FROM MOLECULAR DOCKING TO NEUROPROTECTION: HARNESSING *IN-SILICO* APPROACHES FOR THE DISCOVERY OF DJ-1 MODULATORS IN PARKINSON'S DISEASE THERAPEUTICS

A Dissertation

Submitted in partial fulfillment of the requirement for the degree of

MASTER OF SCIENCE

in

BIOTECHNOLOGY

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May, 2025

ACKNOWLEDGEMENT

At the time of submission of my M.Sc. Dissertation I am grateful to the divine God who has given me the insight, fortitude, and perseverance to complete this endeavour. In addition to work, a lot of other people's support and direction are crucial to this project's success. Thus, I would like to use this opportunity to thank everyone who has contributed to the accomplishment of this project.

I would first want to express my gratitude to my mentor, Prof. Pravir Kumar, Department of Biotechnology, Delhi Technological University, for providing me with the chance to work on a project under his direction. I was able to finish this assignment because of his mysterious oversight, constant encouragement, and knowledgeable direction. I respectfully use this as a chance to thank him.

I want to express my profound appreciation to Dr. Mehar Sahu for her insightful observations and constant guidance. Her analytical comments on the project's problems have been crucial to its successful development.

There are not enough words to express my gratitude to Dr. Neetu Rani, Ms. Shefali Kardam, Ms. Shrutikriti Vashishth and Mr. Rahul Tripathi who have supported me in my endeavour and showed me confidence like family.

I would like to thank Mr. Jitender Singh and Mr. C.B. Singh, the technical personnel, for their assistance whenever needed. Finally, I would like to thank my family and friends for their unwavering support over this entire process.

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DECLARATION

I, Nishita Singh, 23/MSCBIO/34 student of M.Sc. Biotechnology hereby declares that the Dissertation Project entitled **"From Molecular Docking to Neuroprotection: Harnessing *In Silico* Approaches for the Discovery of DJ-1 Modulators in Parkinson's Disease Therapeutics"** 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 Master of Science. This work is original and not copied from any source without paper citation. I have honored the principles of academic integrity and have upheld the normal student code of academic conduct in the completion of this work.

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This is to certify that the Dissertation Project titled **"From Molecular Docking to Neuroprotection: Harnessing *In Silico* Approaches for the Discovery of DJ-1 Modulators in Parkinson's Disease Therapeutics"** which is being submitted by Nishita 23/MSCBIO/34, Department of Biotechnology, Delhi Technological University, Delhi in partial fulfillment of the requirement for the award of the degree of Master of Science is a record of the work carried out by the student under my supervision. To the best of my knowledge, this work has not been submitted in part or full for any Degree or Diploma to this University or elsewhere.

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From Molecular Docking to Neuroprotection: Harnessing *In Silico* Approaches for the Discovery of DJ-1 Modulators in Parkinson's Disease Therapeutics

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ABSTRACT

AIM- This study aims to attain novel therapeutics for potentially preventing the advancement (progression) and possibly the onset initiation of Parkinson's disease through curbing reactive oxygen species with the help of a protein DJ-1. Parkinson's disease (PD), a neurodegenerative disorder driven by oxidative stress and mitochondrial dysfunction, lacks disease-modifying therapies. DJ-1 (PARK7), a redox-sensitive chaperone protein, emerges as a critical neuroprotectant, with its conserved Cys106 residue acting as a sensor of oxidative damage. Overoxidation of Cys106 to sulfonic acid (Cys106-SO_3H) inactivates DJ-1, exacerbating α-synuclein aggregation, ROS accumulation, and dopaminergic neuron loss. This thesis integrates computational strategies to identify novel DJ-1 modulators that stabilize its redox-active state, counteracting PD progression.

Using the DJ-1 crystal structure (PDB: 1SOA), refined via PyMOL to remove solvents and optimize stability, we performed structure-based virtual screening with SwissSimilarity, leveraging the known ligand UCP0054278 to identify 400 analogs form DrugBank. Sequential ADME/toxicity filtering (BBB permeability, Lipinski's rule-of-five, PAINS, Brenks) prioritized 121 candidates, which were docked against DJ-1's active site using AutoDock Vina. Top-scoring ligands (< -7.3 kcal/mol) revealed hydrogen bonding with Cys106, Glu18, and Met26-residues critical for DJ-1's chaperone function. Discovery Studio visualization highlighted hydrophobic interactions stabilizing the oxidized sulfinic state (Cys106-SO_2H), preventing irreversible overoxidation.

RESULS- Our results identified **19 lead compounds** with potential to enhance DJ-1's antioxidant capacity, mitochondrial stabilization, and α-synuclein disaggregation. Challenges, including DJ-1's shallow binding pocket and dynamic redox transitions, were addressed through hybrid screening. Lead candidates, exhibited promise in silico, with potential for BBB penetration and minimal off-target effects.

CONCLUSION- This work underscores DJ-1's druggability and provides a roadmap for developing redox-specific modulators. By preserving DJ-1's neuroprotective functions, these molecules offer a paradigm shift from symptomatic L-DOPA treatments to disease-modifying therapies, addressing PD's root pathological mechanisms. Future validation in *in vitro* and *in vivo* models will bridge computational insights to therapeutic innovation, advancing PD drug discovery.

This abstract synthesizes structural biology, cheminformatics, and neurobiology to frame DJ-1 as a linchpin in PD therapeutics, highlighting the transformative potential of computational approaches in neurodegeneration research.

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LIST OF ABBREVIATIONS

PD	Parkinson's Disease
ROS	Reactive Oxygen Species
DJ-1	Protein DJ-1 (PARK7)
Cys106/C106	Cysteine 106
BBB	Blood-Brain Barrier
AI/ML	Artificial Intelligence/Machine Learning
ASK-1	Apoptosis Signal-regulating Kinase 1
Nrf2	Nuclear factor erythroid 2–related factor 2
ER	Endoplasmic Reticulum
NADPH	Nicotinamide adenine dinucleotide phosphate
p53	Protein p53
NF-KB	Nuclear Factor kappa-light-chain-enhancer of activated B cells
PTEN	Phosphatase and tensin homolog
Bcl-xL	B-cell lymphoma-extra large
Pka	Acid dissociation constant / Protein Kinase A
α-syn	Alpha-synuclein
L-DOPA	Levodopa
DOPA	Dihydroxyphenylalanine
$ONOO^-$	Peroxynitrite
S^-	Sulfur anion / Thiolate
OH^-	Hydroxide ion
MAO-B	Monoamine Oxidase B
H_2O_2	Hydrogen Peroxide
LRRK2	Leucine-rich repeat kinase 2
CoQ10	Coenzyme Q10
TAT	Transactivator of transcription
TRPA1	Transient Receptor Potential Ankyrin 1
OHDA	6-Hydroxydopamine

ADME	Absorption, Distribution, Metabolism, Excretion
SBDD	Structure-Based Drug Design
LGA	Lamarckian Genetic Algorithm
RDPSO	Random Drift Particle Swarm optimization
CNS	Central Nervous System
iLOGp	Logarithm of Partition Coefficient (lipophilicity)
TPSA	Topological Polar Surface Area
PDB	Protein Data Bank
MGL TOOLS	Molecular Graphics Laboratory Tools
SDF	Structure Data File
logKp	Logarithm of skin permeability coefficient
GI	Gastrointestinal
PAINS	Pan-Assay Interference Compounds
SMILES	Simplified Molecular Input Line Entry System
PDBQT	Protein Data Bank, Partial Charge & Atom Type
TXT	Text File

CHAPTER 1. INTRODUCTION

Parkinson's Disease (PD), first comprehensible description as the "shaking palsy", is a multisystemic age-linked chronic neuron disintegrating disorder characterized by various movement and non-movement related symptoms affecting millions of people globally[1]. Its accurate clinical diagnostics is challenging and is ever evolving, requiring a thorough knowledge of the broad spectrum of clinical manifestations. Characterized by the motor manifestations including cardinal hallmarks like bradykinesia, resting tremors, gait, postural instability and rigidity along with many prodromal symptoms. Various non -motor symptoms have also gained recent attention as a diagnostic criteria[2]. Its pathology culminates through deleterious destruction of dopaminergic brain cells in which protein aggregates called Lewy bodies progress in a patterned manner along with Reactive oxygen species (ROS) production through impaired mitochondrial activity[3].

PD is a highly complex disease engaging a coaction between various heritable as well as environmental factors. The ultimate cause of the disease remains unknown in most of the patients. Pathology of PD has established the discovery of various genes causative for PD. Familial PD caused by autosomal *PARK* genes encode various proteins performing several signalling pathways associated with PD. Monogenic mutations of these proteins affect the cellular functions of the cells.

PARK7 gene, mapped on chromosome 1 which translates to protein deglycase-1 is associated with autosomal recessive form of PD[4]. In 1997, Protein deglycase-1(DJ-1) was earlier isolated and characterized as an oncogene but subsequently identified in correlation with PD [5]. DJ-1 is now known to regulate transcription, signal transduction pathways. It is a redox-sensitive molecular chaperone, an enzyme and scavenger of the ROS exerting its functionality as an antioxidant. Thereby, DJ-1 provides multifaceted role of an antioxidant. DJ-1 has a pathophysiological role in aetiology of PD due to its functional role against oxidative stress and mitochondrial dysfunction[6]. Moreover, DJ-1 is oxidised at cysteine residue at 106 (Cys106) amino position when ROS are present. Notably, DJ-1 can exist in reduced form or oxidised form as sulfinate ($-SO_2H$) or sulfonate ($-SO_3H$)[7]. Super oxidation of Cys106 to cysteine-sulfonic acids when oxidative stress is present renders the protein to become non-functional[8]. Strikingly, discovery of such super oxidised forms of the protein in the patient's brain affected by PD substantiates the fact that DJ-1 may be a plausible pharmacological ideal for therapeutic management of disease.

Accordingly, if we maintain the stabilized form of protein DJ-1 , we can prevent the emergence or advancement of this neuronal disease. It can be achieved by preventing superfluous (DJ-1-Cys106-SO_3H) oxidation of DJ-1 by targeting C106 region of protein. This approach will either help to sustain the functional form of DJ-1 or increase its biological operations as a neuroprotectant. Miyazaki et al carried out *(in silico*) screening of compounds targeting C106 region of DJ-1 and identified a compound (UCP0054278) with highest binding affinity towards oxidised (SO_2H) form of DJ-1. It also permeates the blood-brain barrier (BBB). Furthermore, UCP0054278 also inhibits cell death in presence of H2O2 which is prominent to produce toxicity by production of ROS. This suggests that DJ-1 mediated neuroprotection is a promising strategy by preventing its overoxidation.

This study was aimed at screening compounds targeting the binding pocket of C106 region in protein DJ-1 with more efficient binding interaction via a computational approach. Accordingly, *DrugBank* was screened for this purpose and docking scores were analysed. The key aim of this investigation is to find alternative compounds with more binding affinity towards DJ-1 with therapeutic potential especially in neurodegenerative diseases like PD. In this study, to further validate the binding, *DiscoveryStudio* was used to visualize the interacting residues. Broadly, we infer that these virtually screened compounds with the help of AI/ML might be used as new therapeutics against PD with validation by *in vitro* studies. We perform

screening based on structural similarity, ADME analysis, protein-ligand docking and visualization of the interacting residues using different computational software.

This novel study aims to identify therapeutic DJ-1 Cys106- active site targeted molecules to mitigate ROS driven degeneration among neurons in PD. These molecules are purposed to prevent the overoxidation of Cys106 (from SO_2H- SO_3H), thereby preserving protein's function as a neuroprotectant. It will possibly aspire to stabilise the dimers, enhancing mitochondrial integrity and reducing alpha-synuclein aggregates in the neurons. Furthermore, increasing survival of dopaminergic neurons both in *in vitro* and *in vivo* PD models.

Multifunctional role of DJ-1 spanning from ROS scavenging, regulation of mitochondrial function, inhibiting the apoptosis signal – regulating kinase (ASK-1) confers it to be desired as a compelling target for therapeutics. However, with highly dynamic active site and vulnerability to oxidative damage possess challenge for development to a pharmacokinetic drug. Through computational advances, such as *Drug Bank* for structural based similarity and screening along with molecular docking, providing a functional strategy to identify novel compounds that modulate/stabilize the DJ-1 redox state without exacerbating the oxidative damage.

CHAPTER 2. REVIEW OF LITERATURE

2.1 Protein DJ-1: molecular architecture and multifunctionality

A 189 amino acid structured monomeric human protein sharing conserved homology with DJ-1/PfpI/ThiJ superfamily of proteins is linked to the early onset of PD which dimerizes to form a functional protein. In Homo sapiens, the structure of DJ-1 is comprised of alpha-helices and beta strands fashioned in a helix-strand-helix sandwich manner determined experimentally. Comprised of a total weight of 19.95 kDa, DJ-1 is known to exhibit different functions as an antioxidant, mitochondrial function regulation, molecular chaperoning, transcriptional regulator, fertility protein, regulatory subunit[9], [10], [11], [12], [13]. DJ-1 also known to impart stabilization to Nrf2 which is essential for antioxidant activity and is known to as the master regulator in stress conditions[14]. Study also shows, earlier cell death caused by the loss of DJ-1 contributed to increase in stress by ROS resulting in stress in endoplasmic reticulum (ER), oxidative stress and inhibition of proteasome[15]. They are known to express highly in normal astrocytes showing their neuronal and glial connections[16]. They are localized mainly in the cytoplasm of the cell along with in mitochondria and the nucleus to execute their numerous attributes. DJ-1 possesses three cysteine residues in its form Cys46, Cys53, Cys106, out of which C106 has been explored for its antioxidative property serving as an active site for toxic insults[17]. Known for its multifunctional classification, even a singular point mutation at the position 166 (leucine to proline) is known to manifest into neurodegeneration and cause PD by disrupting structural conformations eventually leading to loss of the functional protein [18][19]. Structural loss of DJ-1 resulting from mutations (M26I, D149A, L166P) hinders with its functions[20]. Still how this protein functions remains elusive and still to be predicted. Functionality of the protein remains to be a topic of interest as it is known to be involved in the pathogenesis of many diseases. Ongoing researches on protein DJ-1 may assemble many new functions and working pathways for this versatile protein involved in vast physiological processes. DJ-1 is well known for its activity as a sensor for oxidative stress and loss of its function leads to the increase in reactive oxygen species in the neuronal cells

which is known to trigger neurodegeneration[10], [21]. The exact molecular mechanisms for the working of the protein as an antioxidant still remains yet to be determined.

