IDENTIFYING POTENTIAL SIRT2 INHIBITORS: AN *IN-SILICO* APPROACH TO EXPLORE NOVEL STRATEGIES FOR PARKINSON'S THERAPY

A Dissertation

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by:

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DECLARATION

I, Apurva 23/MSCBIO/11 hereby certify that the work which is being presented in the thesis entitled **"Identifying Potential SIRT2 Inhibitors: An In-silico Approach to Explore Novel Strategies for Parkinson's Therapy"** in partial fulfillment of the requirements for the award of the Degree of Master of Science, submitted in the Department of Biotechnology, Delhi Technological University is an authentic record of my own work carried out during the period from 2023 to 2025 under the supervision of Prof. Pravir Kumar.

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

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CERTIFICATE BY THE SUPERVISOR

This is to certify that the Dissertation Project titled **"Identifying Potential SIRT2 Inhibitors: An In-silico Approach to Explore Novel Strategies for Parkinson's Therapy"** which is being submitted by Apurva 23/MSCBIO/11, 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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"IDENTIFYING POTENTIAL SIRT2 INHIBITORS: AN IN-SILICO APPROACH TO EXPLORE NOVEL STRATEGIES FOR PARKINSON'S THERAPY"

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ABSTRACT

Aim: This study aims to identify and characterize potential SIRT2 inhibitors via computational approach.as an alternate therapeutic approach towards Parkinson's. SIRT2 is positioned second in class III HDAC family of sirtuins and NAD+ (nicotinamide adenine dinucleotide) dependent in nature. SIRT2 plays a crucial role in neurodegeneration leading to diseases like Parkinson's disease (PD). Therefore, the need to discover SIRT2 inhibitors aligns itself with potential therapeutic developments for treating PD. As a result of SIRT2 inhibition, α-synuclein (α-Syn) induced toxicity decreases leading to reduction in cell death. SirReal2 is a native SIRT2 inhibitor. It is situated in the selectivity pocket which is hydrophobic in nature present near the zinc binding domain (ZBD). SirReal2 was taken as the reference ligand for this study. The primary objective is to shortlist better suited compounds for SIRT2 inhibition as potential drug components for Parkinson's therapy in comparison to what was deduced in previous such studies. Here, a large number of compounds will be shortlisted on the basis of structural similarity to SirReal2 which will then be filtered by ADME analysis. As the next step docking studies will be performed to generate binding affinities. This investigation is expected to reveal some compounds with affinities higher than SirReal2. Extended ADME analysis will be employed further to strengthen the fact that these drug like commercial compounds from ZINC database will display greater viability for advancement in the pharmacological field. In contrast with the previous studies on this matter, these probable inhibitors will be shown to be capable of crossing the blood brain barrier making them a better choice. Such analysis of compounds can significantly reduce the time and cost of wet lab research. Conclusively, results from this study will strongly advocate for the analysis of these probable inhibitors inside living systems.

Result: During the course of our study, out of the initial 400 similar compounds, ADME analysis left us with 79 compounds which were all BBB permeable. Docking analysis of these compounds identified seven with binding affinities higher than the reference, SirReal2 (-10.8 kcal/mol). with the highest affinity at -11.4 kcal/mol.

Conclusion: Among the seven identified compounds, Compound 7 emerged as the most effective, with the highest binding affinity and being BBB permeable. We recommend validating these findings through in vivo experiments.

List of Publications

1. Conference paper:

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Apurva[1], Pravir Kumar[1], "Analysis for potential SIRT2 inhibitors through *in silico* assay: Exploring novel strategies for Parkinson's therapy"

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LIST OF ABBREVIATIONS

SNCA	Synuclein Alpha
LRRK2	Leucine-rich Repeat Knase 2
PARK2	Parkinsonism Associated Deglycase 2
SIRT2	Sirtuin 2
AGK2	Acylglycerol Kinase 2
AK-7	Adenylate Kinase 7
DALYs	Disability-adjusted Life Years
SDI	Socio-demographic Index
PINK1	PTEN-induced putative kinase 1
MAO-B	Monoamine oxidase B
COMT	Catechol-O-methyltransferase
DBS	Deep Brain Stimulation
CNS	Central Nervous System
NMR	Nuclear Magnetic Resonance
QSAR	Quantitative Structure-Activity Relationship
ADMET	Absorption, Distribution, Metabolism, Excretion, Toxicity
FOXO3a	Forkhead box protein O3a
SQSTM1	Sequestosome 1
AK-1	Adenylate Kinase 1
IC50	Half Maximal Inhibitory Concentration
c-Myc	Cellular Myc
HTVS	High-Throughput Virtual Screening
SP	Standard Precision
XP	Alpha-Thalassemia-Linked Mental Retardation
GOLD	Vascular Endothelial Growth Factor
ASP	Extra Precision
MOE	Genetic Optimization for Ligand Docking
AMBER	Assisted Model Building with Energy Refinement
CHARMM	Chemistry at HARvard Macromolecular Mechanics

OPLS	Optimized Potentials for Liquid Simulations
PMF	Probability Mass Function
X-CSCORE	Consensus Scoring Functiont
RMSD	Root Mean Square Deviation
ROC	Receiver Operating Characteristic
AUC	Area Under the Curve
GROMACS	GROningen MAchine for Chemical Simulations
NAMD	Nanoscale Molecular Dynamics
MM-PBSA	Molecular Mechanics Poisson-Boltzmann Surface Area
MM-GBSA	Molecular Mechanics/Generalized Born Surface Area
FEP	Free Energy Perturbation
QM	Quantum Mechanics
HTS	High Throughput Screening
ZINC	ZINC is Not Commercial
SMILES	Simplified molecular input line entry system
BBB	Blood-Brain Barrier
SDF	Structural Data File
PDB	Protein Data Bank
PDBQT	Protein Data Bank, Partial charge and Atom Type
CID	Compound Identity Number

1.INTRODUCTION

Parkinson's disease (PD) is a chronic neurodegenerative disorder which is progressive in nature. It majorly affects movement but also involves a series of non-motor symptoms namely mood disorders, cognitive impairment and autonomic dysfunction. Affecting over 10 million individuals worldwide, PD represents a growing public health challenge, particularly in aging populations where incidence is highest. The disorder is neuropathologically characterized by loss of specific type of neurons which are known as dopaminergic (DA) neurons in the region of the brain named substantia nigra pars compacta (SNpc) and the assemblage of α-synuclein (α-Syn) to form fibril like structures which appear as aggregates that further clump up to form protein inclusions termed as Lewy bodies (LB). The exact etiology remains multifactorial, with genetic, environmental, and age-related factors contributing to its onset. Mutations in genes such as SNCA, LRRK2, and PARK2, alongside mitochondrial dysfunction, oxidative stress, and defective proteostasis, are central to PD pathogenesis[1].

Current therapeutic strategies, including the administration of levodopa and dopamine agonists, offer symptomatic relief but fail to modify disease progression or address the underlying neurodegeneration[2] . Therefore, there is a pressing need to construct disease-altering therapies focused on molecular mechanisms at the core of PD. Among various targets, sirtuins—NAD^+-dependent deacetylases—have gained attention for their roles in aging, metabolism, and stress resilience[3] . Of the seven mammalian sirtuins, SIRT2 is particularly intriguing due to its predominant cytoplasmic localization and high expression in vulnerable brain regions such as the striatum and cortex, both critically implicated in PD[4].

SIRT2 controls cellular processes namely microtubule stability, redox homeostasis and progression of the cell cycle. Its dysregulation has been associated with neurodegeneration, where overexpression correlates with enhanced α-synuclein toxicity and dopaminergic neuron loss[5][6]. Notably, SIRT2 promotes the deacetylation of α-tubulin, leading to microtubule destabilization—a process that may exacerbate neuronal vulnerability in PD[7]. Recent studies show that pharmacological inhibition of SIRT2 with agents such as AGK2 and AK-7 confers neuroprotection by enhancing microtubule stability, reducing α-synuclein aggregation, and promoting the clearance of toxic protein species via autophagy and proteasomal pathways[2][8].

Preclinical evidence strongly supports the therapeutic potential of SIRT2 inhibition. For instance, AGK2 has demonstrated efficacy in reducing α-synuclein-mediated cytotoxicity in dopaminergic neurons[5], while AK-7 prolongs survival and preserves motor function in PD mouse models[9]. The neuroprotective mechanisms include reduced neuroinflammation, stabilization of neuronal architecture, and improved proteostasis, positioning SIRT2 as a viable disease-modifying target in PD[10][11].

In light of these insights, in silico drug discovery has emerged as a valuable approach to expedite the identification of novel SIRT2 inhibitors. Computational methods such as molecular docking, molecular dynamics simulations, and pharmacophore modeling enable the rapid screening of large compound libraries by predicting binding affinities and interaction patterns between small molecules and target proteins[12][13]. These techniques reduce the time, cost, and resource demands associated with traditional drug development, while enhancing the precision of candidate selection. Molecular docking, in particular, allows for the visualization of ligand orientation and interaction within the SIRT2 active site, enabling rational optimization of binding specificity and potency[13]. Furthermore, advances in artificial intelligence and machine learning have further refined virtual screening workflows, increasing the predictive accuracy of in silico models in identifying drug-like molecules with favorable pharmacokinetic properties[14].

Given the substantial preclinical evidence supporting SIRT2 inhibition in mitigating PD-related pathology and the growing utility of computational tools in drug discovery, this thesis explores the application of in

silico techniques to identify and evaluate novel SIRT2 inhibitors with potential therapeutic value for Parkinson's disease. By integrating molecular docking simulations with structure-based drug design, the study aims to contribute to the development of effective, mechanism-based interventions that address the unmet clinical needs in PD management.

2. REVIEW OF LITERATURE

2.1 Parkinson's Disease: Pathophysiology and Current Therapies

Parkinson's disease (PD) is a chronic neurodegenerative disorder of progressive nature reportedly marked by motor symptoms which include tremor, bradykinesia, rigidity and postural instability, alongside non-motor manifestations such as autonomic dysfunction, sleep disorders and cognitive impairment. James Parkinson first described it as "shaking palsy" in 1817. Since then our understanding of this complex disorder has evolved significantly, revealing a multifaceted pathology that extends beyond the nigrostriatal system**.** The worldwide prevalence of PD has reached alarming proportions, with approximately 11.77 million people affected as of 2021. This represents a dramatic increase from the estimated 6.1 million cases in 2016 and 8.5 million in 2019, demonstrating a concerning upward trajectory. The rates of incidence, prevalence, and disability-adjusted life years (DALYs) standardized by age have shown consistent increases, with DALYs due to PD rising by 81% since 2000 and mortality more than doubling in the same period. This escalating burden correlates strongly with global aging demographics but also reflects improved diagnostic capabilities and heightened awareness. The socioeconomic impact of PD is substantial, encompassing direct healthcare costs, productivity losses, and caregiver burden. Countries with Socio-demographic Index (SDI) ranging from middle to high values demonstrate significantly noticeable increases in age-standardized incidence rates, reaching 18.49 per 100,000 in 2021 compared to 10.21 per 100,000 in low SDI regions. This disparity likely reflects both genuine epidemiological differences and variations in healthcare access and diagnostic capabilities[15].

