MASTER OF SCIENCE (BIOTECHNOLOGY) [ ANIMAN TRIPATHI ] 2022

# IDENTIFICATION OF PUTATIVE INHIBITORS OF MUTANT LRRK2 INVOLVED IN THE PATHOGENESIS OF PARKINSON'S DISEASE: AN IN-SILICO DRUG RE-PURPOSING APPROACH

A DISSERTATION

SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS

FOR THE AWARD OF THE DEGREE

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MASTER OF SCIENCE

IN

**BIOTECHNOLOGY**


Submitted By:

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**MAY, 2022**

## CANDIDATE'S DECLARATION

I**, Animan Tripathi,** (Roll No.: 2K20/MSCBIO/01) of **M.Sc. Biotechnology,** declare that this work which is presented in this Major Project titled "**Identification of putative inhibitors of mutant LRRK2 involved in the pathogenesis of Parkinson's disease: An *in-silico* drug-repurposing approach**" submitted 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, is original and my own, carried out during a period from 7th January, 2022 to 6th May, 2022, under the supervision of **Prof. Pravir Kumar**.

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Abstract
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**Abstract:**
One of the most common causes of Parkinson's disease are mutations in the enzyme LRRK2 or leucine-rich-repeat kinase 2. Out of all known mutations in this enzyme, the G2019S mutation is the one most commonly studied. This mutation greatly increases the kinase activity and phosphorylates many molecules of an apoptotic nature. We curated a list of 225 FDA approved drugs for the purpose of re-positioning them as an inhibitor for the aberrant LRRK2. Literature presents a substantial correlation between the pathologies of cancer, diabetes, cardiovascular disorders and Parkinson's. Thus, drugs targeting these diseases can be effectively re-purposed for PD on account of shared pathologies. Through molecular docking analysis we identified venetoclax, an anti-cancer drug, as a potent inhibitor of both wild-type LRRK2 and the G2019S mutant. Further analysis showed that venetoclax also inhibits Hsp90, which has a marked anti-apoptotic effect because it targets the enzyme LRRK2 for proteasomal degradation.

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On a more personal note, I express my gratitude towards my family and friends for their love, support and good wishes.

**Animan Tripathi**
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## ABSTRACT

One of the most common causes of Parkinson's disease are mutations in the enzyme LRRK2 or leucine-rich-repeat kinase 2 or LRRK2. Out of all known mutations in this enzyme, the G2019S mutation is the one commonly studied. This mutation greatly increases the kinase activity and phosphorylates many molecules of an apoptotic nature.

In this study we curated a list of various FDA-approved anti-cancer, anti-diabetic and anti-hypertensive drugs to re-purpose them against Parkinson's. Literature shows a correlation in the pathologies of cancer, diabetes, cardiovascular disorders and PD. Thus, these drugs can therefore be repurposed on account of the disease's shared pathology.

By using a molecular docking approach, we identified Venetoclax, a Bcl-2 inhibitor prescribed for the treatment of cancer, as a very potent inhibitor of the G2019S mutant of LRRK2. On further analysis, it showed that Venetoclax, in addition to the G2019S mutant also inhibits Hsp90 (a part of the CHIP ubiquitin E3-ligase system) thereby possessing dual-functionality.

This inhibition of two targets has marked anti-apoptotic effects as it not only helps control the aberrant kinase activity of the G2019S mutant, it also increases the clearance of the mutant LRRK2 through the ubiquitin-proteasome pathway.

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## LIST OF SYMBOLS, ABBREVIATIONS AND NOMENCLATURE

| S.No, | Abbreviation | Full Form |
|---|---|---|
| 1 | LRRK2 | Leucine-rich repeat kinase 2 |
| 2 | FDA | Food and Drug Administration |
| 3 | PD | Parkinson's disease |
| 4 | Bcl-2 | B-cell lymphoma 2 protein |
| 5 | Hsp90 | Heat-shock protein 90 |
| 6 | CHIP | Carboxy-terminus of Hsc 70 interacting protein |
| 7 | E3-ligase | Enzyme 3 ligase |
| 8 | UCHL1 | Ubiquitin carboxy-terminal hydrolase isozyme L1 |
| 9 | SNCA | Synuclein alpha |
| 10 | DJ-1 | A protein deglycase involved in PD |
| 11 | PINK1 | PTEN-induced putative kinase 1 |
| 12 | ATP | Adenosine triphosphate |
| 13 | ROS | Reactive oxygen species |
| 14 | OFF symptoms | Time where symptoms appear between medicine doses |
| 15 | SNpc | Substantia nigra pars compacta |
| 16 | VPS35 | Vacuolar protein-sorting ortholog 35 |
| 17 | PARK genes | Genes where mutations increase susceptibility to Parkinson's |
| 18 | PRKN | Parkin RBR E3 ubiquitin protein ligase |
| 19 | GBA1 | Glucocerebrosidase 1 |
| 20 | TNF- α | Tumor necrosis factor alpha |
| 21 | RNS | Reactive nitrogen species |
| 22 | IL-1β | Interleukin 1β |
| 23 | NMDAR | N-methyl D-aspartate receptor |
| 24 | IL-6 | Interleukin 6 |
| 25 | UPS | Ubiquitin-proteasome system |
| 26 | ATP13A2 | Cation-transporting ATPase 13A2 |
| 27 | ROCO family | Protein family which contains both ROC and COR domains |
| 28 | ANK | Ankyrin domain |

| | | |
|---|---|---|
| 29 | ROC | Ras of Complex domain |
| 30 | COR | C-terminal of Ras of complex |
| 31 | ARM | Armadillo domain |
| 32 | WD40 | 40mer repeat of tryptophan and aspartic acid |
| 33 | LRR | Leucine-rich repeat region of LRRK2 |
| 34 | MKKK | MAP Kinase kinase kinase |
| 35 | MKK | Map Kinase kinase |
| 36 | JNK | Janus Kinase |
| 37 | WAF1/CIP1 | Another name for p21 |
| 38 | SH-SY5Y | A dopaminergic neuroblastoma cell line |
| 39 | TLR | Toll-like receptors |
| 40 | NF-κB | Nuclear factor kappa B |
| 41 | PIAS1 | Protein inhibitor of activated STAT1 |
| 42 | SHP | Small heterodimer partner |
| 43 | XBP1 | X-box binding protein |
| 44 | ERAD protein | Endoplasmic reticulum associated degradation protein |
| 45 | TRIM1 | Tripartite motif family 1 |
| 46 | Fbxl18 | F-box/LRR repeat protein 18 |
| 47 | WSB1 | WD repeat and SOCS box-containing protein 1 |
| 48 | BBB | Blood brain barrier |
| 49 | PDB | Protein data bank |
| 50 | SDF | Spatial data file |
| 51 | GUI | Graphic user interface |
| 52 | PDBQT | Protein data bank (partial charge and atom type) |
| 53 | ADT | AutoDock tools |
| 54 | RMSD | Root mean square distance |
| 55 | DFGΨ | Aspartate-phenylalanine-glutamine motif |
| 56 | CLL | Chronic lymphocytic leukemia |
| 57 | AML | Acute myeloid leukemia |
| 58 | SK-N-SH | A neuroblastoma cell line from which SH-SY5Y is derived |
| 59 | ERK | Extracellular signal-regulated kinase |

| | | |
|---|---|---|
| 60 | 17AAG | 17-N-allylamino-17-demethoxygeldanamycin |
| 61 | IC50 | Half-maximal inhibitory concentration |
| 62 | GLP-1 | Glucagon-like peptide |

# CHAPTER – 1

# INTRODUCTION

## 1.1 BACKGROUND

Parkinson's Disease affected more than 6.2 million people in 2016, and this figure is expected to double by the year 2040 [1]. Both crude and age-standardized prevalence of the disease has increased substantially, with a 145% increase being observed in crude numbers making Parkinson's the fastest growing neurodegenerative disorder [2].

PD is characterized by the appearance of four major symptoms – rigor or rigidity, rest tremors, posture instability and bradykinesia while the loss of nigrostriatal signaling and the presence of misfolded and aggregated α-synuclein which has been sequestered in Lewy bodies or Lewy neurites are the disease's pathological hallmarks [3], [4]. The potential mechanism of disease progression has been proposed as permissive templating or prion-like cell-to-cell transmission of α-synuclein [5].

Multiple risk factors have been discerned which have a hand the pathogenesis of PD making it a multifactorial disease. Age is one of the most important risk factors as the typical age of onset for idiopathic PD is 65-75 years, though 5-10% of the patients also suffer from early-onset PD which manifests before 50 years of age [6], [7].

Men are more susceptible and are known to develop PD a prevalence ratio of 3:2 in comparison to women [2]. Genetics is another important factor affecting disease risk as more than 90 genetic risk loci have been found to be associated with PD through genome-wide association studies [8]. Other modifiable factors like air and water pollutants, pesticide exposure, smoking habits, caffeine consumption, exercise frequency or history of head trauma etc. have been implicated in the pathogenesis of the disease as well [1].

