"TARGETING PINK1 ACTIVATION IN PARKINSON'S DISEASE: UNVEILING THERAPEUTIC POTENTIALS THROUGH DRUG REPURPOSING & IN-SILICO ANALYSIS"

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CANDIDATE'S DECLARATION

I, **Preeti 23/MSCBIO/79** student of M.Sc. Biotechnology hereby declares that the work which is being presented as the Major Project in the Dissertation Project entitled **"Targeting PINK1 Activation In Parkinson's Disease: Unveiling Therapeutic Potentials Through Drug Repurposing & In-silico Analysis"** is submitted by me to the Department of Biotechnology, Delhi Technological University, Delhi in partial fulfillment of the requirements for the award of the Degree of Master of Science in Biotechnology, is an authentic record of my own work carried out during the period from January 2025 to June 2025 under the supervision of Prof. Pravir Kumar.

I have not submitted the matter presented in the report for the award of any other degree of this or any other Institute/University.

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This is to certify that **Preeti 23/MSCBIO/79** student of M.Sc. Biotechnology has submitted her research work presented in this Dissertation Project titled **"Targeting PINK1 Activation in Parkinson's Disease: Unveiling Therapeutic Potentials Through Drug Repurposing & In-silico Analysis"** for the award of Degree of Master of Science in Biotechnology and submitted to the Department of Biotechnology, Delhi Technological University, Delhi under my supervision. This thesis embodies results of original work, and studies are carried out by the student herself and the contents of the thesis do not form the basis for the award of any other degree to the candidate or to anybody else from this or any other University/Institution.

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TARGETING PINK1 ACTIVATION IN PARKINSON'S DISEASE: UNVEILING THERAPEUTIC POTENTIALS THROUGH DRUG REPURPOSING & IN-SILICO ANALYSIS

Preeti

ABSTRACT

Aim: The purpose of this study is to investigate the possibility of targeting PTEN-induced kinase 1 (PINK1), a mitochondrial serine/threonine kinase linked to the etiology of Parkinson's disease (PD). Through the initial stages of mitophagy, PINK1 plays a crucial part in mitochondrial quality control and neuronal degeneration which is linked to its malfunction. This investigation mainly focuses on assessing small molecule modulators that can interact with PINK1, given the pressing need for disease-modifying treatments in Parkinson's disease. The binding interactions of Niclosamide, a known FDA-approved drug, and N-[3-[acetyl(methyl)amino] phenyl]-2,4-difluoro-6-hydroxybenzamide (ZINC ID: Z1571042344), a novel compound found by virtual screening of the ZINC database, were evaluated using molecular docking techniques. To find important residues inside PINK1's functional area, active site prediction was carried out using the PrankWeb server. To determine drug-likeness profiles, pharmacokinetic characteristics, and binding affinity, docking simulations were utilized alongside with ADME studies.

Keywords - Parkinson's Disease, Neurodegenerative disorders, PTEN-induced kinase 1 (PINK1), niclosamide, Molecular Docking

Result: According to the docking studies, Niclosamide has a binding affinity of -8.1 kcal/mol for the predicted PINK1 active site, whereas the novel molecule has a higher binding affinity of -9.6 kcal/mol. Both substances showed higher binding affinity towards the PINK1 functional domain by interacting with important active site residues like Tyr198, Arg201, and Glu203. According to ADME profiling, the new molecule has better pharmacokinetic characteristics, such as full compliance to Lipinski's Rule of Five, strong gastrointestinal absorption and BBB permeability, a decreased molecular weight, and good drug-likeness. In comparison to Niclosamide, it also showed improved water solubility.

Conclusion: The discovery of ZINC1571042344 as a more powerful binder with advantageous drug-like characteristics raises the possibility that it could be used as a substitute lead molecule to target PINK1 in Parkinson's disease. Methods used in this investigation included ADME analysis validation and molecular docking. Thus, suggesting to a successful method for finding new therapeutic agents for Parkinson's disease and offering a positive roadmap for upcoming drug development and experimental validation attempts.

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LIST OF ABBREVIATIONS

PD	Parkinson's Disease
PINK1	PTEN-Induced Kinase-1
ADME	Absorption, Distribution, Metabolism, Excretion
BBB	Blood-Brain Barrier
GI	Gastrointestinal
PPI	Protein-Protein Interaction
ROS	Reactive Oxygen Species
SNCA	Synuclein Alpha
LRRK2	Leucine-Rich Repeat Kinase-2
PARK2	Parkin RBR E3 Ubiquitin Protein Ligase
UPS	Ubiquitin-Proteosome complex
LC3	Microtubule- Associated Protein 1 Light Chain 3
TOMM7	Translocase of Outer Mitochondrial Membrane 7
SOD2	Superoxide Dimutase 2
PDB	Protein Data Bank
SDF	Spatial Data File
CYP	Cytochrome P450
MMP	Mitochondrial Membrane Potential
DAT	Dopamine Transporter

CHAPTER 1

INTRODUCTION

Parkinson's disease (PD) is a progressive neurodegenerative disorder characterized primarily by the degeneration of dopaminergic neurons in the substantia nigra, leading to motor symptoms such as bradykinesia, rigidity, tremors, and postural instability [1]. Despite significant research efforts, existing therapies remain largely symptomatic and fail to address the underlying mechanisms driving disease progression [2]. A key component of the pathophysiology of Parkinson's disease is mitochondrial dysfunction, particularly with respect to impaired mitophagy pathways [3]. PTEN-induced kinase 1 (PINK1), a mitochondrial serine/threonine kinase, is crucial for detecting and removing damaged mitochondria via the PINK1/Parkin-mediated mitophagy pathway [4]. Loss of function which is commonly caused by mutations in PINK1, disrupts mitochondrial quality control, which result in the accumulation of damaged mitochondria, it increased levels of oxidative stress and neuronal degeneration [5]. Therefore, one intriguing therapeutic possibility for the disorder of Parkinson's control is PINK1. For finding novel therapeutic uses of existing medications, drug repurposing is an attractive approach to tackle neurodegenerative diseases considering their high rate of failure and longer development timelines required in de novo drug discovery [6]. The FDA-approved anthelmintic drug - niclosamide, has recently drawn attention because of its ability to change mitochondrial activity and cellular communication pathways [7]. Previous studies have demonstrated that niclosamide affects oxidative stress and mitophagy pathways, in addition to maintaining mitochondrial membrane potential [8]. Based on these characteristics, the binding affinity of Niclosamide for PINK1 was evaluated. However, a computational evaluation of niclosamide revealed a moderate binding affinity (−8.1 kcal/mol), poor water solubility, and limited gastrointestinal (GI) absorption, which limit its overall bioavailability [9]. A virtual screening approach was employed to identify compounds using ZINC database with improved binding efficiency and drug-like characteristics. This screening led to the discovery of N-[3-[acetyl(methyl)amino] phenyl]-2,4-difluoro-6-hydroxybenzamide (ZINC ID: Z1571042344). Subsequent molecular docking investigations revealed that this compound had a higher negative binding affinity for PINK1 (−9.6 kcal/mol) than Niclosamide [10]. After ADME analysis it is found that this new medication exhibited greater water solubility, displayed high GI absorption, and in full compliance with Lipinski's Rule of Five [11]. All of these given characteristics suggest that Z1571042344 may offer superior pharmacokinetic advantages over niclosamide for PINK1-targeting therapeutic uses in Parkinson's disease.

CHAPTER 2

REVIEW OF LITERATURE

2.1 Parkinson's Disease

Parkinson's disease (PD) is a progressive, long-term neurological condition that mostly impacts the motor system. It is acknowledged as the second most common neurological disorder in the world, after Alzheimer's disease. As people age, Parkinson's disease is becoming more prevalent [12]. By 2040, the prevalence of Parkinson's disease (PD), which affects about 1% of those over 60, is expected to increase [13]. Bradykinesia, muscle rigidity, postural instability, and resting tremor are among the many motor symptoms that are clinically suggestive of Parkinson's disease [14]. The loss of dopaminergic neurons in the substantia nigra pars compacta results in striatal dopamine depletion, which is the main cause of these motor deficits [15]. Mood disorders, sleep issues, autonomic dysfunction, and cognitive impairment are examples of non-motor symptoms that often overlap with motor symptoms and can even manifest years before they do, making early detection more challenging [16]. Parkinson's disease is characterized by the presence of Lewy bodies, which are intracytoplasmic inclusions primarily composed of aggregated α-synuclein protein [17]. Although the precise cause of Parkinson's disease is still unknown, genetic and environmental factors have been identified to play a role. A number of genes, including PARK2, PINK1, SNCA, LRRK2, and DJ-1, are linked to Parkinson's disease through mutation. While exposure to environmental pollutants, such as pesticides, has been associated with an increased risk in certain individuals [18,19]. Pathophysiologically, it is believed that oxidative stress, neuroinflammation, impaired protein degradation pathways, and mitochondrial dysfunction are the main causes of the progressive death of dopaminergic neurons [20]. The increasing understanding of the disruption of mitochondrial homeostasis as a critical early event in the etiology of Parkinson's disease [21] highlights the need of mitochondrial-targeted therapeutic methods. Although there are pharmaceutical treatments including levodopa, dopamine agonists, and monoamine oxidase B inhibitors, these mostly address symptoms rather than the underlying cause of the disease [22]. Therefore, it is essential to find disease-modifying therapies that can halt or reverse the neurodegenerative processes associated with Parkinson's disease.