The cardinal pathological hallmark of PD is the degeneration of DA neurons in a progressive fashion in the SNpc region of the brain which further leads to depletion of dopamine in the striatum and subsequent disorganization of circuitry of basal ganglia. By the time motor symptoms manifest clinically, approximately 50-80% of dopaminergic neurons have already been lost, highlighting the substantial compensatory capacity of the nigrostriatal system and the challenge of early diagnosis[16]. The second defining feature is the existence of Lewy bodies - protein inclusions within the neurons, primarily made of misfolded α-Syn aggregates. These pathological structures contain fibrillar α-synuclein arranged in a characteristic pattern with a dense core surrounded by a halo of radiating fibrils[17]. While initially thought to be confined to the substantia nigra, Lewy pathology follows a predictable anatomical progression pattern as described by Braak staging, beginning in the olfactory bulb and enteric nervous system before ascending through the brainstem to eventually reach cortical regions. However, this staging model doesn't apply universally, with approximately 50% of PD cases showing variations in this pattern[18].

The pathogenesis of PD includes various interconnected mechanisms at molecular level that collectively contribute to dysfunction of neurons and their death:
α-Synuclein Misfolding and Aggregation: Native α-synuclein typically exists as an unfolded protein without defined tertiary structure or as stable tetramers resistant to aggregation. In pathological conditions, α-synuclein adopts β-sheet-rich amyloid-like conformations prone to oligomerization and

fibril formation. Recent evidence suggests that soluble oligomeric species, rather than mature fibrils, may represent the most neurotoxic form, capable of "seeding" further aggregation and facilitating pathology spread through the brain[19].

Mitochondrial Dysfunction: Compelling evidence implicates mitochondrial impairment in PD pathogenesis, particularly through complex I deficiency, increased oxidative stress, and compromised calcium buffering. The revelation that MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine), that is metabolized to the complex I inhibitor MPP+, causes parkinsonism provided strong support for the mitochondrial hypothesis. Furthermore, several PD-associated genes, including PINK1 and Parkin, regulate mitochondrial quality control through mitophagy, underscoring the importance of mitochondrial homeostasis in neuronal survival[20].

Neuroinflammation: Microglial activation and neuroinflammatory processes contribute significantly to PD progression. Misfolded α-synuclein can activate microglia, triggering inflammatory responses that exacerbate neurodegeneration. The association between certain autoimmune disorders and increased PD risk, along with the observation that immunosuppressants may reduce PD risk, supports an immunological component in disease pathogenesis[21].

Impaired Protein Clearance: Dysfunction in the pathways of protein degradation, including the autophagy-lysosomal pathway and ubiquitin-proteasome system, compromises the cell's ability to eliminate misfolded proteins, leading to toxic accumulation. This creates a vicious cycle where protein aggregation further impairs clearance mechanisms[22].

Vesicular Trafficking Defects: Disruption of vesicular transport affects neurotransmitter release, protein sorting, and organelle function. α-Synuclein can block endoplasmic reticulum vesicles from reaching the Golgi and impair lysosomal trafficking, contributing to cellular stress and protein accumulation[23].

Current pharmacological interventions for PD primarily focus on symptomatic relief through dopamine replacement or enhancement strategies:

Levodopa: As the gold standard treatment, levodopa (L-DOPA) functions as a precursor of dopamine that passes through the blood-brain barrier and is transformed by aromatic L-amino acid decarboxylase to dopamine. While highly effective for motor symptom control, long-term use is associated with motor fluctuations, dyskinesias, and diminishing efficacy[24].

Dopamine Agonists: These compounds directly stimulate dopamine receptors, bypassing degenerating neurons. They include non-ergot derivatives like pramipexole and ropinirole, which are often used as initial monotherapy in younger patients or as adjuncts to levodopa[25].

Monoamine oxidase B (MAO-B) Inhibitors: Monoamine oxidase B is inhibited by Selegiline and rasagiline, decreasing breakdown of dopamine and prolonging its action. They offer modest symptomatic benefits and may have neuroprotective properties, although the latter remains controversial[26].

COMT Inhibitors: Entacapone and opicapone inhibit catechol-O-methyltransferase, preventing peripheral levodopa metabolism and extending its half-life, thereby reducing "wearing-off" fluctuations[27].

Non-motor Symptom Management: Various medications address specific non-motor manifestations, including antidepressants for mood disorders, anticholinergics for urinary symptoms, and melatonin or other sleep aids for insomnia[28].

Deep Brain Stimulation (DBS): This surgical intervention includes electrode implantations in particular brain regions, typically the globus pallidus interna or subthalamic nucleus, to regulate abnormal activity of the neurons. DBS can significantly improve motor symptoms and reduce medication requirements in appropriately selected patients[29].

Despite advances in symptomatic management, current PD therapies face significant limitations:

Lack of Disease Modification: Existing treatments address symptoms without altering the underlying neurodegenerative process. Consequently, disease progression continues unabated, with gradual worsening of both motor and non-motor manifestations[30].

Motor Complications: Long-term levodopa therapy frequently leads to motor fluctuations ("wearing-off" phenomenon) and dyskinesias, significantly impacting quality of life[31].
Inadequate Non-motor Symptom Control: Many non-motor symptoms, including cognitive impairment, autonomic dysfunction, and sleep disorders, respond poorly to dopaminergic therapy and may even be exacerbated by it[32].
Treatment-Resistant Symptoms: Certain manifestations, particularly postural instability, freezing of gait, and dementia, show limited response to available interventions[33].
Side Effect Burden: Dopaminergic medications can cause significant adverse effects, including impulse control disorders, hallucinations, and orthostatic hypotension[34].

These limitations underscore the urgent need for disease-modifying therapies that target the fundamental pathological processes driving neurodegeneration. Approaches that address protein misfolding, mitochondrial dysfunction, neuroinflammation, or enhance neuroprotective mechanisms represent promising avenues for developing transformative treatment[35]s.

2.2 Drug Discovery Approaches in Neurodegenerative Diseases

Developing effective therapies for neurodegenerative disorders presents unique challenges that have historically resulted in high attrition rates and limited therapeutic breakthroughs. Understanding these challenges and leveraging modern drug discovery approaches is essential for advancing the field.

Drug development for neurodegenerative diseases faces several distinct obstacles:
Blood-Brain Barrier (BBB) Penetration: The BBB's selective permeability restricts the entry of approximately 98% of small molecules and nearly all large molecules into the central nervous system. Successful CNS drugs must possess specific physicochemical properties to facilitate BBB crossing, including appropriate molecular weight (<400-500 Da), lipophilicity (logP 1-4), and limited hydrogen bond donors (<3)[36].
Complex Disease Pathophysiology: Neurodegenerative disorders involve multiple interacting pathological mechanisms, making single-target approaches often insufficient. The intricate interplay between protein aggregation, mitochondrial dysfunction, neuroinflammation, and other processes necessitates multi-faceted therapeutic strategies.
Slow Disease Progression: The typically gradual clinical course of neurodegenerative diseases complicates clinical trial design, requiring longer study durations and larger patient cohorts to demonstrate efficacy. This substantially increases development costs and timelines.
Heterogeneous Patient Populations: Considerable variability in disease manifestation, progression rates, and underlying pathology creates challenges in patient selection and outcome assessment[37].
Limited Translational Validity of Animal Models: Preclinical models often inadequately recapitulate the complex human pathology, leading to poor predictive value for clinical efficacy[38].

Drug discovery methodologies have evolved significantly over recent decades:
Traditional Approaches: Conventional drug discovery relied heavily on phenotypic screening, where compounds were tested directly in cellular or animal models without necessarily understanding their molecular targets. While this approach led to important discoveries, it was resource-intensive and provided limited mechanistic insights.
Target-Based Approaches: The identification of disease-relevant molecular targets enabled more focused screening campaigns, typically employing high-throughput screening (HTS) of compound libraries which have a large size against isolated targets. This approach accelerated the discovery process but sometimes yielded compounds with excellent in vitro activity but poor in vivo efficacy due to inadequate pharmacokinetic properties or off-target effects[39].
Rational Drug Design: Drug design based on structure employs 3-D structural information about target proteins to guide compound optimization. This approach has become increasingly powerful with

advances in structural biology techniques, including X-ray crystallography, cryo-electron microscopy, and NMR spectroscopy[40].

Computational approaches have revolutionized drug discovery, offering cost-effective strategies for identifying promising compounds:

Molecular Docking: This technique predicts affinity of molecules which are small in size to protein targets and the mode of binding, enabling virtual screening of large compound libraries . Docking algorithms evaluate various ligand conformations and orientations within the target binding site, scoring them based on predicted interaction energies[40].

Virtual Screening: Large chemical databases can be computationally screened against target proteins, significantly reducing the number of compounds requiring experimental testing. This approach can be structure-based (using docking) or ligand-based (using known active compounds as templates).

Quantitative Structure-Activity Relationship (QSAR): These models tie in the chemical structures with biological activities, allowing prediction of compound properties and guiding structural optimization.

Pharmacophore Modeling: By identifying essential features necessary for biological activity (hydrophobic regions,hydrogen bond donors/acceptors etc.), pharmacophore models enable the design of novel compounds maintaining these critical elements.

Molecular Dynamics Simulations: These simulations model the time-dependent behavior of molecular systems, providing insights into protein flexibility, ligand binding kinetics, and induced-fit phenomena not captured by static docking approaches[41].

Machine learning (ML) and artificial intelligence (AI) have come out as transformative technologies in drug discovery:

Target Identification: AI algorithms can analyze complex biological datasets to identify novel disease-associated targets and pathways.

Compound Property Prediction: Machine learning models can forecast certain properties namely ADMET (absorption, distribution, metabolism, excretion, toxicity) properties with increasing accuracy, facilitating early elimination of compounds with unfavorable characteristics.

De Novo Drug Design: Generative models can design novel chemical entities with desired properties, expanding chemical space exploration beyond existing libraries[42].

Binding Affinity Prediction: Deep learning approaches are improving the accuracy of binding affinity predictions, addressing limitations of traditional scoring functions[43].

Despite intensive research efforts, the development of disease-modifying therapies for neurodegenerative disorders has seen more failures than successes:

Alzheimer's Disease: Numerous amyloid-targeting therapies failed in late-stage clinical trials, highlighting the complexity of the disease and potential limitations of the amyloid hypothesis. However, recent approvals of monoclonal antibodies targeting amyloid-β represent potential breakthroughs, albeit with modest clinical benefits[44].

Parkinson's Disease: Despite promising preclinical results, potential disease-modifying agents including coenzyme Q10, creatine, and various growth factors have failed to demonstrate significant clinical benefits in PD patients. These disappointments underscore the challenges in translating mechanistic insights into effective therapies[37].