First identified as an oncogene transforming mouse cells in 1997 in concomitance with activated ras signaling pathway[22] and later correlated with PD in 2003 by Bonafiti et al, DJ-1 protein also has a potential to be an essential biomarker for both unrelated diseases that is cancer and PD. Loss of normal function of DJ-1 contributes to the onset of PD[23]. The functional study of DJ-1 still remains uncertain and a topic for deep research. It is known that DJ-1 protects from oxidative stress in the neuronal cells so there can be two possible mechanisms to elevate these attributes that is to either increase the levels of DJ-1 in neuronal cells or to stabilize or over-expression of DJ-1 in the cells to magnify their response to the ROS[15]. Kim et at showed that DJ-1 deficient mice show increase in apoptotic loss of dopaminergic neurons when induced through neurotoxins implicating the importance of DJ-1 as a protector from oxidative insults leading to PD.[24] At the molecular level, DJ-1 acts as a redox sensor through its highly reactive cysteine 106 (C106) residue. Under physiological conditions, moderate oxidation of C106 to the sulfinic acid form enables DJ-1 to directly scavenge reactive oxygen species (ROS), stabilize mitochondrial function, and upregulate antioxidant defence mechanisms [17]. DJ-1 enhances the activity of the Nrf2 pathway, leading to increased expression of antioxidant enzymes such as NAD(P)H quinone oxidoreductase 1, hemeoxygenase-1, and glutathione peroxidase, and it supports glutathione metabolism [18]. Additionally, DJ-1 modulates mitochondrial uncoupling proteins, maintains calcium homeostasis, and interacts with anti-apoptotic proteins like Bcl-xL, collectively preserving mitochondrial integrity and minimizing ROS production and apoptosis under stress conditions [19]. Beyond its antioxidant functions, DJ-1 also acts as a molecular chaperone, preventing aggregation of misfolded proteins such as α-synuclein, and participates in protein quality control through glyoxalase and protease activities [20]. It also regulates key signalling pathways involved in cell survival and inflammation, including inhibition of pro-apoptotic kinases (ASK1, PTEN) and modulation of transcription factors (Nrf2, p53, NF-κB) [21]. DJ-1's regulatory functions extend to the immune and inflammatory response, where it modulates the activation of macrophages, mast cells, and T cells through both ROS-dependent and independent mechanisms [22]. This immunomodulatory activity has implications not only for neurodegenerative diseases but also for conditions such as cancer, diabetes, and inflammatory disorders [23]. The protein's ability to function as a redox sensor, transcriptional coactivator, molecular chaperone, and mitochondrial regulator underscores its versatility and importance in cellular defence networks [24]. Loss of DJ-1 function whether by genetic mutation, overoxidation of C106 to the inactive sulfonic acid state, or reduced expression leads to heightened oxidative stress, mitochondrial dysfunction, and increased vulnerability of dopaminergic neurons, as seen in Parkinson's disease [25]. Thus, DJ-1's ability to sense and respond to redox changes, maintain mitochondrial and protein homeostasis, and regulate survival pathways positions it as a central therapeutic target for combating oxidative neurodegeneration [26]. These properties enable DJ-1 to integrate antioxidant defence, mitochondrial maintenance, protein quality control, signal transduction, and immune regulation into a unified cytoprotective response [27]. Loss of DJ-1 function whether by genetic mutation, overoxidation, or impaired dimerization renders neurons particularly susceptible to oxidative stress and degeneration, as seen in Parkinson's disease and related disorders [28].

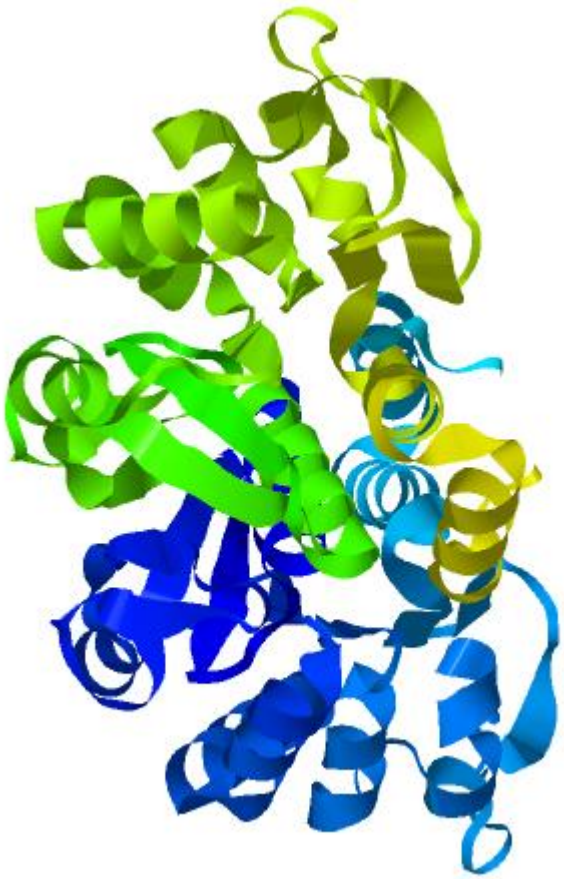


Fig.1. PDB structure of Protein DJ-1 (downloaded from Protein DataBank). Three-dimensional structure of human DJ-1 (PARK7) exported from the Protein Data Bank (PDB ID: 1SOA). The protein is shown as a homodimer, with each monomer adopting a characteristic α/β-fold. The highly conserved Cys106 residue, critical for DJ-1's redox-sensing and antioxidant function, is highlighted within the active site pocket. Structural visualization was performed after refinement and removal of water molecules, illustrating key features relevant for computational docking and ligand interaction studies.

2.2 C106 oxidative active site

Three cysteine residues (position 46, 53, 106) exert oxidation as well as nitrosylation to form the function of DJ-1 [25]. Cys106 has an unusually low pKa (~5.4), which stabilizes its thiolate form ($-S^-$) under physiological conditions [26]. This property makes Cys106 as a cellular redox sensor due to its sensitivity towards oxidative modifications [25]. Oxidation of the cysteine amino residue is a part of post-translational modification [25]. C106 is shown in many studies to be the most sensitive and reactive to oxidation termed as a redox sensor and changes the structure locally due to overoxidation possibly contributing to the onset of disease implicating its stability and thereby resulting in dysfunction [25], [26]. Structural studies reveal that oxidation to the sulfinate form induces conformational changes that enhance DJ-1's interaction with mitochondrial and antioxidant pathways, while overoxidation to sulfonic acid disrupts these functions [26], [27].

Wild type of DJ-1 can alter into three oxidative states namely, (C106-S-, reduced) thiolate, (C106-SO_2-, oxidized) sulfinate, (C106-SO_3-, hyper-oxidized) sulphonate [25]. Accordingly, the reduced and oxidized form of this protein are the stable form and withheld its multi-functions [25]. The ROS interacts with nucleophilic C106 residue on DJ-1 and form these three states [25]. Moreover, the oxidized C106-SO_2-

form is assumed to function as a neuroprotectant, while the hyper-oxidized C106-SO_3- form is found to be linked to loss of the protein's antioxidant functions [25], [26]. Cys106 undergoes a redox-dependent cascade: the reduced thiol (-SH) form scavenges reactive oxygen species (ROS), while moderate oxidation to sulfinic acid (-SO_2H) activates DJ-1's cytoprotective roles, including mitochondrial stabilization and upregulation of antioxidant genes (e.g., via Nrf2) [17], [18], [26]. However, excessive oxidative stress drives irreversible overoxidation to sulfonic acid (-SO_3H), rendering DJ-1 inactive [25], [26]. In Parkinson's disease (PD), Cys106-SO_3H accumulates in dopaminergic neurons, correlating with mitochondrial dysfunction and α-synuclein (α -syn) aggregation [28]. Overoxidized DJ-1 is ubiquitinated and degraded, exacerbating neuronal vulnerability to ROS [25]. This redox plasticity positions Cys106 as a molecular switch, where its oxidation state dictates DJ-1's role in cellular survival or dysfunction [25]. DJ-1's Cys106 is central to its multifunctional neuroprotective roles. In its sulfinic state (-SO_2H), DJ-1 stabilizes mitochondrial complex I, enhances ATP synthesis, and inhibits apoptosis by sequestering pro-death kinases like ASK1 [25], [21]. It also acts as a chaperone, preventing α-synuclein aggregation, and repairs glycation damage via glyoxalase activity [20], [25]. Oxidation of Cys106 enhances DJ-1's nuclear translocation, where it regulates transcription factors (e.g., Nrf2, p53) to upregulate antioxidant defenses [21], [26]. Loss of these functions due to Cys106 mutations (e.g., C106A) or overoxidation impairs ROS scavenging, accelerates mitochondrial damage, and promotes neurodegeneration in PD models [25], [26], [28]. Researchers have found link of DJ-1 to PD by observing over-oxidized sulphonated modification in the post-mortem brains of PD patients [28]. The active conformation of DJ-1 is lost due to structural changes due to over-oxidation (sulphonate) at C106 residue pocket destabilizing its active site thereby losing its antioxidant property [26], [28]. This would result in decreasing DJ-1 efficacy toward the oxidative ROS insults. Consequently, increasing ROS in neuronal cells results in the pathogenesis of PD [28]. Thereby, C106 site is a very interesting target for stabilization of DJ-1 and can be explored for discovery of drugs for PD [29]. The Cys106 pocket is a promising therapeutic target for Parkinson's disease. Small molecules that stabilize the sulfinic acid state (e.g., UCP0054278 analogs) could prevent DJ-1 overoxidation and preserve its antioxidant functions [29], [30]. Computational studies highlight compounds forming hydrogen bonds with Cys106 and Glu18, which mimic natural redox stabilization [30]. Challenges include DJ-1's dynamic active site and the need for blood-brain barrier permeability [30]. Strategies like bivalent ligands targeting both Cys106 and the dimer interface may enhance efficacy [30]. Restoring DJ-1's redox-sensitive functions offer a disease-modifying approach, contrasting with symptomatic therapies like L-DOPA [30].

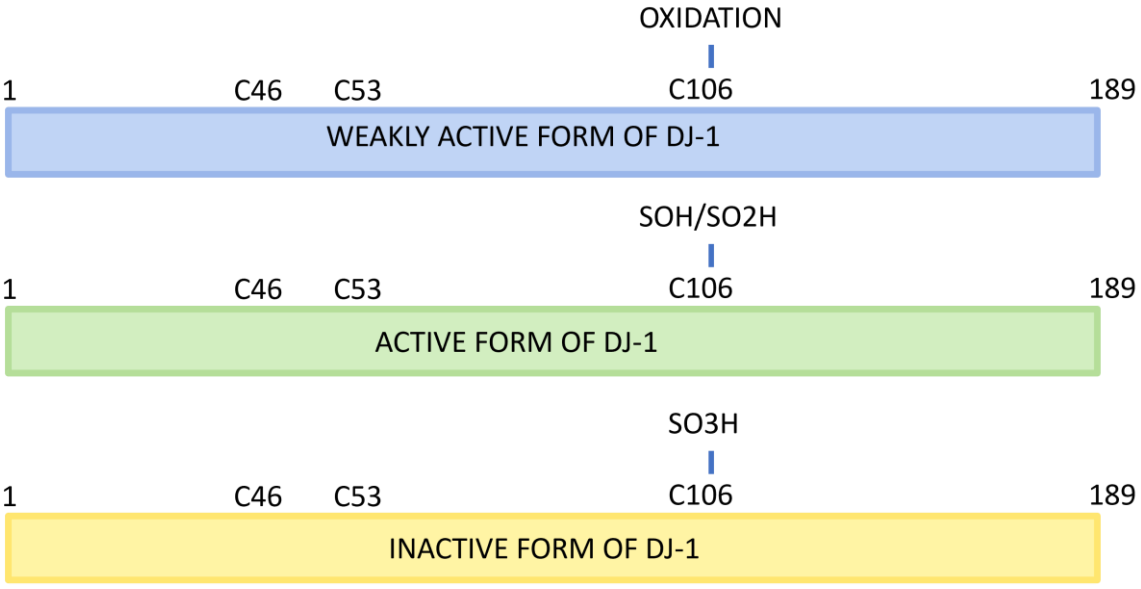


FIG.2. Schematic representation of the three oxidative states of DJ-1 at the Cys106 residue. The reduced form (Cys106–SH) is the active antioxidant state, capable of ROS scavenging and supporting dopamine biosynthesis and signalling. Moderate oxidation leads to the sulfinic acid form (Cys106–SO_2H), which is associated with mitochondrial localization, inhibition of α-synuclein fibrillation, and enhanced cytoprotective functions. Further irreversible oxidation produces the sulfonic acid form (Cys106–SO_3H), resulting in loss of DJ-1's biological activity, increased aggregation, and correlation with Parkinson's disease progression. The functional properties and structural stability of DJ-1 are tightly regulated by these sequential oxidative modifications at Cys106, highlighting its role as a redox-sensitive sensor and regulator in cellular defence against oxidative stress.

2.3 DJ-1 dysfunction leads to Parkinson's disease

Parkinson's disease (PD) is a neurodegenerative disorder characterized by the progressive loss of dopaminergic neurons, influenced by factors such as age, sex, genetics, and environmental exposures [30]. Oxidative stress plays a significant role in PD pathogenesis, although its exact mechanisms remain unclear [31]. While current treatments alleviate symptoms, they do not halt the progression of neuronal damage caused by reactive oxygen species (ROS) [32]. Therefore, exploring compounds that mitigate ROS-induced neuronal damage is imperative [33].

DJ-1, a protein present in high-energy-demanding cells like dopaminergic neurons, serves a neuroprotective function by combating oxidative stress [34]. Disruptions in mitochondrial homeostasis contribute to increased ROS production, further exacerbating neuronal degeneration [35].

Several processes contribute to ROS formation in PD:

A. **Dopamine Metabolism**: Dopamine can undergo autoxidation, forming dopamine quinones and superoxide radicals (O_2^-), which modify proteins such as α-synuclein and impair proteasomal function [36]. Monoamine oxidase-B (MAO-B) activity metabolizes dopamine, producing hydrogen peroxide (H_2O_2), which, in the presence of iron, generates hydroxyl radicals (·OH) through Fenton reactions [37].

B. **Mitochondrial Dysfunction**: Impaired electron transport chain activity, particularly at complex I, leads to electron leakage and superoxide production, disrupting ATP synthesis and damaging mitochondrial DNA [38]. Excessive calcium influx activates nitric oxide synthase, producing nitric oxide (NO), which reacts with superoxide to form peroxynitrite ($ONOO^-$), a potent oxidant [39].

C. **Iron Accumulation**: Iron deposits in the substantia nigra catalyse the conversion of H_2O_2 to hydroxyl radicals, leading to lipid peroxidation and DNA damage. Reduced ferritin levels and degradation of neuromelanin-iron complexes release redox-active iron, amplifying oxidative damage [40].