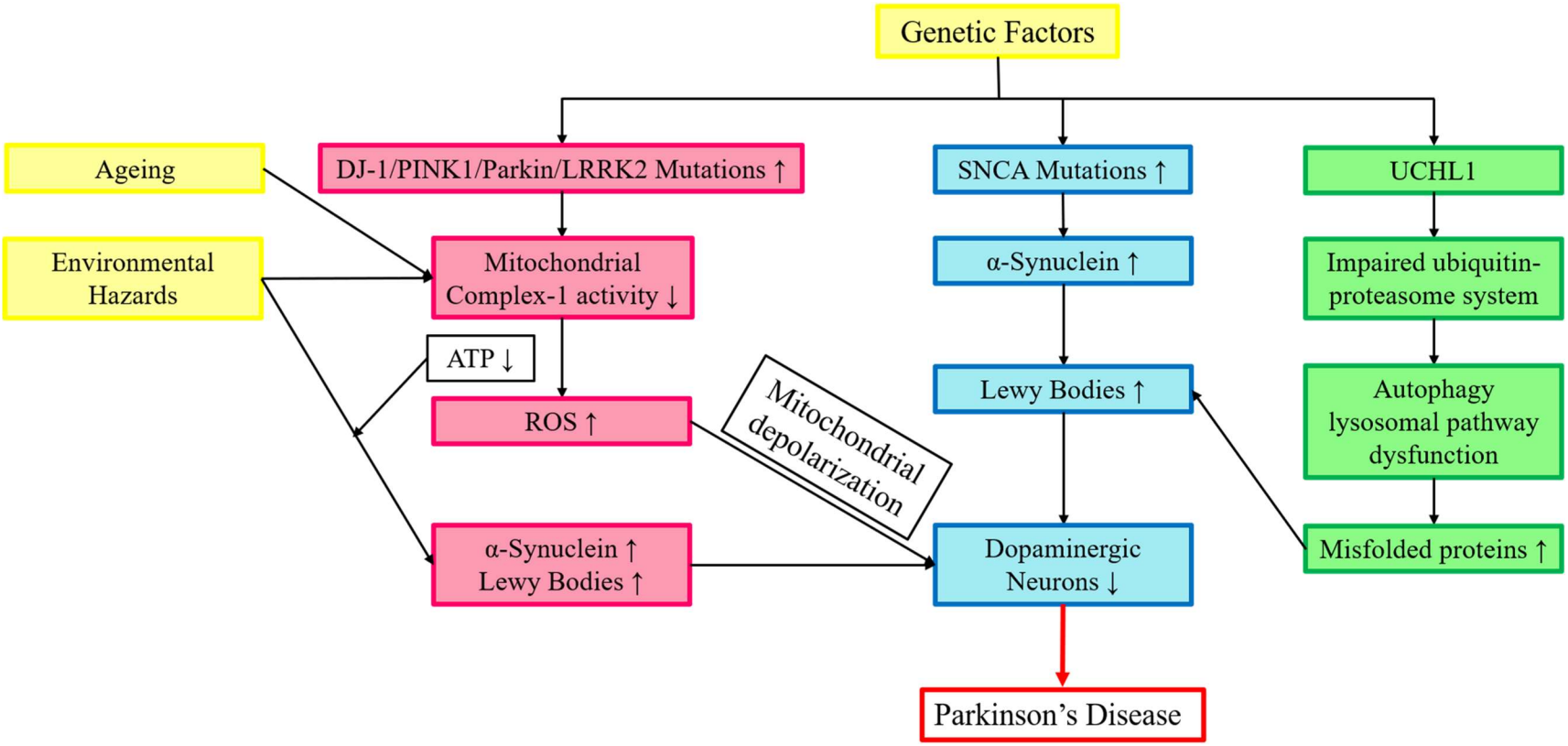


**Figure 1.1: Factors responsible for the development of PD:** Multiple different factors like ageing, environmental factors, mutations in genes associated with PD etc. are responsible for the development of PD in individuals. Due to ageing mitochondrial complex-1 activity is reduced leading to a decrease in the concentration ATP and an increase in the ROS concentration. Similarly, environmental factors are also responsible for increased ROS as well as increase in the concentration of alpha-synuclein and Lewy bodies ultimately also causing neurodegeneration. Mutations in PD associated genes is also a major factor leading to the onset of PD in the individual

## 1.2 PROBLEM STATEMENT

As discussed above, the incidence of Parkinson's is gradually increasing in the populations due to higher life expectancies as people are living longer lives. This proves to be a challenge since PD presently has no cure and the therapies in use only help manage the symptoms but do not treat the underlying cause of the disease.

First synthesized in 1911 by Casimir Funk, Levodopa has remained the standard medication used in the treatment of Parkinson's disease since it gained FDA approval in 1970 [9]. Subsequently, in 1975 the first combined therapy for Parkinson's was introduced which consisted of Levodopa and Carbidopa [10]. In this combined therapy, Levodopa is converted to dopamine by the action of the enzyme dopamine decarboxylase while Carbidopa, a dopamine decarboxylase inhibitor maintains the Levodopa levels inside the brain [11].

Though effective at maintaining the levels of dopamine in the brain, it should not be forgotten that this therapy has certain well-known side-effects like nausea, depression, motor problems, hallucinations and low blood pressure [12], [13]. Also, Levodopa-induced OFF symptoms and dyskinesia still pose a problem to patients as reported in a study where they occurred in 55.9% and 13.5% of the population respectively [14].

Thus, there remains a very urgent need to explore and develop new therapeutic strategies for the treatment of Parkinson's.

## 1.3 OBJECTIVES OF THE STUDY

- To curate a list of FDA-approved anti-cancer, anti-hypertensive and anti-diabetic medication.
- To carry out molecular docking analysis of suitable drugs with LRRK2 to find its inhibitor
- To compare the results with other known inhibitors of LRRK2 and to identify whether LRRK2-induced PD can be treated with re-purposed drugs.

# CHAPTER – 2

# LITERATURE REVIEW

## 2.1 PARKINSON'S DISEASE – INTRODUCTION TO PREVALENCE, CAUSES AND PATHOPHYSIOLOGY

### 2.1.1 Prevalence and Causes

Characterized by tremors, bradykinesia, posture instability and coordination problems, PD has become the world's second most-dominant neurodegenerative disorder right after Alzheimer's [1]. PD affects upwards of 1% of the population above the age of 65 years and this pervasiveness is expected to double by the year 2030 [15]. Currently over 10 million people are living with this disease with over 25 billion dollars being spent each year on its treatment [16]. It impacts multiple neurological pathways in addition to the loss of neurons in one specific region of the mid-brain referred to as the substantia nigra pars compacta (SNpc). Its etiology has been shown to be linked to various factors like gender, genetics, age, environmental factors, nutritional insufficiencies and brain damage [17].

### 2.1.2 Genetic Basis of PD

Till date more than 90 genetic risk loci and over 20 genes have been implicated in the pathophysiology of PD with over 5-10% of the diseased population suffering from a form of PD caused by mutations in these genes [18]. Out of these 20 genes, 11 genes are autosomal dominant (including SNCA, LRRK2, VPS35, PARK3 etc.), and nine are autosomal recessive (including PRKN, PINK1, DJ-1, PARK7 etc.) [19]. Among these, the most commonly implicated ones are SNCA, LRRK2, PRKN, GBA1 and PINK1.

1. SNCA – Overexpression of α-Synuclein causes neurodegeneration in dopaminergic neurons [20]. Mutations like A30P, E46K and A53T when present in the protein impair dopamine storage in neurons and are the main cause for the formation of Lewy bodies [21]. α-Synuclein in its oligomeric form activates the toll-like receptor 2 leading to neuroinflammation through activation of microglial cells and other

inflammatory mediators like TNF- α, complement cascade protein, IL-1β, IL-6, ROS and RNS [22]. Neurotrophic factors, though responsible for preventing α-Synuclein misfolding cannot perform their function adequately in PD brains because of depletion of growth factors thus leading to aggregation and accumulation of α-Synuclein inside the neurons [23].

2. LRRK2 – It is implicated with the highest frequency in both familial and sporadic PD [20]. In addition to SNCA it is the only other gene which when mutated leads to presence of α-Synuclein Lewy bodies in diseased condition [24]. LRRK2 is also involved in lysosomal functioning where it modulates vesicle trafficking and autophagy [25].
3. PINK1 – This gene encodes a serine/threonine protein kinase of a mitochondrial origin and regulates the destruction of dysfunctional mitochondria [29].
4. PRKN – This gene encodes Parkin which is a ubiquitin E3-ligase responsible for the clearance of damaged mitochondria from the cell mainly through the process of autophagy. PRKN mutations are responsible for mitochondrial dysfunction in PD [26]–[28].
5. GBA1 – The mutations N370S and L444P present in GBA1 are risk factors most commonly associated with sporadic, early-onset PD which is accompanied by very rapid decline in cognition [30]. These mutations are also responsible for increased protein aggregation in cell as well as lysosomal malfunction [31].

| **PARK** | **Gene** | **Gene Description** | **Onset** | **Inheritance Type** | |
|---|---|---|---|---|---|
| PARK1/ PARK4 | SNCA | α-Synuclein | Classical PD to early onset with dementia, autonomic dysfunction and rapid progression | Dominant | [32] – [34] |

| | | | | | |
|---|---|---|---|---|---|
| PARK2 | PRKN | Parkin RBR E3 Ubiquitin Ligase | Early onset PD with slow progression, features of dystonia observed | Recessive | [35] |
| PARK4 | UCHL1 | Ubiquitin C-terminal hydrolase L1 | Classical PD till date found in one family only | Dominant | [36] |
| PARK6 | PINK1 | PTEN-induced putative kinase 1 | Early onset PD with slow progression | Recessive | [37] |
| PARK7 | DJ-1 | Parkinsonism-associated deglycase | Early onset PD with slow progression | Recessive | [38] |
| PARK8 | LRRK2 | Leucine-rich repeat kinase 2 | Classical PD with slow progression and less frequent dementia | Dominant | [39] |
| PARK9 | ATP13A2 | Cation-transporting ATPase 13A2 | Early onset PD (in adolescence), atypical parkinsonism with dementia, spasticity and supranuclear palsy | Recessive | [40] |

| | | | | | |
|---|---|---|---|---|---|
| PARK11 | GIGYF2 | GRB10 interacting GYF protein 2 | Classical PD | Dominant | [41] |
| PARK13 | HTRA2 | HtrA serine peptidase 2 | Classical PD | Recessive | [42] |
| PARK14 | PLA2G6 | Calcium-independent phospholipase A2 | Early onset PD with dystonia | Recessive | [43] |
| PARK15 | FBX07 | F-box protein 7 | Early onset PD with pallido-pyramidal syndrome | Recessive | [44] |
| PARK17 | VPS35 | Vacuolar protein sorting-associated protein 35 | Classical PD | Dominant | [45] |
| PARK18 | ElF4G1 | Eukaryotic translation initiation factor 4 gamma | Classical PD | Dominant | [46] |
| PARK19 | DNAJC6 | HSP40 Auxillin | Early onset PD with slow progression | Recessive | [47] |
| PARK20 | SYNJ1 | Synaptojanin | Dystonia and cognitive decline | Recessive | [48] |
| PARK21 | DNAJC13 | Receptor-mediated endocytosis 8 | Classical PD | Dominant | [49] |

| | | | | | |
|---|---|---|---|---|---|
| PARK23 | VPS13C | Vacuolar Protein sorting-associated protein 13C | Early onset PD with rapid progression | Recessive | [50] |

**Table I:** Genes known to be implicated in PD with their description, type of PD caused as well as description of certain key clinical features

### 2.1.3 Oxidative Stress and Mitochondrial Dysfunction in PD

Various studies and experimental models have shown that disruptions in the mitochondria and impaired biogenetics in PD is the cause of increase in ROS levels and calcium levels and, decrease in ATP production as well as excitotoxicity-mediated neuron damage [28]. This dysregulation of the mitochondria affects processes like biogenesis, transport, mitochondrial fusion and fission as well as transport [28], [51]. Accumulation of α-Synuclein inside the mitochondria, its aggregation and accumulation, is stipulated to be one of the main causes of mitochondrial fragmentation [52]. PINK1, DJ-1 and PRKN are also reported as major players in the dysregulation of mitophagy and subsequently neurodegeneration [47], [53], [54].

