the SIRT1 binding site (PDB ID: 4I5I), are presented in

Fig. 4.9. For the leading compounds, the bioavailability score was consistently 0.55 and synthetic accessibility values ranged from 1.32 to 3.41, suggesting realistic prospects for progression. Overall, these properties provide a coherent picture of the drug-like behavior of the screened natural products and offer a practical baseline for future optimization of their therapeutic potential.

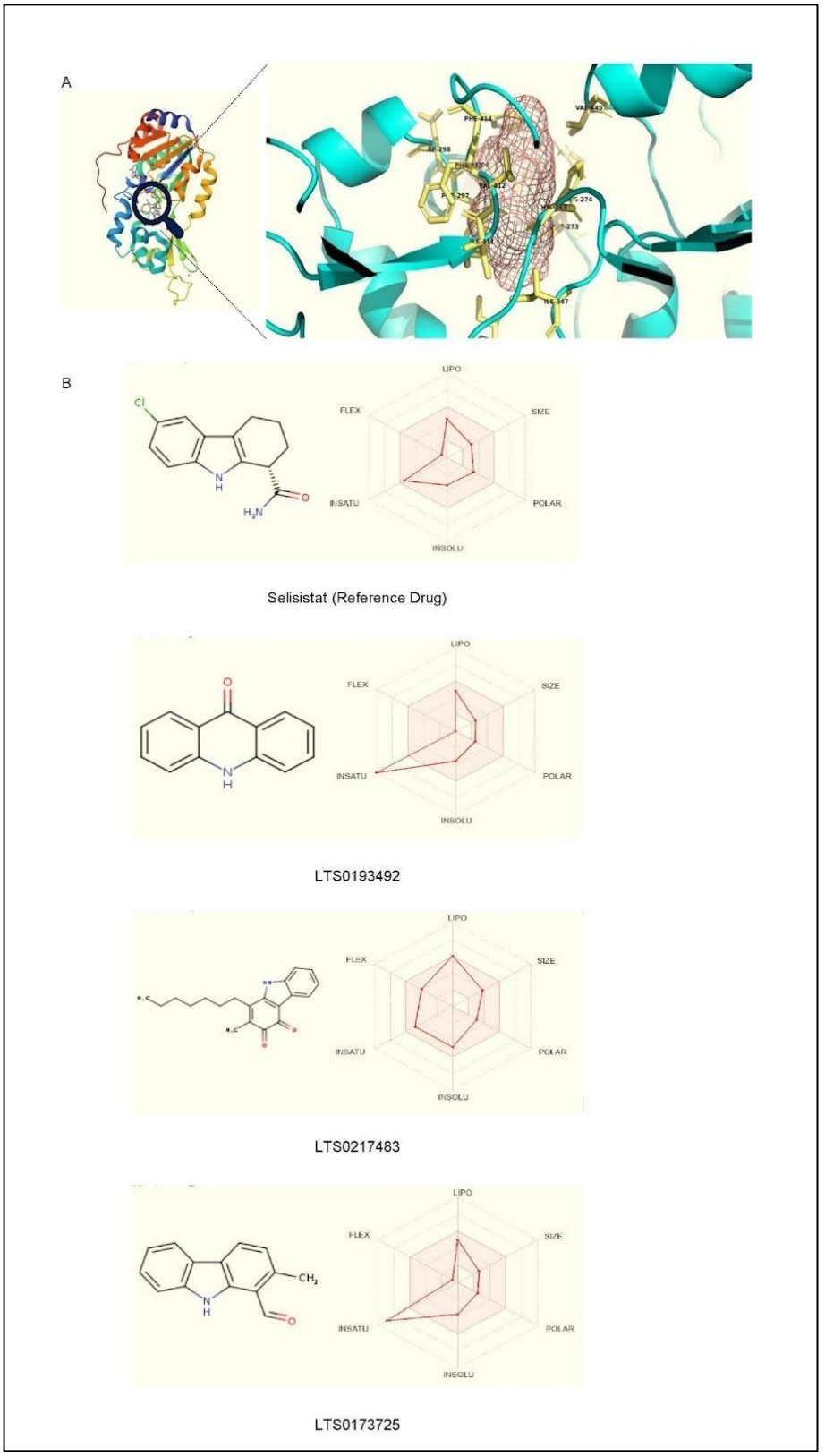
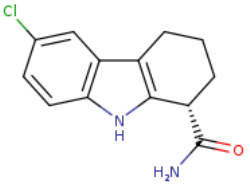
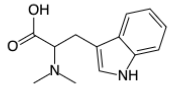
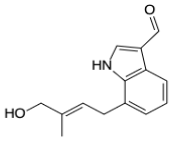
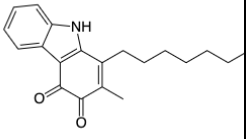
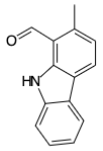
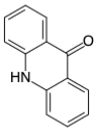
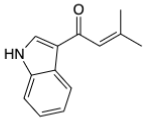
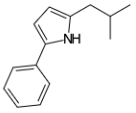
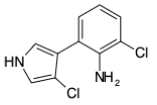


Fig. 4.9. Structural and Physicochemical Profiling of SIRT1 and Selected Ligands. The figure illustrates (A) the 3D ribbon structure of the SIRT1 protein (PDB ID: 4I5I), highlighting the ligand binding site with key interacting residues (yellow sticks) and the binding pocket surface (pink mesh); and (B) the 2D structure and radar plots of Selisistat (reference compound) and selected hit

compounds (LTS0193492, LTS0217483, and LTS0173725). The radar plot visualizes six key physicochemical properties influencing oral bioavailability- lipophilicity (LIPO), size, polarity (POLAR), solubility (INSOLU), saturation (INSATU), and flexibility (FLEX). The pink region represents the optimal range for drug-likeness; closer alignment indicates improved pharmacokinetic potential.

Table 4.2. Physicochemical properties, drug-likeness filters, and docking score of selected compounds

Compound ID	2D Structure	Molecular weight (Da)	TPSA (Å²)	Ali LogS	BBB Permeability	Lipinski Violations	PAINS Alerts	Docking Score (kcal/mol/
Selisistat (Reference)		248.71	58.88	-3.37	Yes	0	0	**-8.345**
LTS0050678		232.28	56.33	-0.65	Yes	0	0	-8.945
LTS0226432		229.27	53.09	-2.94	Yes	0	0	-8.406
LTS0217483		309.4	49.93	-5.87	Yes	0	2	**-9.681**

LTS0173725		209.24	32.86	-3.57	Yes	0	0	**-8.939**
LTS0193492		195.22	32.86	-3.33	Yes	0	0	**-8.814**
LTS0138593		199.25	32.86	-3.54	Yes	0	0	-8.402
LTS0038668		199.29	15.79	-4.03	Yes	0	0	-8.419
LTS0127974		227.09	41.81	-3.45	Yes	0	0	-8.836

4.3.4 Molecular Docking Analysis

A total of 77 natural products were docked against the catalytic domain of SIRT1 (PDB ID: 4I5I) to assess their potential as inhibitors, with Selisistat (EX-527), a clinically validated SIRT1 inhibitor, used as the reference. Docking was carried out in Webina, which provided binding free-energy estimates (ΔG, kcal/mol), where more negative scores correspond to stronger predicted ligand-receptor interactions. Selisistat showed a binding energy of −8.3 kcal/mol, consistent with its known ability to compete with NAD^+ and stabilize an inactive conformation of SIRT1. Several natural derivatives achieved even more favorable docking scores than the reference. On the basis of these energies, a subset of top-ranked molecules

was selected for closer inspection, including compounds with LOTUS IDs LTS0050678, LTS0226432, LTS0217483, LTS0173725, LTS0193492, LTS0138593, LTS0038668, and LTS0127974. From this group, three candidates, LTS0217483, LTS0173725, and LTS0193492, were prioritized as hits for subsequent analyses because of their superior predicted affinity for SIRT1, as illustrated in **Fig. 4.10**.