Huntington's Disease: Antisense oligonucleotides targeting mutant huntingtin showed promise in early clinical studies but faced setbacks in later trials, illustrating the challenges in developing genetic therapies for neurodegenerative disorders[45].

In silico methods offer several distinct advantages in neurodegenerative disease drug discovery:

Cost and Time Efficiency: Computational screening can evaluate millions of compounds in days to weeks, compared to months or years for equivalent experimental screening.

Reduced Ethical Concerns: Virtual approaches minimize the need for animal testing in early discovery phases.
Exploration of Chemical Space: Computational methods can explore vast regions of chemical space not readily accessible through conventional synthesis.
Mechanistic Insights: Molecular modeling provides detailed structural information about protein-ligand interactions, guiding rational optimization strategies.
Integration of Multiple Parameters: Modern in silico approaches can simultaneously consider target binding, BBB permeability, metabolic stability, and other critical parameters.
These advantages make computational drug discovery particularly valuable for neurodegenerative diseases, where traditional approaches have yielded limited success. By efficiently identifying compounds with optimal target engagement and CNS penetration, in silico methods can speed up the development of critically required disease-altering therapies.

2.3 Role of Sirtuins, Especially SIRT2, in PD

Sirtuins have emerged as key regulators of cellular homeostasis with significant implications for neurodegenerative disorders, particularly Parkinson's disease. Their diverse functions in metabolism, stress response, and protein quality control position them as promising therapeutic targets.

Sirtuins are a family of deacetylases that are NAD^+-dependent which remove acetyl groups from lysine residues on various protein substrates, thereby modulating their activity, stability, and interactions. Originally identified in yeast as silent information regulator 2 (Sir2), sirtuins are evolutionarily conserved from bacteria to humans, underscoring their fundamental biological importance[46].
The sirtuin family in mammals includes seven members (SIRT1-7), which vary in their location within the cell, substrate specificity, and enzymatic activities:
SIRT1: Predominantly nuclear, with roles in transcriptional regulation, DNA repair, and metabolism.
SIRT2: Primarily cytoplasmic but shuttles to the nucleus during specific cell cycle phases; functions in cell cycle regulation, microtubule dynamics, and oxidative stress response.
SIRT3, SIRT4, SIRT5: Mitochondrial sirtuins involved in metabolic regulation and oxidative stress management.
SIRT6: Nuclear, regulates DNA repair, telomere maintenance, and glucose homeostasis.
SIRT7: Nucleolar, participates in rRNA transcription and processing.
Beyond deacetylation, certain sirtuins possess additional enzymatic activities, including desuccinylation (SIRT5), demalonylation (SIRT5), and ADP-ribosylation (SIRT4), expanding their regulatory repertoire[47].

SIRT2 is the most abundant sirtuin in the brain, with particularly high expression in myelin-forming oligodendrocytes and postmitotic neurons [48]. Structurally, SIRT2 consists of a core domain of catalytic nature flanked by extensions of N- and C-terminal that regulate its activity and interactions.

Subcellular Localization: It is predominant in cytoplasm during interphase but during G2/M transition, SIRT2 translocates to the nucleus, where it deacetylates histone H4K16 to regulate chromatin condensation. In neurons, SIRT2 localizes to cytoplasmic neurites and growth cones, suggesting roles in neurite outgrowth and synaptic function[49].
Enzymatic Activity: As a robust deacetylase, SIRT2 removes acetyl groups from various substrates including α-tubulin, histones, and transcription factors. This NAD^+-dependent reaction yields deacetylated substrate, nicotinamide, and 2'-O-acetyl-ADP-ribose[50].
Cellular Functions: SIRT2 regulates multiple cellular processes with relevance to neuronal health:

- **Microtubule Dynamics**: Through deacetylation of α-tubulin, SIRT2 modulates microtubule stability, affecting axonal transport, neurite outgrowth, and synaptic plasticity.

- **Cell Cycle Regulation**: SIRT2 controls mitotic progression through interactions with cell cycle regulators, although this function may be less relevant in post-mitotic neurons.
- **Oxidative Stress Response**: By deacetylating transcription factors like FOXO3a, SIRT2 influences the expression of antioxidant enzymes and stress response genes.
- **Metabolism**: SIRT2 regulates glucose and lipid metabolism through effects on insulin signaling and sterol biosynthesis pathways[51].
- **Autophagy**: Recent evidence indicates that SIRT2 modulates autophagic flux, potentially affecting the clearance of protein aggregates in neurodegenerative conditions[52].

SIRT2's expression pattern and functions suggest important roles in both normal neuronal physiology and pathological states:

Developmental Roles: SIRT2 contributes to oligodendrocyte differentiation and myelin formation, processes critical for proper neural circuit establishment.

Aging-Related Changes: SIRT2 expression increases with age, particularly in the brain, potentially contributing to age-related neurological decline[53]. This contrasts with SIRT1, which typically decreases with aging .

Neuropathological Implications: Emerging evidence links SIRT2 dysregulation to various neurodegenerative conditions:

- In Alzheimer's disease, SIRT2 may impair autophagy by promoting microtubule disassembly, thereby hindering the fusion of autophagic vacuoles with lysosomes.
- In Huntington's disease, SIRT2 inhibition reduces mutant huntingtin aggregation and neurotoxicity by modulating sterol biosynthesis pathways.
- In amyotrophic lateral sclerosis, SIRT2 may influence protein aggregation and neuroinflammatory processes, although its precise role remains under investigation[54].

Multiple lines of evidence implicate SIRT2 in PD pathophysiology, particularly through its effects on α-synuclein aggregation and neuronal survival:

α-Synuclein Acetylation and Aggregation: SIRT2 directly interacts with and deacetylates α-synuclein, affecting its aggregation propensity. Decreased SIRT2 levels or activity reduce α-synuclein aggregation in cellular models, suggesting that SIRT2 inhibition may mitigate this key pathological process[55].

Microtubule Dysfunction: By deacetylating α-tubulin, SIRT2 can destabilize microtubules, potentially impairing axonal transport and contributing to neuronal dysfunction. SIRT2 inhibition increases tubulin acetylation, stabilizing the microtubule network and facilitating transport processes essential for neuronal survival[56].

Autophagy Regulation: SIRT2 inhibition enhances autophagic flux, potentially improving the clearance of protein aggregates. In models of α-synuclein aggregation, SIRT2 knockdown decreases the levels of SQSTM1/p62 (an autophagy receptor) and reduces higher-molecular-weight oligomeric species, suggesting enhanced clearance mechanisms[57].

Oxidative Stress Modulation: While SIRT2 can influence oxidative stress responses, its effects appear context-dependent. In some settings, SIRT2 activation promotes antioxidant defense, while in others, SIRT2 inhibition appears protective against oxidative damage[58].

Several experimental studies support the prospect of SIRT2 inhibition as an alternate therapeutic strategy for PD:

Cellular Models: In human neuroglioma cells expressing aggregation-prone α-synuclein, SIRT2 knockdown significantly reduced the percentage of cells with α-synuclein inclusions and increased α-synuclein solubility. These effects correlated with enhanced autophagic clearance of aggregated proteins.

Drosophila Models: Genetic ablation of SIRT2 rescued neurodegeneration in Drosophila expressing human mutant huntingtin, with similar neuroprotective effects observed in models expressing α-synuclein .

Pharmacological Studies: Small-molecule SIRT2 inhibitors, including AGK2 and AK-1, depicted effects of neuroprotective nature in cellular and invertebrate models of neurodegenerative diseases. In Drosophila expressing mutant huntingtin, these compounds significantly improved neuronal survival.

Mechanistic Insights: SIRT2 inhibition appears to exert neuroprotection through multiple mechanisms, including decreased sterol biosynthesis, enhanced autophagy, stabilized microtubule networks, and reduced protein aggregation.

These findings collectively suggest that SIRT2 inhibition displays an optimistic disease-altering strategy for PD, potentially addressing multiple pathological processes simultaneously. However, some studies have reported contradictory results, indicating that SIRT2's role may be context-dependent and that careful consideration of timing, dosage, and specificity will be crucial for therapeutic development.

2.4 Previous SIRT2 Inhibitors: Known Compounds and Limitations

The development of SIRT2 inhibitors has progressed significantly over the past decade, yielding diverse chemical scaffolds with varying potency, selectivity, and pharmacological properties. Understanding the strengths and limitations of existing inhibitors is essential for advancing more effective therapeutic candidates.

Major Classes of SIRT2 Inhibitors

AGK2 and Related Compounds

AGK2, a potent and selective SIRT2 inhibitor, represents one of the earliest compounds specifically developed to target this enzyme:

Chemical Structure: AGK2 features a thiobarbituric acid core with an appended phenyl ring, creating a relatively planar structure that fits within the SIRT2 active site[59].

Binding Mode: While detailed crystallographic data with SIRT2 is limited, biochemical studies suggest AGK2 competes with both the acetylated substrate and NAD^+ cofactor[60].

Potency and Selectivity: AGK2 inhibits SIRT2 with an IC_{50} of approximately 3.5 μM, showing approximately 10-fold selectivity over SIRT1 and minimal activity against SIRT3[61].

Efficacy Data: In PD models of cellular level, AGK2 reduced α-Syn toxicity and protected dopaminergic neurons from MPTP-induced damage. Similar neuroprotective effects were observed in Drosophila models of neurodegeneration[62].

Limitations: Despite its promising activity, AGK2 suffers from limited potency, moderate selectivity, poor aqueous solubility, and unknown BBB permeability, restricting its therapeutic potential[63].

AK-1 and AK-7

These structurally related compounds were developed as alternatives to AGK2 with potentially improved properties:

Chemical Structure: AK-1 and AK-7 contain a sulfobenzoic acid core with various substituents that influence their pharmacokinetic properties[64].

Binding Mode: Like AGK2, these compounds are believed to interact with both substrate and cofactor binding sites, although detailed structural information is lacking[65].

Potency and Selectivity: Both compounds show micromolar potency against SIRT2 with moderate selectivity over other sirtuin isoform[66]s.

Efficacy Data: AK-1 demonstrated neuroprotection in Drosophila models of Huntington's disease, while AK-7 showed improved brain penetration and efficacy in mouse models. Mechanistically, these compounds appear to reduce sterol biosynthesis and protein aggregation[67].
Limitations: While representing improvements over AGK2, these compounds still exhibit suboptimal potency and pharmacokinetic properties for clinical development[68].

SirReal Compounds

The SirReal (Sirtuin-rearranging ligands) series represents a breakthrough in selective SIRT2 inhibition:
Chemical Structure: SirReal2, the prototype of this class, features an aminothiazole core with appended aromatic rings that enable specific interactions with SIRT2[69].
Binding Mode: Crystallographic studies revealed that SirReal2 induces a major conformational change in SIRT2, creating a selectivity pocket not present in other sirtuins. This unique binding mode explains its exceptional selectivity]70\.
Potency and Selectivity: SirReal2 inhibits SIRT2 where the IC_{50} measure remains 140 nM, displaying minimal activity against SIRT1, SIRT3-5, and only slight inhibition of SIRT6 at high concentrations. This places it among the sirtuin inhibitors with highest selectivity reported to date.
Efficacy Data: SirReal compounds effectively increase tubulin acetylation in cellular models and demonstrate antiproliferative effects in cancer cell lines. However, their evaluation in neurodegenerative disease models remains limited[71].
Limitations: Despite excellent potency and selectivity, SirReal compounds have not been extensively characterized for BBB penetration, in vivo efficacy in neurodegeneration models, or detailed pharmacokinetic properties.