Mitochondria regulates the influx and efflux of calcium ions through the ligand-gated glutamate receptors like NMDAR and voltage dependent ion channels [55]. Mitochondria are responsible for the calcium homeostasis in neurons. Any change in calcium homeostasis can lead to alterations in neuronal activity as neurons are entirely dependent on the mitochondria for their energy and ATP requirements [56]. PINK1 and PRKN are two genes which are remotely associated with calcium homeostasis. PINK1 controls calcium efflux while PRKN is responsible for the calcium influx. Thus, deficiency of PINK1 leads to an overload of calcium inside the mitochondria and subsequent production of ROS pushing the cells towards neurodegeneration [53].

PINK1 and PRKN also play an important role adaptive immunity as they suppress antigen presentation by the mitochondria thus suggesting that autoimmune disorders might also be implicated as causes of PD [26].

### 2.1.4 Dysfunction of the Autophagy-Lysosome System

Non-functional proteins are discarded by one of three processes – the UPS, using molecular chaperones or the autophagy-lysosomal system [57]. Autophagy is very important in PD as the process is responsible for delivering aggregated and misfolded proteins as well as defective cell organelles to the lysosome for degradation by either of the three autophagy pathways – macro, micro or chaperone-mediated. Impairment in these three pathways results in accumulation of aggregates of proteins inside the cell which is a marker for PD [58]. Genes like PINK1, PRKN, DJ-1 and UCHL1 help regulate UPS functioning thus mutations in these genes affect chaperone-mediated autophagy in addition to the UPS negatively influencing axonal transport and neuronal function [59].

Effect of mutations in genes like LRRK2, ATP13A2 and GBA1 on autophagy is well studied. In LRRK2 the R1441C and the G2019S mutations decrease autophagic flux [60]. Deficiency of ATP13A2 which is a lysosome pathway regulator is associated with lysosomal dysfunction, buildup of Syn A53T and autophagy impairment which are all responsible for PD pathogenesis [61]. Similarly, mutations in GBA1 are also responsible for accumulation of α-Synuclein due to alterations in the architecture of the lysosome [62].

## 2.2 LRRK2 AND PD

### 2.2.1 General Introduction to LRRK2

Over 25 genetic risk loci and 20 genes have been described as being associated with PD [63] one such gene is ‘dardarin’ or LRRK2. Identified mutations in this kinase are inherited autosomal dominantly and are responsible for both sporadic PD (1-5%) and familial PD (5-13%) [64]. Out of various known mutations, R1441C, R1441G, R1441H, R1628P, Y1699C, G2019S, I2020T and G2385R have been established as pathogenic [64].

LRRK2 is present on chromosome 12 (12p11.2–q13.1) at the PARK8 locus [65]. As a 2527 amino acid protein, it is fairly large and belongs to the ROCO family [66]. It comprises 7 individual domains - the ROC (Ras of Complex) GTPase, COR (C-terminus of ROC) and the Kinase domain (serine/threonine) which jointly form the catalytic center and show dual kinase/GTPase activity, while the ARM (Armadillo), ANK (Ankyrin), LRR (Leucine-rich-repeat) domains present at the protein's N-terminal and the WD40 domain present at the C-terminal are protein-protein interaction domains [64].

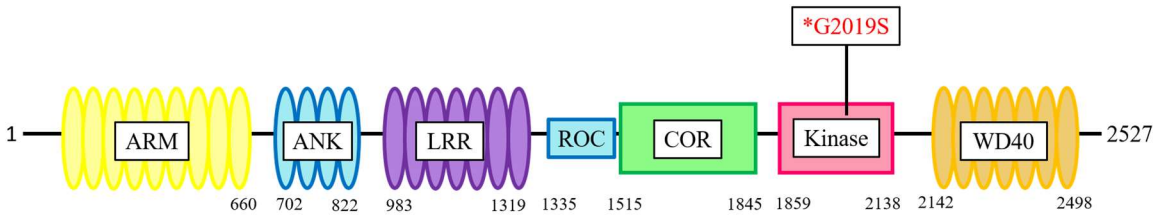


**Figure 2.1**: The domain structure of LRRK2 with its multiple individual domains and the approximate location of the G2019S mutation

### 2.2.2 Effect of Mutations on LRRK2 Functioning

LRRK2 functions as a MKKK [66], [67]. It phosphorylates MKK4/7 and MKK3/6, which regulate the cell's stress response by activating the JNK and p38 pathways respectively [67], [68]. Kinase activity of LRRK2 increases because of the G2019S mutation [67]. This causes hyperphosphorylation of MKK4 at Ser257, ultimately causing apoptosis of neurons through the activation of JNK pathway in mice models [69]. The G2019S mutants also generate a sustained expression of p38 in *C. elegans* [70] and increase the phosphorylation of p53 through induction of $p21^{WAF1/CIP1}$ in SH-SY5Y cells [71]. All these have pro-apoptotic effects.

LRRK2 also phosphorylates ERK, leading to a small but substantial increase in the levels of α-synuclein [72]. This upregulation of α-synuclein activates microglial cells where toll-like receptors (TLRs) can activate the release of inflammation mediators like NF-κB. This NF-κB then activates other pro-inflammatory molecules, leading to neuroinflammation and, ultimately, neuronal death in the Substantia Nigra [73].

The G2019S mutants' increased kinase activity can trigger apoptosis via the ER stress response. Mutated LRRK2 activates the small heterodimer partner (SHP) which stabilizes PIAS1 - a protein bringing about SUMOlyation of XBP1, a transcription factor involved in transcription of chaperones, ERAD proteins and foldases [74]. Because of SUMOlyation, XBP1 cannot perform its duty, and the cell is pushed towards apoptosis.

### 2.2.3 LRRK2 Regulation and Potential for Therapeutic Intervention

Like most other proteins, LRRK2 is also subject to regulation by proteasomal degradation using ubiquitination. It interacts with multiple different ubiquitin E3 ligases including TRIM1, Fbxl18, WSB1 and CHIP [75]–[78]. Except WSB1, which alters its solubility, all other ubiquitin ligases target LRRK2 for proteasomal degradation [77]. One ubiquitin ligase of considerable interest is CHIP. Under normal cellular condition, CHIP controls the LRRK2 levels inside the cell but under mutant conditions clearing is affected, leading to apoptosis through caspase 3 activation [78].

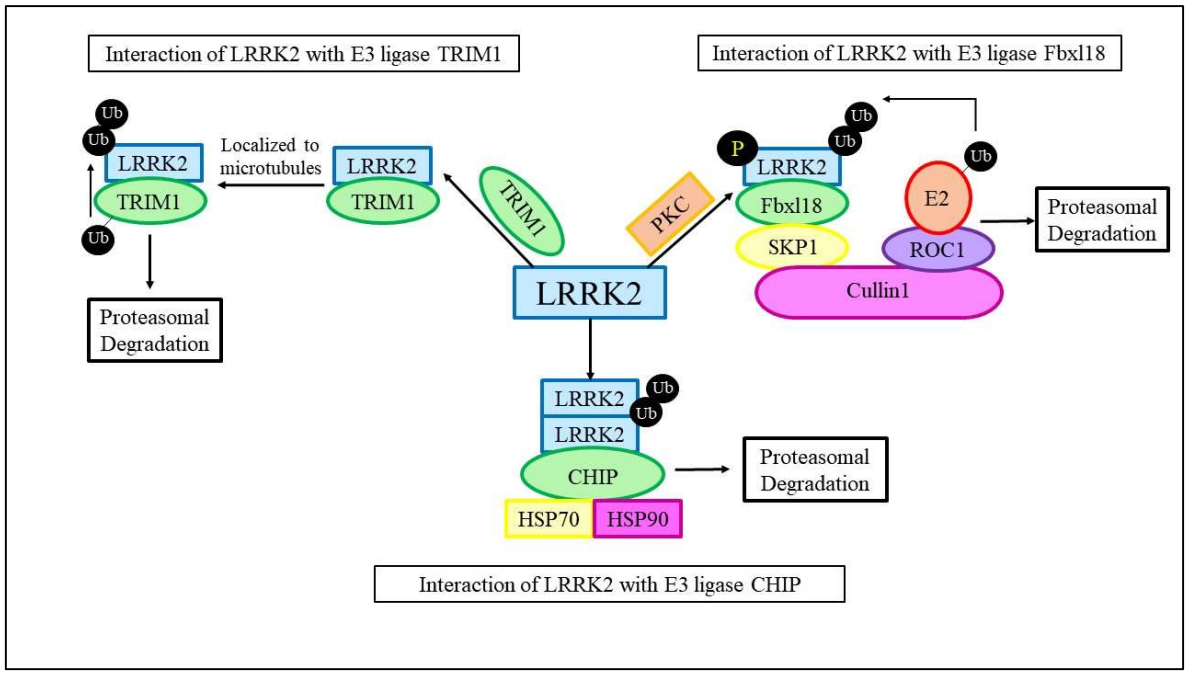


**Figure 2.2**: Interactions of LRRK2 with ubiquitin E3 ligases CHIP, TRIM1 and Fbxl18 target LRRK2 for proteasomal degradation

As is evident, LRRK2 is involved in various different pathways which are localized in multiple regions of the cell. Thus, mutations in its structure are proapoptotic and frequently deadly. Therefore, there is an urgent requirement for a potent LRRK2 inhibitor that can inhibit its aberrant kinase activity. Traditional drug discovery approaches are very labor and time intensive. In this regard, drug repurposing of approved medication with the help of molecular docking software emerges as an efficient and cost-effective methodology.