4.3.5 Interpretation of the Interaction of Hit Compounds with SIRT1

Docking studies showed that all of the selected ligands fit into the SIRT1 nicotinamide-binding pocket and share a common interaction pattern. They form hydrogen bonds with polar residues, engage in π–π contacts with aromatic side chains, and make hydrophobic interactions with residues lining the cavity. Together, these contacts position the ligands so that they obstruct NAD^+ access to the active site, which is a recognized way to inhibit deacetylase activity. Additional van der Waals contributions further improve shape complementarity within the pocket and help to limit unnecessary conformational motion.

Notably, LTS0193492 exhibits the most extensive aromatic stacking interactions (Phe273, Phe297) coupled with polar contacts (Gln345) that contribute to orientation and specificity. LTS0217483 mimics Selisistat's binding mode (Gln108-Phe36) and adds dipole-dipole contacts for enhanced stability. LTS0173725 maintains key polar (Gln108) and aromatic interaction (Phe36) interactions while exploiting additional π-alkyl contacts with hydrophobic residues (Ile174, Phe60), suggesting alternative stabilization strategies. Selisistat's Gln108 hydrogen bond and Phe36 π-π stacking serve as the reference pattern.

MD simulations confirmed interactions persistence with LTS0217483 and LTS0173725, maintaining the highest stability (>60% trajectory time), and LTS0193492 preserving extensive aromatic stacking. MM/PBSA decomposition identified van der Waals contributions (Phe273, Phe414) and electrostatic stabilization (Arg446) as primary binding affinity determinants.

In the active state, SIRT1 binds NAD^+ within the Rossmann-fold pocket while the acetyl-lysine substrate engages the adjacent channel. Cleavage of NAD^+ releases

nicotinamide and generates an ADP-ribosyl (alkyl-imidate) intermediate that resolves to deacetylated substrate and 2'-O-acetyl-ADP-ribose. Our inhibitors occupy the nicotinamide (C-site) pocket overlapping the reported EX-527 pose and sterically misalign the NAD^+ nicotinamide/ribose moiety. This prevents productive cofactor accommodation and intermediate formation, halting the deacetylation reaction and leaving the substrate acetylated. The binding mode supports an NAD^+ site-directed mechanism consistent with predominantly NAD^+ competitive (or mixed) inhibition, which is expected to increase KM, NAD^+ (with a possible decrease in Vmax if a mixed component is present).

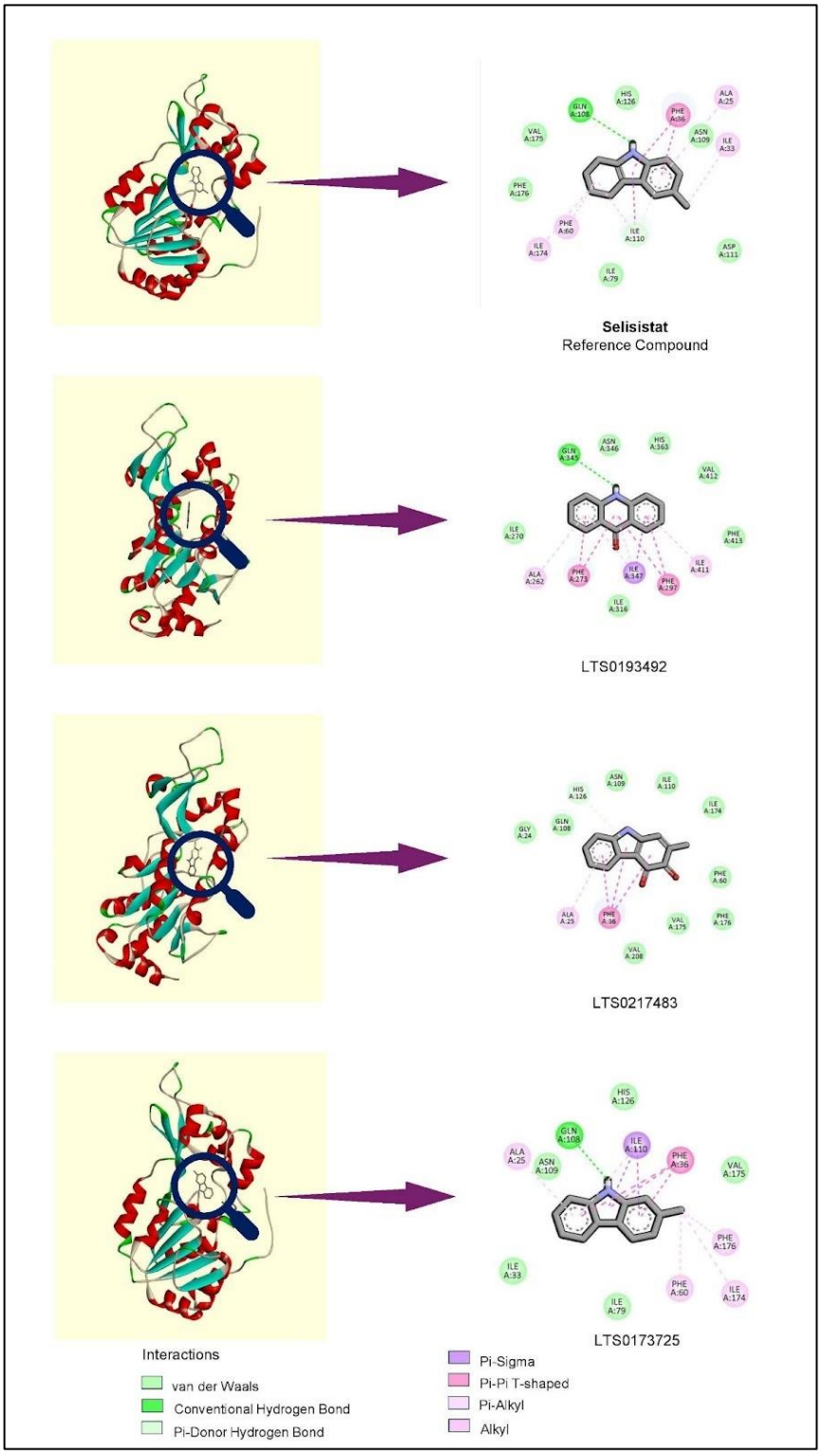


Fig. 4.10. Molecular Docking Analysis of SIRT1 with Selisistat and Natural Compounds. A molecular docking analysis of SIRT1 with Selisistat and the best natural compounds (LTS0193492, LTS0217483, and LTS0173725) reveals meaningful binding interactions, including van der Waals force, hydrogen bonds, π-π stacking, and hydrophobic contacts with crucial active site residues. These interactions demonstrate these compounds' potent SIRT1 inhibitory properties and higher binding affinity.