Tenovin Compounds

Tenovins were initially identified as p53 activators and subsequently shown to inhibit sirtuins:
Chemical Structure: Tenovin-6, the most studied member, contains a disubstituted pyridinyl core linked to a benzoic acid derivative[59].
Binding Mode: Detailed structural information on tenovin binding to sirtuins remains limited, although biochemical studies suggest competition with both substrate and NAD^+[60].
Potency and Selectivity: Tenovin-6 inhibits both SIRT1 and SIRT2 with IC_{50} values of 21 μM and 10 μM, respectively, showing limited isoform selectivity[61].
Efficacy Data: Tenovins have demonstrated significant anticancer activity through p53 activation and disruption of autophagy. Their potential in neurodegenerative diseases has been less extensively explored[62].
Limitations: The dual SIRT1/SIRT2 inhibition profile may complicate their application in neurodegenerative diseases, where SIRT1 activation is generally considered beneficial. Additionally, their moderate potency and unclear BBB penetration limit their therapeutic potential for CNS disorders[63].

Thiomyristoyl Compounds

These mechanism-based inhibitors represent a distinct approach to sirtuin inhibition:
Chemical Structure: Compounds like TM (thiomyristoyl lysine) mimic the structure of myristoylated lysine, a natural SIRT2 substrate.
Binding Mode: These compounds form a stalled intermediate in the sirtuin catalytic cycle, leading to potent and prolonged inhibition.
Potency and Selectivity: TM shows nanomolar potency against SIRT2 with good selectivity over other sirtuin isoforms[66].
Efficacy Data: TM demonstrates broad anticancer effects by promoting c-Myc degradation. Its potential in neurodegenerative diseases remains to be fully explored.

Limitations: The peptide-like nature of these compounds may limit their BBB penetration and oral bioavailability, potentially restricting their application in CNS disorders.

Despite significant progress, existing SIRT2 inhibitors face several limitations that hinder their clinical development:

Potency Challenges: Many reported inhibitors show only moderate potency (micromolar IC_{50} values), which may be insufficient for achieving therapeutic effects at tolerable doses[69].

Selectivity Issues: With the exception of SirReal compounds, most SIRT2 inhibitors demonstrate limited selectivity over other sirtuin isoforms, particularly SIRT1. This lack of selectivity raises concerns about potential off-target effects, especially since SIRT1 activation (rather than inhibition) is generally considered beneficial in neurodegenerative contexts.

Pharmacokinetic Limitations: Many SIRT2 inhibitors possess suboptimal drug-like properties, including:

- Poor aqueous solubility, limiting formulation options and bioavailability.
- Inadequate BBB penetration, a critical requirement for CNS-targeted therapeutics.
- Rapid metabolism and clearance, necessitating frequent dosing.
- Limited oral bioavailability, complicating long-term administration.

Incomplete Biological Characterization: For many inhibitors, comprehensive evaluation in relevant disease models is lacking, particularly regarding:

- Efficacy in mammalian models of neurodegeneration.
- Long-term safety and tolerability.
- Effects on non-target tissues where SIRT2 plays important physiological roles.

Mechanistic Uncertainties: The precise mechanisms through which SIRT2 inhibition confers neuroprotection remain incompletely understood, complicating rational drug optimization..

These limitations highlight the need for next-generation SIRT2 inhibitors with enhanced properties:

Improved Potency: Developing inhibitors with nanomolar or sub-nanomolar potency would enable therapeutic effects at lower doses, potentially reducing off-target effects.

Enhanced Selectivity: Greater selectivity over other sirtuin isoforms, particularly SIRT1, would minimize unintended consequences and provide cleaner pharmacological tools for dissecting SIRT2's specific roles.

Optimized BBB Penetration: Designing compounds with physicochemical properties conducive to BBB crossing is essential for targeting CNS disorders.

Better Pharmacokinetic Profile: Improved metabolic stability, reduced plasma protein binding, and enhanced bioavailability would support convenient dosing regimens and consistent target engagement.

Reduced Toxicity: Minimizing interactions with off-target proteins and addressing structural features associated with toxicity would improve the safety profile[65].

In silico approaches, including molecular docking and virtual screening, offer powerful tools for identifying novel SIRT2 inhibitors with these improved characteristics. By leveraging structural information about SIRT2 and its interactions with known inhibitors, computational methods can efficiently explore chemical space to discover compounds with optimal target engagement and drug-like properties.

2.5 Molecular Docking in Drug Design

Molecular docking has emerged as a cornerstone of modern drug discovery, enabling the rapid and cost-effective identification of promising compounds for experimental validation. This computational approach predicts how small molecules interact with protein targets, providing valuable insights for rational drug design[72].

2.5.1 Basics and Significance

Definition and Purpose: Molecular docking is an *in-silico* tool that foretells the favoured orientation and conformation (pose) of a molecule (ligand) of small size when it is bound to a large molecular target, typically a protein. The primary goals are to:

- Predict the binding mode of potential drug candidates
- Estimate binding affinity between ligands and targets
- Identify key molecular interactions that drive binding
- Screen large compound libraries for active molecules[73].

Historical Development: Docking methodologies have evolved significantly since their inception in the 1980s, progressing from simple rigid-body approaches to sophisticated flexible algorithms that account for both ligand and protein conformational changes[74].

Theoretical Foundation: Docking is fundamentally an optimization problem seeking to identify the lowest-energy configuration of a protein-ligand complex. This process involves:

- Sampling the conformational space of the ligand within the protein binding site
- Evaluating each pose using a scoring function
- Ranking poses based on predicted binding energy or score[75]

Types of Molecular Interactions: Docking algorithms evaluate various non-covalent interactions that contribute to protein-ligand binding:

- Hydrogen bonding: Directional interactions between hydrogen bond donors and acceptors
- Hydrophobic contacts: Favorable interactions between non-polar groups
- π-π stacking: Interactions between aromatic rings
- Salt bridges: Electrostatic interactions between charged groups
- van der Waals forces: Repulsive or attractive forces of weak strength between atoms[76]

2.5.2 Software Tools and Methodologies

Numerous docking programs have been developed, each with distinct algorithms, scoring functions, and capabilities:

AutoDock and AutoDock Vina:

- Open-source programs widely used in academic research
- AutoDock employs a Lamarckian genetic algorithm for the purpose of searching based on conformation
- Vina employs a gradient optimization method, offering improved speed and accuracy
- Both support flexible ligand docking with optional protein flexibility[77]

Glide (Schrödinger):

- Commercial program with hierarchical filtering approach
- Offers a number of levels of precision: Extra Precision (XP), Standard Precision (SP) and High-Throughput Virtual Screening (HTVS)
- Incorporates water displacement effects and entropy penalties
- Glide WS leverages explicit water dynamics from WaterMap for improved accuracy[78]

GOLD (Genetic Optimization for Ligand Docking):

- Employs a genetic algorithm to traverse the conformational space of the ligand
- Offers multiple scoring functions: GoldScore, ChemScore, ASP, and ChemPLP
- Supports protein flexibility through rotatable side chains and active site water molecules

MOE (Molecular Operating Environment):

- Comprehensive suite including docking, pharmacophore modeling, and QSAR
- Employs a pharmacophore-guided docking approach
- Includes induced-fit protocols to account for protein flexibility[79]

PyRx and SwissDock:

- User-friendly interfaces designed for non-experts
- PyRx integrates AutoDock Vina with visualization tools
- SwissDock provides web-based docking with EADock algorithm[80]

2.5.3 Docking Workflow

A typical molecular docking study involves several key steps:

Protein Preparation:

- Extracting the protein structure from a database named Protein Data Bank (PDB) or via homology modeling
- Adding missing atoms and residues to complete the structure
- Assigning protonation states at the desired pH
- Removing unnecessary water molecules and ligands
- Energy minimization to relieve steric clashes

Binding Site Identification:

- Using co-crystallized ligands to define the binding pocket
- Employing cavity detection algorithms for proteins without known ligands
- Blind docking to spot possible binding sites across the entire surface of the protein

Ligand Preparation:

- Generating appropriate 3D structures with correct stereochemistry
- Assigning atomic charges and other parameters
- Creating multiple conformers to account for flexibility
- Optimizing geometry to ensure reasonable starting conformations

Grid Generation:

- Defining a three-dimensional grid around the binding site
- Pre-calculating potential energy values at grid points to accelerate docking

Docking Calculation:

- Sampling ligand poses within the binding site using search algorithms
- Evaluating poses with scoring functions
- Clustering similar poses to identify distinct binding modes

Post-Docking Analysis:

- Visualizing protein-ligand interactions
- Identifying key binding residues and interaction patterns
- Comparing docking results with experimental data when available
- Selecting compounds for experimental validation[81]

2.5.4 Scoring Functions

Scoring functions are mathematical models that estimate the binding affinity between proteins and ligands:

Force Field-Based Functions:

- Perform the summation of physical interaction energies (electrostatic, van der Waals)
- Often include solvation terms to account for desolvation effects
- Examples: AMBER, CHARMM, OPLS

Empirical Scoring Functions:

- Use weighted terms derived from experimental binding data
- Include factors like hydrogen bonding, hydrophobic interactions, and entropic penalties
- Examples: ChemScore, GlideScore, X-Score

Functions based on knowledge:

- Analyze the known protein-ligand complexes statistically to derive potentials
- Based on the frequency of specific atom-pair interactions
- Examples: PMF, DrugScore, ITScore

Consensus Scoring:

- Combine multiple scoring functions to compensate for individual weaknesses
- Often improves enrichment in virtual screening campaigns
- Examples: X-CSCORE, MultiScore[82]

2.5.5 Docking Protocol Validation

Ensuring the reliability of results from docking interactions requires rigorous validation:

Redocking Experiments:

- Removing a co-crystallized ligand and docking it back into the binding site
- Calculating the value of Root Mean Square Deviation (RMSD) between the two poses, i.e., predicted and experimental poses
- RMSD < 2 Å typically indicates successful reproduction of the binding mode

Cross-Docking:

- Docking ligands into same protein structures bound to distinct ligands
- Assesses the ability to account for protein flexibility and induced-fit effects

Enrichment Studies:

- Docking a mixed set of active compounds that are known and presumed inactives (decoys)
- Calculating enrichment factors to compute the ability to discriminate actives from inactives
- Metrics include ROC curves, early enrichment factors and area under the curve (AUC)

Correlation with Experimental Data:

- Comparing predicted binding scores with experimental binding affinities
- Calculating correlation coefficients (R^2, Spearman's ρ) to assess predictive power[83]

2.5.6 Limitations and Challenges

Despite its utility, molecular docking faces several important limitations:

Protein Flexibility:

- Most docking programs treat the protein as largely rigid, neglecting significant conformational changes upon ligand binding
- Induced-fit effects can dramatically alter the binding site geometry
- Methods like ensemble docking and induced-fit protocols partially address this issue but increase computational cost[84]

Water Molecules:

- Explicit molecules of water often play crucial roles in the binding of protein and ligand
- Most docking algorithms oversimplify or ignore water-mediated interactions
- Advanced methods like WaterMap improve water handling but add complexity[85]

Scoring Function Accuracy:

- Current scoring functions struggle to accurately rank compounds by binding affinity
- Entropy contributions are particularly difficult to estimate
- False positives and false negatives remain common in virtual screening[86]

Binding Kinetics:

- Docking typically focuses on equilibrium binding (thermodynamics) rather than association/dissociation rates (kinetics)
- Drug efficacy often depends on binding kinetics, particularly residence time[87]

Allosteric Effects:

- Traditional docking focuses on orthosteric binding sites, potentially missing allosteric opportunities
- Allosteric binding often involves more significant protein conformational changes

2.6 Research Gaps and Study Rationale

Despite significant advances in understanding PD pathophysiology and SIRT2 biology, several critical knowledge gaps remain that justify the current study:

While existing SIRT2 inhibitors have demonstrated promising effects in preclinical models, they face significant limitations:
Insufficient Potency and Selectivity: Most current inhibitors exhibit only moderate potency (micromolar range) and limited selectivity over other sirtuin isoforms, particularly SIRT1. This raises concerns about potential off-target effects, especially since SIRT1 activation is generally considered beneficial in neurodegenerative contexts[88].
Suboptimal Pharmacokinetic Properties: Many existing inhibitors possess poor drug-like characteristics, including limited aqueous solubility, inadequate BBB penetration, and unfavorable metabolic profiles . These properties restrict their potential clinical application for CNS disorders[89].
Incomplete Biological Characterization: For many inhibitors, comprehensive evaluation in relevant disease models is lacking, particularly regarding efficacy in mammalian models of neurodegeneration, long-term safety, and effects on non-target tissues[90].

The chemical diversity of known SIRT2 inhibitors remains relatively limited, with most compounds belonging to a few structural classes:
Restricted Scaffold Exploration: Despite the availability of multiple SIRT2 crystal structures, systematic exploration of diverse chemical scaffolds through structure-based approaches has been limited[91].
Focus on Conventional Binding Modes: Most inhibitor design efforts have targeted the canonical substrate and NAD^+ binding sites, with less attention to unique structural features like the selectivity pocket revealed by SirReal compounds.
Limited Natural Product Investigation: Natural products and their derivatives, which often possess unique structural features and biological activities, remain underexplored as potential SIRT2 inhibitors[92].

The precise mechanisms through which SIRT2 inhibition confers neuroprotection in PD remain incompletely understood:
Substrate Specificity: While α-tubulin and α-synuclein are established SIRT2 substrates relevant to PD, the broader substrate landscape and its implications for therapeutic targeting are not fully characterized.
Pathway Integration: How SIRT2 inhibition affects interconnected pathways involved in PD pathogenesis, including protein aggregation, mitochondrial function, and neuroinflammation, requires further elucidation.
Temporal Considerations: The optimal timing for SIRT2 inhibition in the disease course remains unclear, as does the potential for differential effects depending on disease stage.

Bridging the gap between preclinical findings and clinical application presents significant challenges:
Model Limitations: Existing cellular and animal models incompletely recapitulate the complex, multifactorial nature of human PD, complicating the prediction of clinical efficacy.
Biomarker Development: Reliable biomarkers for target engagement and therapeutic response remain limited, hampering clinical trial design and execution[88][90].

Patient Heterogeneity: The heterogeneous nature of PD, with varying clinical presentations and underlying pathologies, suggests that SIRT2 inhibition may benefit specific patient subgroups more than others.

Given these research gaps, this study employs computational approaches to find out novel inhibitors of SIRT2 with improved properties for potential PD therapy:
Leveraging Structural Insights: By utilizing available SIRT2 crystal structures and molecular docking techniques, this study aims to identify compounds that exploit unique structural features for enhanced potency and selectivity.
Exploring Diverse Chemical Space: Virtual screening of compound libraries that are large and diverse enables the recognition of new scaffolds beyond those previously explored, potentially leading to inhibitors with improved pharmacological properties.
Prioritizing CNS-Penetrant Compounds: Incorporating filters for BBB permeability and favorable CNS drug-like properties addresses a key limitation of existing inhibitors4.
Structure-Based Optimization: Detailed analysis of protein-ligand interactions guides the rational optimization of hit compounds for improved target engagement while maintaining drug-like characteristics.
Providing Foundation for Experimental Validation: The computational predictions generated in this study will inform subsequent experimental testing, accelerating the development of inhibitors of SIRT2 with therapeutic potential of disease-altering nature for PD.

By addressing the research gaps through systematic i*n-silico* approaches, this study aims to advance the development of SIRT2 inhibitors as probable disease-altering therapies for Parkinson's, ultimately contributing to improved treatment options for this devastating neurodegenerative disorder.

The current study addresses significant research gaps by employing in silico methods to identify novel SIRT2 inhibitors with improved properties for potential PD therapy. By leveraging structural insights, exploring diverse chemical space, and prioritizing CNS-penetrant compounds, this work aims to further the growth of disease-altering treatments for this devastating neurodegenerative disorder.Upcoming research should aim to focus on wet lab validation of computational predictions, detailed characterization of mechanism of action, optimization of pharmacokinetic properties, and ultimately translation to clinical applications. The amalgamation of *in-silico* and wet lab approaches, coupled with deeper understanding of disease mechanisms, holds promise for developing effective therapies which have the potential to alter the course of Parkinson's and provide better patient outcomes.

3. METHODOLOGY

3.1. Retrieval of human SIRT2 protein complex

For the purpose of all *in-silico* computations a three dimensional structure of human SIRT2 bound SirReal2 and NAD+ was obtained. We got it from a database named Protein Data Bank (PDB) (https://www.rcsb.org/) using 4RMG as its code. This particular compound structure on PDB was obtained via X-ray diffraction at a resolution of 1.88Å [93].

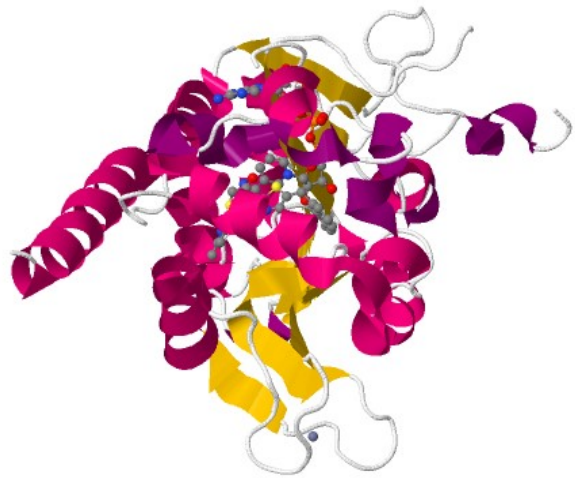


Fig.1. Human Sirt2 in complex with SirReal2 and NAD+ (4RMG)

3.2. Preparation of target protein

The file downloaded from PDB database was in PDB format. AutoDockTools-1.5.7 as a part of MGL Tools was then used to get rid of the water molecules and heteroatoms from the structure, add polar hydrogens and charges along with removing the native ligand from the structure (SirReal2 in this case) [94]. The structure was then saved in PDBQT format.One of the major strengths of AutoDock is the flexibility it offers. Linear regression analysis serves as the basis for its free-energy scoring function [95].

3.3. Selection of ligands

● Reference selection:There are a few SIRT2 inhibitors which have a selective nature that are situated in the hydrophobic pocket close to zinc-binding domain. One of those inhibitors is SirReal2. This co crystallized ligand and inhibitor was thus chosen as the reference [93].

● Database mining: SwissSimilarity is an online tool (http://www.swisssimilarity.ch/) aimed at screening several libraries of small molecules virtually in a ligand based fashion. SwissSimilarity aids in high throughput screening (HTS). This tool was used to mine through ZINC_drug library via structure similarity search based on SirReal2 as query molecule. SMILES was used as the format for query molecule.

● Ligand selection based on ADME analysis: In the term ADME, letter A stands for absorption, D is for distribution, M is for metabolism and E stands for excretion. These criteria play a vital role during research and manufacturing of pharmaceutical compounds. There are some major factors that need to be considered during drug development namely BBB permeability, Lipinski rule of five, PAINS and Brenk. These factors are set as standard requirements which need to be met by the ligands in order to be a part of this study to further carry out molecular docking. SwissADME (http://www.swissadme.ch/) is an internet based tool that was employed to carry out this assessment. These screened compounds were then imported to SwissADME and BBB permeability, Lipinski violations, PAINS and Brenk filters were used which resulted in shortlisted compounds. These compounds were further used for docking analysis.

3.4. Preparation of ligands

The 3D structures of all the shortlisted ligands alongwith the reference ligand - SirReal2 were extracted from a database named PubChem (https://pubchem.ncbi.nlm.nih.gov/) in a format named SDF. These structures were then changed in PDBQT format with the use of OpenBabel GUI.

3.5. Molecular docking

EasyDockVina 2.2 was used to dock SirReal2 and shortlisted compounds with the target protein [96]. EasyDockVina is an easy-to-use and relatively simple program. This tool is designed to allow multiple ligand docking against a particular protein. The directory of folder containing the target protein was entered followed by the directory of folder containing the ligands. A grid box with the location of its center at x = -16.27, y = -26.36 and z = 0.41 was created with the dimensions of 50 Å x 50 Å x 50 Å. Separate output files for each ligand in PDBQT format along with an excel file containing binding affinities of all the ligands was generated as a result of docking.

3.6. Protein-ligand complex analysis

BOVIA Discovery Studio is a software suite developed by Dassault Systems BIOVIA [97]. Discovery Studio 2021 Client was used for the creation of 2D and 3D confirmations of ligand interactions. The ligand output file and target protein file were both in PDBQT format. A structural visualization of the reference ligand - target protein interaction as well as interactions of filtered ligands with protein were carried out followed by its extraction in both 2D and 3D formats. Interacting residues and bonds were major focus of observation.