2.2 Molecular & Genetic Pathogenesis of Parkinson's Disease

A complex disorder such as Parkinson's disease (PD) is result of various interactions of diverse pathogenic mechanisms. The loss of dopaminergic nerve cells in the pars-compacta of the substantia nigra is the disease's defining trait. However, the multiple

pathways that are involved in the pathogenesis of Parkinson's provides a way to understand the condition better but the underlying mechanisms causing neuronal degeneration are complex and still poorly understood [23]. One of the major contributing factors to PD pathogenesis is mitochondrial dysfunction. Mitochondria, essential for ATP production and cellular homeostasis, become impaired early in the disease process. Studies have demonstrated decreased activity of mitochondrial complex I in the substantia nigra of PD patients, leading to bioenergetic failure and increased production of reactive oxygen species (ROS) [24,25]. Overproduction of ROS causes oxidative stress, which damages DNA, lipids, and proteins and increases neural susceptibility [26]. PD has also been linked to abnormalities in the autophagy-lysosomal pathway and ubiquitin-proteasome system, which lead to the buildup of misfolded proteins including α-synuclein [27]. One degenerative feature of Parkinson's disease (PD) is the aggregation of α-synuclein into Lewy bodies, which is believed to disrupt mitochondrial integrity, synaptic function, and cellular trafficking [28]. Mutations in a number of genes, including SNCA (α-synuclein), LRRK2, PARK2 (Parkin), PINK1, and DJ-1, have been linked to both familial and sporadic Parkinson's disease [29]. Notably, mutations in the PINK1 gene interfere with mitophagy, which is the process by which damaged mitochondria are selectively degraded, hence impairing mitochondrial quality control systems [30]. Loss of PINK1 function allows the retention of dysfunctional mitochondria, elevated oxidative stress, and apoptotic death of cells, emphasizing the significance of mitochondrial homeostasis in neuronal survival. Neuroinflammation has emerged as another significant contributor to PD pathology. Pro-inflammatory cytokines are released by activated microglia, which increases oxidative stress and aids in the gradual degeneration of dopaminergic neurons [31]. The combination of multiple pathogenic processes—oxidative stress, inflammation, protein aggregation, decreased autophagy, and mitochondrial dysfunction—highlights the complexity of Parkinson's disease and the necessity of multi-targeted therapeutic strategies.

Table 1: summarizes the key molecular pathways disrupted in Parkinson's disease and highlights major proteins and genes implicated in neurodegeneration. These mechanisms form the pathophysiological basis for therapeutic targeting strategies.

Pathway	Normal Role	Effect in PD	Key Molecules Involved	References
Mitochondrial Function	Generates ATP and regulates ROS	Complex I inhibition ROS accumulation	PINK1, Parkin, Co I, DJ-1	[32]
Mitophagy	Removes damaged mitochondria	Impaired clearance toxic buildup	PINK1, Parkin, LC3 TOMM7	[33]

Oxidative Stress Response	Neutralizes free radicals	Excess ROS → DNA/protein/lipid damage	DJ-1, SOD2, GPx, NRF2	[34]
Ubiquitin-Proteasome System (UPS)	Degrades misfold proteins	Aggregation of a-synuclein	Parkin, UCHL1, SNCA	[35]
Autophagy-Lysosome Pathway	Cellular waste degradation	Blockage worsens protein aggregation	LAMP2A, Beclin-1, LC3	[36]
α-Synuclein Homeostasis	Synaptic vesicle trafficking	Aggregates into Lewy bodies	SNCA (a-synuclein), LRRK2	[37]
Neuroinflammatory Response	Clears debris via glial cells	Chronic inflammation, neuronal loss	TNF-a, IL-1ß, IL-6, microglia	[38]
Calcium Signalling	Maintains neurona excitability	Ca2+ dysregulation triggers apoptosis	L-type Ca2+ channels, calbindin	[39]
Dopaminergic Signalling	Motar control & mood	Loss of dopamine neurons	TH, DAT, D2 receptor	[40]

2.3 Role of PINK1 in Mitochondrial Quality Control

The dysfunction of mitochondrial quality control has attracted a lot of attention among the different pathogenic mechanisms linked to Parkinson's disease. By controlling mitophagy, PTEN-induced kinase 1 (PINK1), a serine/threonine-protein kinase found in mitochondria, is essential for maintaining mitochondrial integrity [41]. PINK1 gets carried to the healthy mitochondria under optimal physiological conditions, and there it is quickly broken down. However, upon mitochondrial damage or depolarization, the import mechanism is disrupted, leading to the accumulation of PINK1 on the outer mitochondrial membrane [42]. Accumulated PINK1 acts as a signalling molecule by recruiting and activating the E3 ubiquitin ligase Parkin from the cytosol [43]. PINK1 phosphorylates both Parkin and ubiquitin at specific serine residues, facilitating the ubiquitination of outer mitochondrial membrane proteins, thereby marking the damaged mitochondria for

degradation via the autophagy-lysosome pathway [44]. This vital quality control system is disrupted by mutations in the PINK1 gene, which leads to oxidative stress buildup, impaired clearance of damaged mitochondria, and eventual neuronal death [45]. Loss of PINK1 function has been shown in experimental models to result in mitochondrial fragmentation, compromised respiration, and heightened susceptibility to cellular stress [46]. The correlation between PINK1 deletion and increased susceptibility to environmental neurotoxins that inhibit mitochondrial complex I provides additional evidence of PINK1's role in mitochondrial defence [47]. Recent studies have shown that PINK1 functions within a broader mitochondrial regulatory network and interacts with numerous proteins that control mitophagy and neuroprotection. Protein–protein interaction (PPI) analysis methods such as STRING have revealed key PINK1 interactors, such as Parkin (PARK2), DJ-1 (PARK7), and mitofusin 2 (MFN2). These interactors collectively control mitochondrial dynamics and cellular stress responses. Understanding this network can help us better understand how PINK1 functions in Parkinson's disease and identify multiple potential targets of intervention. Because of its critical function in maintaining mitochondrial health, PINK1 has emerged as a potential therapeutic target in PD research. Pharmacological activation or stabilization of PINK1-mediated pathways is one potential strategy to counteract mitochondrial malfunction and prevent dopaminergic cell loss. Finding small chemical modulators that can improve PINK1 activity or mimic its impact on mitochondrial quality control systems has been the focus of recent studies [48]. Identifying the role of PINK1 in mitochondrial homeostasis has become critical for studying the molecular pathophysiology of Parkinson's disease and for generating targeted treatment options.

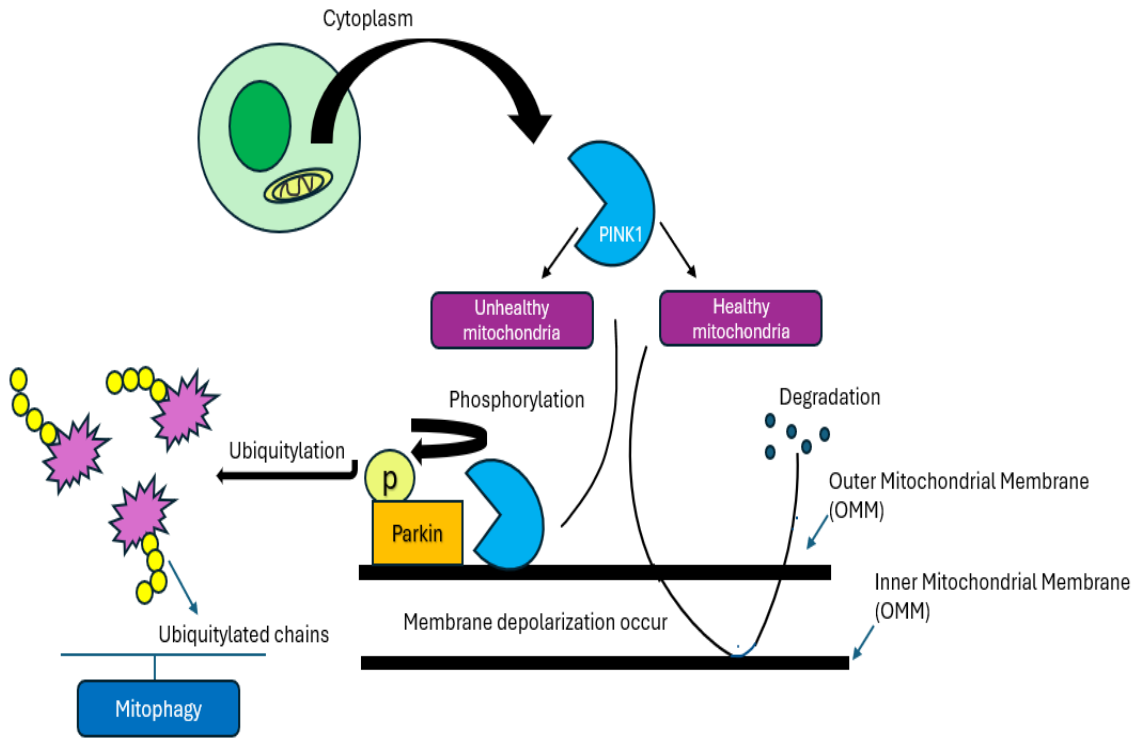

Fig.1. illustrates PINK1-signalling in both healthy and damaged mitochondria. PINK1 is attracted to and degraded by the outer mitochondrial membrane (OMM) when healthy mitochondria are at rest. However, when mitochondria get damage, PINK1 gets stabilized on OMM. There, it phosphorylates ubiquitin at Ser65 and parkin at Ser65, causing damaged mitochondria to be recycled and initiating mitophagy.

2.4 Therapeutic Targeting of Mitochondrial Dysfunction

Since mitochondrial dysfunction has a crucial part in etiology of Parkinson's disease, it has become a desirable target for treatment. By maintaining the sustainability of neurons and their function, mitochondrial homeostasis restoration may be able to stop or reduce the neurodegenerative process [49]. Numerous tactics have been investigated to preserve the integrity and functionality of the mitochondria. The capacity of antioxidants such vitamin E, creatine, and coenzyme Q10 to mitigate the effects of reactive oxygen species (ROS) and lessen oxidative damage has been assessed [50]. The limited effectiveness of therapeutic trials, however, emphasizes the complexity of mitochondrial dysfunction in Parkinson's disease [51]. The creation of new mitochondria, or mitochondrial biogenesis, has also become a potential target for treatment. Researchers have looked into the ability of substances like resveratrol and pioglitazone to activate PGC-1α, a master regulator of mitochondrial biogenesis, which is a peroxisome proliferator-activated receptor [52]. Another interesting approach is to improve mitophagy, which is the process by which damaged mitochondria are selectively cleared. Pharmacological attempts have

concentrated on altering the PINK1/Parkin pathway to improve mitochondrial quality control because of its crucial role in initiating mitophagy [53]. Small molecules that can stimulate Parkin recruitment or stabilize PINK1 on the mitochondrial membrane may be able to reduce neurodegeneration and restore mitophagic flow [54]. Despite these advancements, Parkinson's disease still lacks a specific medication that targets mitochondrial dysfunction. Given the complexity of mitochondrial biology and the multifactorial nature of Parkinson's disease, a complete approach involving antioxidant therapy, biogenesis activation, mitophagy augmentation, and mitochondrial dynamics modification is necessary. Targeting key regulators of mitochondrial quality control, such PINK1, is a targeted strategy with significant therapeutic potential. Advances in molecular docking and virtual screening technologies create new opportunities to identify small molecules that can change these pathways, leading to the development of treatments that change disease.