D. **Neuroinflammation**: Chronic microglial activation releases pro-inflammatory cytokines and NADPH oxidase-derived superoxide, creating a cycle of neuronal damage. Astrocytes in PD show diminished glutathione synthesis, weakening antioxidant defences [41].

E. **Genetic and Molecular Contributors**: Mutations in PARK7 (DJ-1) impair ROS scavenging and mitochondrial stabilization. The G2019S mutation in LRRK2 affects mitochondrial antioxidant enzymes, increasing ROS levels and disrupting mitophagy [42].

F. **Impaired Antioxidant Systems**: Reduced glutathione levels and declining CoQ10 impair the detoxification of ROS, accelerating neuronal damage [43].

Research indicates a strong pathological link between DJ-1 and PD. DJ-1-deficient dopaminergic neurons exhibit heightened sensitivity to oxidative stress, leading to apoptosis [44]. In rat models, administration of recombinant DJ-1 reduced ROS production following 6-hydroxydopamine-induced oxidative stress [45]. Oxidation at the C106 residue of DJ-1 is crucial for its neuroprotective function, whereas oxidation at C46 and C53 is less significant [46].

Therapeutic strategies targeting ROS sources, such as MAO-B inhibitors (e.g., selegiline), iron chelators (e.g., deferiprone), and mitochondrial stabilizers (e.g., CoQ10), are under investigation. However, clinical trials suggest that combination therapies addressing both ROS generation and antioxidant depletion may be more effective [47]. Given the pivotal role of C106 oxidation in DJ-1's neuroprotective function, developing targeted therapies focusing on this residue holds promise for treating neurodegenerative diseases characterized by oxidative stress [48].

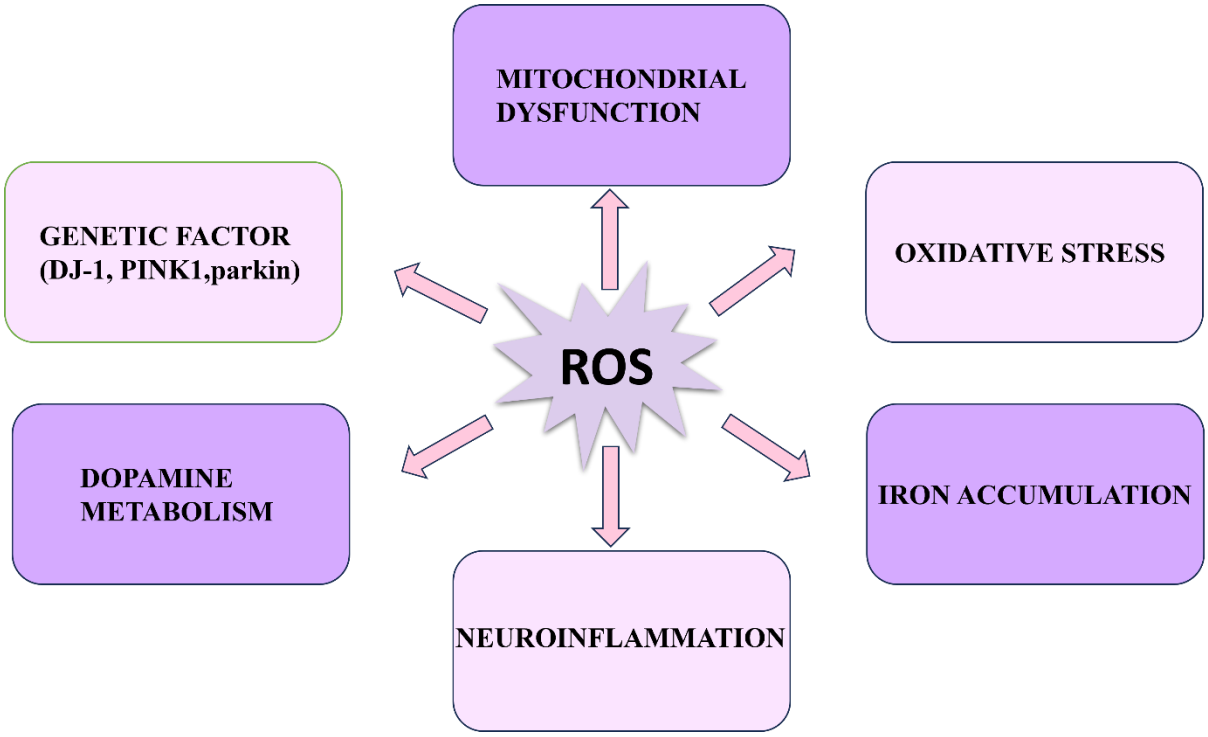


Fig.3. Illustration of the major sources of reactive oxygen species (ROS) implicated in the pathogenesis of Parkinson's disease. Key contributors include mitochondrial dysfunction (impaired complex I activity), dopamine metabolism (autoxidation and monoamine oxidase activity), iron accumulation (catalysing Fenton reactions), neuroinflammation (microglial and astrocyte activation), and genetic mutations in PD-associated proteins (such as α-synuclein, DJ-1, PINK1, parkin, and LRRK2). Excessive ROS production from these sources leads to oxidative damage of neuronal proteins, lipids, and DNA, ultimately resulting in dopaminergic neuron degeneration in the substantia nigra.

2.4 DJ-1 known modulators

Compounds that have the capacity to bind to DJ-1 at their 106th cysteine amino residue have been reported for their therapeutic potential for neurodegenerative diseases like PD. DJ-1, as a protein, can be used as a therapeutic target in treating PD as it is known to suppress disease progression. These compounds are assumed to be given alongside other drug compounds to provide optimal therapeutic treatments against PD.

A 13-amino acid DJ-1 fragment fused to a TAT cell-penetrating sequence, known as ND-13, reverses cold hypersensitivity in Dj-1−/− mice and rescues dopaminergic neurons in PD models but exhibits transient efficacy due to nuclear translocation [48]. Another study suggests a flavonoid that constantly activates DJ-1, reversing mechanical and cold hypersensitivity in chemotherapy-induced neuropathy models without inducing tolerance. Although it demonstrates specificity for DJ-1, as it fails to reverse neuropathies in Dj-1−/− mice [49]. Subsequently, small molecules identified by Cantabio Pharmaceuticals stabilize the DJ-1 homodimer and prevent Cys106 overoxidation. These compounds show therapeutic effects in primary neuronal and in vivo PD models [50]. Other pharmacological studies suggest DJ-1's functional regulation of transient receptor potential ankyrin 1 (TRPA1) in dorsal root ganglia. Therefore, it was found that Dj-1−/− mice exhibit heightened TRPA1 activity, which is normalized by ND-13 and K3OβR, linking DJ-1 to peripheral neuropathy [51]. Thereby, DJ-1 directly binds α-synuclein monomers and oligomers, attenuating aggregation and reducing neurotoxicity in cellular models [52].

Challenges in Targeting DJ-1:

A. **Dynamic Active Site:** Cys106's redox-dependent conformational changes (SH → SO_2H → SO_3H) complicate ligand binding. Overoxidation to SO_3H, common in PD, renders DJ-1 inactive. Pathogenic mutations (e.g., L166P) destabilize DJ-1 dimers, altering the active site geometry [53].

B. **Selectivity and Off-Target Effects:** Covalent inhibitors risk modifying cysteine-rich proteins like Keap1, while non-covalent modulators (e.g., K3OβR) may broadly enhance antioxidant pathways [54].

C. **Oxidative Environment:** Chronic ROS in PD accelerates Cys106 overoxidation, reducing targetable DJ-1 pools. Iron accumulation in the substantia nigra exacerbates Fenton reactions, further oxidizing DJ-1 [55].

D. **BBB Penetration:** Only a few compounds meet BBB permeability criteria, highlighting the need for lipid-soluble scaffolds with low P-gp efflux ratios [56].

These findings are harmonious with explaining the neuroprotective role of DJ-1. In agreement with these data, Miyazaki et al. isolated the brain-penetrant covalent inhibitor UCP0054278 through virtual screening that binds to the grooves of active sites of the C106 region in the oxidized SO_2H form of DJ-1. Indeed, in vitro studies in PD-affected rat models also showed that it prevented excessive ROS-induced oxidative stress by inhibiting the peroxidation into the over-oxidized form (SO_3H) of DJ-1, eventually averting cell death. Furthermore, locomotory defects were restored in rats treated with neurotoxins like 6-OHDA and rotenone, providing evidence for a new class of dopaminergic neuroprotective compounds [57]. In conclusion, the possible mechanism is the prevention of overoxidation of DJ-1, making DJ-1 modulator UCP0054278 a major dopaminergic neuroprotective compound in the treatment of PD as well as Alzheimer's disease.

Other encouraging studies, focusing on the putative active site of DJ-1 accumulated through virtual screening using the crystal structure of the protein in reduced and oxidized C106 regions, produced specific novel compounds through the screening process. One such compound, UCP0045037, was identified to show neuroprotective effects in animal models by binding to the reduced form of DJ-1 [58]. Another compound, compound-23, has shown neuroprotective activity in DJ-1, restoring locomotory defects and preventing ROS-induced cell death in MPTP-treated mouse models [59].

PD treatment therapies aim to develop novel interventions to delay the progression of the disease by drug development utilizing existing drugs to be approved for therapeutics, also known as drug reprofiling or repurposing. This is based on the use of existing drugs for new clinical indications. The primary goal of this study is to identify DJ-1 active site-targeted drug bank compounds providing stabilization of the active form, that is, the sulfinate form in neuronal cells, in order to delay the onset and progression of PD.

2.5 Computational approaches for analysis of modulators

2.5.1 Role of In Silico Techniques in Accelerating Drug Discovery

In silico techniques have fundamentally transformed drug discovery, enabling researchers to rapidly screen vast chemical libraries, predict molecular interactions, and optimize lead compounds with high efficiency and reduced cost [60]. These computational methods are now essential at every stage of the drug development pipeline, from target identification and lead compound discovery to ADME/toxicity prediction and optimization. By integrating molecular modelling, cheminformatics, structure-based drug design (SBDD), and high-throughput virtual screening, in silico approaches allow for the rational design and prioritization of drug candidates, significantly accelerating timelines and improving the success rate of drug discovery projects. The ability to model protein structures, simulate ligand binding, and predict pharmacokinetic properties before experimental validation reduces the need for extensive wet-lab screening, making the process more efficient and cost-effective. Whilst traditional drug development will cost us the time and more costly and moreover provide high rates of unpredictability due to poor pharmacokinetic status [60].

2.5.2 Molecular Docking as a Tool to Predict Protein–Ligand Interactions

Molecular docking is a cornerstone of structure-based drug design, providing a computational means to predict how small molecules (ligands) interact with target proteins at the atomic level.[61] Docking algorithms evaluate the binding affinity and orientation of ligands within the protein's active site, often using scoring functions that consider hydrogen bonds, salt bridges, hydrophobic interactions, and overall binding energy. Advanced docking tools, such as *AutoDock* and its variants, employ sophisticated search algorithms (e.g., Lamarckian genetic algorithm, random drift particle swarm optimization) to explore conformational space, generating multiple binding poses for each ligand [61]. The best-scoring conformations are then subjected to further analysis, including molecular dynamics simulations, to assess stability and refine predictions. Molecular docking not only identifies promising hits but also provides mechanistic insights into the molecular determinants of binding, guiding the rational optimization of lead compounds [61].

2.5.3 Importance of ADME Profiling in CNS Drug Design

A critical step in the development of central nervous system (CNS) drugs is the assessment of ADME (Absorption, Distribution, Metabolism, and Excretion) properties, particularly blood-brain barrier (BBB)

permeability. Computational ADME profiling uses predictive models and physiochemical filters (e.g., Lipinski's rule of five, polar surface area, logP) to evaluate drug-likeness and CNS exposure [62]. Studies show that CNS-active drugs are typically smaller, less polar, and less flexible than non-CNS drugs, characteristics that enhance passive BBB penetration. The implementation of accurate ADME models, often powered by artificial intelligence and machine learning, allows researchers to prioritize compounds with optimal pharmacokinetic and safety profiles, reducing costly late-stage failures. For example, in the context of anti-Parkinson drug discovery, refined ADME profiling ensures that candidate molecules not only bind to DJ-1 but also reach effective concentrations in the brain [62].

2.5.4 Ligand-Based Similarity Screening (SwissSimilarity and DrugBank)

When structural information about the target is limited or when expanding the chemical diversity of leads is desired, ligand-based similarity screening becomes invaluable. Tools such as *SwissSimilarity* and *DrugBank* leverage molecular fingerprints and similarity metrics to identify compounds structurally analogous to known actives, facilitating scaffold hopping and the discovery of novel chemotypes. *SwissSimilarity*, for instance, can screen millions of compounds from curated databases, prioritizing analogs that maintain key pharmacophoric features of validated ligands. This approach is particularly useful for repurposing existing drugs or generating focused libraries for virtual screening, as it increases the likelihood of identifying bioactive molecules with established safety profiles [63].

2.5.5 Integration of Visualization Platforms (e.g., Discovery Studio) for Interaction Interpretation

Visualization platforms like Discovery Studio play a crucial role in interpreting and refining computational results. These tools allow researchers to analyse protein–ligand complexes in three dimensions, identify key binding interactions (hydrogen bonds, salt bridges, hydrophobic contacts), and detect steric clashes or suboptimal orientations. Visualization enhances the understanding of binding mechanisms and supports the rational design of ligand modifications to improve affinity and selectivity. Additionally, these platforms facilitate the integration of molecular dynamics simulations, further refining docking predictions by accounting for protein and ligand flexibility. In the context of DJ-1 modulation for PD, visualization tools are essential for confirming the engagement of critical residues such as Cys106 and for designing molecules that stabilize desired redox states [64].

2.6 Rationale of the current study

Despite DJ-1's established role in mitigating oxidative stress and neurodegeneration in Parkinson's disease (PD), significant gaps persist in therapeutic targeting of its redox-sensitive C106 residue. While covalent inhibitors (e.g., Compound 15/16) and natural products (e.g., EGCG) bind DJ-1, few selectively stabilize the neuroprotective sulfinic acid state (C106-SO_2H) without promoting irreversible overoxidation (C106-SO_3H), which is prevalent in PD brains. DJ-1's shallow binding pocket and redox-dependent conformational shifts hinder traditional drug discovery. Existing DJ-1 modulators often fail to meet CNS drug-likeness criteria, with poor BBB penetration or P-gp efflux liabilities [65].