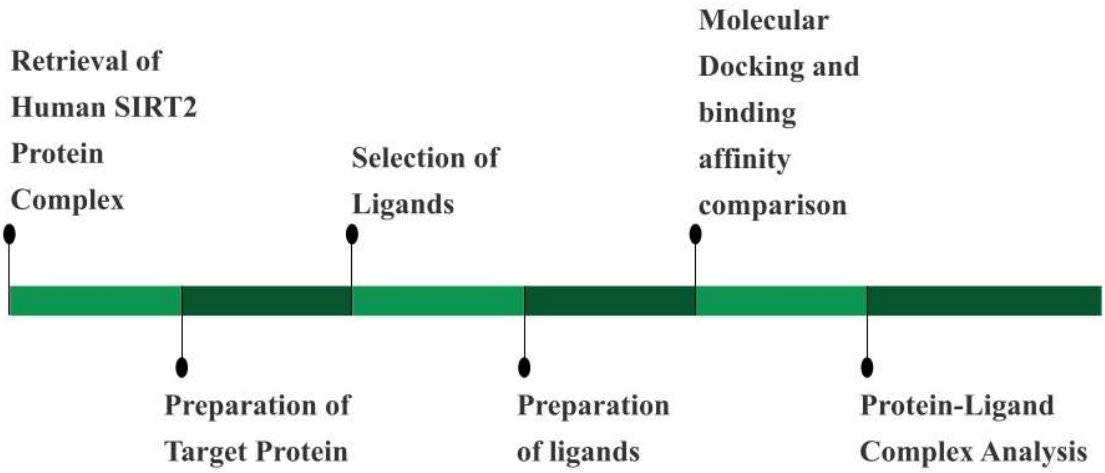


Fig.2. Workflow depicting methodology of molecular docking

4.RESULTS AND DISCUSSION

4.1. Initial screening and ADME analysis

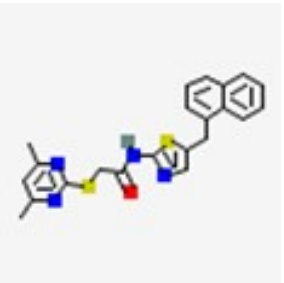
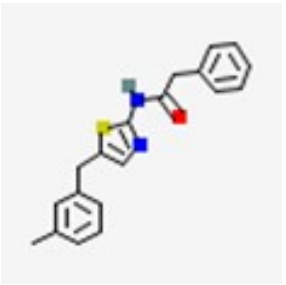
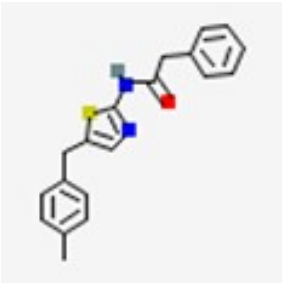
Compounds were shortlisted by SwissSimilarity on the basis of structural similarity. As a result of this search, 400 compounds were obtained which had similarity score above 0.85. These compounds were further filtered through ADME analysis using certain filters like BBB permeability, Lipinski, PAINS and Brenk. This ADME analysis was performed with the help of SwissADME. All the 79 compounds shortlisted were BBB permeable, had zero Lipinski violations, no PAINS alerts and no Brenk alerts.

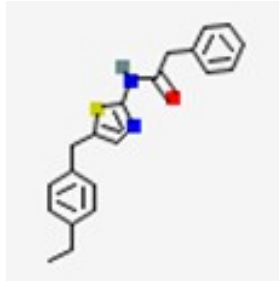
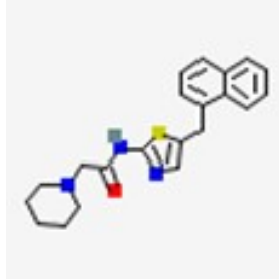
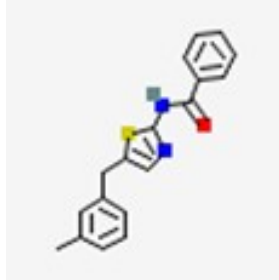
4.2. Docking studies

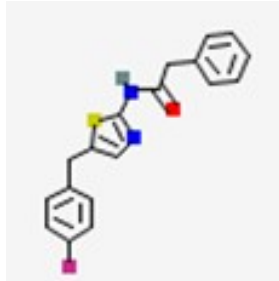
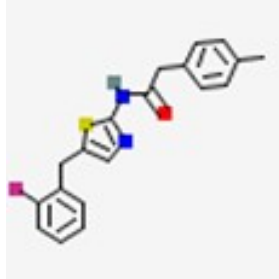
Docking analysis of these 79 compounds provided us with the final seven compounds which had binding affinity more than that of the reference, i.e., SirReal2. The binding affinity of SirReal2 was found to be -

10.8 in kcal/mol. The affinities of binding of these compounds span from -10.9 in kcal/mol to 11.4 in kcal/mol. The docking outcome of Compound 7 with PubChem CID 1664850 demonstrated the largest binding affinity of -11.4 in kcal/mol (Table I).

Table 1:Tabular Representation of 2D Chemical Structures, Binding Affinities and Interactions of Ligands

Compound. No.	PubChem CID	2D Chemical Structure	Binding affinity (kcal/mol)	Interacting residues
Reference	1096292 (SirReal2)		-10.8	Ile232,Phe96, Ile93,Ile169, Pro94.Ala13, Leu138,Leu134, Phe131,Phe234
Compound 1 (*N*-[5-[(3-methylphenyl)methyl]-1,3-thiazol-2-yl]benzamide)	726222		-10.9	Phe119.Ile232, Phe234,Leu138, Phe190,Pro140, Tyr139
Compound 2 (*N*-[5-[(4-fluorophenyl)methyl]-1,3-thiazol-2-yl]-2-phenylacetamide)	779430		-10.9	Phe190,Phe96, Phe119,Phe234, Val233,Ile232, Leu138,Ala135, Leu134,Pro140, Phe143, Tyr139

Compound 3 (2-methyl-*N*-[5-[(4-propan-2-ylphenyl)methyl]-1,3-thiazol-2-yl]propanamide)	2225706		-11.1	Ile232,Tyr139, Phe143,Leu206, Pro140,Phe190, Leu138,Phe96, Ala135,Phe119, Phe234
Compound 4 (*N*-[5-(naphthalen-1-ylmethyl)-1,3-thiazol-2-yl]-2-piperidin-1-ylacetamide)	1527166		-11.1	Ile169,Ile232, Phe190,Pro140, Tyr139,Phe143, Ala135,Leu134, Leu138,Phe96, Phe234
Compound 5 (N-[5-[(3-methylphenyl)methyl]-1,3-thiazol-2-yl]-2-phenylacetamide)	726227		-11.2	Tyr139,Phe143, Phe190,Pro140, Phe234,Ile232, Phe96,Leu134, Leu138,Ala135

Compound 6 (N-[5-[(4-methylphenyl)methyl]-1,3-thiazol-2-yl]-2-phenylacetamide)	724160		-11.2	Ile232,Phe96, Tyr139,Phe143, Leu206,Pro140, Phe90,Leu138, Ala135,Phe234, Phe119
Compound 7 (N-[5-[(2-fluorophenyl)methyl]-1,3-thiazol-2-yl]-2-(4-methylphenyl)acetamide)	16648650		-11.4	Ile118,Phe119, Phe96,Ala135, Leu134,Leu138, Leu206,Phe143, Tyr139,Pro140, Phe190,Phe234, Ile232

4.3. Visualization of docked ligands

BIOVIA Discovery Studio 2021 client was utilized for the visualization of 3D and 2D ligand interactions. Interacting residues were observed for each of the seven compounds as well as the reference (Table I). The 2D ligand interactions of SirReal2 with the target protein are displayed in Fig.3. Remaining figures are 2D representations of interactions between the seven filtered compounds and target protein. Interaction between Compound 1 and the receptor is shown in Fig.4. whereas Fig.5. shows the receptor binding with Compound 2. SIRT2 binds with Compound 3 and Compound 4 in Fig.6. and Fig.7. respectively. Fig.8. represents Compound 5 binding with receptor molecule. Interaction of Compound 6 and Compound 7 with SIRT2 is depicted in Fig.9. and Fig.10. respectively.

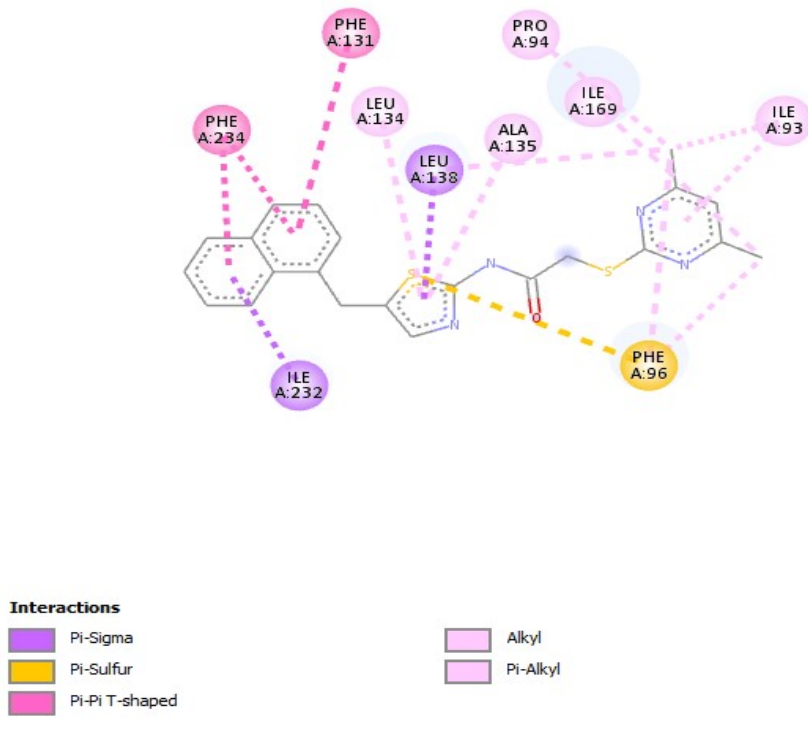


Fig.3. Interactions of SirReal2

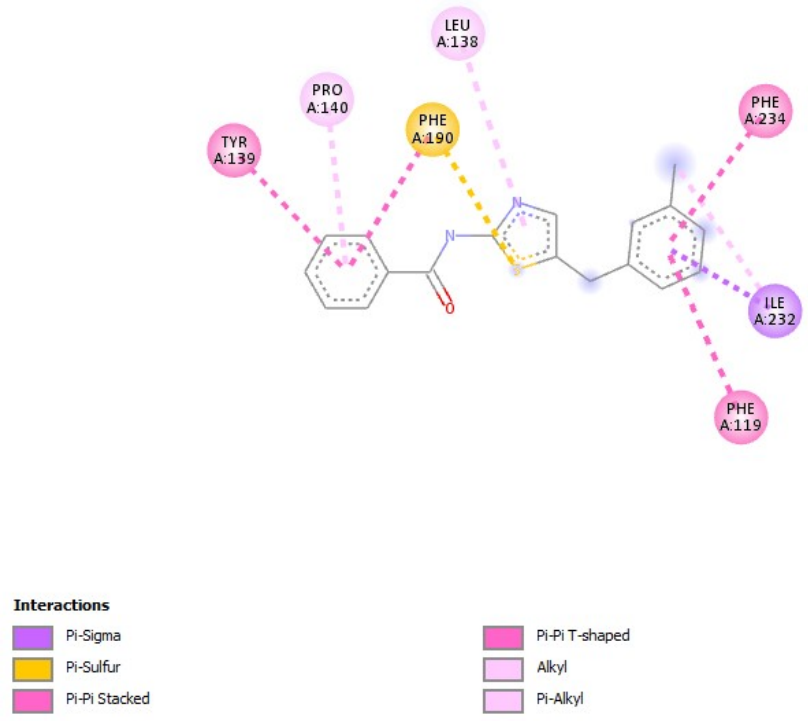


Fig.4. Interactions of Compound 1 (*N*-[5-[(3-methylphenyl)methyl]-1,3-thiazol-2-yl]benzamide)