# CHAPTER – 3

# METHODS

## 3.1 COLLECTION OF DATA

While performing the literature review, it became apparent that there was a substantial correlation between the pathologies of PD and diseases like diabetes, hypertension and cancer. Thus, due to shared pathologies, anti-diabetic, anti-hypertensive and anti-cancer drugs could therefore be repurposed against PD.

A list of 225 FDA-approved drugs (26 anti-diabetic, 64 anti-hypertensive and, 135 anti-cancer drugs) was curated for the express purpose of re-purposing them against the aberrant LRRK2 enzyme. These drugs were further screened using an online BBB permeability predictor (https://cbligand.org/BBB/predictor.php) to check their BBB permeability [79]. Out of the list, only 130 drugs were found to be BBB permeable.

The SDF structures of the BBB permeable drugs were downloaded using PubChem while the protein structures – LRRK2 (https://www.rcsb.org/structure/7LHT) [80], the LRRK2 mutant (https://www.rcsb.org/structure/7LI3) [80] and Hsp90 (https://www.rcsb.org/structure/2QF6) [81] were downloaded from the Protein Databank Bank in the PDB format.

## 3.2 PREPARATION OF RECEPTORS AND LIGANDS

To carry out molecular docking, the PDB structures of the proteins were first processed using AutoDock Tools [82]. The water molecules associated with all three protein structures were removed. Further, polar hydrogens and Kollman charges were also added to the proteins and they were finally saved in the PDBQT format.

Grid maps were then prepared for the proteins with the following specifications:

- LRRK2 – The grid dimensions were 40x40x40 with center at (235.231, 202.282, 212.467)

- LRRK2 mutant – The grid dimensions were 30x30x30 with center at (216.084, 207.581, 236.982)
- Hsp90 – The grid dimensions were 60x60x70 with center at (14.865, 38.596, 7.192)

Similarly, the ligands were also prepared. The SDF structures of all the 130 ligands were converted to the PDB format after using OpenBabel GUI [83] a software used for the interconversion of chemical file formats. The structures were further processed and converted to the PDBQT format using ADT.

### 3.3 MOLECULAR DOCKING USING AUTODOCK VINA

After the proteins and ligands were prepared, they were made to undergo molecular docking using the software AutoDock Vina [84], [85] an *in-silico*, cost-effective and efficient method for the purpose of drug re-purposing. Vina was used as it is approximately 100X faster and more accurate than AutoDock 4.0 in single threaded runs and 7.5 times faster in multi-threaded runs [85]. The 130 BBB permeable drugs were docked with both LRRK2 and the LRRK2 mutant to compare and study any difference between the free energy of binding of the same drug with LRRK2 and the G2019S mutant.

The programming language Perl was used to instruct the program to carry out the molecular docking. To identify suitable hits, the cutoffs for RMSD and free energy of binding were set as less than 1Å and less than -8.0kcal/mol respectively. The hits were then ranked in the descending order of their free energy of binding.

### 3.4 VISUALIZATION AND PROCESSING OF RESULTS USING PYMOL

The results of the molecular dockings using AutoDock Vina were studied, visualized and processed using the software PyMOL. Using PyMOL, the interactions between the drugs and the proteins were studied to determine the amino acid residues that interacted with the drug. These residues were then labelled to make identification easier. Distances between the active sites and the number of hydrogen bonds formed between the receptor and ligand were also studied and labelled.

Finally, the labelled and processed receptor-ligand interactions were saved as publication quality figures.

# CHAPTER – 4

# RESULTS

As specified in the methodology, the compounds which met the cutoff of free energy of binding and RMSD were ranked. Out of the 130 BBB permeable drugs docked with the mutant LRRK2, only 13 met the required cutoffs.

| Compound/Drug | Free Energy of Binding (kcal/mol) |
|---|---|
| Venetoclax | -14.2 |
| Nilotinib | -09.7 |
| Alectinib | -09.3 |
| Olaparib | -09.0 |
| Ponatinib | -09.0 |
| Regorafenib | -08.7 |
| Glimperide | -08.6 |
| Sorafenib | -08.5 |
| Palbociclib | -08.3 |
| Abiraterone | -08.2 |
| Lapatinib | -08.2 |
| Canagliflozin | -08.1 |
| Dabrafenib | -08.1 |

**Table II**: Compounds meeting the required cutoff of free energy of binding and RMSD after docking with the LRRK2 mutant.

From the data obtained it is evident that Venetoclax with RMSD 0.0Å and the free energy of binding as -14.2 kcal/mol in case of the G2019S mutant emerged as a clear winner among the 130 drugs tested. It has the highest free energy of binding among the drugs studied thus indicating high affinity for the kinase domain of the G2019S mutant. Analyzing the results using PyMOL, we find that Venetoclax interacts with Glutamine-2022 in the LRRK2 mutant.

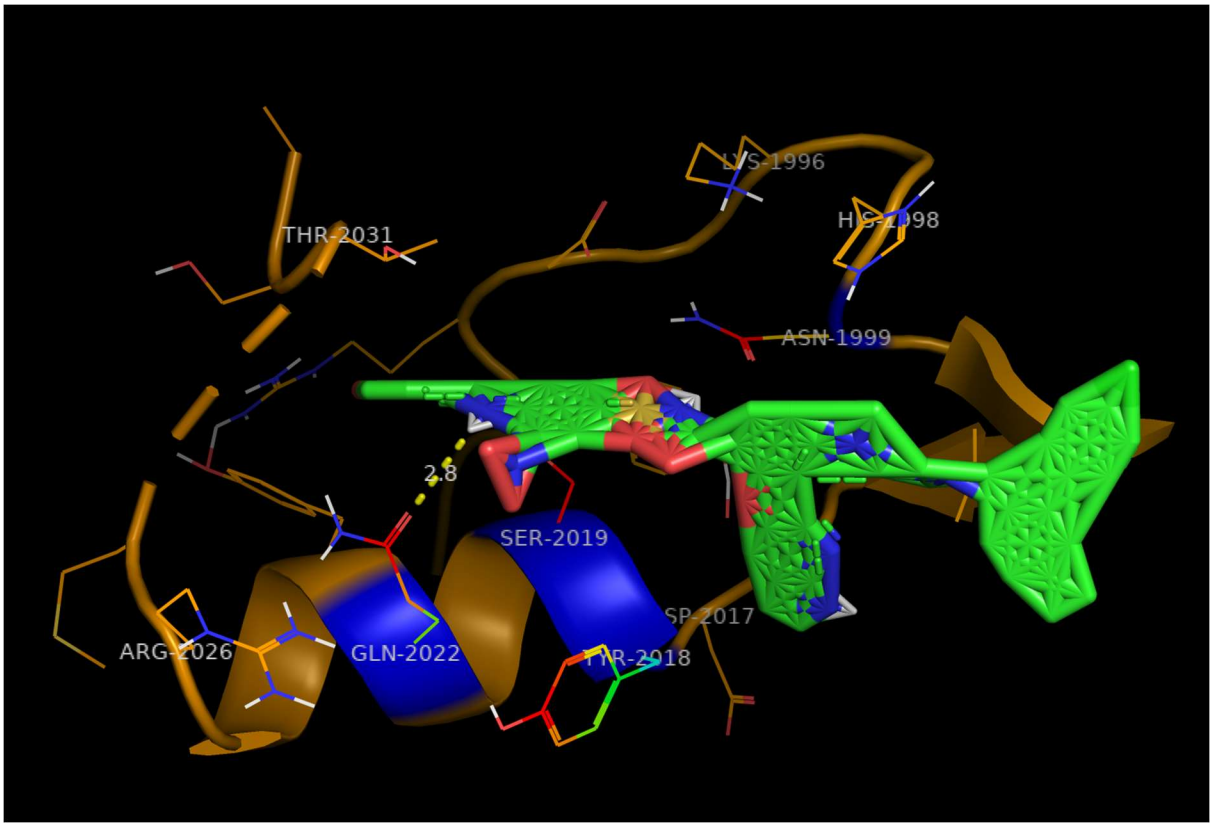


**Figure 4.1**: Venetoclax binds near the DFGΨ motif responsible for regulation of kinase activity and directly interacts with Glutamine-2022 in the G2019S mutant.

Venetoclax (IC50 1.90μM) [86] is a Bcl2 inhibitor sold under the brand name VENCLEXTA® by AbbVie and Roche. Approved by the FDA, it is currently prescribed for the treatment of Chronic Lymphocytic Leukemia (CLL) and Acute Myeloid Leukemia (AML) [87], [88]. It is a targeted treatment as opposed to conventional chemotherapy, and only targets cells where Bcl2 is overexpressed on the surface.

In opposition to other currently known kinase-activity inhibitors of LRRK2, Venetoclax shows a considerably higher docking score meaning that its interactions with LRRK2 are stronger and its IC50 value of 1.90μM (in case of the SK-N-SH, a neuroblastoma cell line) which falls in the range of 1-10μM is correlated with good activity and high efficacy [89].

| Drug/Compound | Free Energy of Binding (kcal/mol) | IC50 (nM) |
|---|---|---|
| Venetoclax | -14.2 | 1900 |
| LRRK2-in-1 | -9.8 | 13 |
| K-252A | -9.1 | 25 |
| Staurosporine | -8.9 | 1 to 40 |
| GO-6976 | -8.6 | 250 |
| RO-31-8220 | -8.1 | 1640 |
| JH-II-127 | -7.9 | 16 |
| Crizotinib | -7.7 | 6.6 |
| czc25146 | -7.6 | 5 |
| Sunitinib | -7.2 | 12 |

**Table III**: A comparison between the free energy of binding and IC50 values of known LRRK2 inhibitors when docked with the G2019S mutant

Inhibition of the mutant LRRK2 kinase by Venetoclax will have a marked antiapoptotic effect. As can be seen from literature referred, the increase in kinase activity was responsible for the phosphorylation and activation of various pro-apoptotic pathways like the MAPK, p53, JNK and the ERK pathway which ultimately lead to neurodegeneration. Due to inhibition of aberrant MKKK activity of LRRK2 by Venetoclax, these pro-apoptotic pathways will not be activated.