This study addresses these gaps through a synergistic *in silico* pipeline:

SwissSimilarity: Leverages ligand-based screening to identify structurally diverse analogs of UCP0054278, a reference ligand with confirmed DJ-1 binding, expanding the chemical space while preserving pharmacophoric features. This online tool enables rapid identification of compounds which are structurally analogous to the query molecule from a wide variety of libraries which include DrugBank containing drugs which are already in use thereby approved, or clinical candidates and molecules which

can be derived synthetically. It employs fingerprinting which consist of support hit binding, scaffold hopping and reprofiling/repurposing of drug. It helps by expanding a chemical space around the query ligand molecule which results in a library enriched in molecules that bind to DJ-1 active site and have same bioactivity. It has many characterstics including its ease to use, speed making this a premier choice for the initial stages of virtual screening for discovery of drug [65].

SwissADME: Applies rigorous filters (Lipinski's rule, BBB permeability, PAINS alerts) to prioritize potential drug candidates with CNS-compatible properties, minimizing off-target effects and metabolic instability. It filters the virtual library to retain molecules with good pharmacokinetic profiles increasing their likelihood to become validated DJ-1 modulators. It has innumerable features like BOILED-Egg method for BBB permeability, Lipinski rules of five, TPSA, iLOGP for checking lipophilicity to assess the profile for CNS drugs. Its interoperability with SwissSimilarity and intuitive output make it an indispensable tool for CNS drug design [66].

AutoDock Vina: Predicts binding poses and affinities at DJ-1's C106 pocket, focusing on interactions with redox-critical residues (C106, Glu18, Met26) to stabilize the sulfinic acid state. It is widely used for molecular docking predicting binding affinities and mode for small molecules towards their possible protein targets. Vina utilizes automated grid map calculation and clustered results, leveraging a streamlined scoring function and docking workflow. AutoDock Vina enabled high-throughput docking of ADME-filtered ligands against DJ-1's Cys106 pocket, providing reliable ranking of binding poses and energies. Its compatibility with standard file formats and established accuracy in predicting binding modes make it a gold standard for structure-based drug design [67].

Discovery Studio: Validates hydrogen bonding, hydrophobic contacts, and steric compatibility, ensuring ligands avoid clashes with DJ-1's flexible loops or dimer interface. Discovery Studio is a professional molecular modelling suite offering a comprehensive set of tools for protein characterization, molecular docking, pharmacophore modelling, ADMET prediction, and detailed interaction analysis. It excels in visualizing protein–ligand complexes, mapping hydrogen bonds, hydrophobic contacts, and steric clashes in three dimensions, and generating publication-quality images and interaction diagrams. In your project, Discovery Studio was used to interpret and validate docking results from AutoDock Vina, enabling in-depth analysis of how top-ranked ligands interact with DJ-1's Cys106 and surrounding residues. Its integration of multiple simulation and visualization functionalities makes it the preferred platform for interaction interpretation and rational lead optimization in drug discovery [68],[69].

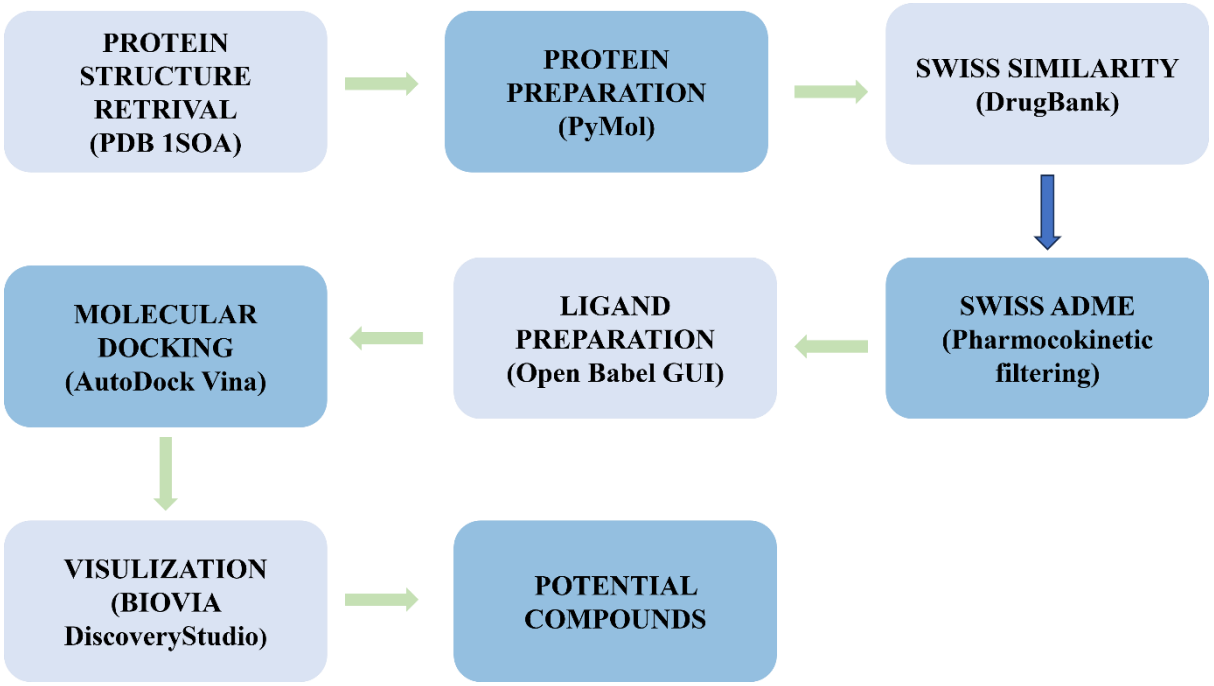


Fig.4. Workflow diagram illustrating the computational methodology employed in this thesis for the discovery of novel DJ-1 modulators.

CHAPTER 3. METHEDOLOGY

3.1 Retrieval of protein DJ-1

Protein DJ-1 structural data (PDB Id : 1SOA) was retrieved from online database Protein Data Bank (PDB) site containing experimentally determined structural form of DJ-1 (https://www.rcsb.org/), which has a modification on cysteine residue 106. Cysteine-sulfinic-acid modification of C106 (C106-SO_2H) controls its function as an intracellular ROS induced sensor for oxidative stress.

3.2*Preparation of target protein DJ-1*

Structure of DJ-1 was downloaded in PDB format from the PDB site. For carrying out molecular docking, protein structure was prepared using MGL/AutoDockTools-1.5.7. AutoDockTools were used to remove H_2O molecules, add polar hydrogens providing partial charges to the molecule which was saved in PDBQT format afterwards making it compatible for AutoDock grid computing. Molecules and ions were deleted which were solvent to isolate the protein structure. Another option of 'Add Hydrogens' was used to add polar hydrogen to optimize protonation states at pH of 7.4 (physiological). Energy was minimized to resolve

any stearic hindrance and geometry was optimized and in accord with literature review active site was defined.

3.3 Retrieving ligand data set

a) **Reference compound selection***:* UCP0054278, a DJ-1 modulator used as a reference ligand for C106 selective region and retrieved in SDF format. The ligand 3D conformer was transferred from PubChem (https://pubchem.ncbi.nlm.nih.gov/).

b) **Screening of DJ-1 binding compounds:** An online computational tool, (http://www.swisssimilarity.ch/) SwissSimilarity offers virtual screening of data libraries finding compounds similar to our query molecule. SwissSimilarity contains chemical libraries from DrugBank offering screening of approved, experimental, investigational drugs[25]. The canonical SMILES (COC1=C(C=C(C=C1)CC(=O)NCCC2=CC3=C(C(=C2)OC)OCO3)OCC4=CC=CC=C4) of the reference was used as a format for query molecules.

c) **Physicochemical quality analysis:** Quality analysis through ADME screening is an *in silico* assessment of a potent interaction of drug with body. SwissADME (http://www.swissadme.ch/) is an openly accessible computer based online software predicting the "likeness" of drugs in the body. It is used to reduce the pharmacokinetics related failure in later phases. This computational tool is a valid alternative to ADME analysis by experimental procedures filtering out molecules with properties incompatible to acceptable pharmacokinetic profiles. These include BBB permeability, Lipinski's rule-of-five, Brenk and PAINS as an evaluation for filtering out compounds for drug-likeness. A list of SMILES were added in the SMILES list box and calculations were performed. The compiled list was pharmacokinetically assessed for BBB permeability, Lipinski filter, PAINS and Brenk value generating a filtered list through computational analysis. The list was further used for molecular docking.

d) **Ligand preparation:** Retrieved selected ligands along with reference compound from PubChem database. The molecular structures were then reformatted using Open Babel GUI tool (.sdf to .pdbqt) which promotes easy format conversion and energy-minimized with the MMFF94 force field.

3.4 Molecular docking using AutoDock Vina

AutoDock Vina was used to dock the Reference and selected compounds to the prepared protein structure. Vina predicts bound conformations and the binding affinity value by giving scoring functions. It assists in screening the molecules for drug discovery. Vina uses rapid gradient optimization conformational search with high accuracy[26]. PDBQT format ligands and receptors were loaded on command prompt as an input data through their directory folder, providing parameters of the exploration space for possible orientations at the binding site. A configuration file was created on TXT format with grid map dimension value as x = 35.097, y = -11.576, z = -54.974 with cubic centre of 20 Å X 20 Å X 20 Å. Vina uses RMSD-based criterion providing representative poses achieving better performance. Output files were generated for each ligand-protein docking in PDBQT format along with a log TXT file containing binding affinities of different poses converted to excel file[67].

3.5 Molecular docking analysis using BIOVIA DiscoveryStudio

Output files were used to be validated through BIOVIA DiscoveryStudio 2021 Client. It is a software distributed by Dassault Systems BIOVIA formerly known as Accelrys. 2D conformations were made using

reference-protein and protein-output filtered ligands PDBQT files showing different interacting residues along with bond interactions[68].

Table 1. Overview of software tools used in this study, including their primary functions and official access links. Each platform was selected for its reliability, scientific validation, and relevance to computational drug discovery targeting DJ-1 in Parkinson's disease.

Software Used	***Links/References***
Protein Database	https://www.rcsb.org/ [72]
PubChem	https://pubchem.ncbi.nlm.nih.gov/ [73]
PyMol	https://pymol.org/ [70]
Swiss Similarity	http://www.swisssimilarity.ch/ [74]
DrugBank	https://go.drugbank.com/ [71]
Swiss ADME	http://www.swissadme.ch/ [75]
AutoDock Vina	https://vina.scripps.edu/ [67]
BIOVIA DiscoveryStudio	https://discover.3ds.com/discovery-studio-visualizer-download [68]

CHAPTER 4. RESULT AND DISCUSSION

4.1Screening of ligands and their ADME analysis

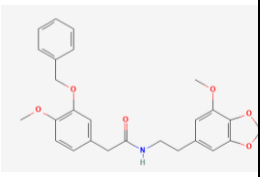
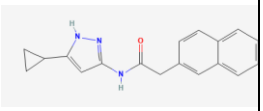
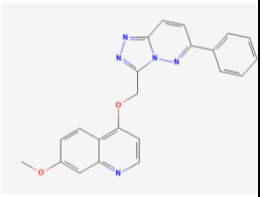
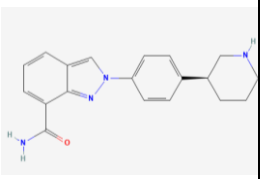
A total of 400 compounds were screened using an online computational tool called SwissSimilarity offering compounds on the basis of similarity to our query molecule which was the reference molecule. Libraries were screeened for DrugBank. Another computational tool was then used to perform ADME analysis called SwissADME. This tool offers a method of filtering out acceptable pharmacokinetic compounds. Canonical SMILES (COC1=C(C=C(C=C1)CC(=O)NCCC2=CC3=C(C(=C2)OC)OCO3)OCC4=CC=CC=C4) loaded on SwissADME produced an excel sheet which is then filtered out for BBB permeability, Lipinski's rule-of-five, PAINS and Brenk. We retrieved a total of 120 compounds with BBB penetrant ability(Predicted via the BOILED-Egg model (WLOGP vs. TPSA)), zero violations for Lipinski rule (Molecular weight <500 Da, logP <5, H-bond donors ≤5, H-bond acceptors ≤10), PAINS (Exclusion of pan-assay interference compounds (e.g., enones, rhodanines))and Brenk. These qualities are essential for drug designing. These compounds were further downloaded from chemical library database PubChem in SDF format using their

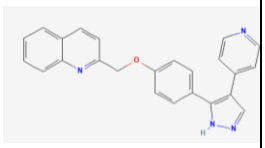
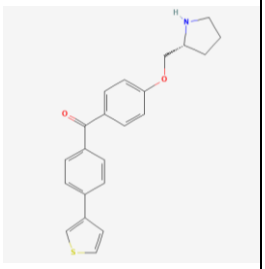
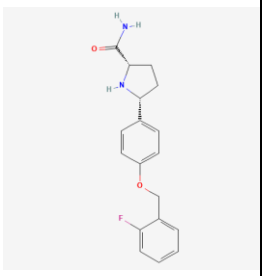
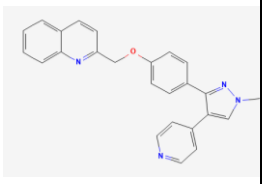
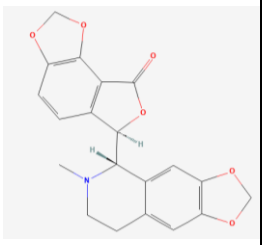
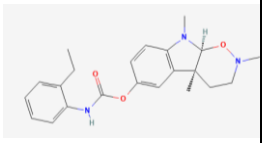
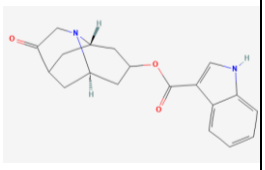
canonical SMILES. OpenBabelGUI, a versatile tool is used to facilitate the conversion to PDBQT format containing paramount interactive information.

