"FROM DOCKING TO DYNAMICS: REPURPOSING FDA-APPROVED DRUGS AS USP13 INHIBITORS FOR PARKINSON'S DISEASE"

Thesis submitted
in Partial Fulfilment of the Requirements for the
Degree of

MASTER OF SCIENCE
in
BIOTECHNOLOGY

by

ASHISH
2k22/MSCBIO/14

Under the Supervision of
Prof. PRAVIR KUMAR
Professor and Dean IA
Delhi Technological University

DEPARTMENT OF BIOTECHNOLOGY
DELHI TECHNOLOGICAL UNIVERSITY
(Formerly Delhi College of Engineering)
Bawana Road, Delhi - 110042

June, 2024

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CANDIDATE'S DECLARATION

I, **Ashish**, bearing Roll No. 2K22/MSCBIO/14 hereby certify that the work which is being presented in the thesis entitled "**From Docking to Dynamics: Repurposing FDA-Approved Drugs as USP13 Inhibitors for Parkinson's Disease**" in partial fulfillment of the requirement 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 January 2024 to May 2024 under the supervision of Prof. Pravir Kumar.

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

Candidate's Signature

This is to certify that the student has incorporated all the corrections suggested by the examiner in the thesis and the statement made by the candidate is correct to the best of our knowledge.

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Certified that **Ashish** (2K22/MSCBIO/14) has carried out their search work presented in this thesis entitled "**From Docking to Dynamics: Repurposing FDA-Approved Drugs as USP13 Inhibitors for Parkinson's Disease**" for the award of **Master of Science** from Department of Biotechnology, Delhi Technological University, Delhi under my supervision. The thesis embodies results of original work, and studies are carried out by the student herself and the contents of the thesis do not form the basis for the reward of any other degree to the candidate or to anybody else from this or any other University/Institution.

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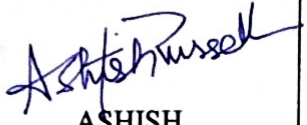

ASHISH

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From Docking to Dynamics: Repurposing FDA-Approved Drugs as USP13 Inhibitors for Parkinson's Disease