Inhibition of LRRK2 also prevents SUMOlyation of the protein XBP1. The inhibition of LRRK2 by Venetoclax stops the activation of the protein SHP and will therefore not be able to increase its concentration. This decreased concentration of SHP prevents SUMOlyation of XBP1 by PIAS1.

XBP1 which is a transcription factor is therefore free to enter the nucleus and help transcribe RNAs responsible for proteins like chaperones, foldases and ERAD proteins which will either help in the refolding of the mutated LRRK2 or will ultimately mark it for degradation.

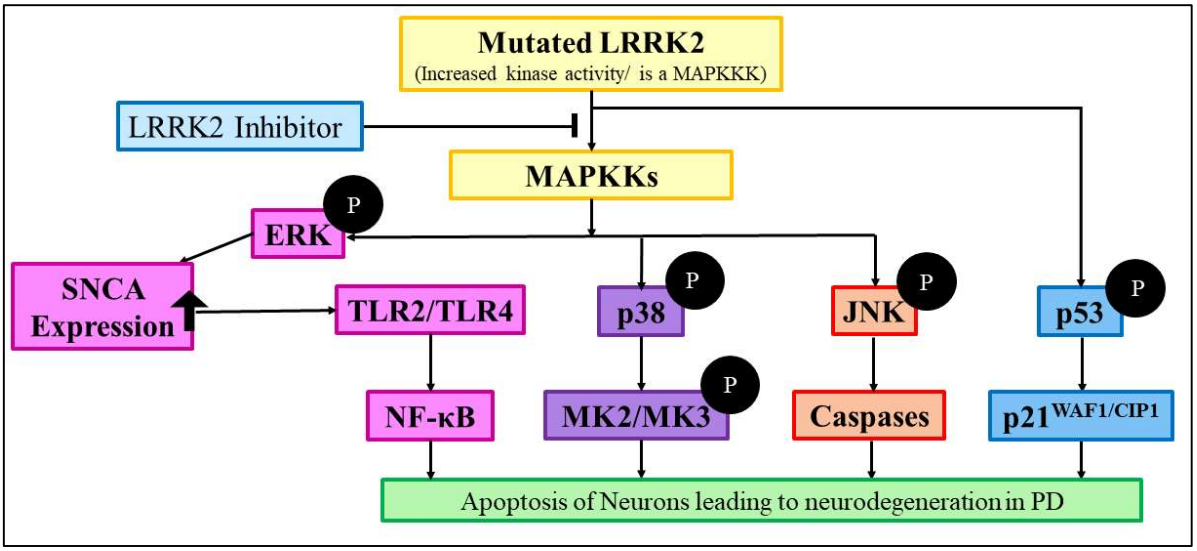


**Figure 4.2**: The G2019S mutant, a known MKKK, is responsible for the activation of various pro-apoptotic pathways like the ERK, p53, JNK and MAPK due to increased kinase activity.

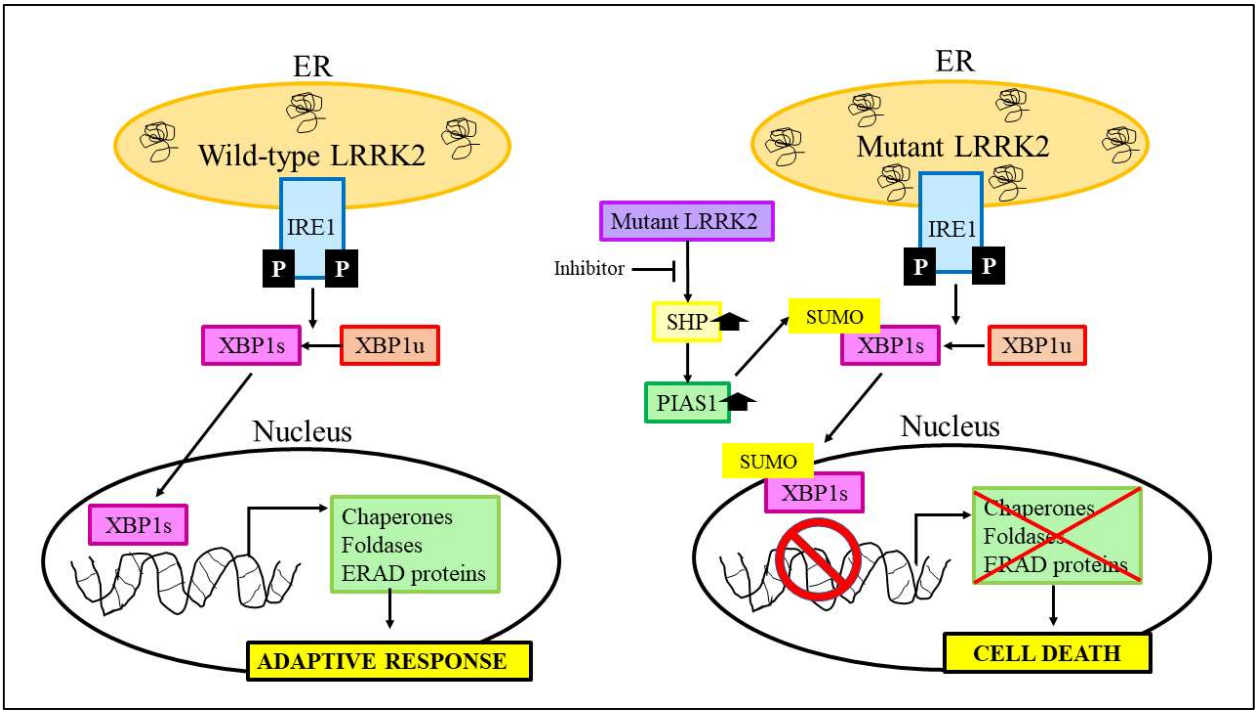


**Figure 4.3**: Inhibition of mutated LRRK2 prevents stabilization of PIAS1 thus preventing SUMOlyation of XBP1 and triggering the adaptive response leading either to refolding or degradation of the mutant enzyme

Inhibition of Hsp90, a protein involved in the CHIP ubiquitin E3 ligase pathway leads to an increase in the concentration of CHIP and increase in proteasomal degradation through the ubiquitination pathway. Studies have shown that using 17AAG as a Hsp90 inhibitor increased the degradation of mutant LRRK2 by increasing CHIP concentration [78].

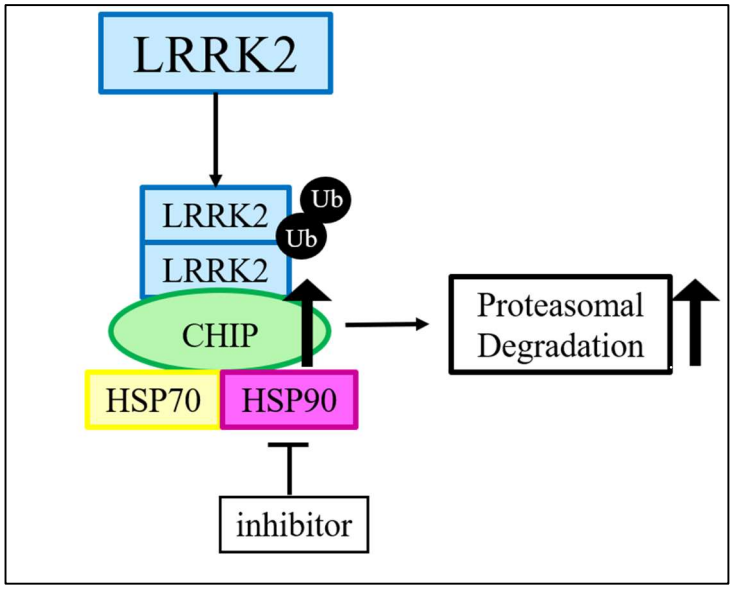


**Figure 4.4**: Inhibition of Hsp90 increases CHIP concentration thereby increasing its clearance using the ubiquitin-proteasome system

To check whether Venetoclax possesses dual functionality and can inhibit Hsp90 as well, molecular docking analysis between the two was also carried out using the methodology described above. When analyzed using PyMOL, the results showed that Venetoclax is a very good inhibitor of Hsp90 (free energy of binding = -13.0kcal/mol and RMSD 0.0Å) and interacts with Asparagine-106.

Thus, Venetoclax proves to be an excellent candidate that can be repurposed as a neuroprotectant against the G2019S mutant of LRRK2 as shown by in-sillico analysis because of its high free energy of binding, high IC50 and dual functionality as an inhibitor.