4.3.6 Molecular Dynamics Simulation

After docking and virtual screening, a small set of natural compounds with close structural similarity to Selisistat was taken forward to molecular dynamics simulations. Each SIRT1 complex was simulated for 100 ns so that changes in binding geometry and protein motion could be followed over time. This allowed assessment not just of the starting poses, but of how specific contacts formed, broke, or persisted, and how stable the overall complexes remained during the trajectory. In HD-relevant simulations, the leads LTS0217483, LTS0173725, and LTS0193492 all stayed engaged with the SIRT1 binding site and showed behaviour consistent with the earlier ADMET and drug-likeness filters. On this basis, the combined docking MD pipeline offers a reasonable starting point for selecting these molecules for experimental testing and, if their activity is confirmed, for considering them in longer-term therapeutic development.

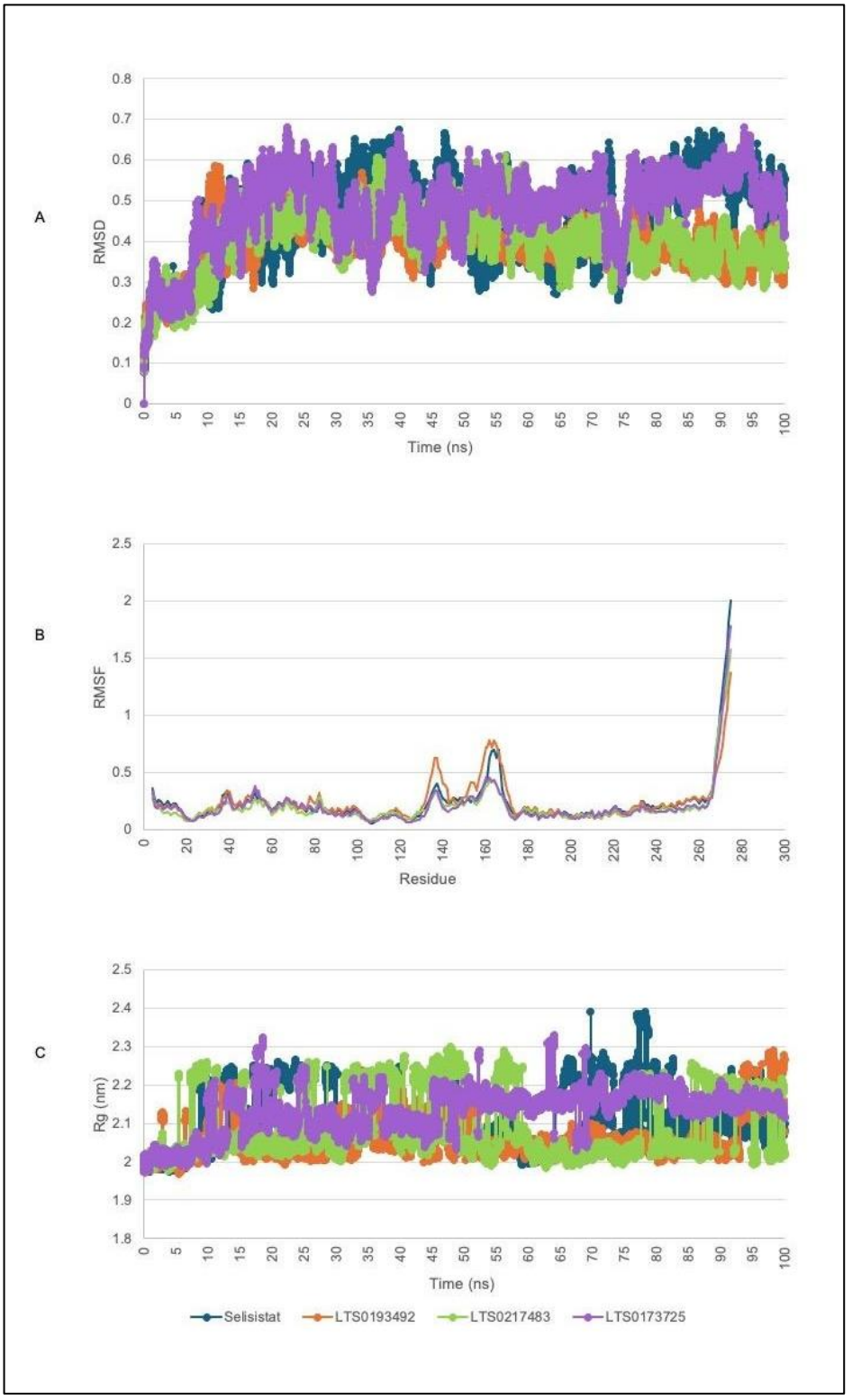


Fig. 4.11. Molecular dynamics simulation analysis of SIRT1 complexes with Selisistat and three hit natural compounds (LTS0193492, LTS0217483, LTS0173725) over 100 ns. (**A**) RMSD plots show stable protein backbone trajectories for all complexes, with average deviations of ~0.39-0.47 nm, indicating stable binding. (**B**) RMSF analysis reveals moderate residue fluctuations (0.12-0.17 nm), mainly at terminal and loop regions, suggesting ligand binding does not induce significant

local flexibility. (**C**) Radius of gyration remains consistent (2.01-2.08 nm), confirming overall protein compactness and structural integrity.

4.3.6.1 Root Mean Square Deviation (RMSD)

To track global structural changes, the C-α backbone RMSD was followed over the full 100 ns simulations for the Selisistat and natural compound complexes. All four systems showed an initial rise in RMSD during the first 0-20 ns, consistent with relaxation and equilibration of the structures, after which the curves plateaued. This levelling-off behavior, also visible in **Fig. 4.11.**, indicates that each system settled into a stable conformational state for the remainder of the trajectory.

After equilibration, each complex showed a somewhat distinct stability profile. The LTS0217483 complex maintained RMSD values in the range of approximately 0.3-0.4 nm with relatively small fluctuations, suggesting a tightly stabilized structure. Selisistat, in comparison, remained between about 0.4 and 0.6 nm and displayed moderate variation. LTS0193492 behaved similarly to Selisistat, with marginally higher stability than LTS0217483 but no major deviations. The LTS0173725 complex was more flexible, with RMSD values spanning roughly 0.4-0.7 nm before settling near a stable value around 70 ns.

Ligand RMSD traces further supported the notion of stable binding. None of the ligands left the binding pocket, and all systems reached equilibrium within roughly 10-15 ns. Average ligand RMSD values were around 0.45 nm for Selisistat, ~0.35 nm for LTS0217483, ~0.40 nm for LTS0193492, and ~0.55 nm for LTS0173725. Among these, LTS0217483 showed the smallest fluctuations, consistent with a more stable protein-ligand complex. It is important to note, however, that ligand RMSD mainly reports on positional stability and does not by itself quantify binding affinity; in this work, it is therefore used as an indicator of interaction stability rather than as direct proof of stronger binding.

4.3.6.2 Root Mean Square Fluctuation (RMSF)

Root-mean-square fluctuation was used to map flexible and rigid regions of SIRT1 by tracking the average positional deviation of individual residues over the MD

trajectories. For the complexes with Selisistat, LTS0193492, LTS0217483, and LTS0173725, the RMSF traces followed broadly similar patterns, with pronounced peaks around residues 150-170 and 265-270. These segments correspond to loop regions adjacent to the NAD^+-binding and substrate-interacting domains and are expected to retain a degree of flexibility that supports catalytic rearrangements. Mean RMSF values were 0.17 nm for Selisistat, 0.18 nm for LTS0193492, 0.15 nm for LTS0217483, and 0.16 nm for LTS0173725. Notably, the LTS0217483 complex showed the lowest overall fluctuations, suggesting enhanced stabilization of these functionally important regions and a catalytic environment that may be particularly favorable for inhibitor binding.