2.5 Small Molecule Modulators of PINK1

Because PINK1 lays down great significance for maintaining mitochondrial integrity and assisting in the removal of mitochondria with damage, therapeutic methods that walk down targeting of PINK1 activity have garnered heightened interest. The usage of small molecule modulators which leads to an increase in PINK1 activity is an interesting technique for combating against mitochondrial dysfunction, which is associated with Parkinson's disease [55]. The capacity of a variety of small molecules to activate or stabilize PINK1 signalling pathways has been a subject of investigation. For instance, it has been shown that kinetin and its derivatives stand upon the factor of an improved mitochondrial quality control by enhancing PINK1-mediated phosphorylation processes [56]. A modified nucleotide known as kinetin triphosphate (KTP) can directly activate PINK1 without attempting to depolarize the mitochondrial membrane, attracting Parkin and triggering mitophagy [57]. In a similar line, the FDA-approved anthelmintic drug Niclosamide has showed potential as a great mitochondrial function modulator. Although Niclosamide was originally developed to treat parasitic infectious conditions, investigations have demonstrated that it also maintains mitochondrial membrane potential and leads to activation of PINK1-Parkin-mediated mitophagy [58]. These findings consequently suggest that current drug compounds may be changed, which can modify mitochondrial dynamics and hence avoid dopaminergic neurodegeneration. Despite these promising results, discovering potent and selective small molecule PINK1 activators still remains difficult. The structural properties of PINK1 and mitochondrial integral complexity have impeded the identification of direct modulators [59]. Consequently, virtual screening and computational drug discovery methods have been applied increasingly to sort through vast chemical libraries and hence identifying new molecules which have a candidature of having strong binding affinity for PINK1. Developments in ADME profiling systems, active site prediction tools, and approaches through molecular docking have facilitated the rapid evaluation of potential modulators. Screening against curated chemical databases such as ZINC [60] is one of the potential strategies for discovering lead compounds, which hold the capacity of targeting PINK1 and restoring

mitochondrial homeostasis. The discovery of new small compounds using in silico methods is a significant and crucial step in developing disease-modifying therapeutics for Parkinson's disease.

Table 2: Represent small molecule modulators known to influence PINK1-mediated mitophagy.

Compound Name	Mechanism/Effect	Therapeutic Relevance	Reference
Kinetin	Enhances PINK1 stability and autophosphorylation	Restores mitophagy in PINK1-deficient cells	Hertz et al., 2013
Kinetin Triphosphate (KTP)	Activates PINK1 independent of mitochondrial depolarization	Promotes Parkin recruitment and mitophagy	Hertz et al., 2013
Niclosamide	Stabilizes mitochondrial membrane potential, indirectly activates PINK1	Enhances mitophagy and inhibits neuroinflammation	Fonseca et al., 2012
Celastrol	Induces mitochondrial stress and activates PINK1-Parkin pathway	Neuroprotective in PD models	Wang et al., 2015
Urolithin	Improves mitophagy via PINK1-Parkin axis	Enhances mitochondrial turnover in aging muscle and neurons	Ryu et al., 2016
PINK1 Activating Peptide	Direct mimetics of PINK1 phosphorylation sequence	Under investigation in experimental systems	Youle & van de 2012

Several small molecules have been reported to enhance PINK1 stability or mitophagy (Table 2.1). These agents represent a starting point for designing or identifying novel modulators using in silico approaches.

2.6 Niclosamide as a Potential Neuroprotective Agent

Niclosamide is a very well-known medication that has an anthelmintic nature that was initially licensed by the Food Development Authority for the treatment of infections associated with tapeworm. In recent years, however, this molecule has received increasing interest as a result of its biological effects showing pleiotropy, in addition to its antiparasitic activity [61]. Niclosamide's capacity to affect crucial signalling pathways, such as Wnt/β-catenin, mTORC1, NF-κB, and STAT3, has led to its potential repurposing in cancerous conditions, metabolic disorders, and neurological illnesses [62]. Importantly, niclosamide has been shown to aid in preservation of mitochondrial homeostasis. Investigations on preclinical basis have demonstrated that niclosamide may cause depolarization of mitochondrial membranes under regulation, activating the PINK1-Parkin pathway and promoting mitophagy [63]. Niclosamide may protect neurons against damage due to oxidation and mitochondrial stress, two main pathogenic characteristics of Parkinson's disease, by facilitating the clearance of mitochondria showing defects. Niclosamide has been shown to reduce neuroinflammation by inhibiting the NF-κB signalling cascade, which consequently contributes to the progress of the Parkinson's disease [64]. Niclosamide is seen as a promising option for neuroprotective treatment because of it lays down its foundation under multifunctional pharmacological profile, which targets both neuroinflammatory pathways and mitochondrial dysfunction. Despite these promising outcomes, niclosamide shows a number of disadvantages in relevance to its pharmacokinetic profile. Under oral administration, the compound has low systemic bioavailability due to weak gastrointestinal absorption and water solubility [65]. Furthermore, ability of Niclosamide to reach neural targets in acceptable quantities may be limited by its variable blood-brain barrier permeance and a very short half-life [66]. These disadvantages henceforth, lay down the foundation stone leading to importance of discovering or developing other compounds which show superior pharmacokinetic characteristics while retaining the beneficial mitochondrial-targeting properties of niclosamide. In this context, molecular docking and virtual screening offer approaches under calculated conditions which identifies replacement molecules with higher binding affinities and drug-like features suitable for Parkinson's disease therapeutic development.

2.7 Virtual Screening and Molecular Docking in Drug Discovery

Approaches based on traditionality which help in discovering new medications is often cumbersome, resource-intensive, and riddled with failure. Whereas, Computational approaches based on molecular docking and virtual screening have significantly evolved as critical components of drug development pipelines in the current modern era in response to these challenges [67]. Virtual screening is the practice of examining large chemical libraries with a rapid effect, to identify potential ligands that are most likely to bind to a particular biological target. This computational strategy hence speeds up the discovery of lead compounds, significantly, while eliminating the need for extensive experimental screening [68]. Both, the approach based on ligand and the structure-based

virtual screening approach, are often used, the latter predicts favourable binding interactions on the basis of analysis of target protein's three-dimensional structure. Molecular docking is a significant and important tool for structure-based virtual screening. Its goal is to assess the complex's binding affinity and predict the preferred orientation of a small molecule when attached to a protein target [69]. Algorithms predicated on docking insert the ligand within the target protein's active site and evaluate binding modes by using scoring techniques that consider features such as hydrogen bonding, hydrophobic interactions, electrostatic complementarity, and steric fit [70]. The determination of the specific binding pocket or active site on the target protein is critical to the accuracy of docking investigations. Active site prediction systems, such as PrankWeb, SiteMap, and CASTp, were developed to assist researchers in identifying potential ligand-binding sites based on structural features and pocket shape [71]. These strategies function by focussing the docking process to physiologically significant locations, hence improving the effectiveness and dependability of virtual screening campaigns. Docking and virtual screening are now much predictive and employed on a high frequency in view of the fact that algorithms' complexity and processing power have increased. Several potential therapeutic candidates have been identified or optimized using these approaches, therefore, highlighting their importance in early-stage drug development [72]. Structure-based virtual screening against critical targets such as PINK1 is a strategy of great potential for discovering novel small molecules capable of altering mitochondrial quality control pathways in Parkinson's disease. Active site prediction, molecular docking, and in silico ADME profiling, work together to provide effective chemical selection for subsequent experimental validation.

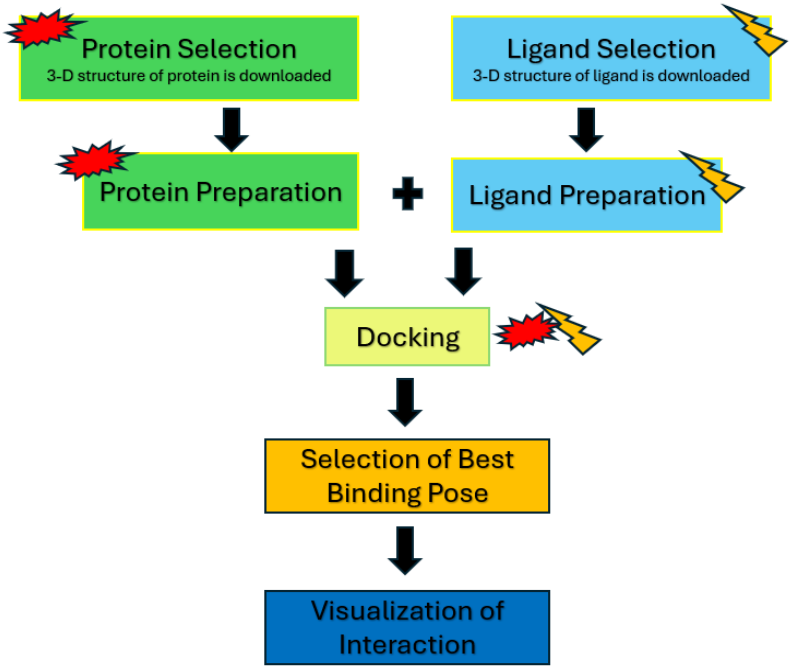

Fig.2. Flowchart showing Overall steps involving molecular docking

2.8 Application of ZINC Database in Drug Discovery

Access to large and diverse chemical libraries is typically required for the synthesis of novel bioactive molecules. The ZINC database ("ZINC Is Not Commercial") is a popular tool for virtual screening in drug development programs [73]. It provides a free, carefully curated range of commercially available chemicals designed specifically for use in in silico screening. ZINC includes millions of chemicals in ready-to-dock 3D formats, allowing researchers to rapidly screen biological targets utilizing structure-based virtual screening. Each molecule is tailored for docking by ensuring correct protonation states, tautomers, and 3- Dimensional conformations during physiological conditions [74]. The database is an effective tool for lead finding and optimization, since it is regularly updated to reflect the availability of compounds from various producers. ZINC's extensive filtration capabilities are one of its central advantages. Researchers can customize compound subsets based on molecular weight, lipophilicity (LogP), the number of rotatable bonds, and sticking to drug-likeness guidelines such as Lipinski's Rule of Five [75]. Drug development is quickened by the capacity to choose compounds with favourable pharmacokinetic characteristics prior to docking. Virtual screening of compounds based on structural properties in case of ZINC compounds has been beneficial in the context of neurodegenerative disorders such as Parkinson's disease in identifying molecules which have the candidature of targeting mitochondrial pathways, oxidative stress regulators, and neuroinflammatory mediators [76]. Scientists may successfully rank pharmaceuticals from the ZINC database for further biological investigation by combining molecular docking, ADME/Tox profiling, and active site prediction approaches. For identification of alternatively small compounds which have a higher binding affinity for PINK1, the current study employed a virtual screening strategy using the ZINC database. Molecular docking studies and pharmacokinetic studies led to the discovery of a new chemical, N-[3-[acetyl(methyl) amino] phenyl]-2,4-difluoro-6-hydroxybenzamide (ZINC ID: Z1571042344). It had higher docking scores and more drug-like properties than the reference chemical, niclosamide. Working upon the ZINC database exemplifies how resources based on computational technologies might accelerate early-stage drug research and expand the number of candidate molecules for challenging therapeutic targets such as PINK1.