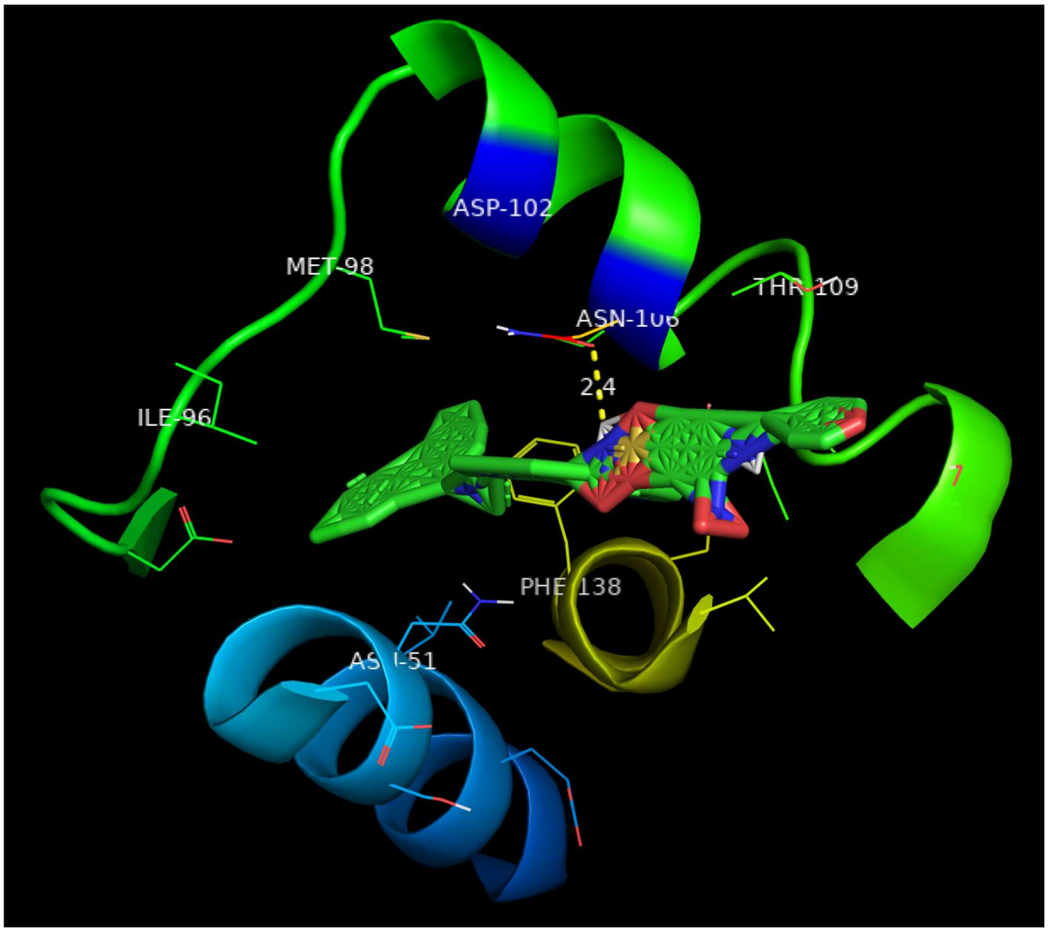


**Figure 4.5**: Venetoclax binds with Hsp90 at the ATP-binding domain and interacts with Asparagine-106

# CHAPTER – 5

# DISCUSSION

Parkinson's disease has no known cure and therapies used for symptom-management have not progressed much since the early 1970's when levodopa, a dopamine precursor was introduced to the market thus creating a dire dearth of drugs that can be used for the treatment of PD. One solution to this problem can be found in the form of drug re-purposing or drug repositioning as it is a cost and time-effective method that has been employed for the search of new drugs. Multiple such efforts are currently underway, in varying degrees of progress, to re-purpose extant, FDA approved drugs against Parkinson's disease. A few examples include GLP-1 (in Phase III clinical trials) [90], ursodiol (under clinical trials) [90] and others like ambroxol and ceftriaxone which have already been approved for use against PD associated dementia [91].

Drug re-purposing is also being carried out against LRRK2 and two other anti-cancer drugs Crizotinib and Sunitinib [92] have been recently shown to inhibit LRRK2. But, as is evident from Table III, their free energy of binding and therefore affinity for binding to the enzyme is not very high.

Earlier, discovering new drugs was a very time and labor-intensive process which required a large amount of capital but, in recent years, thanks in part to advances in computing, this process has been considerably simplified. Hundreds upon hundreds of compounds can be screened computationally against the desired protein/receptor by using molecular docking software. Which dramatically reduces the time required for pre-clinical analysis to test drug efficacy and hastening the entire process.

Using a similar computational drug re-purposing approach, we found out that Venetoclax acts as a potent inhibitor of the G2019S mutant of LRRK2. It shows immense promise as a therapy against LRRK2-induced PD on account of its very high docking score (free energy of binding) and IC50 value in comparison to other well-known and commonly studied inhibitors like LRRK2-in-1.

The results of inhibition of the enzyme will be quite favorable as it will turn the many pro-apoptotic pathways activated by the G2019S mutant anti-apoptotic.

The results also showed that Venetoclax possesses dual functionality as it not only inhibits LRRK2 but Hsp90 as well, which increases the degradation of the mutant enzyme via the ubiquitin-mediated proteasome system.

# CHAPTER – 6

# CONCLUSION

The preliminary study carried out identified a novel LRRK2 G2019S mutant inhibitor, Venetoclax. Originally a Bcl-2 inhibitor which we have tried to re-purpose for LRRK2 mutant-induced PD, it showed a considerably high free energy of binding when compared with other well-known and commonly studied inhibitors.

The study also helped to show that Venetoclax is a multi-functional drug that not only inhibits LRRK2 but also inhibits Hsp90 thus playing a dual role in its bid to prevent neurodegeneration.

Future studies, both *in vitro* (on cell lines such as SH-SY5Y) or *in vivo* (on PD mice models) need to be carried out to validate the results obtained.

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## LIST OF PUBLICATIONS

1. A. Tripathi and P. Kumar, "Identification of Putative LRRK2 Inhibitors in the Pathogensis of Parkinson's Disease: A Drug-Repurposing Approach," *2021 5th International Conference on Information Systems and Computer Networks (ISCON)*, 2021, pp. 1-4, doi: 10.1109/ISCON52037.2021.9702406.

# APPENDIX

## A.1 CODE FOR PERFORMING MOLECULAR DOCKING USING AUTODOCK VINA

File Edit View

```
#!/usr/bin/perl
print"ligand_file:\t";
$ligfile=<STDIN>;
chomp $ligfile;
open (FH,$ligfile)||die "Cannot open file\n";
@arr_file=<FH>;

for($i=0;$i<@arr_file;$i++)
{
print"@arr_file[$i]\n";
@name=split(/\./,@arr_file[$i]);
}
for($i=0;$i<@arr_file;$i++)
{
        chomp @arr_file[$i];
        print"@arr_file[$i]\n";
        system("vina.exe --config configdj1.txt --ligand @arr_file[$i] --log @arr_file[$i]_log.log");
}
```

**(A)**

File Edit View

```
C:\Users\ANIMAN>cd /

C:\>cd "Autodock"

C:\Autodock>cd "WORKSPACE"

C:\Autodock\WORKSPACE>perl -v

C:\Autodock\WORKSPACE>dir /B >ligand.txt

C:\Autodock\WORKSPACE>perl Vina_windows.pl
ligand_file:ligand.txt
```

**(B)**

**Figure A.1**: **Code for performing molecular docking using AutoDock Vina**: Two different sets of code was required to carry out molecular docking using AutoDock Vina as it does have a GUI and requires code in the programming language Perl for it to run. **(A)** – This code is the Vina_windows.pl file that appears in (B). It instructs the Vina software about what data files pertaining to the receptor have to be used to get the correct grid map for a successful docking. **(B)** – It is the code required to run to the actual docking experiment via the command prompt center in the computer.

## A.2 MOLECULAR DOCKING RESULTS OF COMPOUNDS MENTIONED IN TABLE II AND III

### Venetoclax

Output will be venetoclax_out.pdbqt
Detected 8 CPUs
Reading input ... done.
Setting up the scoring function ... done.
Analyzing the binding site ... done.
Using random seed: 1220466624
Performing search ... done.
Refining results ... done.

| mode | affinity (kcal/mol) | dist from best mode rmsd l.b. | rmsd u.b. |
|---|---|---|---|
| 1 | -14.2 | 0.000 | 0.000 |
| 2 | -14.1 | 1.384 | 2.648 |
| 3 | -13.8 | 3.831 | 12.389 |
| 4 | -13.5 | 5.217 | 14.856 |
| 5 | -13.4 | 1.789 | 3.719 |
| 6 | -13.3 | 3.769 | 12.220 |
| 7 | -13.0 | 5.300 | 15.078 |
| 8 | -13.0 | 3.217 | 4.907 |
| 9 | -12.8 | 3.534 | 12.407 |

Writing output ... done.

### Nilotinib

Output will be Nilotinib_out.pdbqt
Detected 8 CPUs
Reading input ... done.
Setting up the scoring function ... done.
Analyzing the binding site ... done.
Using random seed: -713296740
Performing search ... done.
Refining results ... done.

| mode | affinity (kcal/mol) | dist from best mode rmsd l.b. | rmsd u.b. |
|---|---|---|---|
| 1 | -9.7 | 0.000 | 0.000 |
| 2 | -9.3 | 7.100 | 13.275 |
| 3 | -8.9 | 2.298 | 2.671 |
| 4 | -8.9 | 4.843 | 6.722 |
| 5 | -8.8 | 7.170 | 12.059 |
| 6 | -8.8 | 2.670 | 5.168 |
| 7 | -8.7 | 4.302 | 11.730 |
| 8 | -8.5 | 4.565 | 11.364 |
| 9 | -8.5 | 3.915 | 5.464 |

Writing output ... done.

### Alectinib

Output will be alectinib_out.pdbqt
Detected 8 CPUs
Reading input ... done.
Setting up the scoring function ... done.
Analyzing the binding site ... done.
Using random seed: -1262510312
Performing search ... done.
Refining results ... done.

| mode | affinity (kcal/mol) | dist from best mode rmsd l.b. | rmsd u.b. |
|---|---|---|---|
| 1 | -9.3 | 0.000 | 0.000 |
| 2 | -9.1 | 2.709 | 11.202 |
| 3 | -8.6 | 1.736 | 2.096 |
| 4 | -8.4 | 3.359 | 11.308 |
| 5 | -8.3 | 4.078 | 4.665 |
| 6 | -8.1 | 4.528 | 5.482 |
| 7 | -8.1 | 4.934 | 11.683 |
| 8 | -8.0 | 2.000 | 2.351 |
| 9 | -7.8 | 2.950 | 3.980 |

Writing output ... done.

### Olaparib

Output will be Olaparib_out.pdbqt
Detected 8 CPUs
Reading input ... done.
Setting up the scoring function ... done.
Analyzing the binding site ... done.
Using random seed: -755212400
Performing search ... done.
Refining results ... done.

| mode | affinity (kcal/mol) | dist from best mode rmsd l.b. | rmsd u.b. |
|---|---|---|---|
| 1 | -9.0 | 0.000 | 0.000 |
| 2 | -8.7 | 4.107 | 5.118 |
| 3 | -8.6 | 3.129 | 7.755 |
| 4 | -8.6 | 1.713 | 2.190 |
| 5 | -8.5 | 2.929 | 3.711 |
| 6 | -8.5 | 2.660 | 9.719 |
| 7 | -8.4 | 2.585 | 3.737 |
| 8 | -8.3 | 2.937 | 3.654 |
| 9 | -8.3 | 1.921 | 2.454 |

Writing output ... done.