4.3.6.3 Radius of Gyration (Rg)

In the MD runs, overall protein compactness was followed by calculating the radius of gyration (Rg), which reflects how mass is distributed around the protein's center and thus gives a read-out of tertiary-structure integrity. Lower and less variable Rg values point to a tighter, more rigid conformation, whereas higher values and larger swings indicate greater flexibility. Over the 100 ns trajectories, the Selisistat complex showed an average Rg of roughly 2.14 nm. The LTS0193492 complex maintained a slightly lower mean Rg of 2.08 nm with only small fluctuations, suggesting a more compact and conformationally stable protein. By contrast, the complexes with LTS0217483 and LTS0173725 were more heterogeneous, with Rg values ranging between about 2.1 and 2.3 nm, consistent with increased structural. Taken together, these observations suggest that LTS0193492 induces the highest degree of protein compaction among the tested ligands, which may be linked to stronger binding and more stable protein–ligand interactions, and thus supports its consideration as a lead candidate.

4.3.6.4 Hydrogen Bond Analysis

Moreover, the hit compounds formed hydrogen bonds with the target protein, and both their persistence and number reflected ligand-protein stabilisation. As shown in **Fig. 4.12**., Selisistat maintains a low but steady hydrogen-bonding profile, typically 1-2 bonds over most of the trajectory. LTS0173725 exhibits the strongest profile, most often sustaining 2-3 bonds with transient increases up to

~5-6, indicative of robust polar engagement. LTS0193492 generally maintains 1-2 bonds with occasional short-lived increase, consistent with a shallower pose. LTS0217483 shows an intermediate behaviour, frequently maintaining 2-3 bonds for extended periods while dynamically exchanging contacts.

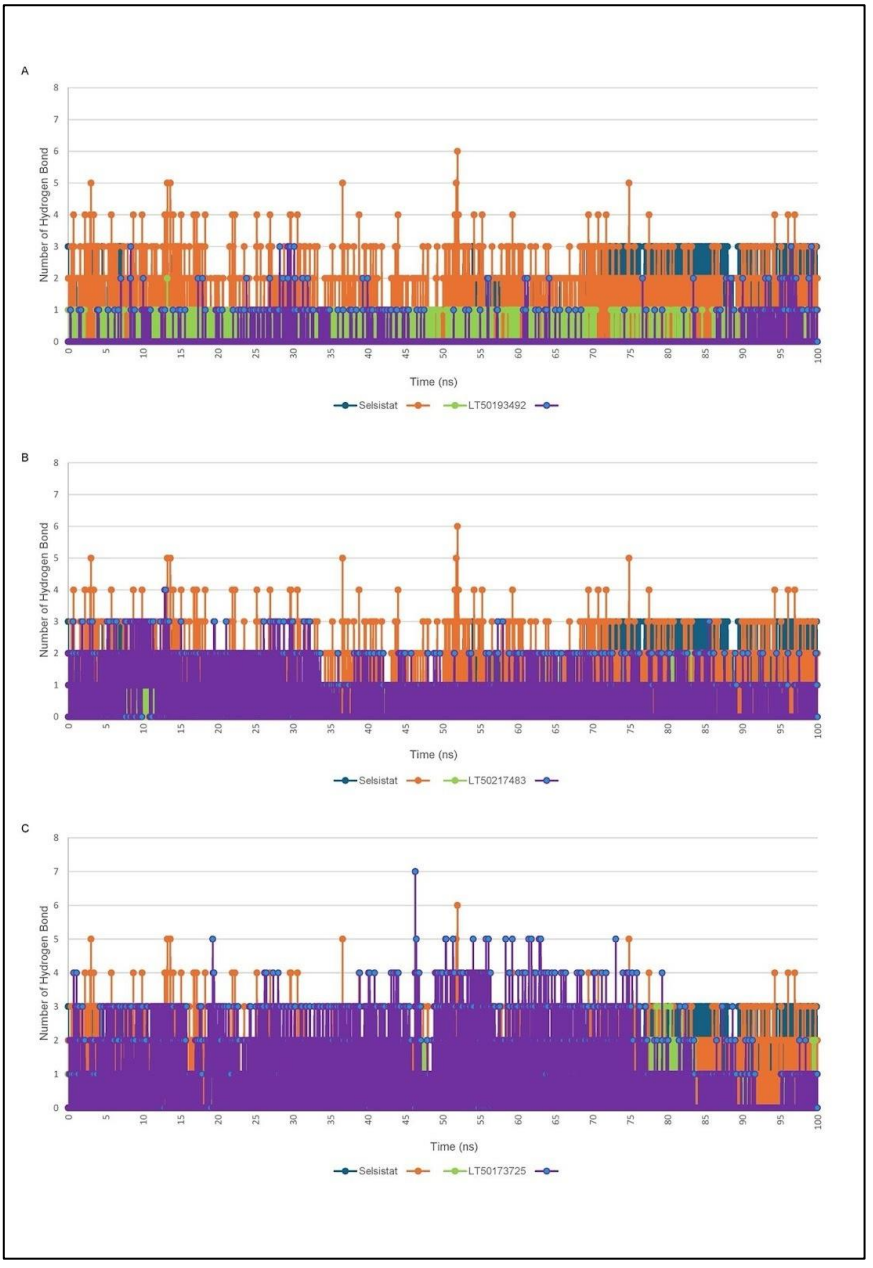


Fig. 4.12. Hydrogen Bond Dynamics of SIRT1-Ligand Complexes During MD Simulation. The time-resolved hydrogen bond dynamics between SIRT1 and Selisistat from the best hot natural compounds over 100 ns of MD simulations. **(A)** Selisistat and LTS0217483, **(B)** Selisistat and LTS0173725, and **(C)** Selisistat and LTS0193492. Among the top hits, LTS0217483 formed up to six hydrogen bonds, while LTS0173725 and LTS0193492 maintained dynamic but frequent interactions, suggesting favourable binding affinities with SIRT1.

4.3.6.5 MM/PBSA Analysis

MM energy decomposition analysis was carried out using the g_mmpbsa tool to analyze the binding interactions of SIRT1 with Selisistat and three natural compounds selected by the assay group (LTS0173725, LTS0193492, and LTS0217483) along a 100 ns molecular dynamics trajectory. Analysis included van der Waals, electrostatic, and total interaction energies. The optimum van der Waals interactions were found for LTS0217483, with energies in the range < -200 kcal/mol during the simulation, predicting good hydrophobic binding. Accordingly, electrostatic energy profiles revealed that LTS0217483 and LTS0193492 possessed more electrostatic interactions (-100 to -150 kcal/mol) than Selisistat and LTS0173725. Combining the two interaction modes, the lowest total MM energy was found for LTS0217483 (approx. -350 kcal/mol), predicting the strongest and most energetically favourable binding profile, as shown in **Fig. 4.13** and Table 4.3. These findings validate the optimum binding affinity and stability of LTS0217483, consistent with previous docking and MD simulation outcomes.