ASHISH

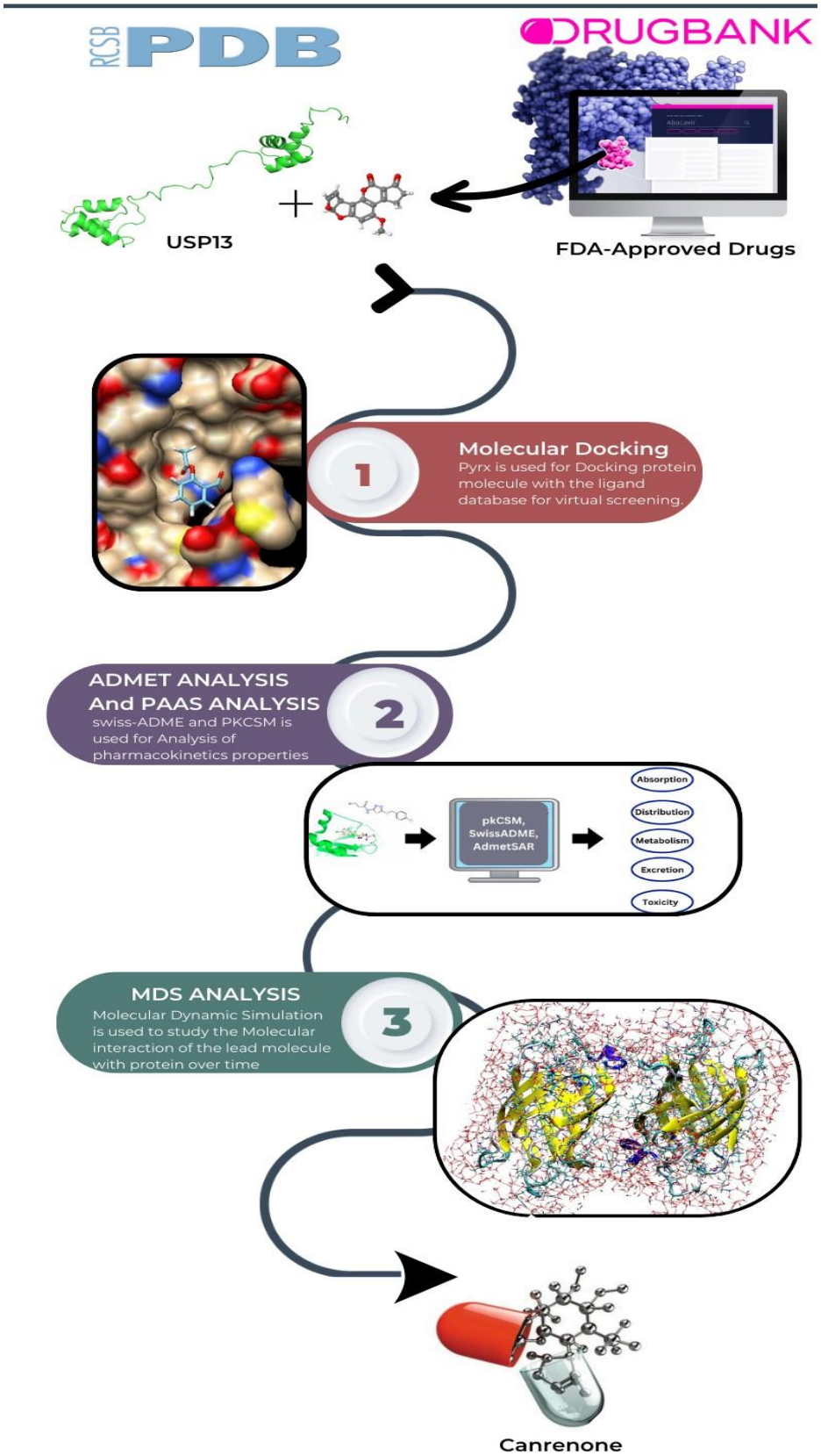
ABSTRACT

The aggregate term neurodegenerative diseases (NDD) refer to a group of conditions typified by a slow and steady decline in the structure and functionality of the nervous system. These disorders affect a large proportion of the world's population. They severely impair both motor and non-motor skills. One typical example of such an illness is Parkinson's disease (PD). It is classified as dominant NDD. It typically affects those over 65. Degeneration of dopamine-producing neurons in the Substantia Nigra par compacta (SNpc) a particular area of the brain important for regulating a variety of symptoms, including bradykinesia postural instability, stiffness and tremors is one prominent sign of PD. Lewy bodies are a major pathogenic feature of PD and they are present in dopaminergic neurons. The primary component of those bodies is alpha-synuclein a protein that causes aberrant buildup and impairs nerve cell function in pathological situations. There is currently no proven treatment for PD. Despite several experimental efforts, no cure exists. The mainstay of current therapy is L-dopa. It acts by increasing brain dopamine levels to alleviate symptoms. This approach highlights the urgent need for novel curative methods. Yet it does not permanently halt the illness from progressing. The current study investigates Ubiquitin-Specific Protease 13's (USP13) potential for treatment in this regard. USP13 is classified as a deubiquitinase It plays an essential role in the ubiquitin-proteasome system. The system disrupts malformed and damaged proteins. In PD USP13 prevents the precise decline of alpha-synuclein by removing ubiquitin tag, thus preventing its degradation and leading to the accumulation of undesirable protein groups. The imbalance in protein level is harmful. This is especially true in the dopaminergic neurons which worsens the neurodegenerative condition. Due to the significant role of USP13 in PD, concentrating on this enzyme provides a novel way of treating this condition. To rapidly identify a possible inhibitor of USP13, the study uses a drug repositioning method using an FDA-approved medicinal product. Drug repositioning is an efficient and economical strategy involving the repositioning of an existing medicinal product for new healthcare purposes. This technique exploits the significant safety and pharmacokinetic properties of the above-mentioned medicinal product, which contributes to speeding up the process of developing new medicines. Sophisticated pharmacoinformatic tools were used to examine the selection of FDA-approved medicines to see if they could block USP13. The method uses computational techniques to predict the method by which a substance could sufficiently bind to the USP13 enzyme. Canrenone has been identified as a candidate for the capability of favourably binding USP13. Canrenone is considered to be an aldosterone antagonist and was designed to have beneficial pharmacokinetic and pharmacodynamic properties, as well as the ability to penetrate the blood brain barrier, which is essential