2.9 ADME Profiling in Drug Development

In order to ensure the success of lead optimization and lower late-stage clinical failures, early assessment of the pharmacokinetic characteristics of candidate molecules—abbreviated ADME (Absorption, Distribution, Metabolism, and Excretion)—is essential in contemporary drug discovery [77]. A molecule's capacity to pass through biological membranes and enter the systemic circulation after oral administration is determined by its absorption profile. Gastrointestinal absorption and bioavailability are influenced by variables such molecular weight, hydrogen bonding ability, lipophilicity (LogP), and polar surface area [78For neurodegenerative therapies that target the tissues of the central

nervous system (CNS), distribution characteristics, such as the capacity to pass through the blood-brain barrier (BBB), are especially crucial. A molecule's chemical stability and susceptibility to biotransformation by hepatic enzymes, including cytochrome P450 isoforms, are influenced by metabolism. Rapid clearance, the production of hazardous metabolites, or drug-drug interactions might result from unfavourable metabolic profiles [79]. Excretion controls the drug's removal from the body and influences toxicity profiles and dosage schedules, primarily through the renal or biliary systems. SwissADME and other in silico ADME prediction technologies are now essential for early-stage drug development. SwissADME offers a quick and thorough assessment of bioavailability scores, drug-likeness according to Lipinski's Rule of Five, and pharmacokinetic characteristics [80]. Utilizing such platforms allows researchers to prioritize compounds with favorable ADME profiles even before experimental validation, thereby increasing the efficiency and success rate of drug discovery projects. In the present study, candidate molecules from the ZINC Lead-like subset were filtered through SwissADME to select compounds exhibiting optimal physicochemical properties conducive to drug development. The parameters considered included molecular weight between 250–500 Da, LogP values within acceptable ranges, minimal hydrogen bond donors and acceptors, and compliance with Lipinski’s criteria. This strategy ensured that the selected compounds, including the novel molecule identified, possessed not only strong binding affinity towards PINK1 but also favorable pharmacokinetic characteristics suitable for potential therapeutic application in Parkinson’s disease.

CHAPTER-3

METHODLOGY

3.1 Selection of Protein PINK1 & ligand molecules

Information on the target protein and reference molecule is obtained by reviewing the literature. Initially, the literature was collected webserver like PubMed (https://pubmed.ncbi.nlm.nih.gov/) & Google Scholar (https://scholar.google.com/). The mitochondrial serine/threonine-protein kinase PINK1 was selected as the therapeutic target based on its central role in mitophagy and mitochondrial quality control. Swisssimilarity search (http://www.swisssimilarity.ch/) is used to collect all the compounds that are similar to niclosamide from the ZINC database (https://zinc.docking.org/), specifically from the ZINC lead like subset. The ZINC database is often utilized to screen the compounds. The database yielded a list of 328 chemicals, which were then further filtered using several criteria like medication bioavailability, BBB permeability, and the Lipinski rule of five. After applying the filter, we obtained 63 molecules. There were notable structural similarities between these compounds and niclosamide.

3.2 Retrieval of target protein and ligand

The three-dimensional crystal structure of human PINK1 retrieved from the Protein Data Bank (PDB ID: 8UYF) in .pdb format. The structure was visualized and validated for completeness using BIOVIA Discovery Studio Visualizer.
The 3D structures of the ligands are downloaded from PubChem in the SDF format (https://pubchem.ncbi.nlm.nih.gov/).

3.3 Protein & Ligand preparations

Protein is prepared using AutoDock4, which removes water and adds the required Kollman charges. Only hydrogens are then added to the polar. The .pdbqt format is used to store the protein structure.
All ligand molecules in .sdf format and were transformed into.pdb format using Open Babel in order to perform docking. AutoDock4 adds polar hydrogen, computes Gasteiger charges, combines non-polar hydrogen, and imports the ligands in a.pdb file. There was no change made to the default torions option. The files are saved in the .pdbqt format. The receptor and ligand grid map has been created with the following dimensions: x = 70; y = 62; z = 70, and the center x = 82.219; y = 103.925; z = 110.357.

3.4 Active Site Prediction

It is crucial to identify protein's active site for understanding molecular interactions, as the active site plays a key role in the protein's function. Accurate prediction of these binding sites allows for more efficient drug design by guiding the selection of potential ligands that can interact with the protein's active site. There are several computational techniques and tools available to predict these binding sites.

The PrankWeb server (version 3.0) was used to predict the probable active site residues of the PINK1 protein. The PDB structure was uploaded, and the top-ranked binding pocket based on ligandability score was selected for molecular docking. Predicted residues were noted and matched with the ligand binding residues post-docking to validate specific interactions.

3.5 Molecular Docking

Molecular docking is a computer technique that predicts how well ligands will attach to receptor proteins. Docking is done with AutoDock Vina. It is a molecular docking application that is openly accessible. Compared to other docking systems, it was thought to be more efficient. It computes the grid map quickly and provides docking results. Docking is done on command prompt, and a Config.txt file is generated using the grid.txt file. An out.pdbqt file containing the interactions and a log.txt file containing the docking scores are produced. When the docking process is complete, a list of the best binding molecules is generated based on the binding affinity, and the best molecule is recommended for further study. The reference molecule, niclosamide, has a binding energy of 8.1 kcal/mol.

3.6 Protein-ligand Interactions analysis

After the docking process was completed, all of the target-ligand interaction structures were captured in the out.pdbqt file and converted to PDB format. To evaluate every encounter, BIOVIA Discovery Studio (version v25.1.0.24284) was utilized.

3.7 Pharmacokinetic and Toxicity Prediction (ADME/ProTox)

SwissADME (http://www.swissadme.ch/), an open-access online software, was used for analyzing pharmacokinetic properties of compounds. ADME analysis is used for each of the 63 drug compounds that were selected from 328 drug candidate. The main criteria used in the evaluation was water solubility, lipophilicity, high GI absorption, blood-brain barrier permeability, violations of Lipinski's Rule, and bioavailability.

Toxicity profiling was conducted using ProTox 3.0, focusing on hepatotoxicity, carcinogenicity, and LD_{50} class prediction to assess safety and tolerability.

3.8 Network Analysis of Protein–Protein Interactions (PPI)

To grasp the broader biological context of PINK1, STRING database (v12.0) was used to generate a protein–protein interaction network. The query was set as "PINK1" (Homo sapiens) with a high-confidence score threshold (≥0.7). A maximum of 10 interactors was selected to visualize the core network. Evidence sources included experimental data, curated databases, and co-expression. The network was analyzed to identify PINK1's interaction partners relevant to mitophagy, oxidative stress, and neuronal survival

Table 3: List of common tools used in Virtual Screening and Molecular Docking in Drug Discovery

Category	Tools/Database	Function	Link
Compound Libraries	ZINC	Database of purchasable compounds for virtual screening	https://zinc.docking.org
	DrugBank	Drug information & repurposing database	https://go.drugbank.com
	PubChem	Public database of chemical molecules	https://pubchem.ncbi.nlm.nih.gov
Ligand Preparation	Open Babel	Format conversion and molecule optimization	https://openbabel.org
Protein Preparation	Pymol	Visualization and ligand manipulation	https://pymol.org
	AutoDock4	Flexible docking of ligands into target proteins	https://autodock.scripps.edu/download-autodock4/
Active site prediction	Ft server	Predicts active/binding sites on proteins	https://ftsite.bu.edu/cite
	PrankWeb	Predicts active/binding sites on proteins	https://prankweb.cz
Molecular Docking	AutoDock	Flexible docking of ligands into target proteins	https://autodock.scripps.edu/download-autodock4/

	AutoDock Vina	Docking of ligands into target proteins	https://vina.scripps.edu/downloads/
Visualization & analysis	Discovery Studio visualizer	Ligand-protein interaction visualization	https://discover.3ds.com/discovery-studio-visualizer-download
	Chimera	Protein/ligand complex rendering and RMSD	https://www.cgl.ucsf.edu/chimera
Pharmacokinetics & Toxicology Prediction	SwissADME	Predicts drug-likeness, GI absorption, BBB, etc.	http://www.swissadme.ch
	pkCSM	In silico toxicity profiling	http://biosig.unimelb.edu.au/pkcsm
	Pro Tox	Predicts ADME/Tox using graph-based signatures	https://tox.charite.de/protox3/
Protein Network & pathway Mapping	STRING	Protein-protein interaction network visualization	https://string-db.org/

CHAPTER - 4
RESULT

4.1 Molecular docking Result

When the 63 compounds evaluated were docked against the PINK1 protein, the molecular docking study showed a significant range in binding affinities. The reference chemical, niclosamide, exhibited a binding energy of -8.1 kcal/mol. According to the findings, some BBB-permeable medications interact with the active area of the target protein very little, while others interact with it somewhat. Stronger expected affinities were shown by a number of analogues and structurally related molecules, with binding energies as low as –9.6 kcal/mol. These variations in docking scores imply that the chosen candidates have better ligand-receptor interactions than the reference medication.