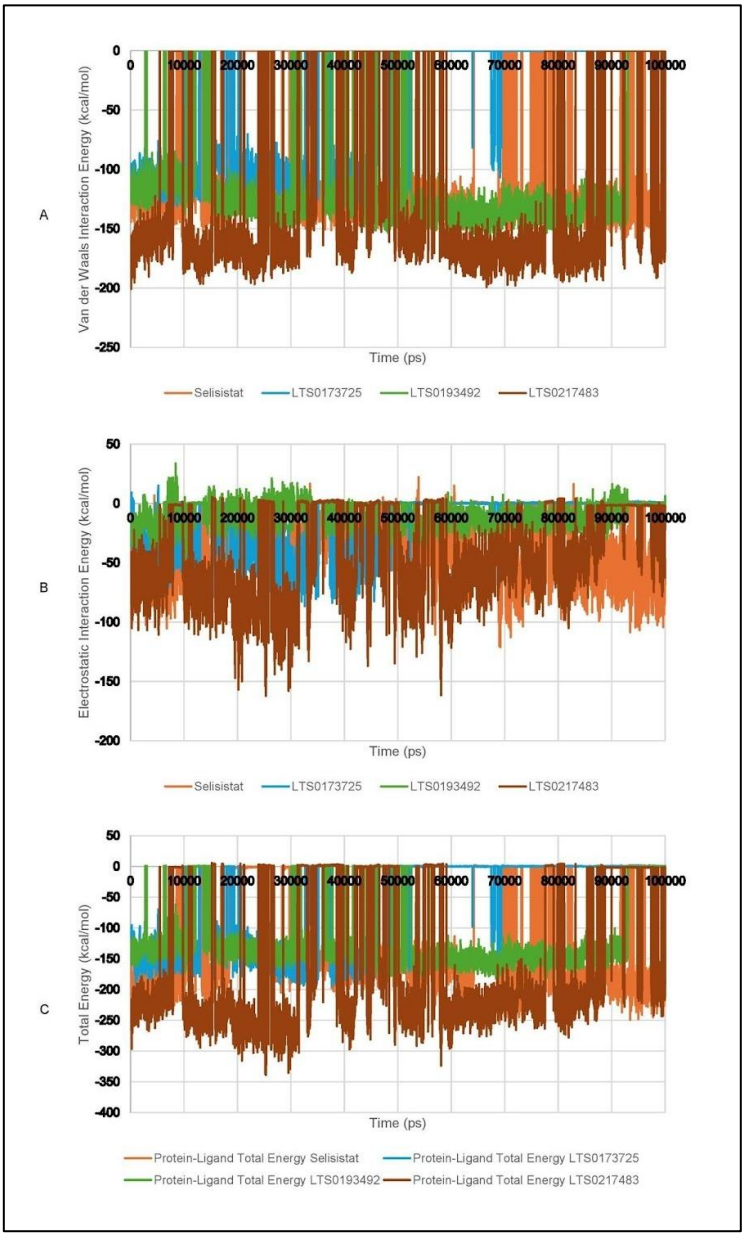


Fig. 4.13. MM/PBSA Interaction Energy Analysis of SIRT1-Ligand Complexes. This figure shows van der Waals **(A)**, electrostatic **(B)**, and total interaction energies **(C)** of the protein with four ligands, namely Selisistat, LTS0193492, LTS0173725, and LTS0217483, during the 100 ns molecular dynamics simulation, computed by MM/PBSA. The van der Waals and electrostatic graphs show the specific contributions of non-covalent and polar interactions, respectively, and the total interaction energy reflects the combined contribution to binding stability. Considering the tested ligands, the most stable and favourable binding profile occurs for LTS0217483, suggesting strong affinity and stable interaction with the targeted protein.

Table 4.3. MM/PBSA Interaction Energies and Binding Strength

Compound	Van der Waals Energy (kcal/mol)	Electrostatic Energy (kcal/mol)	Total Interaction Energy (kcal/mol)	Binding Strength (kcal/mol)
Selisistat	-128.7±8.1	-81.5±10.7	-210.2±13.5	Weakest
LTS0217483	-170.2±5.8	-140.7±7.1	-355.4±9.6	**Strongest, most stable**
LTS0193492	-153.8±6.2	-132.4±8.3	-326.1±10.2	Strong
LTS0173725	-134.5±7.6	-89.2±9.9	-223.7±12.3	Moderate

CHAPTER 5

CONCLUSION

LIMITATION OF THE STUDY

FUTURE PROSPECTIVE

Chapter 5
CONCLUSION, LIMITATION OF THE STUDY, AND FUTURE PROSPECTIVE

5.1 Discussion

Neurodegenerative disorders emerging from this work appear more molecularly complex than suggested by their classical clinical descriptions. In place of a single linear cascade, the data point to several partially overlapping pathogenic routes that collectively drive disease progression. Across different NDDs, the same core disturbances repeatedly appear, including persistent oxidative stress, mitochondrial failure, loss of transcriptional control, impaired proteostasis, and a long-lasting inflammatory response. In Chapter 1, neuroinflammation was introduced as an active driver of pathology, and the current findings support this view by showing that microglia and astrocytes do not simply respond to injury but help shape a chronic inflammatory milieu. Prolonged glial activation, with sustained release of cytokine, chemokines, and ROS, increases the vulnerability of neurons to additional-linked gene signatures and the upstream regulatory circuits that control them; therefore, it becomes central for designing interventions that can modify, rather than only alleviate, the disease course.

The present thesis uses a deliberately integrated computational pipeline to connect inflammatory biomarkers with putative drug targets in NDDs. As described in Chapter 2, transcriptomic profiling was first used to detect shifts in gene expression linked to immune signaling, extracellular matrix remodeling, transcriptional programs, and neurotransmitter handling. Subsequent pathway enrichment analysis showed that these genes cluster in several major immune and inflammatory pathways, indicating broad disturbance of neuroimmune homeostasis rather than isolated pathway defects. In particular, signaling modules related to NF-κB, TNF, IL-17, chemokine networks, and NOD-like receptors were over-represented, all of which have established roles in neuroinflammatory responses, neuronal death, synaptic instability, and blood–brain barrier dysfunction. The high degree of overlap between these pathways suggests that

neuroinflammation in NDDs operates as a coordinated network of signals, creating multiple points of amplification and crosstalk.

Within this framework, CXCL1, FOXC2, CLEC2B, OGN, and SLC6A12 emerged as robust differentially expressed genes that satisfy the criteria for inflammation-associated biomarkers in NDDs and therefore address the first objective of the study. CXCL1 encodes a chemokine that attracts and activates immune cells, and its increased expression in this dataset is in line with persistent microglial recruitment in affected brain tissue. FOXC2, a transcription factor with known roles in vascular and metabolic regulation, may couple inflammatory signaling to changes in neurovascular integrity and endothelial gene programs. CLEC2B is involved in immune recognition and intracellular signaling, so altered levels could point to impaired detection and clearance of damaged or misfolded proteins. OGN, an extracellular matrix protein, suggests remodeling of the local neural environment and changes in neuron–glial interactions. SLC6A12, a transporter important for osmolyte balance and aspects of neurotransmission, may influence how resilient neurons are when exposed to inflammatory or excitotoxic stress. Taken together, these five markers link immune activation, metabolism, and structural homeostasis, illustrating how inflammatory and degenerative processes intersect in NDDs.

In the PD part of this work, attention was shifted to DJ-1 (PARK7), which was chosen as the second main therapeutic target. DJ-1 is a redox-responsive protein that helps to buffer oxidative stress, supports mitochondrial health, and shapes inflammatory signaling; when its function is compromised, oxidative damage increases, and dopaminergic neurons are more likely to degenerate. Within a drug-repurposing framework, a selected group of FDA-approved drugs was therefore docked against DJ-1 to look for favorable binding. Among these, Oxatomide and Levocabastine repeatedly showed stable, low-energy interactions with residues important for DJ-1 activity. These docking results suggest that the two drugs might stabilize or modulate DJ-1 in ways that could help counter PD-related molecular defects. The reproducibility of these results across multiple docking platforms strengthens the reliability of the predicted interactions. Given

their established safety profiles and favorable pharmacokinetic properties, these compounds represent promising candidates for repurposing as DJ-1 modulators. Stabilization of DJ-1 function may mitigate oxidative stress and suppress inflammatory amplification, thereby offering neuroprotective benefits in PD.