for a drug designed to treat neurodegenerative disorders. Canrenone's promise was further confirmed by examination using the forecasting PASS study and MD simulation. The PASS investigation revealed Canrenone's numerous physiological activities, which contributed to its potential for curative use. To assess the stability and robustness of the Canrenone's interaction with USP13, an MD simulation was used. The findings suggest that Canrenone, as opposed to the currently available USP13 inhibitor Spautin-1, has a higher binding affinity to USP13. Canrenone could be a better PD therapy. In conclusion, using usp13 as a target for the treatment of PD is highlighted by this work. The research proposes Canrenone as a potential USP13 inhibitor which exhibits higher efficiency and constructive pharmacokinetic properties when used in combination with an already approved medicinal product. To accelerate the introduction of a new treatment for PD, the investigation uses a unique method of integration of docking, PASS investigation, as well as MD simulation. That analysis shows significant progress in the search for improved treatment in the fight against the disabling effects of PD as well as in improving the welfare of people affected by the current NDD.

Keywords: Neurodegenerative diseases, Parkinson’s disease, USP13, Canrenone

GRAPHICAL ABSTRACT



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LIST OF ABBREVIATIONS

PD	Parkinson's Disease
AD	Alzheimer's Disease
DUB	Deubiquitinase
USP13	Ubiquitin Specific Potease13
BBB	Blood-Brain Barrier
NDD	Neurodegenerative Diseases
DR	Drug Repurposing
NMS	Non-Motor Symptoms
SNpc	Substantia Nigra Pars Compacta
CBD	Corticobasal Degeneration
MSA	Multiple System Atrophy
PSP	Progressive Supranuclear Palsy
DLB	Dementia with Lewy Bodies
LB	Lewy bodies
UPS	Ubiquitin–Proteasome Pathway
MD	Molecular Dynamics
RMSD	Root Mean Square Deviation
RMSF	Root Mean Square Fluctuations
PDF	Probability Distribution Function
ETC	Electron Transport Chain

CHAPTER 1

INTRODUCTION

1.1Background

Cognitive downturn, trouble speaking, impaired movement, and other indications are among the many neurological illnesses that fall under the umbrella term of neurodegeneration. A prevalent pathogenic characteristic observed in many disorders is the reduction in the overall amount of neuronal cells in the CNS. For each ailment, the clinical symptoms depend on the particular areas involved. Both common and uncommon conditions are included in the category of neurodegenerative diseases (NDDs). These include multiple system atrophy (MSA), , amyotrophic lateral sclerosis (ALS), Huntington's disease (HD), frontotemporal dementia (FTD), dementia with Lewy bodies (DLB), corticobasal degeneration (CBD), and spinal muscular atrophy [1]. Numerous factors, such as genetic predispositions, abnormalities in cell signaling pathways, neuronal apoptosis, inflammatory responses, aggregation of proteins, mitochondrial dysfunction, oxidative stress, deterioration, gender differences, genetic mutations, ethnicity, exposure to the environment, and external influences, are associated with NDDs [2], [3]. Alzheimer's Disease (AD) and PD are common neurodegenerative illnesses that share clinical manifestations, including bradykinesia, bodily stiffness, depression, shakes, and related psychosocial problems. In the hippocampus and cortical areas, AD is defined by the death of neurons that causes symptoms such as memory loss, mental impairment, and eventually mortality [4], [5]. Many people worldwide are afflicted with various NDDs. For example, since 1990, the number of people with AD and PD has grown by more than double. Alpha-synuclein in PD and beta-amyloid in AD are two examples of the aberrant aggregation or incorrect assembly of peptides that are the most prevalent pathophysiology connected to NDDs [6].

PD is a neurological condition and is the second most prevalent progressive neurodegenerative illness that worsens over time and is typified by tremors, bradykinesia, or sluggish movement, and postural instability. Since the disease's eponymous physician's description in the early 1800s, PD has been known to exist, called as "Shaking Palsy" and "paralysis agitans" at times [7]. A significant turning point in the etiopathogenesis of PD was Frederick Lewy's discovery in 1912 of

intracytoplasmic inclusion bodies, or Lewy bodies(LB). Following the establishment of the crucial link between dopamine insufficiency and PD in 1957, research by Arvid Carlsson and Oleh Hornykiewicz made substantial progress toward understanding the function of dopamine deficiency in PD. This was further supported by the first clinical study, which showed that intravenous levodopa improved PD patients' symptoms in 1961, and George Cotzias' later invention of high-dosage levodopa treatment in 1967 [8]. PD typically affects 1% to 2% of those 65 years of age and beyond and most cases are sporadic; in people 80 years of age and above, the frequency rises to 4% [9]. There are currently no proven neuroprotective drugs or medical treatments for PD [10]. Even with continued investigation from several angles, the illness is still incurable. Therefore, developing novel and more effective drugs that target PD-related pathways is a key goal. Creating a novel medication from scratch is a priced-out and tedious operation. Typically, it takes thirteen to fifteen years and costs two to three billion dollars [11].