### Ponatinib

Output will be Ponatinib_out.pdbqt
Detected 8 CPUs
Reading input ... done.
Setting up the scoring function ... done.
Analyzing the binding site ... done.
Using random seed: -1882291216
Performing search ... done.
Refining results ... done.

| mode | affinity (kcal/mol) | dist from best mode rmsd l.b. | rmsd u.b. |
|---|---|---|---|
| 1 | -9.0 | 0.000 | 0.000 |
| 2 | -9.0 | 2.013 | 2.536 |
| 3 | -8.8 | 4.748 | 6.839 |
| 4 | -8.8 | 5.369 | 11.710 |
| 5 | -8.7 | 2.811 | 3.546 |
| 6 | -8.6 | 4.854 | 7.415 |
| 7 | -8.6 | 3.082 | 3.941 |
| 8 | -8.5 | 4.812 | 6.117 |
| 9 | -8.3 | 4.254 | 11.872 |

Writing output ... done.

### Regorafenib

Output will be Regorafenib_out.pdbqt
Detected 8 CPUs
Reading input ... done.
Setting up the scoring function ... done.
Analyzing the binding site ... done.
Using random seed: -197289668
Performing search ... done.
Refining results ... done.

| mode | affinity (kcal/mol) | dist from best mode rmsd l.b. | rmsd u.b. |
|---|---|---|---|
| 1 | -8.7 | 0.000 | 0.000 |
| 2 | -8.6 | 1.859 | 2.831 |
| 3 | -8.6 | 1.735 | 2.383 |
| 4 | -8.5 | 5.400 | 6.518 |
| 5 | -8.5 | 2.908 | 3.654 |
| 6 | -8.4 | 3.410 | 4.301 |
| 7 | -8.1 | 3.183 | 3.986 |
| 8 | -8.0 | 2.389 | 3.687 |
| 9 | -7.9 | 2.227 | 3.312 |

Writing output ... done.

### Glimperides

Output will be Glimperide_out.pdbqt
Detected 8 CPUs
Reading input ... done.
Setting up the scoring function ... done.
Analyzing the binding site ... done.
Using random seed: -411975640
Performing search ... done.
Refining results ... done.

| mode | affinity (kcal/mol) | dist from best mode rmsd l.b. | rmsd u.b. |
|---|---|---|---|
| 1 | -8.6 | 0.000 | 0.000 |
| 2 | -8.4 | 3.699 | 5.755 |
| 3 | -8.2 | 3.758 | 5.919 |
| 4 | -8.0 | 3.989 | 11.370 |
| 5 | -8.0 | 3.308 | 4.922 |
| 6 | -7.9 | 3.697 | 5.923 |
| 7 | -7.8 | 2.698 | 12.215 |
| 8 | -7.7 | 3.585 | 4.873 |
| 9 | -7.6 | 5.920 | 7.097 |

Writing output ... done.

### Sorafenib

Output will be Sorafenib_out.pdbqt
Detected 8 CPUs
Reading input ... done.
Setting up the scoring function ... done.
Analyzing the binding site ... done.
Using random seed: -1568126632
Performing search ... done.
Refining results ... done.

| mode | affinity (kcal/mol) | dist from best mode rmsd l.b. | rmsd u.b. |
|---|---|---|---|
| 1 | -8.5 | 0.000 | 0.000 |
| 2 | -8.3 | 2.466 | 3.218 |
| 3 | -8.3 | 5.421 | 6.484 |
| 4 | -8.3 | 2.262 | 2.810 |
| 5 | -8.2 | 1.844 | 2.989 |
| 6 | -8.2 | 3.260 | 4.012 |
| 7 | -8.1 | 6.742 | 12.192 |
| 8 | -8.0 | 2.393 | 3.304 |
| 9 | -7.9 | 2.138 | 2.742 |

Writing output ... done.

### Palbociclib

Output will be Palbociclib_out.pdbqt
Detected 8 CPUs
Reading input ... done.
Setting up the scoring function ... done.
Analyzing the binding site ... done.
Using random seed: -228195456
Performing search ... done.
Refining results ... done.

| mode | affinity (kcal/mol) | dist from best mode rmsd l.b. | rmsd u.b. |
|---|---|---|---|
| 1 | -8.3 | 0.000 | 0.000 |
| 2 | -8.2 | 1.978 | 3.904 |
| 3 | -8.2 | 2.178 | 3.511 |
| 4 | -8.0 | 1.578 | 1.923 |
| 5 | -7.8 | 3.369 | 4.721 |
| 6 | -7.8 | 3.611 | 9.053 |
| 7 | -7.8 | 3.717 | 9.700 |
| 8 | -7.5 | 3.449 | 9.053 |
| 9 | -7.4 | 3.319 | 5.509 |

Writing output ... done.

## Abiraterone

Output will be abiraterone_out.pdbqt
Detected 8 CPUs
Reading input ... done.
Setting up the scoring function ... done.
Analyzing the binding site ... done.
Using random seed: 1542230052
Performing search ... done.
Refining results ... done.

| mode | affinity (kcal/mol) | dist from best mode rmsd l.b. | rmsd u.b. |
|---|---|---|---|
| 1 | -8.2 | 0.000 | 0.000 |
| 2 | -8.2 | 1.639 | 2.955 |
| 3 | -8.0 | 1.511 | 1.886 |
| 4 | -7.9 | 3.840 | 7.732 |
| 5 | -7.8 | 4.100 | 8.035 |
| 6 | -7.7 | 3.876 | 8.256 |
| 7 | -7.1 | 2.148 | 2.828 |
| 8 | -7.0 | 3.974 | 5.664 |
| 9 | -7.0 | 4.859 | 8.838 |

Writing output ... done.

## Lapatinib

Output will be Lapatinib_out.pdbqt
Detected 8 CPUs
Reading input ... done.
Setting up the scoring function ... done.
Analyzing the binding site ... done.
Using random seed: -1992023152
Performing search ... done.
Refining results ... done.

| mode | affinity (kcal/mol) | dist from best mode rmsd l.b. | rmsd u.b. |
|---|---|---|---|
| 1 | -8.2 | 0.000 | 0.000 |
| 2 | -8.2 | 3.376 | 8.120 |
| 3 | -7.9 | 5.704 | 8.326 |
| 4 | -7.8 | 2.206 | 2.851 |
| 5 | -7.8 | 4.571 | 6.857 |
| 6 | -7.6 | 4.565 | 6.344 |
| 7 | -7.3 | 3.752 | 8.838 |
| 8 | -7.3 | 3.789 | 8.293 |
| 9 | -7.3 | 2.376 | 3.432 |

Writing output ... done.

## Canagliflozin

Output will be Canagliflozin_out.pdbqt
Detected 8 CPUs
Reading input ... done.
Setting up the scoring function ... done.
Analyzing the binding site ... done.
Using random seed: 2135226564
Performing search ... done.
Refining results ... done.

| mode | affinity (kcal/mol) | dist from best mode rmsd l.b. | rmsd u.b. |
|---|---|---|---|
| 1 | -8.1 | 0.000 | 0.000 |
| 2 | -8.0 | 3.621 | 5.637 |
| 3 | -8.0 | 5.125 | 9.155 |
| 4 | -8.0 | 3.256 | 4.987 |
| 5 | -7.9 | 1.391 | 1.873 |
| 6 | -7.8 | 4.564 | 7.959 |
| 7 | -7.8 | 4.927 | 8.749 |
| 8 | -7.7 | 3.688 | 5.291 |
| 9 | -7.7 | 3.109 | 4.788 |

Writing output ... done.

## Dabrafenib

Output will be Dabrafenib_out.pdbqt
Detected 8 CPUs
Reading input ... done.
Setting up the scoring function ... done.
Analyzing the binding site ... done.
Using random seed: 29750968
Performing search ... done.
Refining results ... done.

| mode | affinity (kcal/mol) | dist from best mode rmsd l.b. | rmsd u.b. |
|---|---|---|---|
| 1 | -8.1 | 0.000 | 0.000 |
| 2 | -8.0 | 2.601 | 6.931 |
| 3 | -8.0 | 2.605 | 7.501 |
| 4 | -8.0 | 1.800 | 2.677 |
| 5 | -8.0 | 2.601 | 7.473 |
| 6 | -8.0 | 2.552 | 6.814 |
| 7 | -7.9 | 3.235 | 7.936 |
| 8 | -7.9 | 1.874 | 2.485 |
| 9 | -7.8 | 3.722 | 8.390 |

Writing output ... done.

## LRRK2-in-1

Output will be lrrk2_in1_out.pdbqt
Detected 8 CPUs
Reading input ... done.
Setting up the scoring function ... done.
Analyzing the binding site ... done.
Using random seed: 415575592
Performing search ... done.
Refining results ... done.

| mode | affinity (kcal/mol) | dist from best mode rmsd l.b. | rmsd u.b. |
|---|---|---|---|
| 1 | -9.8 | 0.000 | 0.000 |
| 2 | -9.7 | 0.810 | 1.460 |
| 3 | -9.6 | 1.401 | 1.705 |
| 4 | -9.1 | 1.586 | 3.116 |
| 5 | -9.0 | 2.526 | 12.377 |
| 6 | -8.9 | 2.689 | 11.894 |
| 7 | -8.7 | 4.033 | 12.272 |
| 8 | -8.1 | 2.865 | 11.660 |
| 9 | -8.1 | 6.688 | 10.800 |

Writing output ... done.

## K-252A

Output will be K252a_out.pdbqt
Detected 8 CPUs
Reading input ... done.
Setting up the scoring function ... done.
Analyzing the binding site ... done.
Using random seed: -1134997720
Performing search ... done.
Refining results ... done.

| mode | affinity (kcal/mol) | dist from best mode rmsd l.b. | rmsd u.b. |
|---|---|---|---|
| 1 | -9.1 | 0.000 | 0.000 |
| 2 | -8.8 | 2.209 | 5.440 |
| 3 | -8.3 | 1.700 | 7.602 |
| 4 | -8.2 | 1.512 | 2.191 |
| 5 | -7.5 | 2.190 | 3.523 |
| 6 | -7.4 | 2.227 | 7.530 |
| 7 | -7.3 | 2.112 | 5.876 |
| 8 | -7.2 | 3.179 | 7.466 |
| 9 | -7.2 | 2.334 | 6.040 |

Writing output ... done.