This work integrates ligand-based selection from a large natural products space with structure-based modelling of the human SIRT1 catalytic core to prioritize CNS-relevant chemotypes that stably occupy the NAD^+ C-site and engage conserved catalytic residues. Using Selisistat (EX-527) as a reference scaffold, we filtered LOTUS compounds for oral drug-likeness and BBB permeability, prioritized candidates by docking to the 4I5I structure, and subjected the best complexes to 100ns molecular dynamics with interaction-energy evaluation. Three natural derivatives, LTS0217483, LTS0173725, and LTS0193492, emerged as top hits.

Global conformational metrics support stable, target-engaged binding across all complexes. Backbone RMSD values remained within typical stability ranges without evidence of pocket escape or unfolding, and Rg values indicated sustained compactness of the catalytic domain. RMSF profiles localized flexibility to loop segments that border the NAD^+ and substrate channels, while the binding site remained comparatively rigid. Among the hits, LTS0217483 displayed slightly lower and steadier RMSD/RMSF than Selisistat, suggesting enhanced stabilization of local microenvironments important for catalysis.

To go beyond what static docking can show, residue-specific contacts were analyzed along the MD trajectories. In each system, at least one protein–ligand hydrogen bond persisted for a substantial part of the simulation, indicating that key polar interactions were not just artefacts of the starting poses. Selisistat repeatedly contacted the catalytic His363-Asp298 pair, in line with its reported mechanism of SIRT1 inhibition. LTS0173725 also formed recurring hydrogen bonds with His363 but, unlike Selisistat, extended into the substrate-binding loop, engaging Tyr280 and aromatic residues such as Phe273 and Phe414, a pattern suggestive of dual anchoring. LTS0217483 behaved somewhat differently: its

contacts with residues near the catalytic site, including Gln345 and Asn346, were more dynamic yet remained present throughout the trajectory. In contrast, LTS0193492 interacted mainly with residues at the pocket edge and formed fewer long-lived contacts with catalytic residues, consistent with a weaker stabilization of the active site. Overall, ligands that repeatedly engage conserved catalytic residues show greater dynamic stability and are more likely to have functionally relevant.

The energetic analysis supports these interaction patterns. Among the tested compounds, LTS0217483 showed the most favorable total interaction energy, arising from a well-balanced contribution of van der Waals and electrostatic terms, and this is compatible with its stable positioning within the catalytic site. LTS0193492 also achieved relatively strong interaction energies, but these were lower than those of LTS0217483, in keeping with its more peripheral binding mode. LTS0173725 yielded intermediate interaction energies, suggesting that it has promising features but may benefit from further structural optimization.

Although SIRT1 has attracted intense interest as a therapeutic target, progress with small-molecule inhibitors in the clinic has been modest. Selisistat, the only selective SIRT1 inhibitor tested in HD patients, produced limited clinical benefit. Several other inhibitors, such as sirtinol, splitomicin, inauhzin, cambinol, and tenovin-6, suffer from drawbacks including poor solubility, limited selectivity, and off-target toxicity. The natural product-derived scaffolds identified in this work, therefore, provide an attractive alternative starting point. These compounds show favorable CNS-relevant properties and, as demonstrated by the MD simulations, make stable contacts with conserved residues in the SIRT1 catalytic site. In particular, LTS0217483 and LTS0173725 reproduce key mechanistic features of clinically explored inhibitors while introducing new chemical architectures that could be refined in future lead-optimization efforts.

At the same time, these findings remain computational and require experimental confirmation. Priority should be given to determining biochemical potency and kinetic parameters, establishing selectivity across the sirtuin family, and obtaining

structural data to validate the predicted binding modes. In parallel, cellular assays and neuroprotective studies in HD-relevant models, together with pharmacokinetic profiling and blood-brain barrier assessments, will be essential to judge the true translational potential of these candidates.

In this study, molecular biomarker findings are connected directly to the selection of structure-based candidate drugs for NDDs. The transcriptomic and pathway analyses converged on a set of inflammation-related genes CXCL1, FOXC2, CLEC2B, OGN, and SLC6A12 whose joint dysregulation links immune activity with metabolic control and neuron-glial communication during disease progression. Using these insights as a backdrop, a DJ-1-centred repurposing screen identified Oxatomide and Levocabastine as candidates able to influence redox balance and inflammatory signaling in PD, and both ligands showed consistent, stable binding in repeated computational tests. In the SIRT1 arm of the work, LTS0217483 emerged as a strong natural-product-derived hit; MD simulations indicated sustained engagement of the catalytic region together with favorable interaction energies, supporting its selection as a lead compound. Taken together, the results demonstrate how an integrated computational workflow can transition from gene-level signatures to concrete molecules that warrant experimental follow-up. The framework links disease-associated molecular signatures with potential therapeutic targets. **Fig. 5.1.** highlights a promising lead compound identified through in silico analysis. However, experimental validation is required to confirm these findings. Such studies are necessary to advance SIRT1-targeted therapeutic development.