The strategy known as "drug repurposing" (sometimes known as "drug repositioning"), which Ashburn and Thor initially covered in 2004, has drawn interest in this situation [12]. The term "drug repurposing" describes the process of finding new uses and functions for pharmaceuticals that are currently available on the market. This approach's main benefit is that currently available medications have preclinical data and established clinical features, such as pharmacokinetics, pharmacodynamics, and toxicity), which reduces development risk. Consequently, repurposed drugs can quickly enter late-stage clinical trials, significantly cutting down on development costs and time [13]. USP13 has a role in the development and accumulation of LB, which is a build-up of alpha-synuclein in the brain. The "tags" of ubiquitin attached to alpha-synuclein that indicate its pending breakdown are eliminated by this enzyme. Consequently, alpha-synuclein hazardous aggregates accumulate because they are not effectively removed [14]. Since USP13 is involved in the process of removing ubiquitin tags from alpha-synuclein, which causes toxic aggregates to build up and Lewy bodies to form, targeting USP13 offers a potential treatment approach for Parkinson's disease. Consequently, using FDA-approved medications in a different way to inhibit USP13 may be a realistic and effective therapy strategy, utilizing the safety and efficacy profiles already in place to accelerate the research process.

1.2 Objective

- To examine whether USP13 may be used as a therapeutic target to treat PD.
- To find new USP13 inhibitors with better effectiveness than currently available drugs like Spautin-1.
- Modify FDA-approved medications to specifically target USP13 to hasten the creation of PD treatment options.
- Utilize PyRx's sophisticated molecular docking analysis to explore a library of FDA-approved medications and find substances that have strong binding capabilities for USP13.
- Verify the stability and dependability of ligand-protein interactions by using MDS and PASS Analysis to validate the results.

CHAPTER 2

LITERATURE REVIEW

2.1 Parkinson’s Disease (PD)

PD is a chronic NDD of the nervous system that can cause motor-related dysfunction such as tremors, decreased pace, rigidity, gait impairment, and lack of balance may occur. Along with these physical issues, people with the condition may also have trouble with digestion, mood, thinking, and other related conditions of PD [15]. As people age, the prevalence of Parkinson's disease increases: 1% of those over 65 and 3% of those over 80 are affected by the disorder [16]. Important elements like genetics and environment have an impact on the reasons for the emergence of this illness. PET and SPECT (single-photon emission computed tomography) are diagnostic tools for Parkinson's disease because they can reveal changes in the presynaptic dopaminergic pathway [17]. However, the patient's history and physical examination are the primary factors for diagnosing PD. Nevertheless, these sophisticated methods are capable of distinguishing PD patients from individuals in good health, with a sensitivity of more than 95%. PD therapies available today are mostly aimed at symptom management, not slowing the illness's development. The combination of levodopa and a peripheral decarboxylase inhibitor is still the most effective treatment [18]. Modern symptomatic medicine in the US has concentrated on increasing levodopa's duration of action by blocking COMT and changing the availability and make-up of older drugs such as apomorphine and selegiline [19]. This calls for the identification of novel targets and development of targeted therapeutic approaches, and diagnostic and treatment modalities. Several medications that may be useful for treating PD have been found thanks to advancements in the rational and methodical repurposing of pharmaceuticals [20].

2.1.1 Motor and Nonmotor Symptoms (NMS)

The standard method for medically diagnosing PD is diminished motor skills. Key characteristics that move from asymmetric initial presentation to bilateral participation comprise bradykinesia, stiffness, tremor, and unstable posture. A mask-like expression on the face, talking and swallowing problems, festination (fast shuffling movements with a forward-flexed posture), and micrographia are supplementary motor indications