## Staurosporine

Output will be staurosporine_out.pdbqt
Detected 8 CPUs
Reading input ... done.
Setting up the scoring function ... done.
Analyzing the binding site ... done.
Using random seed: 1823607816
Performing search ... done.
Refining results ... done.

| mode | affinity (kcal/mol) | dist from best mode rmsd l.b. | rmsd u.b. |
|---|---|---|---|
| 1 | -8.9 | 0.000 | 0.000 |
| 2 | -8.7 | 1.984 | 5.219 |
| 3 | -7.3 | 2.202 | 3.655 |
| 4 | -7.2 | 2.603 | 7.071 |
| 5 | -7.1 | 3.065 | 7.928 |
| 6 | -6.9 | 2.610 | 7.516 |
| 7 | -6.9 | 2.486 | 5.640 |
| 8 | -6.9 | 2.254 | 5.013 |
| 9 | -6.8 | 2.077 | 3.085 |

Writing output ... done.

## GO-6976

Output will be go6976_out.pdbqt
Detected 8 CPUs
Reading input ... done.
Setting up the scoring function ... done.
Analyzing the binding site ... done.
Using random seed: -1100691320
Performing search ... done.
Refining results ... done.

| mode | affinity (kcal/mol) | dist from best mode rmsd l.b. | rmsd u.b. |
|---|---|---|---|
| 1 | -8.6 | 0.000 | 0.000 |
| 2 | -8.5 | 1.441 | 5.680 |
| 3 | -8.2 | 2.479 | 5.627 |
| 4 | -7.6 | 2.752 | 5.611 |
| 5 | -7.6 | 2.241 | 4.284 |
| 6 | -7.5 | 3.204 | 7.904 |
| 7 | -7.5 | 2.822 | 6.277 |
| 8 | -7.4 | 2.478 | 3.715 |
| 9 | -7.3 | 3.040 | 7.630 |

Writing output ... done.

## RO-31-8220

Output will be ro318220_out.pdbqt
Detected 8 CPUs
Reading input ... done.
Setting up the scoring function ... done.
Analyzing the binding site ... done.
Using random seed: 238907124
Performing search ... done.
Refining results ... done.

| mode | affinity (kcal/mol) | dist from best mode rmsd l.b. | rmsd u.b. |
|---|---|---|---|
| 1 | -8.1 | 0.000 | 0.000 |
| 2 | -7.5 | 1.699 | 2.074 |
| 3 | -7.2 | 3.490 | 7.995 |
| 4 | -6.8 | 3.162 | 7.689 |
| 5 | -6.6 | 2.308 | 2.677 |
| 6 | -6.4 | 3.564 | 7.487 |
| 7 | -6.3 | 3.269 | 7.508 |
| 8 | -6.3 | 3.261 | 4.428 |
| 9 | -6.1 | 3.937 | 5.777 |

Writing output ... done.

JH-II-127

Output will be jhii127_out.pdbqt
Detected 8 CPUs
Reading input ... done.
Setting up the scoring function ... done
Analyzing the binding site ... done.
Using random seed: -320821552
Performing search ... done.
Refining results ... done.

| mode | affinity (kcal/mol) | dist from best mode rmsd l.b. | rmsd u.b. |
|---|---|---|---|
| 1 | -7.9 | 0.000 | 0.000 |
| 2 | -7.8 | 0.706 | 2.127 |
| 3 | -7.4 | 5.764 | 11.580 |
| 4 | -7.3 | 7.435 | 13.480 |
| 5 | -7.3 | 4.257 | 7.127 |
| 6 | -7.3 | 5.574 | 11.566 |
| 7 | -7.1 | 6.681 | 12.707 |
| 8 | -7.1 | 5.473 | 11.456 |
| 9 | -7.1 | 5.408 | 11.312 |

Writing output ... done.

Crizotinib

Output will be crizotinib_out.pdbqt
Detected 8 CPUs
Reading input ... done.
Setting up the scoring function ... done.
Analyzing the binding site ... done.
Using random seed: -2087626172
Performing search ... done.
Refining results ... done.

| mode | affinity (kcal/mol) | dist from best mode rmsd l.b. | rmsd u.b. |
|---|---|---|---|
| 1 | -7.7 | 0.000 | 0.000 |
| 2 | -7.7 | 1.773 | 2.888 |
| 3 | -7.7 | 1.668 | 2.307 |
| 4 | -7.6 | 2.590 | 4.083 |
| 5 | -7.5 | 3.294 | 4.773 |
| 6 | -7.3 | 3.890 | 5.992 |
| 7 | -7.2 | 3.340 | 4.806 |
| 8 | -7.2 | 1.337 | 1.707 |
| 9 | -7.1 | 1.216 | 2.159 |

Writing output ... done.

czc25146

Output will be Czc25146_out.pdbqt
Detected 8 CPUs
Reading input ... done.
Setting up the scoring function ... done.
Analyzing the binding site ... done.
Using random seed: 1787040000
Performing search ... done.
Refining results ... done.

| mode | affinity (kcal/mol) | dist from best mode rmsd l.b. | rmsd u.b. |
|---|---|---|---|
| 1 | -7.6 | 0.000 | 0.000 |
| 2 | -7.6 | 1.812 | 2.073 |
| 3 | -7.3 | 2.683 | 3.630 |
| 4 | -7.3 | 3.108 | 5.611 |
| 5 | -7.2 | 2.272 | 2.720 |
| 6 | -7.0 | 4.062 | 6.519 |
| 7 | -7.0 | 3.611 | 8.037 |
| 8 | -6.9 | 2.175 | 2.790 |
| 9 | -6.8 | 9.013 | 15.632 |

Writing output ... done.

Sunitinib

Output will be sunitinib_out.pdbqt
Detected 8 CPUs
Reading input ... done.
Setting up the scoring function ... done.
Analyzing the binding site ... done.
Using random seed: -104979688
Performing search ... done.
Refining results ... done.

| mode | affinity (kcal/mol) | dist from best mode rmsd l.b. | rmsd u.b. |
|---|---|---|---|
| 1 | -7.2 | 0.000 | 0.000 |
| 2 | -7.2 | 1.611 | 1.949 |
| 3 | -7.2 | 1.526 | 1.994 |
| 4 | -6.8 | 2.481 | 3.304 |
| 5 | -6.7 | 1.940 | 2.705 |
| 6 | -6.7 | 2.117 | 2.721 |
| 7 | -6.6 | 8.891 | 10.726 |
| 8 | -6.4 | 3.632 | 9.319 |
| 9 | -6.4 | 5.093 | 11.342 |

Writing output ... done.

**Figure A.2**: Free energy of binding of various ligands in ten different conformations when docked with G2019S mutant LRRK2

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**Through HOD Biotechnology**

**SUBJECT – Declaration of fulfillment of University's requirements for the award of M.Sc. Biotechnology**

Respected Sir,

I, **Animan Tripathi**, final year student of **M.Sc. Biotechnology**, Roll No. **2K20/MSCBIO/01**, Department of Biotechnology, Delhi Technological University, hereby declare that the work which is presented in the thesis titled "**Identification of putative inhibitors of mutant LRRK2 involved in the pathogenesis of Parkinson's disease: An *in-silico* drug-repurposing approach**" submitted in partial fulfillment of the requirements of the degree of Master of Science in Biotechnology is an authentic record of my own work done under the guidance of my supervisor, **Prof. Pravir Kumar**.

The work attached with this declaration complies with the University's requirements and is my own and original work. The overall similarity is 15%, out of which 10% is from my own publications. The plagiarism report has been attached. Further, the work attached to this declaration has not been submitted in full or partial for the satisfaction of degree in another university.

Animan

Yours sincerely,

Animan Tripathi
2K20/MSCBIO/01
M.Sc. Biotechnology
Semester - 4

## CANDIDATE'S DECLARATION

I, **Animan Tripathi,** (Roll No.: 2K20/MSCBIO/01) of **M.Sc. Biotechnology,** declare that this work which is presented in this Major Project titled "**Identification of putative inhibitors of mutant LRRK2 involved in the pathogenesis of Parkinson's disease: An *in-silico* drug-repurposing approach**" submitted 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, is original and my own, carried out during a period from 7th January, 2022 to 6th May, 2022, under the supervision of **Prof. Pravir Kumar**.

I also declare that this work has not previously formed the basis for the award of any Degree or other similar title or recognition.

This work has been communicated in an IEEE conference with Scopus indexed proceedings.

**The details of which are as follows:**

**Title of Paper**: "Identification of Putative LRRK2 Inhibitors in the Pathogenesis of Parkinson's Disease: A Drug Re-purposing Approach"
**Names of Authors**: Animan Tripathi and Pravir Kumar
**Name of the Conference**: 2021 5th International Conference on Information Systems and Computer Networks (ISCON)
**Conference date with Venue**: 21st October, 2021, GLA University, Mathura
**Registration for the conference**: Completed
**Status of the Paper (Accepted/Published/Communicated)**: Published
**Date of Paper Communication**: 28th July, 2021
**Date of Paper Acceptance**: 7th September, 2021

**Date of Paper Publication**: 14th February, 2022

Animan

Place: Delhi

Date: 04/05/2022

**ANIMAN TRIPATHI**

**(2K20/MSCBIO/01)**

## SUPERVISOR CERTIFICATE

To the best of my knowledge, the work titled "**Identification of putative inhibitors of mutant LRRK2 involved in the pathogenesis of Parkinson's disease: An *in-silico* drug re-purposing approach**" has not been submitted anywhere else either in part or in full for any Degree or Diploma at this University or elsewhere. I further certify that the publication and indexing information given by the student is correct.

Pravir Kumar
04/08/2022

Place: Delhi

Date:

**Prof. Pravir Kumar**

**(SUPERVISOR)**

Head of Department

Department of Biotechnology

Pravir Kumar
04/08/2022

HOD BIOTECHNOLOGY

PROF. PRAVIR KUMAR

Head of the Department

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On a more personal note, I express my gratitude towards my family and friends for their love, support and good wishes.

Animan

**Animan Tripathi**

**2K20/MSCBIO/01**