The structural analogues of Niclosamide that were examined were chosen for their drug-likeness and capacity to alter mitochondrial pathways associated with PINK1 activity. To ensure the docking site's biological relevance, all ligands were docked into the top-ranked active site pocket determined via PrankWeb analysis.

Table 4 summarizes the docking data, which include the chemical formula, Docking score, and PubChem CID for each ligand. Interestingly, a number of potential compounds showed binding energies that were better than Niclosamide's, indicating improved interaction with the PINK1 binding pocket. Stronger thermodynamic stability inside the PINK1 active site was shown by the ZINC-screened compounds, such as CID 75439760 and CID 18132625, which showed binding energies of –9.6 kcal/mol and –9.4 kcal/mol, each. In order to develop prospective treatment leads, our results provide a solid foundation for further examination of pharmacokinetic characteristics, toxicity, and molecular interaction profiles.

Table 4: Binding Energy of drugs Interacting with PINK1

S.NO.	PubChem CID	Chemical formula	BE
Reference Drug	4477	C13H8Cl2N2O4	-8.1
1	487723	C13H9ClFNO2	-7.3
2	487719	C14H12ClNO3	-7.5
3	18105434	C15H13ClN2O3	-8.4
4	3747508	C14H9ClN2O2	-7.5
5	18104278	C14H12ClNO3	-7.4
6	18104520	C14H12ClNO3	-7.4

7	5274024	C13H9ClFNO2	-7
8	5274029	C14H12ClNO3	-6.8
9	123627	C13H11ClN2O3	-7.5
10	236652	C14H9ClN2O2	-7.8
11	24248148	C14H12ClNO3	-7.6
12	3011449	C13H11ClN2O2	-7.4
13	3011448	C13H11ClN2O2	-6.8
14	18165335	C15H12ClNO2	-7.9
15	689241	C15H12ClNO3	-8.4
16	3849798	C14H9BrN2O2	-7.9
17	99822506	C15H12ClFN2O3	-7.7
18	47288964	C13H11ClN2O3	-7.8
19	30217750	C14H14ClN3O2	-7.5
20	71975956	C15H10Cl2N2O3	-9.1
21	18132625	C17H15ClN2O3	-9.4
22	3726082	C14H9BrN2O2	-8.2
23	18106757	C13H11ClN2O2	-7.4
24	30345691	C11H10ClN3O2	-6.8
25	17424661	C15H14ClNO4	-7.6
26	18165326	C16H14ClNO4	-8.6
27	28393005	C13H11ClN2O2	-7.6
28	71914775	C13H10ClFN2O3	-7.7
29	99822353	C14H11F2NO2	-6.9
30	18165326	C15H12ClNO2	-8.3
31	29564701	C13H11ClN2O2	-78
32	71970515	C11H11BrClNO3	-7.3
33	86801955	C14H13ClN2O2	7.1
34	54677857	C17H13ClN2O3	-8.6
35	11500237	C15H12ClNO3	-8.1
36	3315263	C11H9ClN2O3	-7.1
37	39334538	C15H12ClNO3	7.4
38	86792346	C14H13ClN2O3	-7.4
39	95767128	C16H15ClN2O3	-8.5
40	4141605	C14H10ClN3O2	-8.1
41	30272268	C14H14ClN3O2	-7.5

42	100615452	C15H12F2N2O3	-8.8
43	54678199	C17H13ClN2O3	-7.7
44	71867366	C11H13BrClNO3	-5.7
45	99585239	C16H16FNO3	-8.4
46	78957317	C12H14ClNO3	-7.5
47	4346684	C12H14Cl2N2O2	-7.7
48	75439760	C16H14F2N2O3	-9.6
49	86801916	C13H10F2N2O2	-7.2
50	4807069	C14H11ClFNO2	-8.3
51	54860879	C14H14N2O3	-7.4
52	71885130	C15H16ClN3O2	-7.5
53	16368954	C15H14ClNO3	-8.1
54	99632398	C17H17FN2O3	-8.4
55	51100221	C16H14ClNO3	-8.6
56	2482080	C15H14ClNO2	-8.4
57	95287132	C12H16ClNO2S	-6.6
58	28392486	C15H14ClNO2	-7.9
59	95287133	C12H16ClNO2S	-6.9
60	18106066	C15H14ClNO3	-8
61	71974245	C16H13F3N2O3	-8.0
62	71980475	C15H14ClNO2	7.4
63	91649861	C14H13FN2O3	-7.3

4.1.1 Top-Ranked Compounds Based on Binding Affinity

Out of a total of 63 compounds docked against the PINK1 protein, the top eight molecules exhibiting the most favorable binding affinities were shortlisted based on their docking scores. Out of these compounds PubChem CID: 75439760 is selected for further analysis due to their significantly lower binding energies compared to the reference drug, Niclosamide (PubChem CID: 4477), which recorded a binding energy of –8.1 kcal/mol.

Table 5: Represent compounds having Binding affinities between -8.3 to -9.6 kcal/mol

S.No.	Compound CID	Evaluated △ G (kcal/mol)
Reference compound	4477	-8.1
1.	18165326	-8.3
2.	2482080	-8.4
3.	95767128	-8.5
4.	51100221	-8.6
5.	100615452	-8.6
6.	71975956	-9.1
7.	18132625	-9.4
8.	75439760	-9.6

4.2 Visualization of Interaction of top 8 compounds with PINK1 using BIOVIA

Table 6: Binding Affinities and Interacting Residues of Top 8 Docked Compounds with PINK1

S.No.	Compound CID	Evaluated △ G (kcal/mol)	Interacting Residues
Reference compound	4477	-8.1	Lys B:365, Lys B:237, Tyr B:212, Glu A:376, Arg B:213
1.	18165326	-8.3	Asp B:235, Arg B:213, Asp A:213, Leu B:362, Tyr B:212, Glu A:376
2.	2482080	-8.4	Lys B:365, Lys B:209, Glu A:376, Arg B:239, Cys B:363.
3.	95767128	-8.5	Lys A:380, Glu A:376, Tyr B:212, Arg B:213, Lys B:209,

4.	51100221	-8.6	Arg B:239, Glu A:376, Arg B:213, Tyr B:212, Lys B:365, Lys B:237, Asp B:235
5	100615452	-8.6	Cys B:363, Tyr B:212, Asp B:235, Glu A:376, Arg B:213
6	71975956	-9.1	Cys B:363, Glu A:376, Tyr B:212, Lys A:380
7	18132625	-9.4	Arg B:239, Tyr B:212, Asp B:235, Tyr A:380
8.	75439760	-9.6	Lys A:380, Lys B:209, Tyr B:212, Glu A:376, Cys B:363

After molecular docking the ligands showed preferential binding inside a pocket bounded by crucial residues Tyr212, Glu376, Lys380, Cys363, and Arg239. These residues closely match the active binding location that PrankWeb analysis anticipated. The discovered pocket's biological significance is further supported by Tyr212's frequent involvement in all docking outcomes. As a result, the docking contacts can support the chemicals' possible medicinal applications.

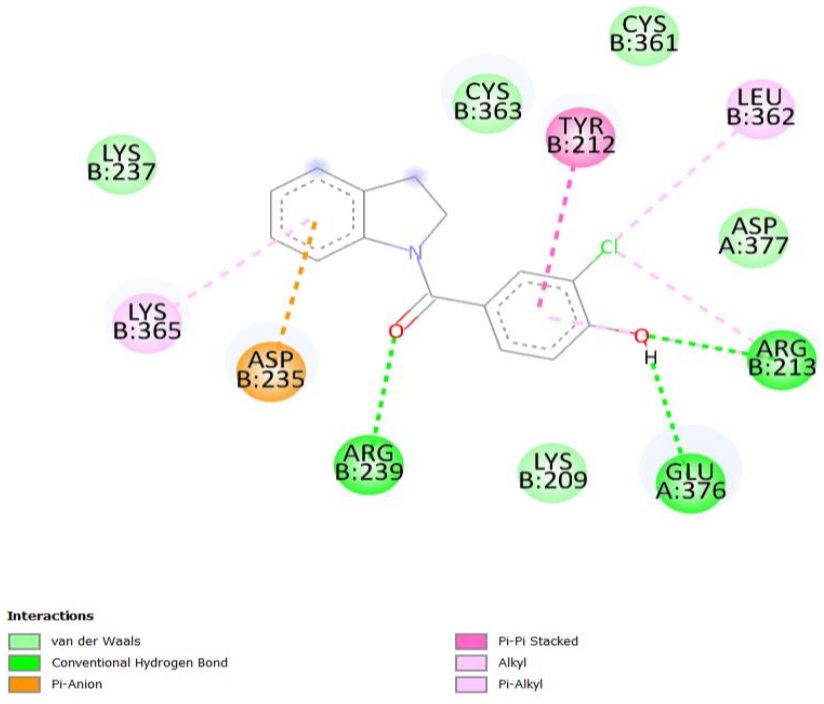

Fig.3. 2D representation of the binding interaction between PubChem CID: 18165326 and PINK 1. Binding interactions includes Van der Waals forces, pi-alkyl, alkyl, hydrogen bonds, and pi-anion-like interactions

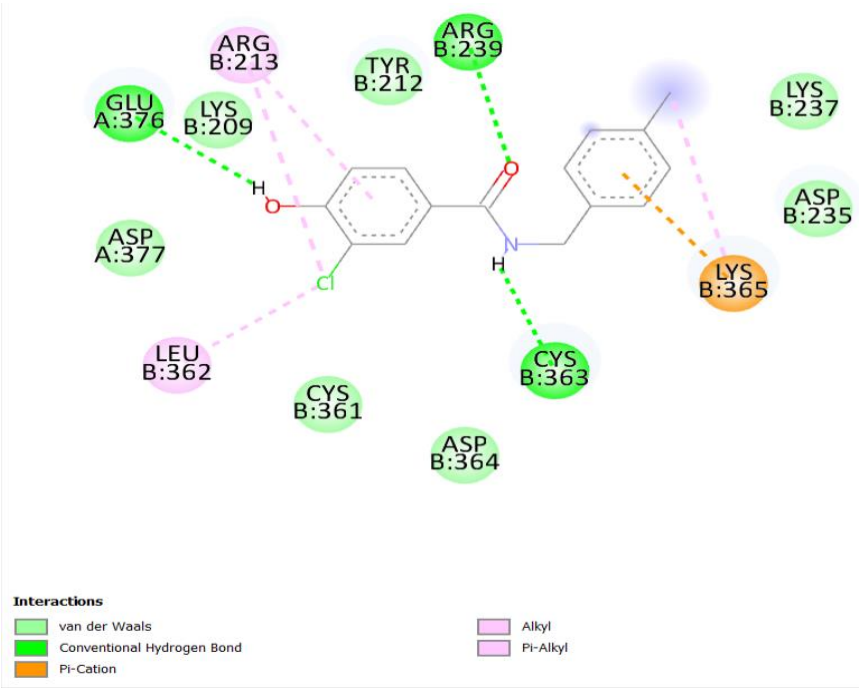

Fig.4. Two-dimensional illustration of the binding interaction between PINK 1 and PubChem CID: 2482080. Binding interactions encompass a range of intermolecular forces, including Van der Waals forces, pi-alkyl, alkyl, pi-donor hydrogen bonds, and pi-cation-like interactions.