[21]. All people with PD experience both Motor and NMS and the incidence of NMS increases with the illness's progression. Patients in the late stages frequently have 6–10 NMS. These symptoms are the most resource-intensive for healthcare settings, often go undiagnosed and untreated, and have a significant effect on the results experienced by the patient. NMS raises the risk of handicaps, lowers the standard of living, and necessitates sustained care [22]. Though little is known about the origins of NMS, the explanations for dysfunctional movement in PD are clear. There is likely more than one cause for these symptoms than the basal ganglia. The key characteristic of NMS is the fact that they might occur years before motor symptoms do. Examples of these symptoms include sadness, constipation, olfactory impairments, and rapid-eye-movement sleep behavior disruption. They may, nevertheless, also appear simultaneously with or after the commencement of motor anomalies [23]. In subsequent years NMS may be used to diagnose PD early and begin neuroprotective treatment earlier. The goal of ongoing investigations with sizable cohorts of elderly people who appear to be in excellent condition is to enable this kind of prompt assessment [24]

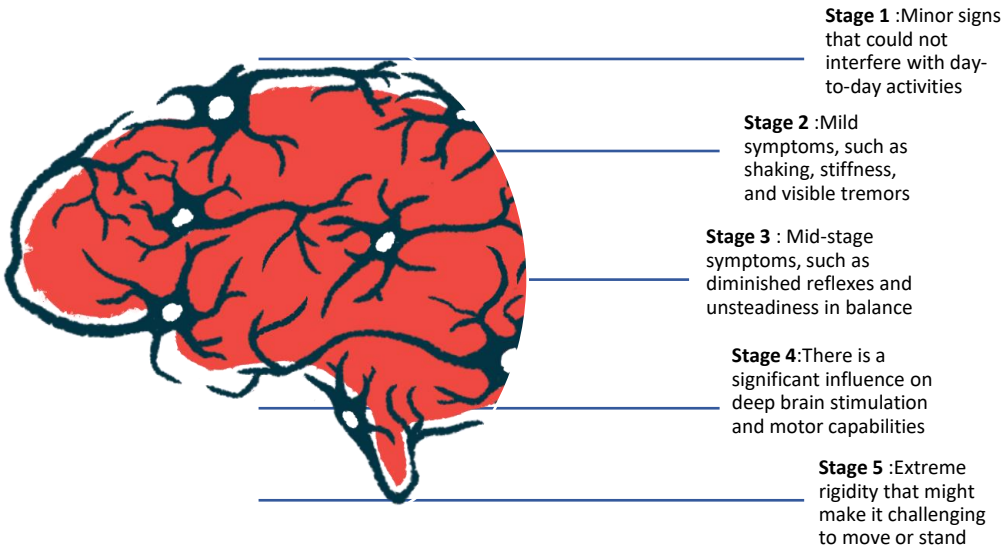


Figure 2.1. Various Stages in Parkinson's Disease

2.1.2 Animal Models and Neurotoxin-Induced Mechanisms in PD

With the use of animal models, research into the pathogenesis of PD has advanced dramatically. Initially, models included the injection of reversible blockers of

dopamine release (vesicular monoamine-transporter inhibitor reserpine), dopamine synthesis (tyrosine hydroxylase inhibitor α-methyl-p-tyrosine), or dopamine receptor blockers (haloperidol) [25]. Subsequent models used toxins such as systemically given MPTP or locally injected 6-hydroxydopamine (6-OHDA) to cause lasting damage in dopaminergic neurons. In humans, MPTP's harmful effects were first noted in the early 1980s [26], [27]. Although MPTP is not poisonous in and of itself, monoamine oxidase type B (MAO-B) in the brain metabolizes it to produce the toxin MPP+. The dopaminergic cells of substantia nigra pars compacta (SNpc) preferentially absorb MPP+, which then causes Disruption of complex I of the ETC, ultimately leading to the death of cells Both 6-OHDA, which enhances oxidative stress, and MPTP, which hinders mitochondrial complex I, play a role in the mechanisms contributing to the pathophysiology of PD [28].

2.1.3 Types

Parkinsonian symptoms that appear earlier to one reaches forty years of age are referred to as early-onset Parkinson's disease (EOPD). About 3–5% of all PD cases are related to it [29]. Idiopathic Parkinson's disease can resemble other neurodegenerative illnesses. Among them are CBD, MSA, PSP, and DLB [30]. Although the Parkinson's majority of PD cases are idiopathic, there are recognized hereditary and environmental risk factors. In most groups, males are twice as likely as women to get PD. There is evidence that female sex hormones have a protective impact [31]. Large-scale, uniform epidemiological data on PD are absent from India [32]. By 2030, there will be more than 50% more persons with PD due to longer lifespans[33]. Although this projection is only an approximation based on population increase in the future, it emphasizes the significant cost that PD and other neurological diseases can have on society.