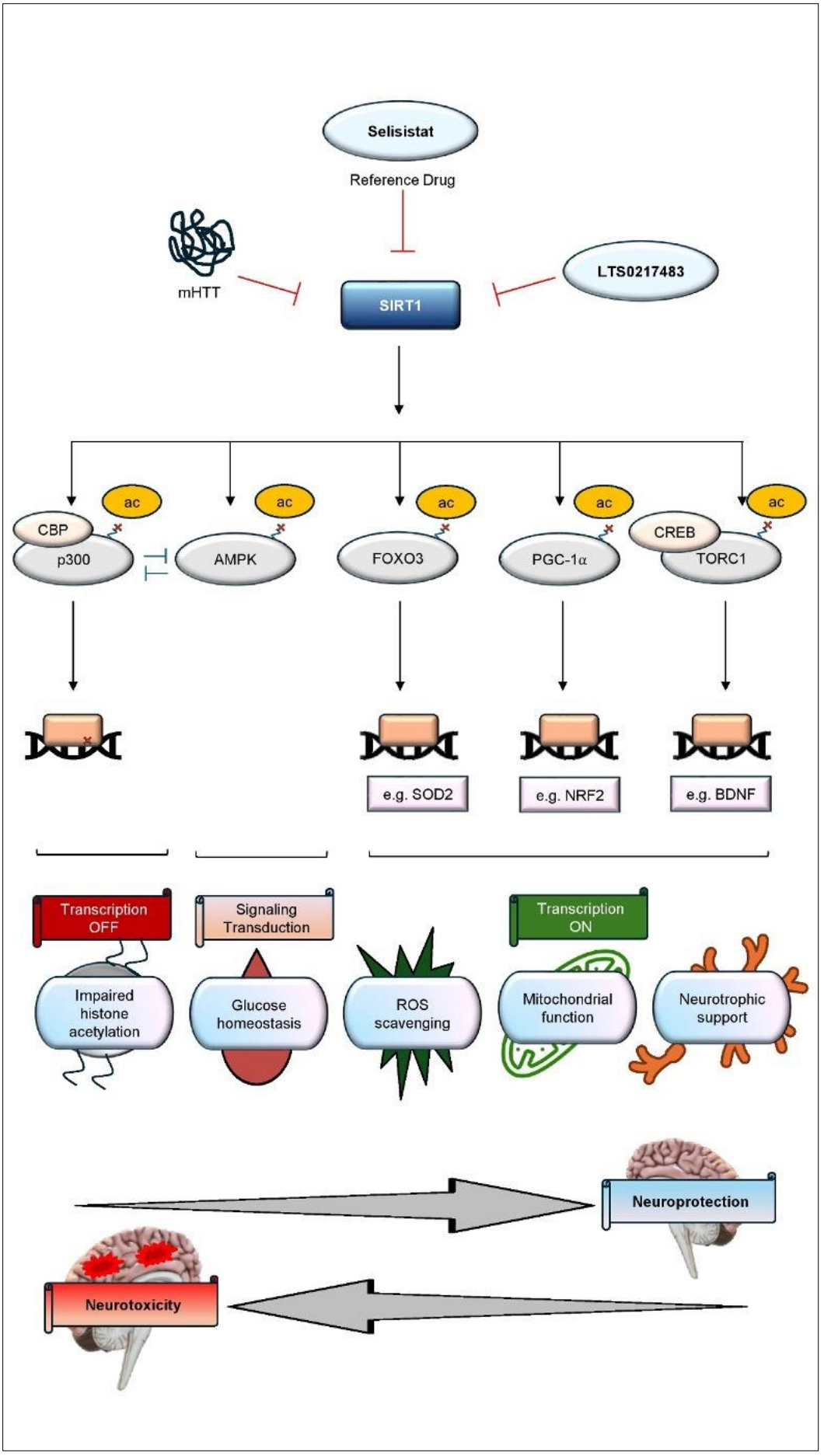


Fig. 5.1. Mechanistic pathway of SIRT1 inhibition in Selisistat and potential implications for neuroprotection in HD. The reference SIRT1 inhibitors Selisistat and compound

LTS0217483 inhibit SIRT1 activity induced by mutant huntingtin (mHTT). SIRT1 inhibitors enhance the acetylation of multiple downstream targets, including CBP/p300, AMPK, FOXO3, PGC-1α, and CREB/TORC1. This cascade gives rise to transcriptional modulation: transcriptional suppression, associated with impaired histone acetylation and aberrant glucose homeostasis, and transcriptional activation involving oxidative stress response transcription factors (e.g., SOD2 by FOXO3), mitochondrial function transcription factors (e.g., NRF2 by PGC-1α), and neurotrophic signaling (e.g., BDNF by CREB). The combined effect alters the equilibrium from neurotoxicity to neuroprotection by improving ROS scavenging, mitochondrial performance, and neurotrophic assistance.

5.2 Limitations of the Study

There are certain limitations to the current study that must be acknowledged. The overall workflow is entirely computational and depends on multiple software platforms, parameters, and filtering criteria for virtual screening, docking, MD, and ADMET prediction, which means that small variations in tools or settings could lead to modest differences in ranking or interaction patterns of the candidate compounds. In addition, all proposed drugs and associated genes remain unvalidated at the experimental level; comprehensive in vitro and in vivo studies are still required to confirm target engagement, clarify the precise mechanism of action, and verify BBB permeability, pharmacokinetics, and potential off-target effects. Only through such wet-lab validation will it be possible to determine the true therapeutic efficacy and safety of the prioritized candidates and to map their downstream and upstream impact on the complex signaling networks involved in neurodegeneration.

5.3 Future Perspective

- By targeting inflammation-associated molecular regulators and redox-sensitive proteins, the findings of this study provide a strong basis for the development of novel therapeutic strategies for NDDs. The investigation of interconnected inflammatory, metabolic, and neuronal pathways opens new avenues for improved therapeutic management of NDDs.

- This study has identified several potential inflammatory and disease-associated biomarkers (CXCL1, FOXC2, CLEC2B, OGN, and SLC6A12), as well as key therapeutic targets such as DJ-1 and SIRT1, which can be further explored for the development of targeted and disease-modifying interventions in NDDs.

- The objectives and computational methodology adopted in this study are highly useful for identifying the therapeutic potential of repurposed drugs and natural compounds in combating NDDs, thereby supporting precision-driven drug discovery and future translational research.

This work is most useful for showing how more targeted, mechanism-based treatments for NDDs might be developed. NDDs place a growing burden on patients, families, and health services, so approaches that join biomarker discovery with structure-guided repurposing and natural-compound screening are attractive. The strategy explored here could help identify drugs that offer real benefit with fewer side effects and lower long-term costs. As populations age and NDDs become more common, such computational pipelines may also simplify parts of the drug-development process and support more tailored treatment decisions, improving day-to-day management of these disorders. Ultimately, the translational outputs of this work have the potential to enhance patient quality of life, alleviate socioeconomic burdens, and support sustainable healthcare planning in the context of managing NDD.

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LIST OF PUBLICATIONS

Cumulative impact factor of all publications = 19.5

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PUBLICATIONS FROM THESIS

1. **Kardam, S.,** & Kumar, P. "In Silico Identification of Natural SIRT1 Inhibitors through Molecular Docking, Dynamics Simulation, and MM/PBSA". Cell Biochemistry and Biophysics (2025). https://doi.org/10.1007/s12013-025-01886-0 **(SCIE IF 2.5)**

2. **Kardam, S.,** Ambasta, R. K., & Kumar, P. "Overview of pro-inflammatory and pro-survival components in neuroinflammatory signalling and neurodegeneration". Ageing research reviews vol. 100 (2024): 102465. https://doi.org/10.1016/j.arr.2024.102465 **(SCIE IF 12.4)**

OTHER PUBLICATIONS

3. **Rani, N.,** Kaushik, A., Kardam, S., Kag, S., Raj, V. S., Ambasta, R. K., & Kumar, P. (2024). Reimagining old drugs with new tricks: Mechanisms, strategies and notable success stories in drug repurposing for neurological diseases. Progress in Molecular Biology and Translational Science. https://doi.org/10.1016/BS.PMBTS.2024.03.029 **(IF 4.6)**

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1. **Kardam, S.,** and P. Kumar, "In Silico Repurposing of FDA Drugs for DJ-1-Linked Parkinson's Disease," 2025 International Conference on Biomedical Engineering and Sustainable Healthcare (ICBMESH), Manipal, India, 2025, pp. 1-5, doi: 10.1109/ICBMESH66209.2025.11182200

2. S. Kardam and P. Kumar, "Navigating the Dual Challenges: Antimicrobial Resistance and Parkinson's Disease," 3rd International Conference on "*Antimicrobial Resistance, Novel Drug Discovery and Vaccine Development: Challenges and Opportunities*", Delhi, India 2024. (Poster Presentation) **[Poster presentation]**

WORKSHOP

1. Karyashala High End Workshop on "*Exploring Potentials of Alternative Models in Toxicology" at Jamia Hamdard*, New Delhi, on all days from 27th February to 4th March 2024, from 9:00 AM to 6:00 PM.

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EDUCATION/TRAINING

INSTITUTION /UNIVERSITY	POSITION	YEAR(s)	FIELD OF STUDY
Delhi Technological University (Formerly Delhi College of Engineering), Delhi	Ph.D.	January 2023-present	Neurodegenerative Diseases, Neuroscience, and Neuroinflammation
Sharda University, Greater Noida, India	M.Sc.	2021	Microbiology
NIMS University	B.Sc.	2018	Medical Laboratory Technology

PERSONAL STATEMENT

My scientific research is dedicated to leveraging computational approaches to understand and develop therapies for neurodegenerative disorders, with a focus on Parkinson's and Huntington's diseases. I utilize in silico drug repurposing, molecular docking, molecular dynamics simulations, MM/PBSA free energy calculations, and transcriptomic data analysis to identify promising therapeutic targets and candidate molecules. I have published in SCIE-indexed journals and presented my work at international conferences. I am deeply committed to integrating computational strategies, multi-omics data, and translational neuroscience to address critical unmet challenges in neurodegeneration.