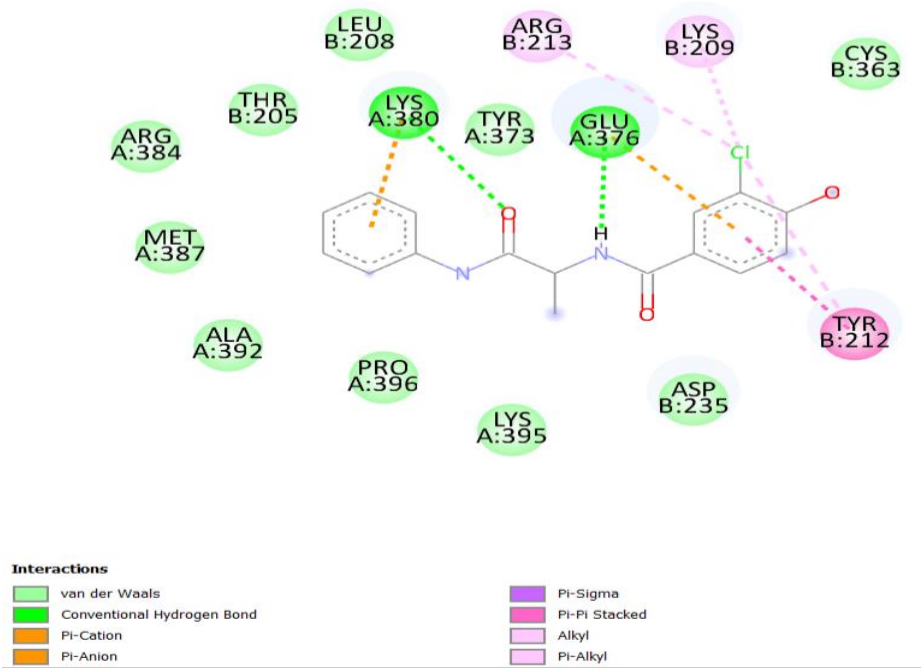

Fig.5. The binding interaction between PINK 1 and PubChem CID: 95767128 is shown in two dimensions. Among the several types of intermolecular forces observed in binding interactions are van der Waals forces, pi-alkyl, alkyl, pi-donor hydrogen bonds, pi-pi stacking, pi-sigma, pi-cation, and pi-anion-like interactions.

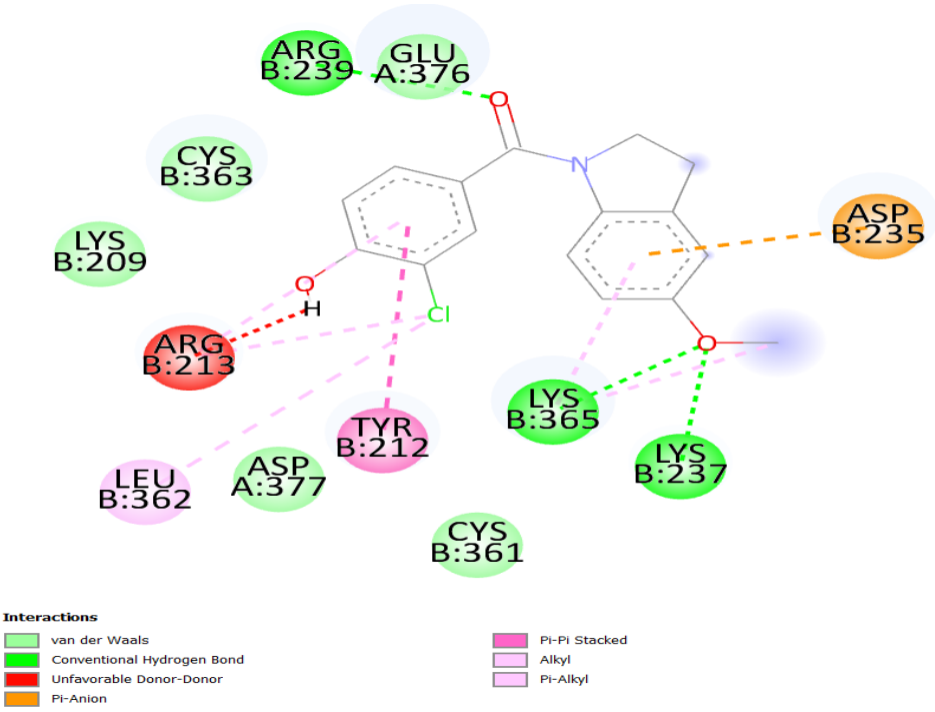

Fig.6. A two-dimensional representation of the binding interaction between PubChem CID: 51100221 and PINK 1. Examples of intermolecular forces that are illustrated through binding interactions include van der Waals forces, pi-alkyl, alkyl, pi-donor hydrogen bonds, pi-pi stacking, and pi-anion-like interactions.

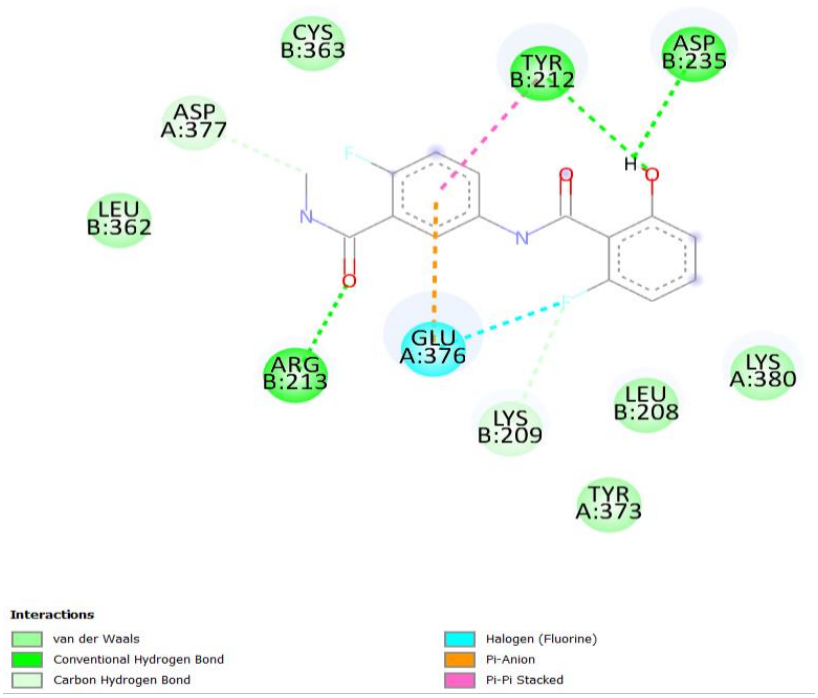

Fig.7. A 2D representation of the binding interaction between PINK 1 and PubChem CID: 100615452. Binding interactions reveal a wide range of intermolecular forces, including hydrogen bonds, van der Waals forces, and pi-anion-like interactions.

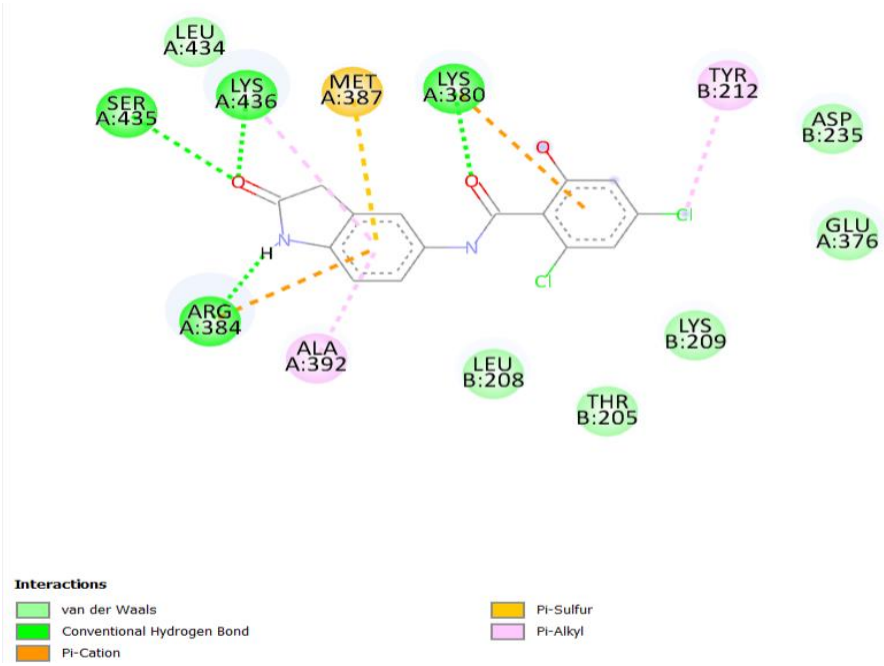

Fig.8. A 2D representation of the binding interaction between PINK 1 and PubChem CID: 71975956. These interactions involve a variety of intermolecular forces, including as hydrogen bonds, pi-alkyl, pi-sulfur, pi-cation, and Van der Waals forces.