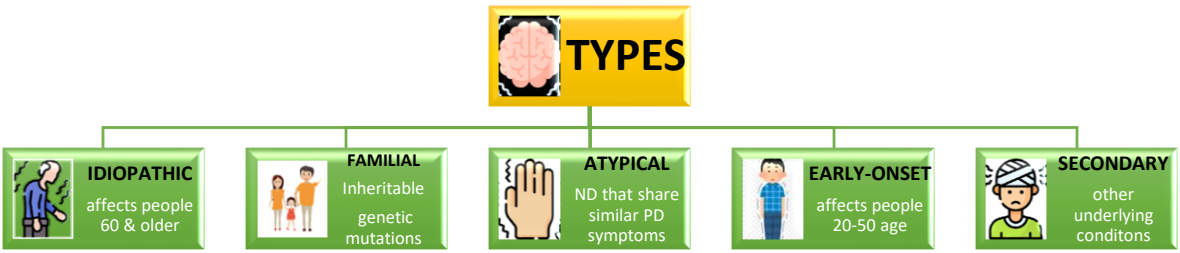


Figure 2.2. Types of PD

2.1.4 Pathology

PD is identified by the pathology of dopamine-releasing neurons deteriorating or dying in the substantia nigra, together with the production of Lewy bodies (LB), clumped, insoluble alpha-synuclein is a primary component of LB, the characteristic cellular

inclusions, a pathological signature that is specific to these dopaminergic neurons[34]. Misfolded ubiquitin proteins, which are essential for protein recycling, are overproduced in Lewy bodies LBs. A dysfunctional ubiquitin-proteasome system (UPS) is the cause of this buildup [35]. Pathologically speaking, PD is characterized by characteristic anomalies in the pontine locus ceruleus and SNpc of the brain. Gliosis, neuronal loss, and depigmentation are some of these anomalies. About 60–70% of the SNpc's neurons have deteriorated by the time symptoms of PD appear [36]. The brainstem's cholinergic and monoaminergic neurons as well as the olfactory system's neurons are where LB dysfunction first manifests. The limbic and neocortical parts of the brain are affected as the condition worsens. By the time the illness reaches its latter stages, only the SNpc exhibits the first reduction in dopaminergic neurons [37], [38]. This neuronal loss marks the first step toward the development of early motor symptoms in PD and facilitates medical assessment. It usually appears later in the course of the disease, but degeneration of nondopaminergic neurons also occurs [39].