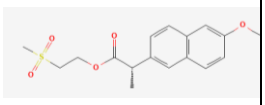
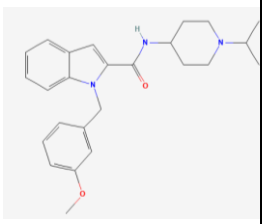
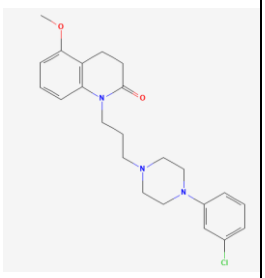
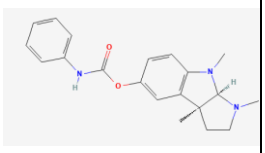
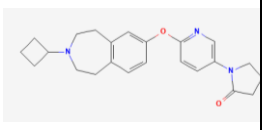
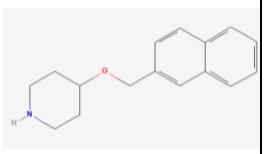
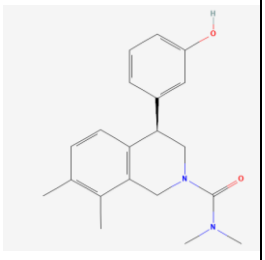
4.2 Molecular docking: Binding mechanism of DJ-1 to modulators

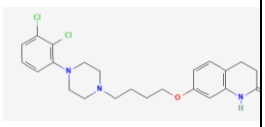
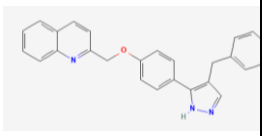
AutoDock Vina was used for site-specific molecular docking of reference and other 120 compounds with our target protein DJ-1. Grid box parameterization was Cys106 centered (x = 35.097, y = -11.576, z = -54.974 with cubic centre of 20 Å X 20 Å X 20 Å) with 0.375 spacing. Understanding the physical interaction between ligand and protein molecule is essential for computational drug designing. Lamarckian Genetic Algorithm (LGA) with 100 runs per ligand. The binding affinity of the reference molecule with protein DJ-1 came out to be -7.3 kcal/mol. Out of 120 compounds, 19 compounds had more negative binding energy than the reference compound (UCP0054278)(PubChem CID : 42596919) (TABLE II). Compound 1 demonstrated the topmost affinity (-8.4 kcal/mol) amidst other compounds for receptor protein.

TABLE II LIGANDS SHOWING BEST DOCKING SCORES WITH PROTEIN IN CONTRAST WITH THE REFERENCE COMPOUND ALONG WITH THEIR BINDING AFFINITIES AND INTERACTING RESIDUES

Compound No.	DrugBank Id	Compound Name	PubChem CID	Binding Affinity(kcal/mol)	Structural diagram
Reference/ UCP0054278	-	2-[3-(Benzyloxy)-4-methoxyphenyl]-N-[2-(7-methoxy-2H-1,3-benzodioxol-5-yl)ethyl]acetamide	42596919	-7.3	
Compound A	DB06944	N-(5-Cyclopropyl-1H-pyrazol-3-yl)-2-(2-naphthyl)acetamide	449087	-8.4	
Compound B	DB08079	7-Methoxy-4-((6-phenyl-[1,2,4]triazolo[4,3-b]pyridazin-3-yl)methoxy)quinoline	24864821	-8.3	
Compound C	DB11793	Niraparib	24958200	-8.1	

Compound D	DB08386	2-{[4-(4-pyridin-4-yl-1H-pyrazol-3-yl)phenoxy]methyl}quinoline	11610553	-8.1	
Compound E	DB07237	{4-[(2R)-pyrrolidin-2-ylmethoxy]phenyl}(4-thiophen-3-ylphenyl)methanone	44129624	-8.0	
Compound F	DB11706	Raxatrigine	16046068	-7.9	
Compound G	DB08387	Mardepodect	11581936	-7.9	
Compound H	DB11562	Bicuculline	10237	-7.7	
Compound I	DB06525	Ganstigmine	9823294	-7.7	
Compound J	DB00757	Dolasetron	3033818	-7.5	

Compound K	DB12398	Naproxen Etemesil	25170420	-7.5	
Compound L	DB07973	N-(1-Isopropylpiperidin-4-YL)-1-(3-methoxybenzyl)-1H-indole-2-carboxamide	6540267	-7.5	
Compound M	DB05422	Opc-14523	9892540	-7.5	
Compound N	DB04892	Phenserine	192706	-7.5	
Compound O	DB15120	1-(6-((3-Cyclobutyl-2,3,4,5-tetrahydro-1H-benzo[D]azepin-7-YL)oxy)pyridin-3-YL)pyrrolidin-2-one	9976892	-7.5	
Compound P	DB15038	Litoxetine	65650	-7.4	
Compound Q	DB07064	(4R)-4-(3-Hydroxyphenyl)-N,N,7,8-tetramethyl-3,4-dihydroisoquinoline-2(1H)-carboxamide	6857690	-7.4	

Compound R	DB01238	Aripiprazole	60795	-7.4	
Compound S	DB08384	2-({4-[4-(pyridin-4-ylmethyl)-1H-pyrazol-3-yl]phenoxy}methyl)quinoline	44141871	-7.4	

4.3 Docked ligands visualization

BIOVIA Discovery Studio2021 was used for visualizing the docked ligands along with target receptor protein providing their 2D conformations along with interacting residues and bond interactions. Interacting residues of ligands and the reference molecule is given in TABLE II. The reference compound positioned itself in the active pocket of the C106 region interacting with various residues such as Leu77, Asn76, Arg48, His126, Pro158, Cys106 where 4 conventional hydrogen bonds were formed.

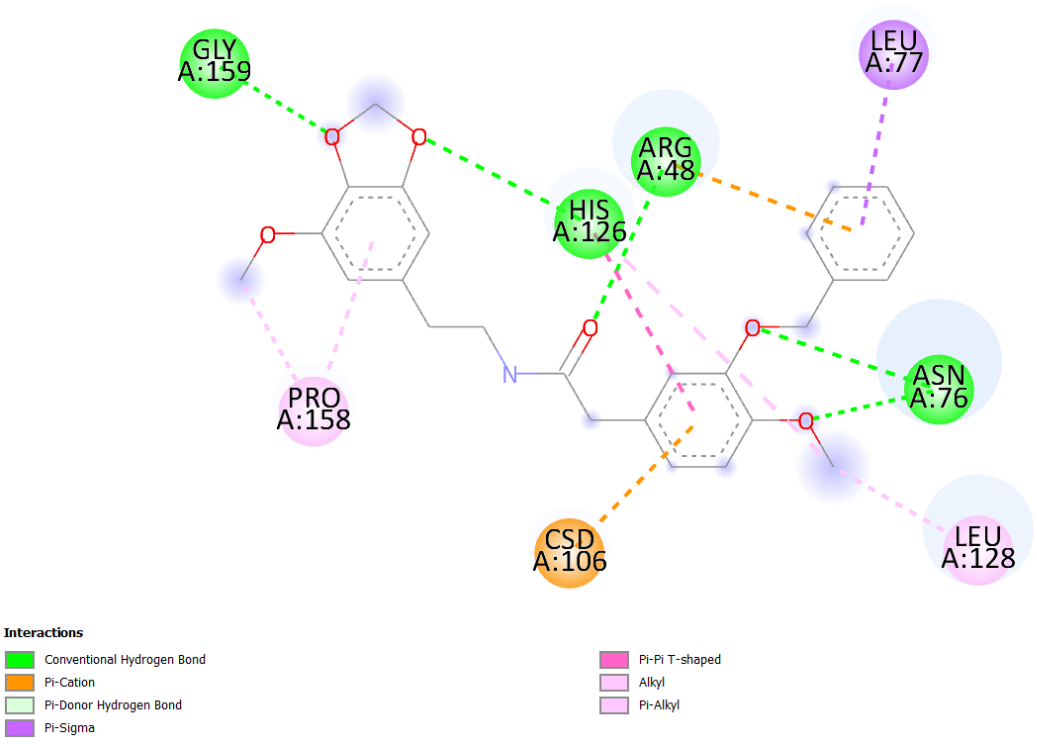


Fig. 5. Interactions of reference compound : UCP0054278 with DJ-1

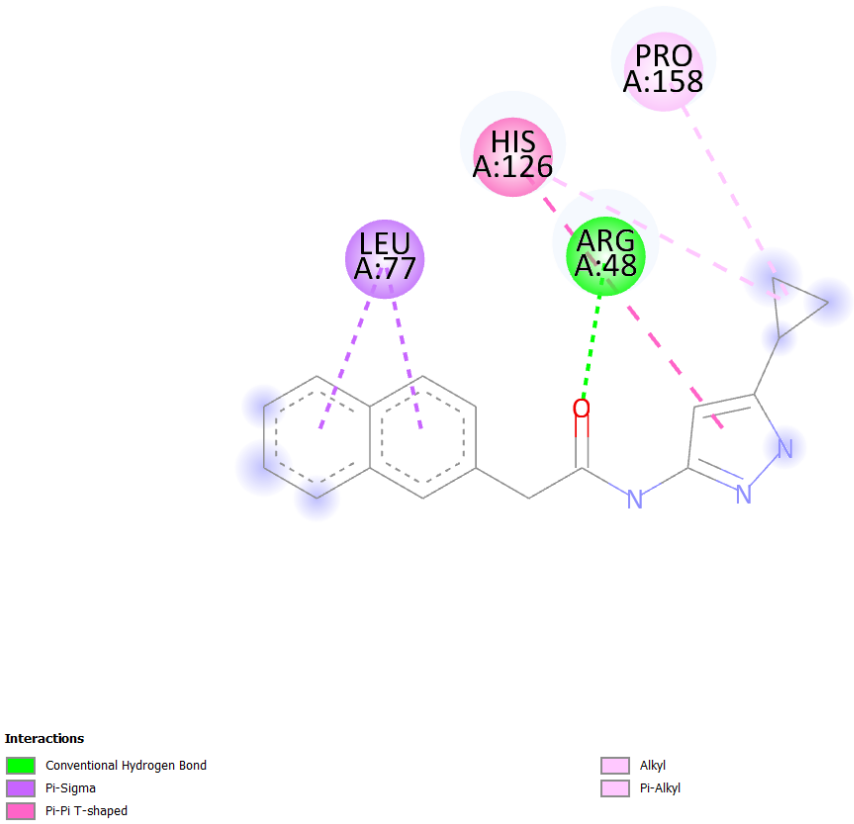


Fig. 6. Interactions of compound A [N-(5-Cyclopropyl-1H-pyrazol-3-yl)-2-(2-naphthyl)acetamide]

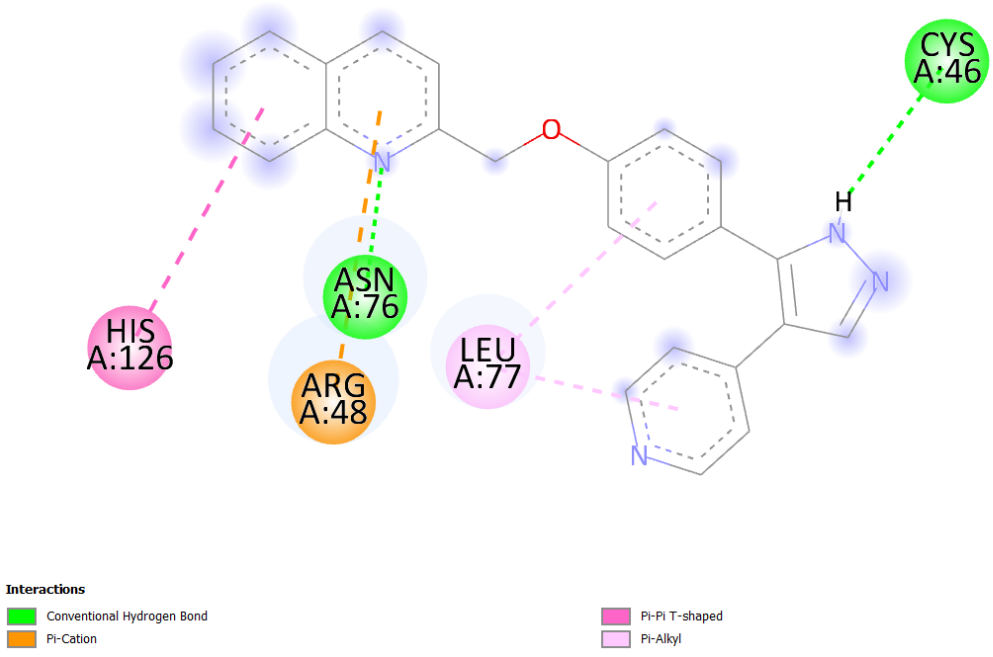


Fig. 7. Interactions of compound B [7-Methoxy-4-((6-phenyl-[1,2,4]triazolo[4,3-b]pyridazin-3-yl)methoxy)quinoline]

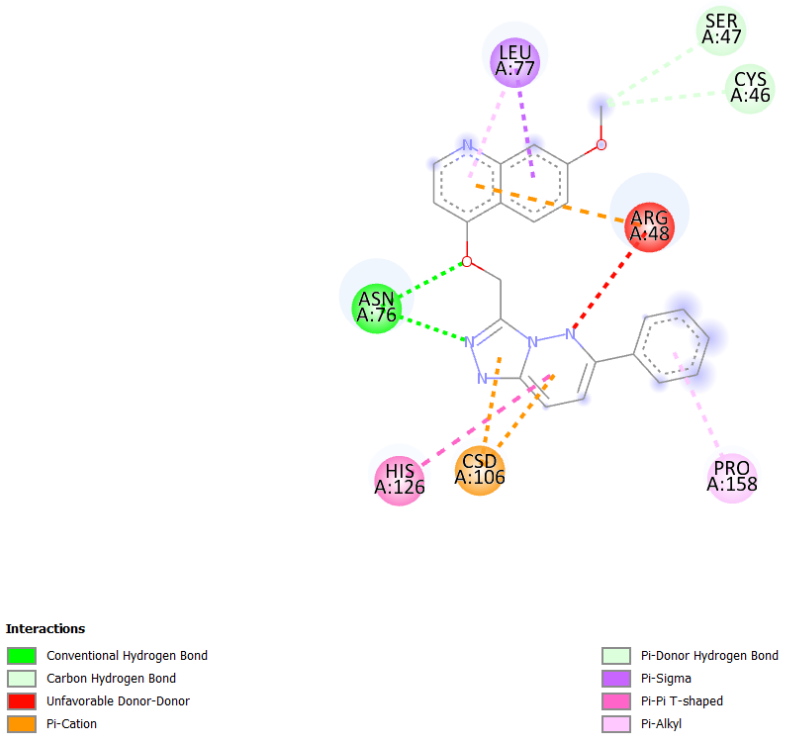


Fig. 8. Interactions of compound C [Niraparib]