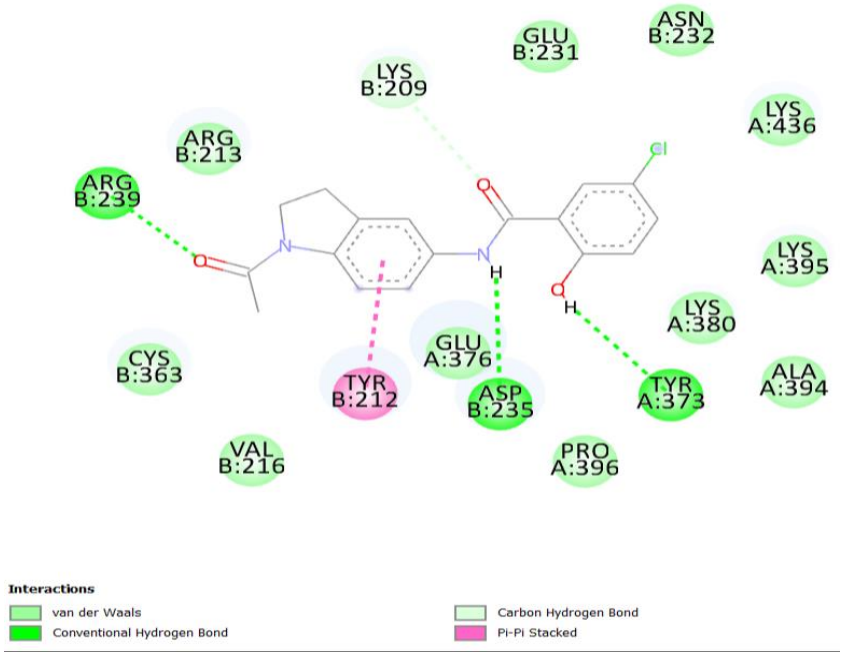

Fig.9. A two-dimensional representation of the binding interaction between PINK 1 and PubChem CID: 75439760. Binding interactions involve a variety of intermolecular forces, including van der Waals forces, pi-pi stacking, and hydrogen bonding.

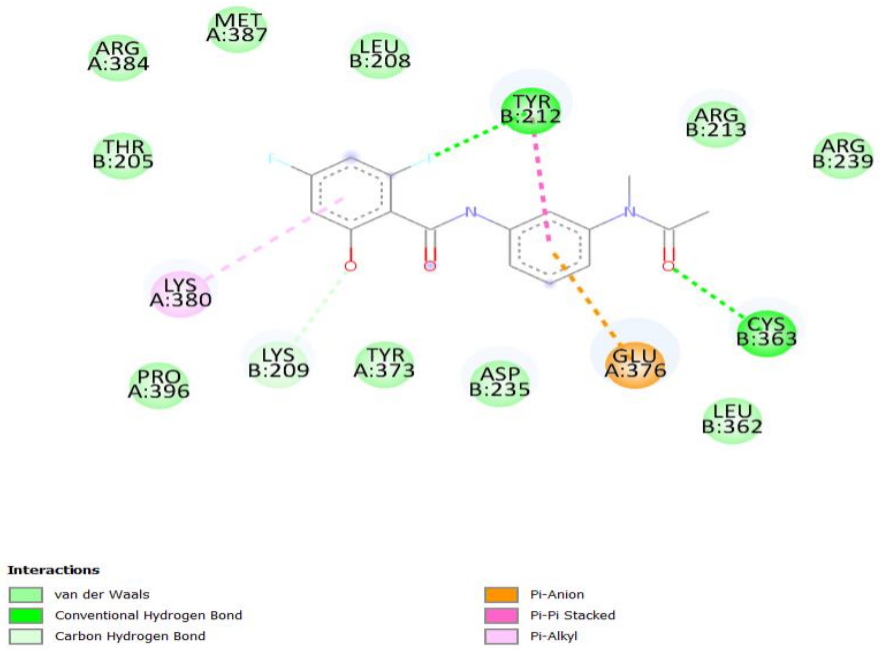

Fig.10. The binding interaction between PINK 1 and PubChem CID: 18132625 is shown in two dimensions. Van der Waals forces, pi-alkyl, pi-donor, hydrogen bonds, and pi-cation-like interactions are examples of intermolecular forces that are included in binding interactions.

4.3 Protein-Protein Interaction (PPI) Analysis

Using the STRING database (v12.0), a network of protein–protein interactions (PPIs) was constructed. with a confidence score threshold of 0.700 in order to evaluate the functional landscape of PINK1 and its interacting partners in Parkinson's disease. There are maximum of 20 proteins per shell, including both first- and second-shell interactors.

The result shows a highly interconnected and biologically significant connections network in (Figure 11), which had 42 nodes and 238 edges—much more than the predicted number of edges (54). As seen by the average node degree of 11.6 in the network, the majority of proteins were linked to a significant number of other proteins. It is highly likely that nearby proteins interact with one another, as indicated by the average local clustering coefficient of 0.661. Crucially, the PPI enrichment p-value was less than 1.0e-16, suggest that the discovered network is heavily enriched for functional relationships rather than being the result of chance.

4.3.1 PPI Network Statistics for PINK1 via STRING-DB

Table 7: Represent the network stats of PINK1

Parameter	Value
Number of nodes	42
Number of edges	238
Expected number of edges	54
Average node degree	11.6
Average clustering coefficient	0.661
PPI enrichment p-value	<0.1e-16

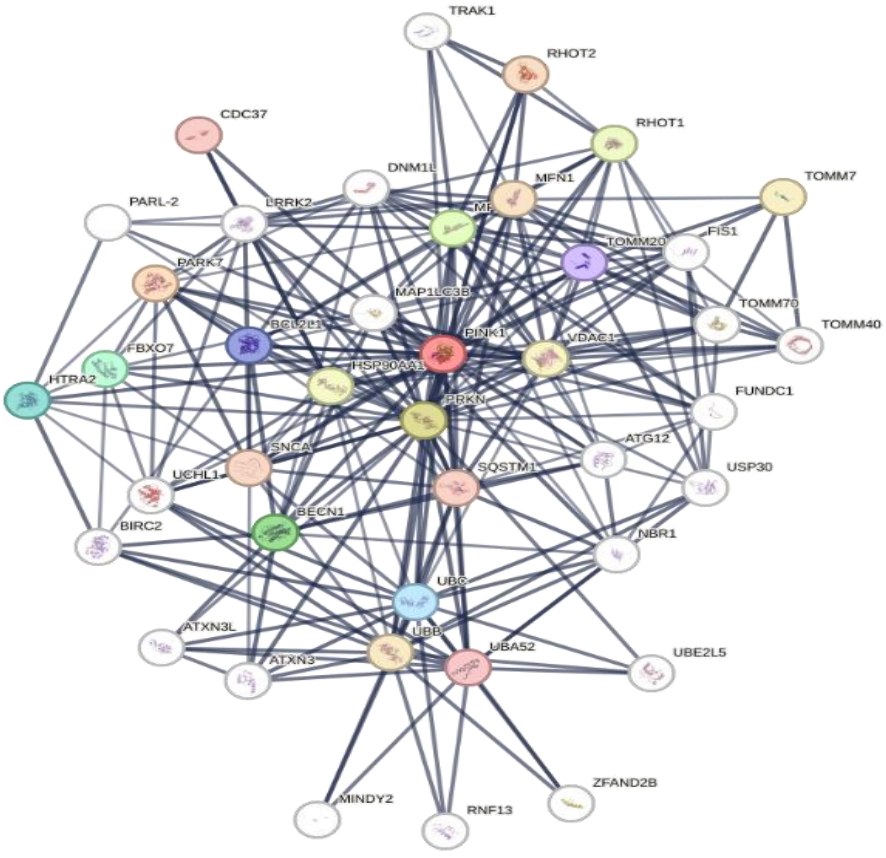

Fig.11. PPI analysis of PINK1 using STRING-DB

4.4 ADME Prediction Results

The pharmacokinetic profiles of both the reference compound (Niclosamide) and the ZINC compound (Z1571042344) which shows highest negative binding energy were evaluated using the SwissADME web server. Parameters assessed included Lipinski's Rule of Five compliance, gastrointestinal (GI) absorption, blood–brain barrier (BBB) permeability, bioavailability score, and key physicochemical properties relevant to drug-likeness.

4.4.1 Lipinski's Rule of Five:

Both compounds complied with Lipinski's criteria, indicating good oral bioavailability potential. No violations were observed for molecular weight, LogP, hydrogen bond donors, or hydrogen bond acceptors in either molecule.

4.4.2 Gastrointestinal Absorption:

The ZINC compound exhibited high GI absorption, which supports favorable oral uptake. In contrast, Niclosamide showed low GI absorption, aligning with its known limited oral bioavailability.

4.4.3. Blood–Brain Barrier (BBB) Permeation:

Since the ZINC molecule was projected to be BBB permeant, it may be able to reach the central nervous system, which is an essential prerequisite for treating Parkinson's disease. The direct CNS activity of niclosamide was anticipated to be limited due to its expected non-permeability.

4.4.4. Bioavailability Score:

Both medications showed a moderate bioavailability score of 0.55, suggesting that systemic absorption might occur with appropriate dosage methods.

4.4.5 Other Properties:

The ZINC compound displayed superior membrane permeability due to its decreased Topological Polar Surface Area (TPSA) compared to Niclosamide. For drug-like activity, both compounds showed balanced lipophilicity with acceptable LogP values.

4.4.6 Interpretation:

Overall, the ZINC compound surpassed Niclosamide in terms of pharmacokinetic parameters, particularly BBB permeability and gastrointestinal absorption. Because of these characteristics, it is a better candidate as a CNS-active drug in the treatment of Parkinson's disease as a result of mitochondrial dysfunction.

Table 8: Represent the ADME analysis using SwissADME

Property	Niclosamide (Ref. Drug)	ZINC1571042344
Lipinski's Rule of 5	Violation - 1	0
GI absorption	Low	High
BBB permeability	No	Yes
Bioavailability score	0.55	0.55
Drug-likeness	Moderate	Good
Water solubility	Poor	Moderate

4.5 Toxicity Prediction Results

To access the toxicity profile of the selected ZINC compound (Z1571042344), the ProTox-3.0 web server is used. The drug was tested for toxicity endpoints, multiple organ toxicity, and possible metabolism by cytochrome P450 enzymes.