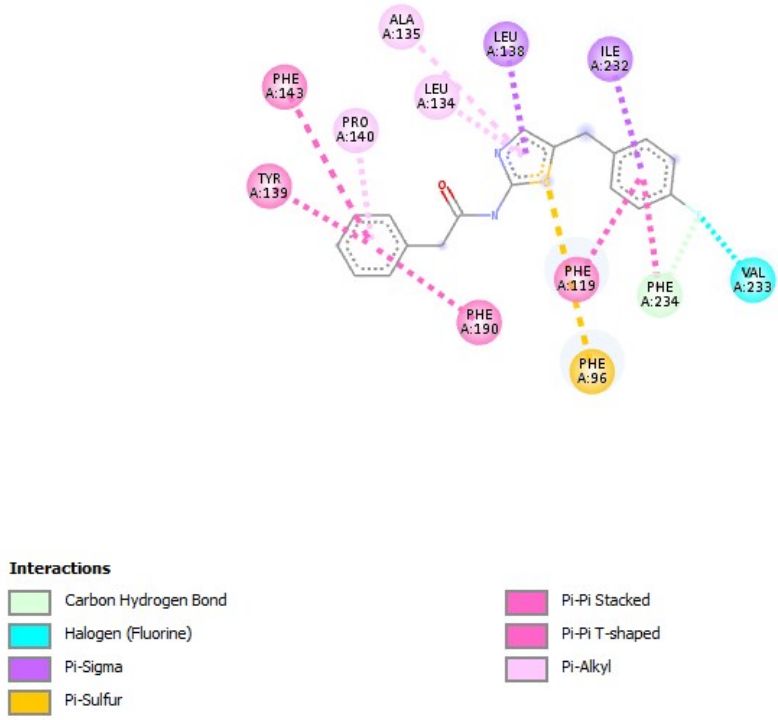


Fig.5. Interactions of Compound 2 (N-[5-[(4-fluorophenyl)methyl]-1,3 thiazol-2-yl]-2-phenylacetamide)

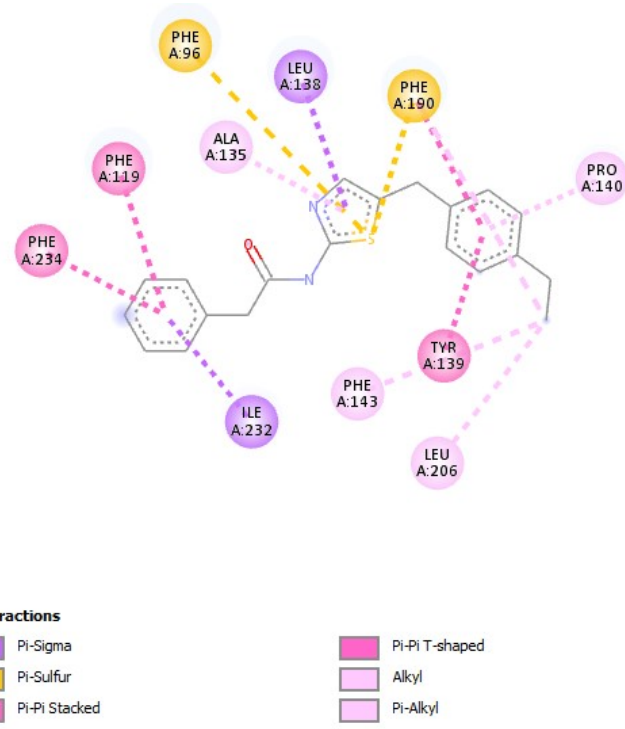


Fig.6. Interaction of Compound 3 (2-methyl-N-[5-[(4-propan-2 ylphenyl)methyl]-1,3-thiazol-2-yl]propanamide)

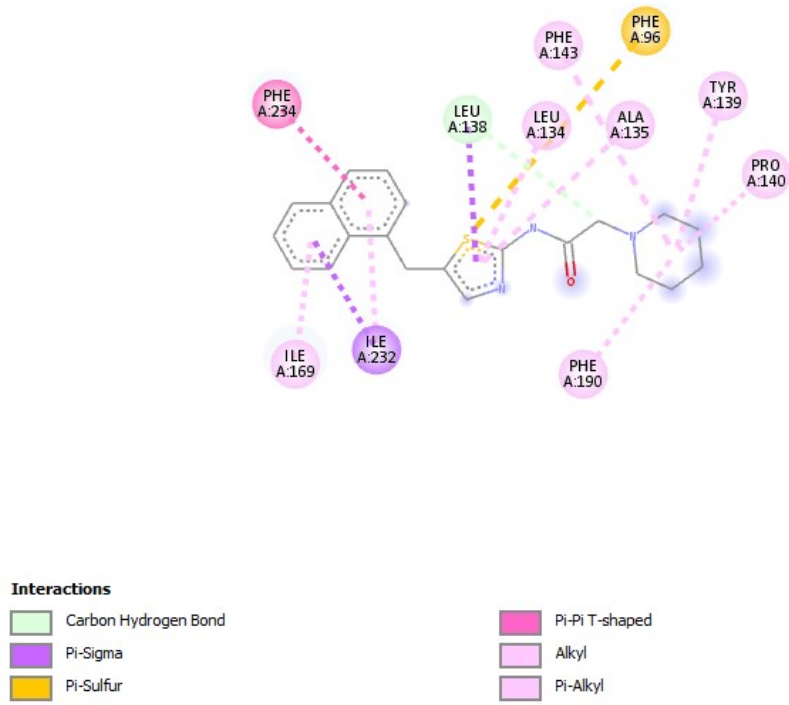


Fig.7. Interactions of Compound 4 (N-[5-(naphthalen-1-ylmethyl)-1,3 thiazol-2-yl]-2-piperidin-1-ylacetamide)

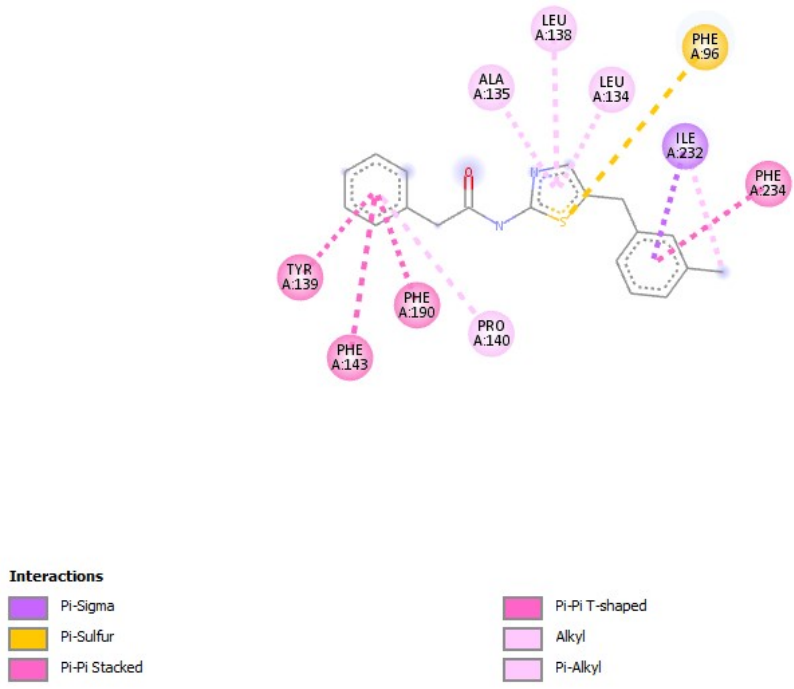


Fig.8. Interactions of Compound 5 (N-[5-[(3-methylphenyl)methyl]-1,3 thiazol-2-yl]-2-phenylacetamide)

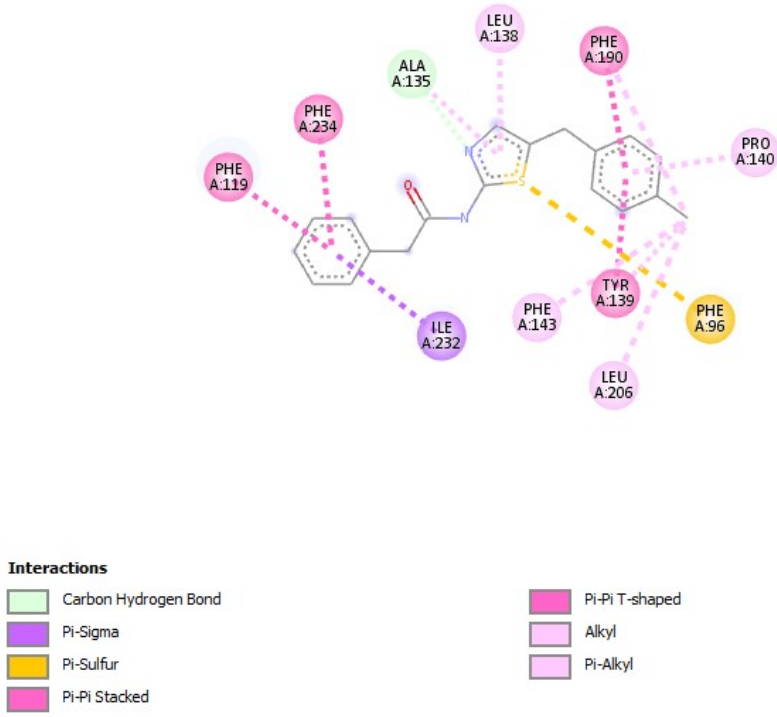


Fig.9. Interactions of Compound 6 (N-[5-[(2-fluorophenyl)methyl]-1,3 thiazol-2-yl]-2-(4-methylphenyl)acetamide)