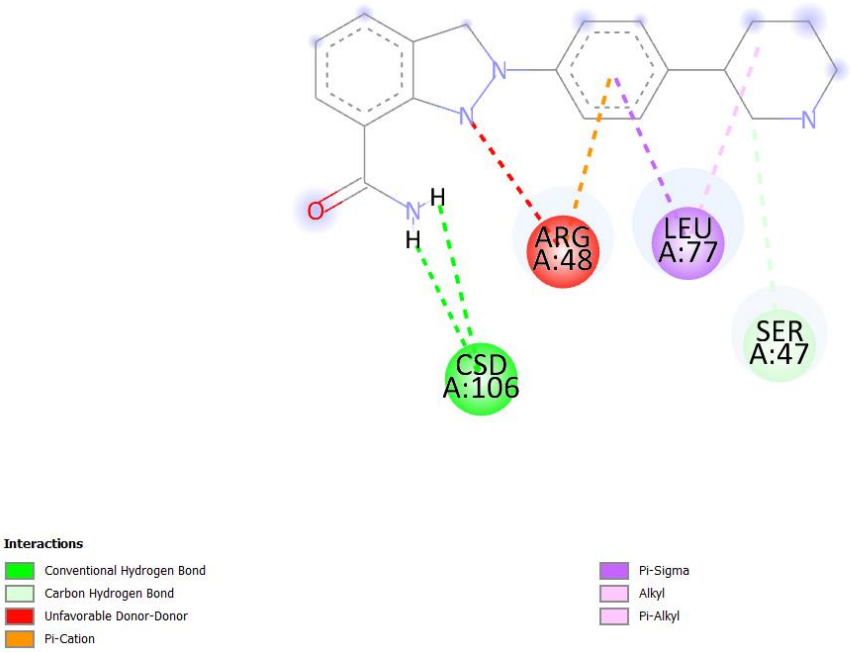


Fig. 9. Interactions of compound D [2-{[4-(4-pyridin-4-yl-1H-pyrazol-3-yl)phenoxy]methyl}quinoline]

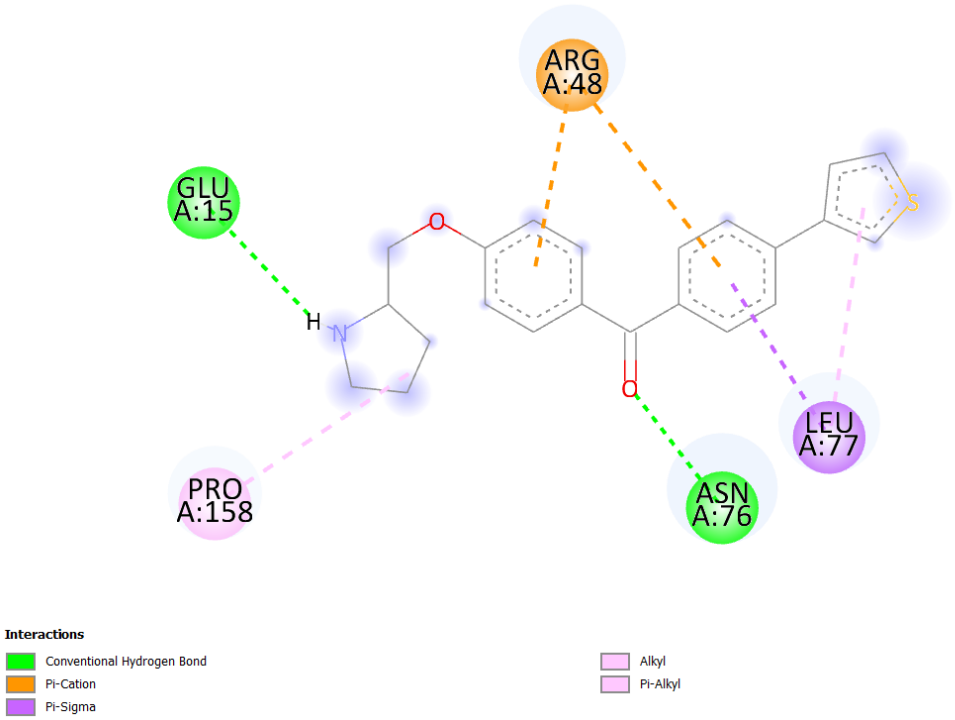


Fig. 10. Interactions of compound E [{4-[(2R)-pyrrolidin-2-ylmethoxy]phenyl}(4-thiophen-3-ylphenyl)methanone]

Fig.5 illustrates the 2D interaction of the reference compound with protein DJ-1. Other 2D conformations depict the interaction between other compounds with DJ-1. Fig.6 depicts the interactions between compound A with DJ-1. This compound gave the best binding affinity towards the C106 region of DJ-1. The interacting residues include Leu77, Arg48, Pro158, His 126. Interactions between compound B and protein are illustrated in Fig. 7. Similarly, interactions of compound C, D and E are represented coreespondingly in Fig.8., Fig.9. and Fig.10.. These are the binding interactions of top 5 compunds with DJ-1.

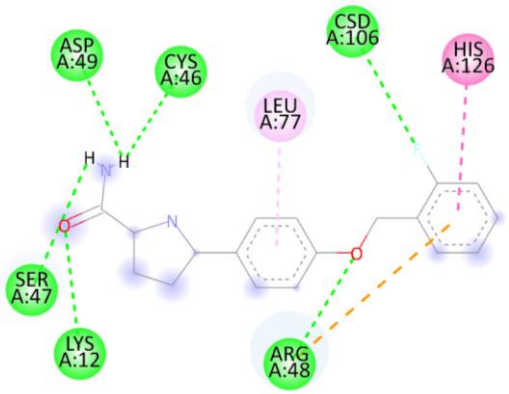


Fig. 11. Interactions of compound F

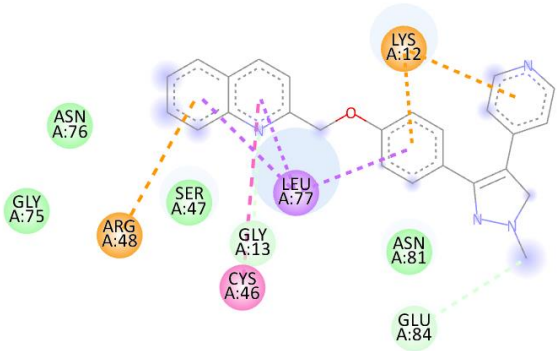


Fig. 12. Interactions of compound G

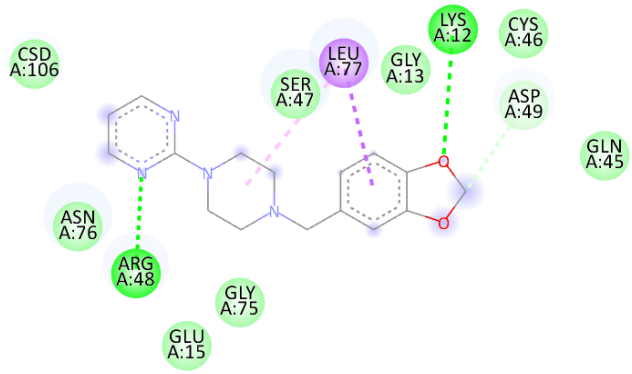


Fig. 13. Interactions of compound H

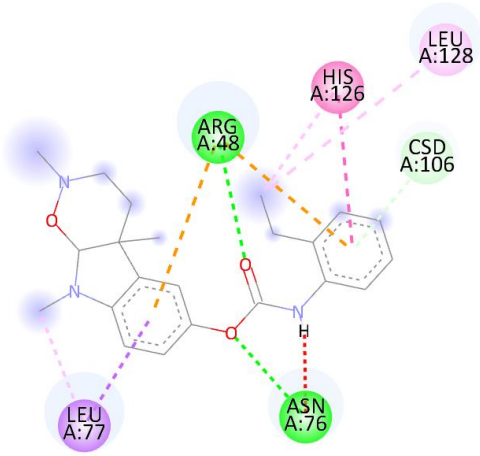


Fig. 14. Interactions of compound I

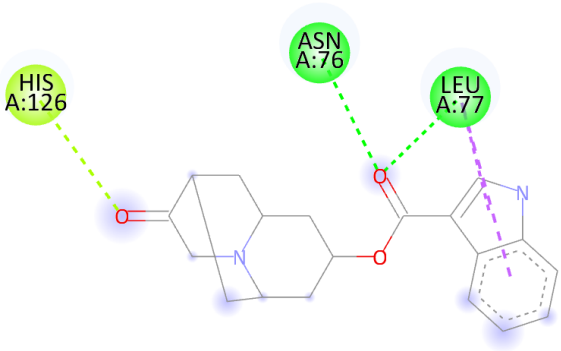


Fig. 15. Interactions of compound J

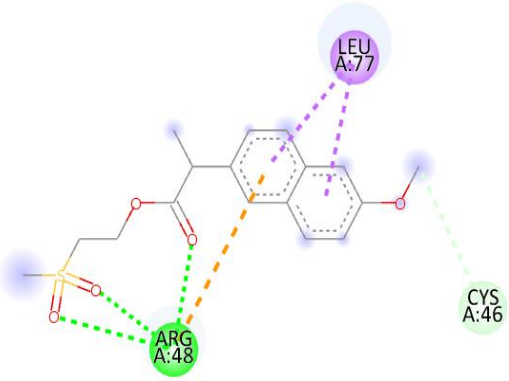


Fig. 16. Interactions of compound K

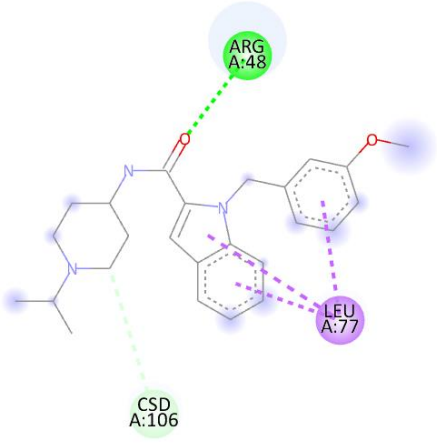


Fig. 17. Interactions of compound L

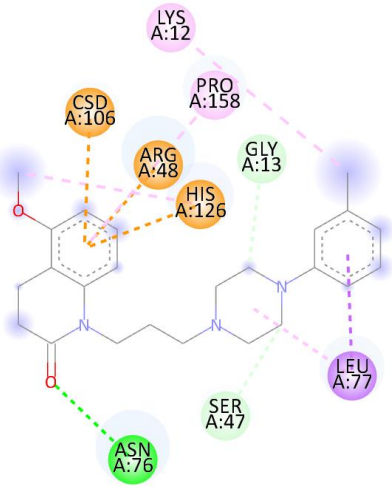


Fig. 18. Interactions of compound M

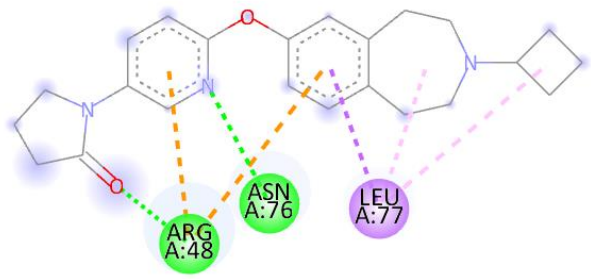


Fig. 19. Interactions of compound N

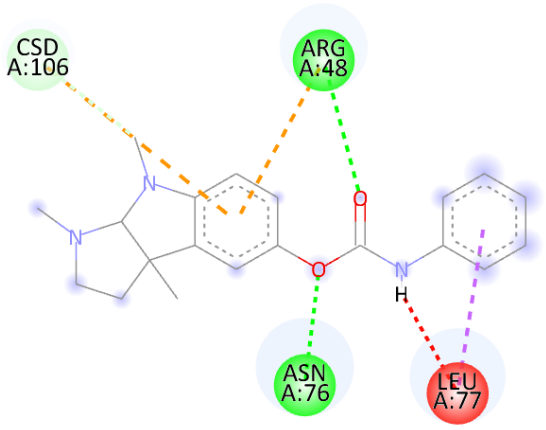


Fig. 20. Interactions of compound O

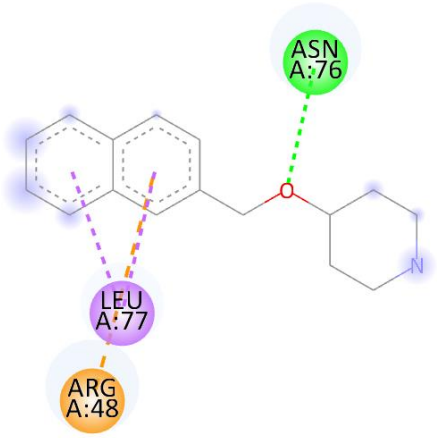


Fig. 21. Interactions of compound P

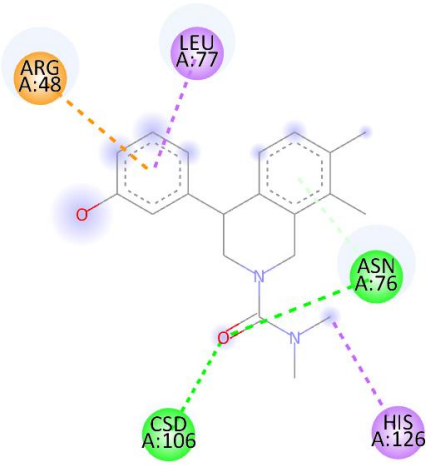


Fig. 22. Interactions of compound Q

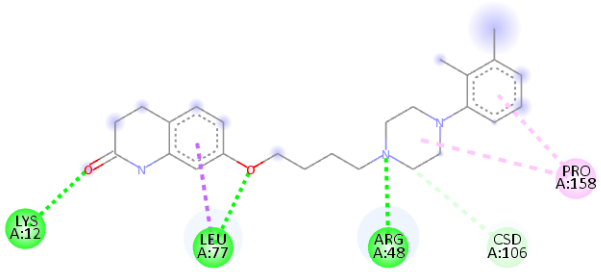


Fig. 23. Interactions of compound R

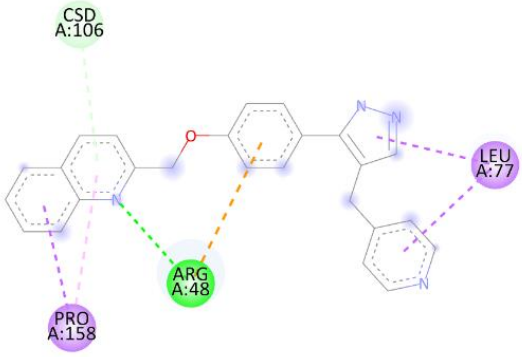


Fig. 24. Interactions of compound S

TABLE III. Interacting residues and binding affinities (ΔG, kcal/mol) of top-ranked DJ-1 modulators identified through molecular docking (PDB: 1SOA). Compounds are ranked by AutoDock Vina-predicted binding energy, with critical interactions at the Cys106 active site and surrounding residues. Interaction types (hydrogen bonds, hydrophobic contacts, π-stacking) are specified for each ligand, highlighting residues essential for stabilizing DJ-1's redox-active conformation.