According to the results, the drug has been predicted to be reactive for hepatotoxicity (probability: 0.51), neurotoxicity (0.55), nephrotoxicity (0.65), and respiratory toxicity (0.59). This suggests that there may be safety concerns unique to certain organs at greater concentrations or after prolonged exposure. To support its therapeutic potential, it was expected to be inactive for cardiotoxicity (0.80), carcinogenicity (0.60), mutagenicity (0.68), and cytotoxicity (0.72). This suggests that it has no systemic genotoxic or cytotoxic risks.

In terms of pharmacological relevance, the drug candidate exhibited active blood–brain barrier (BBB) penetration (0.58), which is a crucial characteristic for neurotherapeutics that target illnesses of the central nervous system (CNS), including Parkinson's disease.

The necessity for additional in vivo validation and dosage optimization is highlighted by the clinical toxicity prediction of active (0.64).

Furthermore, metabolism prediction indicated that it was inactive against CYP1A2, CYP2C19, CYP3A4, and CYP2E1, but likely interacted with CYP2C9 and CYP2D6. This suggests a possible drug-drug interaction and partial hepatic metabolism, although there is little chance of cytochrome-related clearance problems. Together, these expectations suggest that even if the chemical exhibits some organ-specific toxicities, its favourable BBB permeability and lack of mutagenic and carcinogenic potential encourage its ongoing assessment as a possible molecule for the treatment of Parkinson's disease.

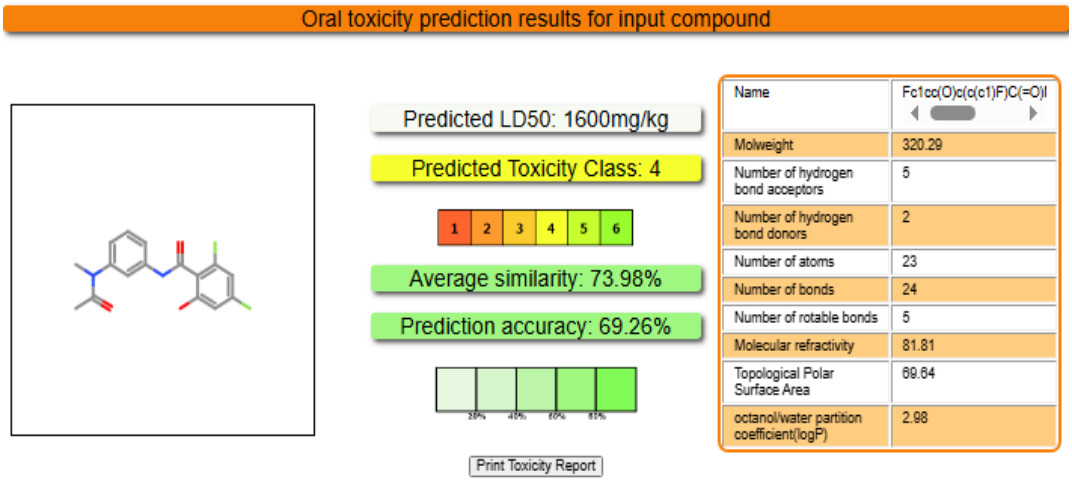

Fig.12. Oral Toxicity Prediction Report

Table 9: ProTox-3.0 - ZINC1571042344 Toxicology Prediction

Toxicity Prediction of Z1571042344	Column1	Column2	Column3
Classification	**Target**	**Prediction**	**Probability**
Organ toxicity	Hepatotoxicity	Active	0.51
Organ toxicity	Neurotoxicity	Active	0.55
Organ toxicity	Nephrotoxicity	Active	0.65
Organ toxicity	Respiratory toxicity	Active	0.59
Organ toxicity	Cardiotoxicity	Inactive	0.80
Toxicity end points	Carcinogenicity	Inactive	0.60
Toxicity end points	Immunotoxicity	Active	0.63
Toxicity end points	Mutagenicity	Inactive	0.68

Toxicity end points	Cytotoxicity	Inactive	0.72
Toxicity end points	BBB-barrier	Active	0.58
Toxicity end points	Clinical toxicity	Active	0.70
Toxicity end points	Nutritional toxicity	Inactive	0.81
Tox21-Stress response pathways	Mitochondrial Membrane Potential (MMP)	Active	0.62
Metabolism	Cytochrome CYP1A2	Active	0.65
Metabolism	Cytochrome CYP2C19	Inactive	0.78
Metabolism	Cytochrome CYP2C9	Active	0.59
Metabolism	Cytochrome CYP2D6	Inactive	0.74
Metabolism	Cytochrome CYP3A4	Inactive	0.58
Metabolism	Cytochrome CYP2E1	Inactive	1

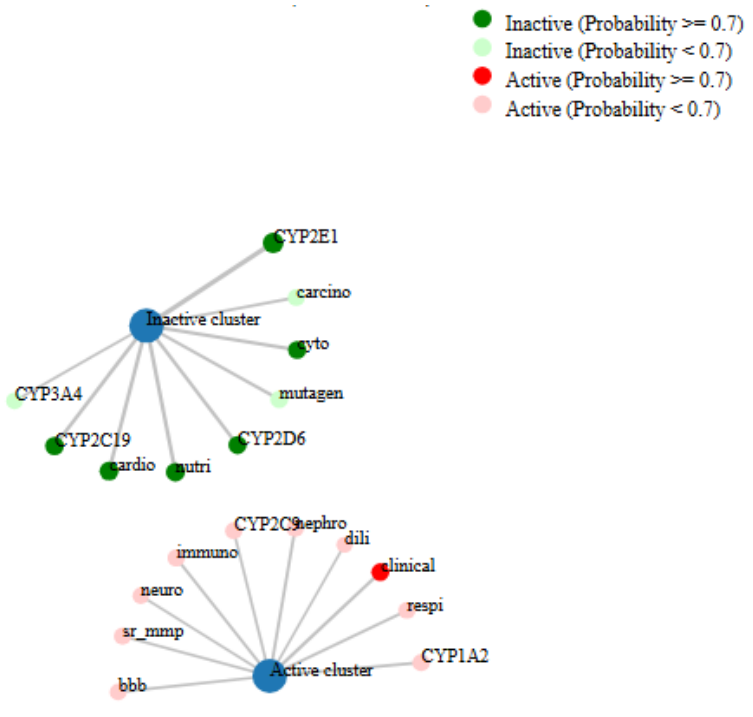

Fig.13. Show The relationship between the proposed molecule (Z1571042344) and expected activities is depicted in the network graphic.

CHAPTER-5
CONCLUSION & DISCUSSION

Parkinson's disease (PD) continues to pose a substantial global health burden, with currently available therapies offering only symptomatic relief and failing to modify the underlying neurodegenerative processes. Among the pathological mechanisms implicated in PD, mitochondrial dysfunction—particularly defective mitophagy—plays a critical role. Given PINK1's involvement in mediating mitochondrial quality control through mitophagy, it presents a compelling therapeutic target.

This study highlights how well computational drug discovery methods work to find possible novel therapies for Parkinson's disease (PD), with an emphasis on the PINK1 protein, which is essential for preserving mitochondrial integrity. Using the ZINC database and virtual screening techniques, N-[3-[acetyl(methyl)amino] phenyl]-2,4-difluoro-6-hydroxybenzamide (ZINC ID: Z1571042344) was shown to be a potential candidate chemical. This compound had a higher binding affinity for PINK1 (-9.6 kcal/mol) than Niclosamide, the reference medication (-8.1 kcal/mol).

The effectiveness of computational approaches in early-stage drug discovery is demonstrated by the use of in silico tools, such as AutoDock Vina for docking, SwissADME for pharmacokinetics, ProTox-3.0 for toxicity profiling, and STRING for protein interaction analysis. These methods make it possible to prioritize medication candidates in a time and money-efficient manner.

According to docking studies, both ligands interact with essential residues in the PINK1 active site (Tyr198, Arg201, Glu203), exhibiting accurate binding and target selectivity. Positive characteristics of Z1571042344's ADME (absorption, distribution, metabolism, and excretion) profile included greater solubility compared to Niclosamide, high absorption through the GI tract and also have the ability to pass through the blood-brain barrier. It also adhered to Lipinski's Rule of Five. These features imply that it could be able to overcome Niclosamide's primary disadvantages, which include limited water solubility and low blood-brain barrier (BBB) permeability, which limits its efficacy in disorders affecting the central nervous system.

Despite toxicity projections that suggested possible organ-specific issues such hepatotoxicity, neurotoxicity, and nephrotoxicity, the chemical showed no mutagenic or carcinogenic effects. This offers credibility to the drug's potential as a safer treatment option. Additionally, PINK1's importance in mitochondrial function and potential as a therapeutic target were validated by analysis of protein-protein interaction networks. This

analysis shows that Z1571042344 is a promising lead chemical, highlighting the necessity of adopting in silico tools to accelerate early-phase drug discovery.
However, the study is limited because it only uses computer estimations. Despite the encouraging in-silico results, Z1571042344's expected toxicities to organs such as the liver, kidney, and nervous system necessitate careful monitoring through in vitro and in vivo assessments. Furthermore, it's possible that other candidates with potentially superior qualities were overlooked due to the extremely narrow scope of compound screening (63 compounds). The lack of evaluation for off-target interactions and downstream effects on the PINK1-Parkin pathway is another disadvantage, particularly in light of the system's critical role in mitochondrial regulation and neuronal survival.

Therefore, experimental validation of Z1571042344's binding efficacy and mitophagy-enhancing properties should be part of future study. In vitro studies should be conducted first, followed by in vivo animal models to evaluate neuroprotection and toxicity. Its therapeutic viability may be further improved by structural optimization that minimizes anticipated side effects while maintaining the compound's pharmacological advantages. The focus of this work on drug repurposing is in keeping with current developments in neurotherapeutic development, where conventional drug discovery is lengthy and has high failure rates.

Niclosamide's known safety profile makes it still drug of interest, but its drawbacks highlight the need for better substitutes like Z1571042344. A multi-targeted therapy approach combining related proteins like Parkin or DJ-1 may improve overall efficacy, according to the inclusion of protein-protein interaction (PPI) analysis, which provides insightful information about the biological context of PINK1. By suggesting a novel lead drug and confirming an extensive computational methodology, this thesis ultimately contributes significantly to the field of Parkinson's disease research, setting the stage for further investigations that may result in treatments that are clinically feasible.

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