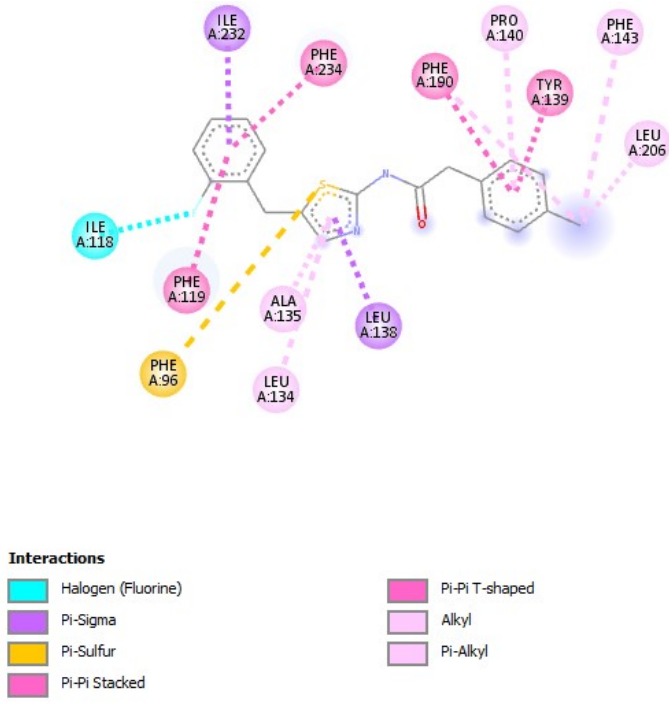


Fig.10. Interactions of Compound 7 (N-[5-[(2-fluorophenyl)methyl]-1,3 thiazol-2-yl]-2-(4-methylphenyl)acetamide)

4.4. Detailed ADME analysis

The results of SwissADME analysis for these seven compounds depicted favourable observations for multiple drug-likeliness factors. All these compounds were BBB permeable with zero Lipinski violations rendering them useful for drug development. There were zero PAINS alerts for all seven of these compounds which meant that chances of false positives were considerably less. Zero Brenk alerts also solidified this observation which was consistent with all seven compounds. Further ADME evaluation utilized filters like TPSA value, GI absorption. Consensus log P value and logKp value which were also considered to aid in determining the viability of the results (Table II). In this study, molecular docking as a method of in-silico analysis was aimed at studying interactions between target protein and ligands at the atomic level. Docking uses computational methods for ligand-protein complex prediction [98]. This study was successful in its aspect to find a replacement for SirReal2 as seven compounds with better binding affinities were discovered.

Table 2. ADME Analysis of Compound 1-7

S. No	BBB permeable	Lipinski violation	TPSA value	Consensus logP	Gastrointestinal absorption (GI)	log Kp (cm/s)
1	Yes	0	High	3.9	High	-5.03
2	Yes	0	High	3.91	High	-5.37
3	Yes	0	High	4.24	High	-4.94
4	Yes	0	High	3.77	High	-5.28
5	Yes	0	High	3.93	High	-5.16
6	Yes	0	High	3.96	High	-5.16
7	Yes	0	High	4.25	High	-5.2

5. CONCLUSION

This study has demonstrated the power and potential of *in silico* drug discovery for neurodegenerative diseases by identifying seven novel SIRT2 inhibitors with superior binding affinities and drug-like properties compared to established reference compounds, most notably SirReal2. Employing a comprehensive computational pipeline that integrated molecular docking, ADME property prediction, and AI-driven virtual screening, the research screened a diverse library of drug-like molecules and pinpointed candidates with optimal pharmacological profiles. The lead compound, **N-[5-[(2-fluorophenyl)methyl]-1,3-thiazol-2-yl]-2-(4-methylphenyl)acetamide**, exhibited a binding energy of -11.4 kcal/mol, surpassing SirReal2 (-10.8 kcal/mol) and forming strong interactions with key residues in SIRT2's hydrophobic selectivity pocket, including Ile232, Phe234, and Tyr139. Notably, all shortlisted compounds demonstrated the ability to pass through the BBB, an essential requirement for drug development targeting CNS and a major shortcoming of many previously reported SIRT2 inhibitors such as AGK2 and Tenovin-6. Additionally, these candidates showed no violations of Lipinski's rule of five and were free from PAINS and Brenk alerts, indicating a low risk of off-target effects and favorable drug-likeness.

The findings from this study reinforce the therapeutic promise of SIRT2 inhibition as a disease-altering strategy for PD, targeting both α-Syn aggregation and microtubule instability-two interrelated processes that underlie dopaminergic neuron loss and disease progression. By stabilizing SIRT2's conformation and potentially blocking NAD^+-dependent deacetylation of pathological substrates, these compounds may offer neuroprotective benefits that go further than symptomatic relief, addressing the molecular mechanisms of PD. While the computational predictions are robust and supported by detailed structural and pharmacokinetic analyses, it is acknowledged that experimental validation through *in vitro* enzymatic assays, cellular neuroprotection studies, and *in vivo* efficacy testing in animal models remains essential to confirm these results and further refine the candidates' safety and selectivity profiles. Moreover, future research should include molecular dynamics simulations to capture protein flexibility and induced-fit effects, as well as broader selectivity screening against the sirtuin family and other NAD^+-dependent enzymes to minimize off-target risks. In summary, this work not only advances the field of SIRT2-targeted therapeutics for Parkinson's disease but also exemplifies the efficiency and impact of *in-silico* drug discovery in speeding up the recognition of promising lead compounds for complex CNS disorders. The insights and candidates generated here lay the groundwork for subsequent experimental and clinical development, with the ultimate goal of delivering disease-altering therapeutics that can slow or halt the progression of Parkinson's.

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List of Publications

1. Conference paper:

Apurva and P. Kumar, "In silico Analysis for Potential SIRT2 Inhibitors," 2024 2nd International Conference on Advances in Computation, Communication and Information Technology (ICAICCIT), Faridabad, India, 2024, pp. 474-479, doi: 10.1109/ICAICCIT64383.2024.10912162. keywords: {Drugs;Technological innovation;Costs;Toxicology;Inhibitors;Medical treatment;Machine learning;Compounds;Zinc;Diseases;SIRT2;Parkinson's disease;HDAC;α-Synuclein;SirReal2;ZBD;ADME;Binding affinity;Ligand interactions;AI;ML;in silico analysis;computational tools},

2. Poster:

Apurva[1], Pravir Kumar[1], "Analysis for potential SIRT2 inhibitors through *in silico* assay: Exploring novel strategies for Parkinson's therapy"

Presented at: SNCI, Jamia Hamdard, New Delhi

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CERTIFICATE

---------- OF PARTICIPATION ----------

This is to certify that Apurva of Delhi Technological University, New Delhi has successfully presented a paper titled In Silico Analysis For Potential Sirt2 Inhibitors

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28th -29th November 2024

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Abstract:
SIRT2 is positioned second in class III HDAC family of sirtuins and NAD+ (nicotinamide adenine dinucleotide)dependent in nature. SIRT2 plays a crucial role in neurodegeneration leading to diseases like Parkinson's disease (PD). Therefore, the need to discover SIRT2 inhibitors aligns itself with potential therapeutic developments for treating PD. As a result of SIRT2 inhibition, α-synuclein (α-Syn) induced toxicity decreases leading to reduction in cell death. SirReal2 is a native SIRT2 inhibitor. It is situated in the selectivity pocket which is hydrophobic in nature present near the zinc binding domain (ZBD). SirReal2 was taken as the reference ligand for this study. The primary objective was to shortlist better suited compounds for SIRT2 inhibition as potential drug components for Parkinson's therapy in comparison to what was deduced in previous such studies. Here, a large number of compounds were shortlisted on the basis of structural similarity to SirReal2 which were filtered by ADME analysis. As the next step docking studies were performed to generate binding affinities. This investigation revealed seven such compounds with affinities higher than SirReal2. Extended ADME analysis strengthened the fact that these drug like commercial compounds from ZINC database displayed greater viability for advancement in the pharmacological field. In contrast with the previous studies on this matter, these probable inhibitors were in fact capable of crossing the blood brain barrier making them a better choice. In silico analysis incorporates artificial intelligence (AI) via machine learning (ML) as a part of various computational tools used in the study. Such analysis of compounds can significantly reduce the time and cost of wet lab research. Conclusively, results from this study strongly advocate for the analysis of these probable inhibitors inside living systems.
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SECTION I.
Introduction
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This is to certify that Prof./Dr./Ms./Mr. Ms. Apurva
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Education

- AUG 2023 - JUN 2025 (Expected)
  M. Sc Biotechnology, Delhi Technological University
  CGPA (Aggr.) - 9.72/10
- NOV 2020 - JUN 2023
  B. Sc Life Sciences, Daulat Ram College, University of Delhi
  CGPA - 8.73/10

Skills and Interests

· Molecular Docking · MEGA X · Gene sequence data analysis(data curation,phylogenetic construction etc.) · Primer design · Cell culturing · Advanced writing and researching sk communication skills

SDPs, Workshops and Internships

[1.] March 23, 2023 to April 08, 2023
Host institute - Daulat Ram College
Supervisor - Dr. Jyoti Singh
Topic - In-House Skill Development Program on Unveiling the Animal World in Behavioural way which included skills like rearing aphid colony, handling model organisms like rat/mice etc.

[2.] Date - October 28, 2023 and October 29, 2023
Conducted by - Ethical Edufabrica Pvt. Ltd.
Host institute - Delhi Technological University
Topic - Offline Workshop on Molecular Biology and Biochemistry Techniques including BLAST, Sanger sequencing, Primer design and Gene sequence data analysis (Data curation, Phylogenetic tree construction etc.)

[3.] December 21, 2023 to January 04, 2024
Host institute - Environmental and Industrial Biotechnology lab, Delhi Technological University
Supervisor - Prof. Jai Gopal Sharma
Topic - Internship and hands-on training involving culturing of *Bacillus brevis* for microplastic degradation, HPLC etc.

[4.] Date - October 2024
Platform - Udemy
Instructor - Muhammad Dujana
Topic - Online certification course titled "Bioinformatics; Learn Docking & Mol Dynamics Simulation"

[5.] Date - April 17, 2025 to April 15,2025
Host Institute - Jamia Hamdard
Organized by - Society for Neurochemistry India (SNCI) – Delhi Local Chapter
Topic - "Translational Neurochemistry: Bridging Basic Science and Clinical Applications"
- Hands-on training in cell culture, western blotting, and model organisms
- Visits to zebrafish and high-end neurobiology facilities
- Lectures by experts from IIT Delhi, AIIMS, NBRC, NII, and more
- Focused sessions on translational tools and therapeutic applications

List of Publications

1. Conference paper:

Apurva and P. Kumar, "In silico Analysis for Potential SIRT2 Inhibitors," 2024 2nd International Conference on Advances in Computation, Communication and Information Technology (ICAICCIT), Faridabad, India, 2024, pp. 474-479, doi: 10.1109/ICAICCIT64383.2024.10912162. keywords: {Drugs;Technological innovation;Costs;Toxicology;Inhibitors;Medical treatment;Machine learning;Compounds;Zinc;Diseases;SIRT2;Parkinson's disease;HDAC;α-Synuclein;SirReal2;ZBD;ADME;Binding affinity;Ligand interactions;AI;ML;in silico analysis;computational tools},

2. Poster:

Apurva[1], Pravir Kumar[1], "Analysis for potential SIRT2 inhibitors through *in silico* assay:
Exploring novel strategies for Parkinson's therapy"

Presented at: SNCI, Jamia Hamdard, New Delhi