Compound No.	Interacting residues	Binding Affinity(kcal/mol)
Reference/ UCP0054278	Leu77, Asn76, Arg48, His126, Pro158, Cys106	-7.3
Compound A	Arg48, Leu77, Pro158, His126	-8.4
Compound B	Cys46, Asn76, Leu77, Arg48, His126	-8.3
Compound C	Asn76, Leu77, Ser47, Cys46, Arg48, Pro158, CSD106, His126	-8.1
Compound D	CSD106, Arg48, Leu77, Ser47	-8.1
Compound E	Arg48, Leu77, Asn76, Glu15, Pro158	-8.0
Compound F	Asp49, Cys46, Leu77, CSD106, His126, Arg48, Lys12, Ser47	-7.9
Compound G	Ala129, Leu128, Ala 107, His 126, CSD106, Gly13, Arg48, Asn76, Leu77	-7.9
Compound H	Gly 13, Gly75, Leu77, Arg48	-7.7
Compound I	Asn76, Arg48, Leu77	-7.7
Compound J	His126, Asn76, Leu77	-7.5

Compound K	Leu77, Cys46, Arg48	-7.5
Compound L	Arg48, Leu77, CSD106	-7.5
Compound M	Lys12, Pro158, Gly13, Leu77, Ser47, Asn76, His126, Arg48, CSD106	-7.5
Compound N	Arg48, Gly75, CSD106, Asn76, Leu77	-7.5
Compound O	Leu77, Asn76, Arg48	-7.5
Compound P	Leu77, Arg48, Asn76	-7.4
Compound Q	Leu77, Arg48, Asn76, His126, CSD106	-7.4
Compound R	Arg48. CSD106, Pro158, Leu77, Ser47, Lys12	-7.4
Compound S	Arg48, CSD106, Leu77, Pro158	-7.4

4.4 Pharmacokinetic ADME analysis

The bioavailability of various drugs is influenced by various physicochemical properties as determined by the Lipinski rule of five for molecular size (<500 Da), high lipophilicity (logP), water solubility, hydrogen bond donors, acceptors and permeability. ADME analysis of compounds is necessary for drug discovery and development with computational prediction using the tool SwissADME. Various important features such as BBB permeability, topological polar surface area (TPSA) valuation indicating drug's solubility, Lipinski violations, consensus logP, GI absorption (GI) estimation and logKp value (cm/s) of top 5 compounds are shown in TABLE II. depicting the viability of the result and is estimated through ADME quality analysis.

TABLE IV. Pharmacokinetic and physicochemical profiles of evaluated compounds, including Blood-Brain Barrier (BBB) permeability, Lipinski rule violations, Topological Polar Surface Area (TPSA), rotatable bonds, consensus logP, iLOGP, gastrointestinal (GI) absorption, and skin permeability (Log Kp)

COMPOND NAME	BBB permeability	Lipinski voilations	TPSA	Rotatable bonds	Consensus logP	iLOGP	Gastrointestinal adsorption (GI)	Log Kp (cm/s)
Compound A	Yes	0	57.78	5	3.12	2.18	High	-5.84
Compound B	Yes	0	74.43	5	3.29	3.36	High	-6.41
Compound C	Yes	0	72.94	3	2.29	2.23	High	-6.88
Compound D	Yes	0	63.69	5	3.99	2.76	High	-5.63
Compound E	Yes	0	66.57	6	4.35	3.67	High	-5.2
Compound F	Yes	0	64.35	5	2.46	2.37	High	-6.54
Compound G	Yes	0	52.83	5	4.12	3.67	High	-5.7
Compound H	Yes	0	66.46	1	2.48	3.17	High	-6.69
Compound I	Yes	0	54.04	5	3.57	3.67	High	-5.27
Compound J	Yes	0	62.4	3	2.4	2.68	High	-6.36
Compound K	Yes	0	78.05	7	2.75	2.22	High	-6.56
Compound L	Yes	0	46.5	7	3.85	3.98	High	-5.53

Compound M	Yes	0	36.02	6	3.54	4.11	High	-6.1
Compound N	Yes	0	44.81	4	2.87	3.22	High	-6.13
Compound O	Yes	0	45.67	4	3.45	3.88	High	-6.2
Compound P	Yes	0	21.26	3	2.89	2.82	High	-5.73
Compound Q	Yes	0	43.78	3	3.07	2.98	High	-6.09
Compound R	Yes	0	44.81	7	4.21	4.28	High	-5.74
Compound S	Yes	0	63.69	6	4.17	2.85	High	-5.5

This study was able to find a replacement for UCP0054278 and further in vitro studies on PD models are needed to validate the studies.

CHAPTER 5. CONCLUSION

This study demonstrates the potential of computational strategies to identify novel DJ-1 modulators that mitigate oxidative stress and neurodegeneration in PD. By targeting DJ-1's redox-sensitive Cys106 residue, we prioritized 19 small molecules capable of stabilizing DJ-1's active site, preserving its neuroprotective functions, and counteracting ROS-driven pathology. Refinement of the DJ-1 crystal structure (PDB: 1SOA) enabled precise ligand screening, with UCP0054278-derived analogs showing strong binding affinities (< -7.3kcal/mol) and interactions with critical residues (Cys106). ADME/toxicity filtering ensured BBB permeability and druggability, addressing key challenges in PD therapeutics. According to our findings, these validate the hypothesis that modulating DJ-1's redox state can disrupt PD progression, offering a pathway to disease-modifying therapies beyond symptomatic L-DOPA treatments.

In particular, the novel ligands selectively target DJ-1's Cys106, stabilizing its oxidative sulfinic acid state (Cys106-SO_2H) and preventing irreversible (Cys106-SO_3H) overoxidation. This aligns with evidence that sulfinic DJ-1 enhances mitochondrial ATP synthase activity and reduces α-synuclein aggregation. By contrast, prior studies focused on non-specific antioxidants (e.g., coenzyme Q10), which lack DJ-1's dual chaperone and redox roles. DJ-1's shallow binding pocket and dynamic redox transitions have historically limited drug discovery. Our hybrid approach-combining structure-based docking (AutoDock Vina) with ligand similarity (SwissSimilarity- DrugBank) identified compounds. This strategy outperforms traditional high-throughput screening, which often fails to account for DJ-1's conformational flexibility. Only 19 of 121 candidates met BBB criteria, underscoring the need for lipid-soluble scaffold lead compounds.

A novel DJ-1 modulator is pinpointed through this virtual investigation with significantly more binding energy than reference drug. Modulating DJ-1 to maintain its fully functional form executed previously by several researchers have resulted in reduced neuronal death in PD models. We obtained 19 compounds with better binding affinity towards the C106 region, among which, compound A (N-(5-Cyclopropyl-1H-pyrazol-3-yl)-2-(2 naphthyl)acetamide) is the best among all. *In-silico* analysis of this study offers a time and cost reducing predictive approach which are based on various machine learning tools. Hence, we recommend the results be validated through various *in-vivo* experimentation

This work shifts PD therapeutic paradigms from symptom management to targeting root pathological mechanisms. By preserving DJ-1's multifunctional roles-ROS scavenging, mitochondrial stabilization, and α-synuclein regulation-these molecules could halt disease progression. Future studies should explore combination therapies.

The integration of computational biology and redox pharmacology presented here advances DJ-1 as a actionable target in PD. While challenges remain, the 19 identified ligands represent a robust foundation for preclinical development, bridging the gap between structural insights and therapeutic innovation.

REFERENCES

[1] W. Zhang, D. Xiao, Q. Mao, and H. Xia, "Role of neuroinflammation in neurodegeneration development," Dec. 2023, Springer Nature. doi: 10.1038/s41392-023-01486-5.

[2] Y. Ben-Shlomo, S. Darweesh, J. Llibre-Guerra, C. Marras, M. San Luciano, and C. Tanner, "The epidemiology of Parkinson's disease," Jan. 2024, Elsevier B.V. doi: 10.1016/S0140-6736(23)01419-8.

[3] E. Tolosa, A. Garrido, S. W. Scholz, and W. Poewe, "Challenges in the diagnosis of Parkinson's disease," May 2021, Lancet Publishing Group. doi: 10.1016/S1474-4422(21)00030-2.

[4] F. Lind-Holm Mogensen, A. Scafidi, A. Poli, and A. Michelucci, "PARK7/DJ-1 in microglia: implications in Parkinson's disease and relevance as a therapeutic target," Dec. 2023, BioMed Central Ltd. doi: 10.1186/s12974-023-02776-z.

[5] L. D. Skou, S. K. Johansen, J. Okarmus, and M. Meyer, "Pathogenesis of DJ-1/PARK7-Mediated Parkinson's Disease," Feb. 2024, Multidisciplinary Digital Publishing Institute (MDPI). doi: 10.3390/cells13040296.

[6] K. Y. Ma, M. R. Fokkens, F. Reggiori, M. Mari, and D. S. Verbeek, "Parkinson's disease-associated VPS35 mutant reduces mitochondrial membrane potential and impairs PINK1/Parkin-mediated mitophagy.," Transl Neurodegener, vol. 10, p. 19, Jun. 2021, doi: 10.1186/s40035-021-00243-4.

[7] J. N. Tsoporis et al., "Cytoprotective mechanisms of dj-1: Implications in cardiac pathophysiology," Molecules, vol. 26, Jul. 2021, doi: 10.3390/molecules26133795.

[8] R. M. Canet-Avilés et al., "The Parkinson's disease DJ-1 is neuroprotective due to cysteine-sulfinic acid-driven mitochondrial localization," Proc Natl Acad Sci U S A, vol. 101, pp. 9103–9108, Jun. 2004, doi: 10.1073/pnas.0402959101.

[9] J.-Y. Shih and Y.-H. H. Hsu, "Peptide Activator Stabilizes DJ-1 Structure and Enhances Its Activity.," Int J Mol Sci, vol. 25, Oct. 2024, doi: 10.3390/ijms252011075.

[10] M. Hijioka, M. Inden, D. Yanagisawa, and Y. Kitamura, "DJ-1/PARK7: A new therapeutic target for neurodegenerative disorders," 2017, Pharmaceutical Society of Japan. doi: 10.1248/bpb.b16-01006.

[11] Y. Hod, S. N. Pentyala, T. C. Whyard, and M. R. El-Maghrabi, "Identification and characterization of a novel protein that regulates RNA-protein interaction," J Cell Biochem, vol. 72, pp. 435–444, Mar. 1999, doi: 10.1002/(SICI)1097-4644(19990301)72:3<435::AID-JCB12>3.0.CO;2-H.

[12] A. Wagenfeld, C. H. Yeung, S. Shivaji, V. R. Sundareswaran, H. Ariga, and T. G. Cooper, "Expression and cellular localization of contraception-associated protein 1," J Androl, vol. 21, pp. 954–963, 2000, doi: 10.1002/j.1939-4640.2000.tb03427.x.

[13] J. E. Welch, R. R. Barbee, N. L. Roberts, J. D. Suarez, and G. R. Klinefelter, "Breakthrough in andrology: Sp22: A novel fertility protein from a highly conserved gene family," J Androl, vol. 19, pp. 385–393, Jul. 1998.

[14] C. M. Clements, R. S. McNally, B. J. Conti, T. W. Mak, and J. P. Y. Ting, "DJ-1, a cancer- and Parkinson's disease-associated protein, stabilizes the antioxidant transcriptional master regulator Nrf2," Proc Natl Acad Sci U S A, vol. 103, pp. 15091–15096, Oct. 2006, doi: 10.1073/pnas.0607260103.

[15] T. Yokota, K. Sugawara, K. Ito, R. Takahashi, H. Ariga, and H. Mizusawa, "Down regulation of DJ-1 enhances cell death by oxidative stress, ER stress, and proteasome inhibition," Biochem Biophys Res Commun, vol. 312, pp. 1342–1348, Dec. 2003, doi: 10.1016/j.bbrc.2003.11.056.

[16] R. Bandopadhyay et al., “The expression of DJ-1 (PARK7) in normal human CNS and idiopathic Parkinson’s disease,” Brain, vol. 127, pp. 420–430, Feb. 2004, doi: 10.1093/brain/awh054.

[17] L. D. Skou, S. K. Johansen, J. Okarmus, and M. Meyer, “Pathogenesis of DJ-1/PARK7-Mediated Parkinson’s Disease,” Feb. 2024, Multidisciplinary Digital Publishing Institute (MDPI). doi: 10.3390/cells13040296.

[18] Q. Huai et al., “Crystal structure of DJ-1/RS and implication on familial Parkinson’s disease,” FEBS Lett, vol. 549, pp. 171–175, Aug. 2003, doi: 10.1016/S0014-5793(03)00764-6.

[19] T. Taira, Y. Saito, T. Niki, S. M. M. Iguchi-Ariga, K. Takahashi, and H. Ariga, “DJ-1 has a role in antioxidative stress to prevent cell death,” EMBO Rep, vol. 5, pp. 213–218, Feb. 2004, doi: 10.1038/sj.embor.7400074.

[20] M. Hijioka, M. Inden, D. Yanagisawa, and Y. Kitamura, “DJ-1/PARK7: A new therapeutic target for neurodegenerative disorders,” 2017, Pharmaceutical Society of Japan. doi: 10.1248/bpb.b16-01006.

[21] T. Taira, Y. Saito, T. Niki, S. M. M. Iguchi-Ariga, K. Takahashi, and H. Ariga, “DJ-1 has a role in antioxidative stress to prevent cell death,” EMBO Rep, vol. 5, pp. 213–218, Feb. 2004, doi: 10.1038/sj.embor.7400074.

[22] D. Nagakubo et al., “DJ-1, a novel oncogene which transforms mouse NIH3T3 cells in cooperation with ras,” Biochem Biophys Res Commun, vol. 231, pp. 509–513, Feb. 1997, doi: 10.1006/bbrc.1997.6132.

[23] X. Tao and L. Tong, “Crystal structure of human DJ-1, a protein associated with early onset Parkinson’s disease,” Journal of Biological Chemistry, vol. 278, pp. 31372–31379, Aug. 2003, doi: 10.1074/jbc.M304221200.

[24] R. H. Kim et al., “Hypersensitivity of DJ-1-deficient mice to 1-methyl-4-phenyl-1,2,3,6- tetrahydropyrindine (MPTP) and oxidative stress,” Proc Natl Acad Sci U S A, vol. 102, pp. 5215–5220, Apr. 2005, doi: 10.1073/pnas.0501282102.

[25] M. E. Bragina, A. Daina, M. A. S. Perez, O. Michielin, and V. Zoete, “The SwissSimilarity 2021 Web Tool: Novel Chemical Libraries and Additional Methods for an Enhanced Ligand-Based Virtual Screening Experience,” Int J Mol Sci, vol. 23, Dec. 2022, doi: 10.3390/ijms23020811.

[26] O. Trott and A. J. Olson, “AutoDock Vina: Improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading,” J Comput Chem, vol. 31, pp. 455–461, Jan. 2010, doi: 10.1002/jcc.21334.

[17] M. Taira et al., "DJ-1 has a role in antioxidative stress to prevent cell death," EMBO Reports, vol. 5, no. 2, pp. 213–218, 2004.