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3. Rani, N., Kaushik, A., **Kardam, S.,** Kag, S., Raj, V. S., Ambasta, R. K., & Kumar, P. (2024). Reimagining old drugs with new tricks: Mechanisms, strategies and notable success stories in drug repurposing for neurological diseases. Progress in Molecular Biology and Translational Science. https://doi.org/10.1016/BS.PMBTS.2024.03.029 **(SCIE IF 4.6)**

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REFERENCES

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Review Article

Overview of pro-inflammatory and pro-survival components in neuroinflammatory signalling and neurodegeneration

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ABSTRACT

Neurodegenerative diseases (NDDs) are identified by the progressive deterioration of neurons and a subsequent decline in cognitive function, creating an enormous burden on the healthcare system globally. Neuroinflammation is an intricate procedure that initiates the immune response in the central nervous system (CNS) and significantly impacts the expansion of NDDs. This study scrutinizes the complicated interaction between neuronal degeneration and neuroinflammation, with an appropriate emphasis on their reciprocal impacts. It also describes how neuroinflammatory reactions in NDDs are controlled by activating certain pro-inflammatory transcription factors, including p38 MAPK, FAF1, Toll-like receptors (TLRs), and STAT3. Alternatively, it evaluates the impact of pro-survival transcription factors, such as the SOCS pathway, YY1, SIRT1, and MEF2, which provide neuroprotective protection against damage triggered by neuroinflammation. Moreover, we study the feasibility of accommodating drug repositioning as a therapeutic approach for treating neuroinflammatory disorders. This suggests the use of existing medications for novel utilization in the treatment of NDDs. Furthermore, the study intends to reveal novel biomarkers of neuroinflammation that contribute fundamental observation for the initial detection and diagnosis of these disorders. This study aims to strengthen therapy interference and augment patient outcomes by combining ongoing data and evaluating novel therapeutic and diagnostic approaches. The goal is to devote the growth of an effective strategy to reducing the impact of neuroinflammation on neuronal protection in NDDs.

1. Introduction

Neurodegenerative disorders (NDDs) are complex disorders characterized by the progressive loss of neurons and the gradual deterioration of several parts of the nervous system. As the elderly population increases, the prevalence of these diseases is also on the rise. Genetic alterations cause the malfunctioning of proteins, linking them to Parkinson's disease (PD), Alzheimer's disease (AD), spinal muscular atrophy, Huntington's disease (HD), spinocerebellar ataxia, and prion disease among the most common neurological disorders. The exact etiology of these disorders remains uncertain; however, it is hypothesized that a multifaceted interplay between genetic, epigenetic, and ecological variables may be involved. Currently, no effective therapies can slow, halt, or prevent these diseases. However, research on the molecular mechanisms underlying the pathogenesis of NDDs is ongoing. Neuroglia plays a fundamental role in the pathophysiology of these diseases, and lifestyle changes can enhance their neuroprotective capabilities (Ciaccio and Agnello, 2023; Zaib et al., 2023; Verkhratsky and Butt, 2023). Neuroinflammation is an umbrella term for inflammation of the nervous system, mainly the central nervous system (CNS). Numerous complex disorders, including neuropsychiatric disorders, cancer, and type 2 diabetes, have been interlinked with it (Asslih et al., 2021).

In NDDs, pro-inflammatory transcription factors, including AD and PD, play a crucial role (Jin et al., 2019). The nucleotide-binding oligomerization domain (NOD), leucine-rich repeat (LRR), and NLR family pyrin domain-containing protein 3 (NLRP3) inflammasome complex is interlinked with pro-inflammatory signalling in PD and AD (Cornut et al., 2020). Inflammasomes are sophisticated molecular complexes that show significant inflammatory properties and are strictly regulated at the transcriptional stage. Neuroinflammation biomarkers are widely

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ORIGINAL PAPER

In Silico Identification of Natural SIRT1 Inhibitors through Molecular Docking, Dynamics Simulation, and MM/PBSA

Shefali Kardam[1] · **Pravir Kumar**[1]

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Abstract

Huntington's disease (HD) is a progressive neurodegenerative disorder caused by mutations in the huntingtin (HTT) gene, leading to transcriptional dysregulation, mitochondrial dysfunction, and neuronal loss. Sirtuin 1 (SIRT 1), a NAD^+-dependent deacetylase, can be neuroprotective in gene expression. Selective SIRT1 inhibition may restore transcriptional balance, making it a potential therapeutic strategy. To identify natural product-derived SIRT1 inhibitors with potential therapeutic relevance for HD. Selisistat (EX-527), a selective SIRT1 inhibitor, was used as a reference to retrieve 1401 structurally similar compounds (Tanimoto similarity ≥68%) from the LOTUS natural products database. Drug-likeness and ADMET properties were evaluated, followed by molecular docking against the catalytic domain of SIRT1 (PDB ID: 4I5I). Top hits underwent 100 ns molecular dynamics (MD) simulations in GROMACS 2023.3, and binding free estimation via MM/PBSA. Three compounds, LTS0217483, LTS0173725, and LTS0193492, showed higher binding affinities than Selisistat. LTS0217483 had the most favourable total binding energy (-183.52 kJ/mol) and maintained stable interactions with key catalytic residues. Hydrogen bond persistence analysis demonstrated consistent ligand-protein contacts. An integrated computational approach identified LTS0217483 as a promising natural SIRT1 inhibitor for potential HD treatment. Future work will focus on compound optimisation, quantitative structure-activity relationship (QSAR) modelling, and experimental validation in cellular and animal HD models. A computerised drug discovery system has identified potential SIRT1 inhibitors that can treat Huntington's disease. The Tanimoto was used to search for the similarity of 276,518 natural products from the LOTUS database in the presence of Selisistat as a reference, and 1401 compounds were retrieved. An in-silico method to predict AMDET properties was used, and the molecules that satisfied Lipinski's rule were found to be the lead set. These were also excluded from the BBB permeability, Brenk, and PAINS filters. Subsequently, molecular docking and molecular dynamics simulations were performed, and MM/PBSA binding free energy calculations were conducted to validate the thermodynamic stability of the protein-ligand complexes. The compound LTS0217483 was chosen because it has the best potential inhibitor, as it has a good binding capability and a more stable configuration that could be a future drug target.

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In Silico Repurposing of FDA Drugs for DJ-1-Linked Parkinson's Disease

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Shefali Kardam ; Pravir Kumar All Authors

Abstract:
In this study, we aimed to present a novel repurposing strategy using FDA-approved anti-inflammatory drugs from Drug Bank as plausible therapeutics against the abnormal Parkinson's DJ-1 gene. One such function of DJ-1, also known as the PARK7 gene, is neuroprotection, which protects cells against oxidative stress and inflammation. We identified compounds that potentially inhibit the PD-related mutant DJ-1 (2R1T) protein. We discovered effective inhibitors against the mutant DJ-1 gene (2R1T): Oxatomide and Levocabastine, using a comprehensive molecular docking analysis. Our data suggest that administering Oxatomide and Levocabastine may ameliorate the progression in wild-type human neural cells but reduce their impact on mutant DJ-1-induced PD pathogenesis. This repurposing approach could delineate a pathway for developing future medications and personalized therapy options to investigate existing anti-inflammatory drugs currently on the market and determine whether they can be developed targeting specific genetic factors associated with neurodegenerative diseases.

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