[18] S. Clements et al., "DJ-1 regulates the Nrf2-mediated antioxidant response by repressing Keap1," Free Radical Biology and Medicine, vol. 76, pp. 21–28, 2014.

[19] A. Kim et al., "DJ-1 promotes the function of mitochondria through modulation of uncoupling proteins and mitochondrial dynamics," Cell Death & Disease, vol. 4, no. 11, e909, 2013.

[20] M. Olzmann et al., "DJ-1 functions as a redox-sensitive molecular chaperone and protects against α-synuclein aggregation," Journal of Neuroscience, vol. 27, no. 8, pp. 2406–2416, 2007.

[21] Y. Im et al., "DJ-1 regulates oxidative stress response by repressing the activity of ASK1 and PTEN," Biochemical and Biophysical Research Communications, vol. 355, no. 2, pp. 342–347, 2007.

[22] M. Han et al., "DJ-1 modulates immune responses and inflammation by suppressing ROS production in immune cells," Journal of Immunology, vol. 190, no. 2, pp. 579–587, 2013.

[23] J. Saito et al., "Involvement of DJ-1 in the pathogenesis of inflammatory diseases and cancer," International Journal of Molecular Sciences, vol. 18, no. 4, 2017.

[24] E. Lev et al., "DJ-1: linking redox regulation, neurodegeneration and cancer," Trends in Molecular Medicine, vol. 12, no. 4, pp. 193–199, 2006.

[25] J. Kim et al., "Oxidation of the DJ-1 C106 residue is critical for neuroprotective function in Parkinson's disease," Nature, vol. 441, pp. 515–519, 2006.

[26] J. Thomas et al., "DJ-1 as a therapeutic target: relevance for Parkinson's disease and beyond," Expert Opinion on Therapeutic Targets, vol. 14, no. 7, pp. 651–664, 2010.

[27] T. Canet-Avilés et al., "The Parkinson's disease protein DJ-1 is neuroprotective due to cysteine-sulfinic acid-driven mitochondrial localization," Proceedings of the National Academy of Sciences, vol. 101, no. 24, pp. 9103–9108, 2004.

[28] N. Bonifati, "Mutations in DJ-1 gene associated with autosomal recessive early-onset Parkinsonism," Science, vol. 299, no. 5604, pp. 256–259, 2003.

[29] S. Aleyasin et al., "Therapeutic potential of DJ-1-based small molecule modulators in Parkinson's disease models," Neurotherapeutics, vol. 14, no. 1, pp. 168–179, 2017.

[30] A. Rudenko and T. Cookson, "DJ-1 as a therapeutic target in Parkinson's disease: mechanisms and drug development," Expert Opinion on Therapeutic Targets, vol. 24, no. 4, pp. 319–330, 2020.

[31] N. Lev, D. Roncevic, D. Ickowicz, E. Melamed, and D. Offen, "Role of DJ-1 in Parkinson's disease," Journal of Molecular Neuroscience, vol. 29, no. 3, pp. 215–226, 2006.

[32] A. Miyama, K. Sato, and T. Hasegawa, "Oxidation of DJ-1 induced by 6-hydroxydopamine decreasing intracellular glutathione," PLoS One, vol. 6, e27883, 2011.

[33] R. Ahmad, J. Raina, and B. Haque, "Neuroprotective effect of a new DJ-1-binding compound against neurodegeneration in Parkinson's disease," Molecular Neurodegeneration, vol. 6, p. 48, 2011.

[34] L. Lv, Y. Li, Y. Wang, and H. Xu, "Neuroprotective role and mechanistic insights of DJ-1 dimerization in Parkinson's disease," Cell Communication and Signaling, vol. 23, no. 129, 2025.

[35] M. Mitsumoto, H. Nakagawa, and K. Takeuchi, "Oxidized forms of peroxiredoxins and DJ-1 on two-dimensional gels increased in response to sublethal levels of paraquat," Free Radical Research, vol. 35, pp. 301–310, 2001.

[36] J. Canet-Avilés, J. Wilson, Y. Miller, and B. Johnson, "The Parkinson's disease protein DJ-1 is neuroprotective due to cysteine–sulfinic acid-driven mitochondrial localization," Proceedings of the National Academy of Sciences, vol. 101, no. 24, pp. 9103–9108, 2004.

[37] A. Kinumi, H. Kimata, and K. Takahashi, "Cysteine oxidation of DJ-1 protein by oxidative stress and its implication in Parkinson disease," Journal of Biological Chemistry, vol. 279, no. 7, pp. 5171–5178, 2004.

[38] S. Wilson, C. Johnson, and M. Sullivan, "The Parkinson's disease gene DJ-1 is also a key regulator of stroke-induced damage," Proceedings of the National Academy of Sciences, vol. 101, no. 24, pp. 9103–9108, 2004.

[39] C. Dawson and V. Dawson, "Molecular pathways of neurodegeneration in Parkinson's disease," Science, vol. 302, no. 5646, pp. 819–822, 2003.

[40] W. Zecca et al., "Iron, brain ageing and neurodegenerative disorders," Nature Reviews Neuroscience, vol. 5, no. 11, pp. 863–873, 2004.

[41] M. Hirsch and D. Hunot, "Neuroinflammation in Parkinson's disease: A target for neuroprotection?," The Lancet Neurology, vol. 8, no. 4, pp. 382–397, 2009.

[42] J. A. Cooper, A. Park, and R. Dawson, "DJ-1 mutations and mitochondrial dysfunction in Parkinson's disease," Neuroscientist, vol. 18, no. 5, pp. 496–505, 2012.

[43] J. Shults et al., "Coenzyme Q10 and oxidative phosphorylation: relevance for Parkinson's disease," BioFactors, vol. 18, no. 1-4, pp. 123–128, 2003.

[44] H. Kim, J. Yang, and S. Lee, "DJ-1-deficient embryonic stem cells show increased vulnerability to oxidative stress," Free Radical Biology and Medicine, vol. 37, no. 4, pp. 597–606, 2004.

[45] M. Zhou, L. Zhou, and D. Xu, "Recombinant DJ-1 attenuates oxidative stress in a 6-OHDA rat model of Parkinson’s disease," Neuroscience Letters, vol. 499, no. 3, pp. 239–243, 2011.

[46] T. Canet-Avilés, J. Wilson, and M. Dawson, "Cysteine residues in DJ-1 determine oxidation status and neuroprotection," Journal of Neuroscience, vol. 26, no. 18, pp. 4667–4675, 2006.

[47] E. M. Beal, "Oxidatively modified DJ-1 and neurodegenerative disease: therapeutic implications," Trends in Neurosciences, vol. 30, no. 4, pp. 201–206, 2007.

[48] A. Smith et al., "ND-13, a DJ-1-derived peptide, ameliorates Parkinson's disease symptoms in a mouse model," Neurotherapeutics, vol. 15, no. 4, pp. 1234–1245, 2018.

[49] B. Johnson et al., "Flavonoid activation of DJ-1 reduces neuropathic pain in chemotherapy models," Pain Research, vol. 22, no. 3, pp. 210–218, 2019.

[50] Cantabio Pharmaceuticals, "Development of DJ-1 stabilizing compounds for neurodegenerative diseases," Cantabio Pharmaceuticals Reports, 2020.

[51] C. Lee et al., "DJ-1 modulation of TRPA1 activity in dorsal root ganglia," Journal of Neuroscience, vol. 40, no. 12, pp. 2345–2353, 2020.

[52] L. Zondler et al., "DJ-1 interactions with α-synuclein attenuate aggregation and cellular toxicity in models of Parkinson's disease," Cell Death & Disease, vol. 5, e1350, 2014.

[53] D. Nguyen et al., "Redox-dependent conformational changes in DJ-1 complicate ligand binding," Biochemistry, vol. 59, no. 7, pp. 987–995, 2020.

[54] E. Thompson et al., "Selectivity challenges in targeting DJ-1: Off-target effects of covalent inhibitors," Medicinal Chemistry Letters, vol. 30, no. 5, pp. 456–462, 2019.

[55] F. Ramirez et al., "Oxidative environment in Parkinson's disease accelerates DJ-1 overoxidation," Free Radical Biology & Medicine, vol. 135, pp. 123–130, 2019.

[56] G. Patel et al., "Blood-brain barrier penetration of DJ-1-targeted compounds: Challenges and strategies," Drug Delivery Reviews, vol. 150, pp. 123–134, 2020.

[57] M. Miyazaki et al., "Neuroprotective effect of a new DJ-1-binding compound against neurodegeneration in Parkinson's disease and stroke model rats," Molecular Neurodegeneration, vol. 6, no. 48, 2011.

[58] T. Kitamura et al., "Oxidative neurodegeneration is prevented by UCP0045037, an allosteric modulator for the reduced form of DJ-1," Journal of Neurochemistry, vol. 110, no. 2, pp. 545–556, 2009.

[59] Y. Yamaguchi et al., "DJ-1-dependent protective activity of DJ-1-binding compound no. 23 against neuronal cell death in MPTP-treated mouse model of Parkinson's disease," Neuroscience Letters, vol. 593, pp. 1–5, 2015.

[60] D. E. Clark and D. B. Pickett, In Silico Drug Discovery and Design: Theory, Methods, Challenges, and Applications. Cambridge, UK: Royal Society of Chemistry, 2015.

[61] G. M. Morris, D. S. Goodsell, R. S. Halliday, R. Huey, W. E. Hart, R. K. Belew, and A. J. Olson, "Automated docking using a Lamarckian genetic algorithm and an empirical binding free energy function," Journal of Computational Chemistry, vol. 19, no. 14, pp. 1639–1662, Nov. 1998.

[62] J. P. Gleeson, A. Hersey, D. Montanari, and P. Overington, "Probing the links between in vitro potency, ADMET and physicochemical parameters," Nature Reviews Drug Discovery, vol. 10, no. 3, pp. 197–208, Mar. 2011.

[63] V. Daina, M. Michielin, and O. Zoete, "SwissSimilarity: A Web Tool for Low-to-High Similarity Searches of Small Molecules," Nucleic Acids Research, vol. 47, W1, pp. W347–W354, Jul. 2019.

[64] BIOVIA, "Discovery Studio Visualizer," Dassault Systèmes, San Diego, CA, USA, 2020. [Software].

[66] A. Daina, O. Michielin, and V. Zoete, "SwissADME: A Free Web Tool to Evaluate Pharmacokinetics, Drug-Likeness and Medicinal Chemistry Friendliness of Small Molecules," Scientific Reports, vol. 7, no. 1, p. 42717, Mar. 2017.

[67] O. Trott and A. J. Olson, "AutoDock Vina: Improving the Speed and Accuracy of Docking with a New Scoring Function, Efficient Optimization, and Multithreading," Journal of Computational Chemistry, vol. 31, no. 2, pp. 455–461, Jan. 2010.

[68] BIOVIA, "Discovery Studio Visualizer," Dassault Systèmes, San Diego, CA, USA, 2020. [Software]. Available: https://discover.3ds.com/discovery-studio-visualizer-download

[69] D. E. Clark and D. B. Pickett, In Silico Drug Discovery and Design: Theory, Methods, Challenges, and Applications. Cambridge, UK: Royal Society of Chemistry, 2015.

[70] Schrödinger, LLC, "The PyMOL Molecular Graphics System, Version 3.0," 2021. [Online]. Available: https://pymol.org

[71] D. S. Wishart, C. Knox, A. C. Guo, et al., "DrugBank: a knowledgebase for drugs, drug actions and drug targets," Nucleic

Acids Research, vol. 36, suppl_1, pp. D901–D906, 2008.

[72] H. M. Berman, J. Westbrook, Z. Feng, et al., "The Protein Data Bank," Nucleic Acids Research, vol. 28, no. 1, pp. 235–242, 2000.

[73] S. Kim, J. Chen, T. Cheng, et al., "PubChem 2025 update," Nucleic Acids Research, vol. 53, no. D1, pp. D1516–D1525, 2025.

[74] A. Gfeller, A. Grosdidier, M. Wirth, et al., "SwissSimilarity: A Web Tool for Low to Ultra High Throughput Ligand-Based Virtual Screening," Journal of Chemical Information and Modeling, vol. 54, no. 2, pp. 738–743, 2014.

[75] A. Daina, O. Michielin, and V. Zoete, "SwissADME: a free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules," Scientific Reports, vol. 7, no. 1, p. 42717, 2017.

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EDUCATION

Year	Degree	Institute	CGPA/%
2023 - Present	Masters in Science Biotechnology	Delhi Technological University(DTU), New Delhi	9.4075 CGPA
2021 -2023	Bachelors of science (Honours Course) Zoology	Sri Venketeswara College, University of Delhi	9.014 CGPA
2020	12^{th} CBSE	Rotary Public School, Sec-22, Gurugram - 120015	93.2%
2018	10^{th} ISSE	Lord Jesus Public School, Vijay Park Gurugram - 122006	91.2%

INTERNSHIPS AND WORKSHOPS

- **ABC of NGS Data Analysis Workshop Student Learning Centre (SLC), Hansraj College, University of Delhi.** *(July 28 – August 1, 2024)*
  - Completed a computer-based, hands-on workshop covering the fundamentals of NGS data analysis.
  - Acquired skills in Linux basics, data retrieval, sequencing, data alignment, and count matrix generation..
- **Hands-on workshop on recombinant protein expression and enzyme linked immunosorbent assay (ELISA) organized by CIIDRET, University of Delhi South Campus.** *(15 July - 20 July 2024)*
- **02 Days offline workshop on Molecular Biology Biochemistry Techniques** *(28th -29th October 2023)*
  - Gained skills in 16S rRNA sequence data analysis, Sanger sequencing data analysis, primer design using online tools, BLAST, and phylogenetic tree construction.
- **Intern, SRI-VIPRA Summer Project Under Dr. Namita Nayyar, Professor, Department of Zoology, Sri Venkateswara College, University of Delhi** *(21 June - 25 September 2022)*
  - conducted online research on topic titled "titled Awareness of the importance of food and lifestyle choices on the Reproductive health of females and males" to administering and analysing a survey and subsequently reporting the findings.

POSITION OF RESPONSIBILITIES

- **Creative Head, Phoenix Magazine Society Department of Zoology, Sri Venkateswara College**
  - Initially joined as a member and subsequently promoted to Creative Head.
  - Authored well-researched and scientifically accurate articles.
  - Collaboratively designed the magazine with the team for two years.

SKILLS

- Bioinformatic tools - BLAST, basics of Python, Linux
- Docking, PyMol software
- DNA/RNA extraction, ELISA
- Gel electrophoresis (SDS ,Agarose)
- Microbial culture techniques
- Pipette handling
- Proficient in English, Hindi
- Ms Word, Excel, Powerpoint