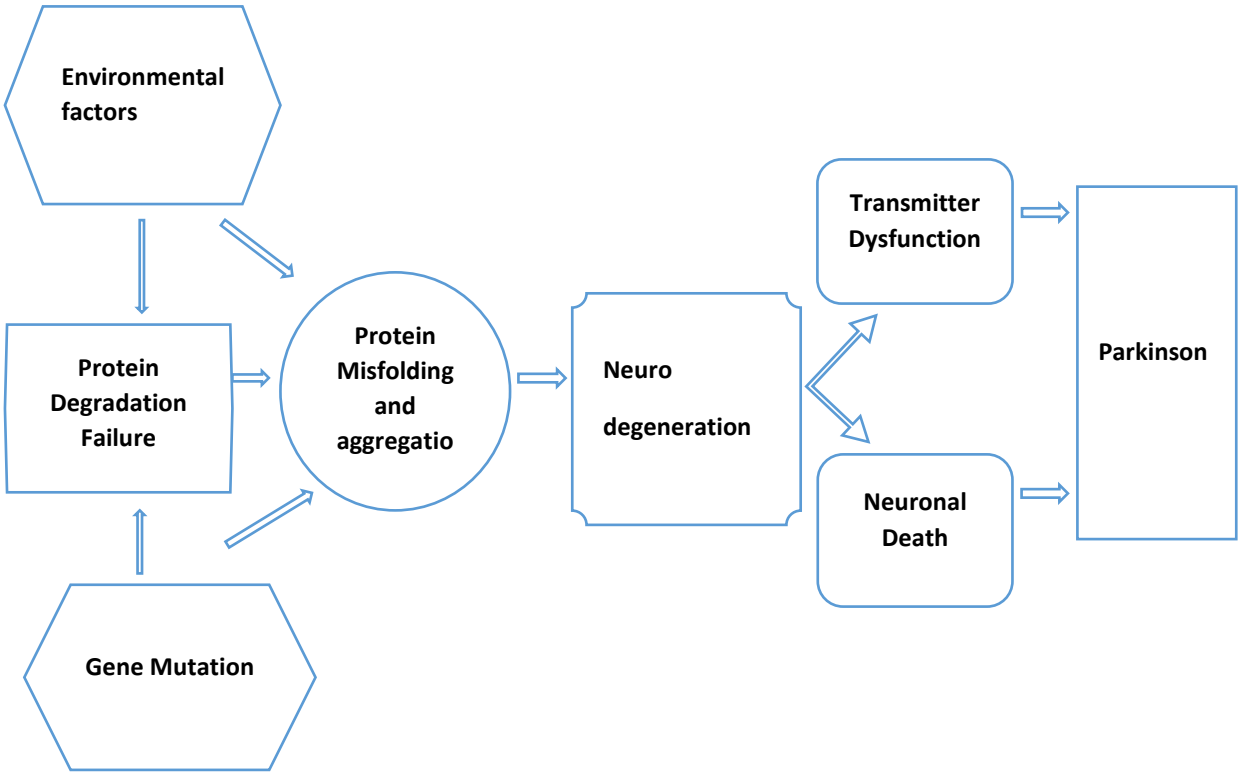


Figure 2.3. Pathogenesis of PD

2.1.5 Genetic and Environmental Factors

PD may have a genetic basis, according to initial twin studies and the discovery of several families with dominant and recessive Mendelian inheritance patterns. The ground-breaking finding of the first PD-related gene, α-synuclein (SNPCA), in 1997 resulted from this area of inquiry [40]. Encoding the protein α-synuclein, the SNPCA gene is one of the most important genetic factors in PD. The condition is largely caused by changes in the SNPCA gene, such as mutations and higher gene dosage from duplications and triplications [41]. Genetic manifestations of PD are present in 5–10% of cases. A total of a minimum of 17 distinct gene mutations that are autosomal have been linked to familial PD. These mutations affect both men and women equally [42]. The major genes identified as causative in PD include PARK2, LRRK2/PARK8, SNPCA-PARK1/PARK4, PINK1/PARK6, DJ1 (PARK7), UCH-L1, and ATP13A2 [43], [44], [45]. Parkin and LRRK2 are probably among the most prevalent genetic connections between late-onset PD and PD, however, GBA mutations are also a significant cause of risk [46]. Additionally, PD damages the systems such as the ubiquitin-proteasome system that break down mutant or improperly folded proteins. An unusual kind of oxidative stress caused by free radical-generating species, which results in neuronal degeneration, and mitochondrial malfunction are further dysfunctional processes linked to PD [47]. Strain caused by oxidation and the damage that it causes has come to light as important factors in some ways, involving the idea that elevated chemical and enzymatic dopamine oxidation leads to the generation of free radicals, and the processes behind toxins such as 6-hydroxydopamine (6-OHDA) and paraquat, and confirmation from post-mortem and medical trials [48], [49], [50]. The pathophysiological relevance of complex I inhibition—a mechanism shared by other dopaminergic neuron poisons like rotenone and annonacin—was brought to light by the finding of MPTP neurotoxicity through its metabolite MPP+ [51], [52]. It was discovered very rapidly that the SNpc included a tissue- and disease-specific inhibition of complex I, albeit it was also present in the muscle and platelets of patients with PD [53]. The biggest contributing element to PD risk is age, with the average onset occurring around 50 to 60 years. Additionally, two other important risk factors are family history, indicating a genetic link, and exposure to pesticides [54]. The varying prevalence of PD globally suggests that environmental and genetic factors, as well as ethnic differences, may all contribute to the disease's pathogenesis [55].

2.2 Protein Degradation Pathways

The ubiquitin-proteasome system (UPS) and the lysosome peptide cleaving mechanism, including chaperone-mediated autophagy (CMA), microautophagy, and macroautophagy, which is also known as the autophagy-lysosome pathway (ALP), are the two main methods used by eukaryotic cells to degrade protein.

2.2.1 The Autophagy–Lysosomal Pathway

Enzymes found in degradative organelles called lysosomes are essential to the disposal system of the cell. They participate in the materials' breakdown that comes from different degradation processes [56]. Autophagy functions both under normal and stressful circumstances and is essential to a cell's ability to survive. It destroys intracellular pathogens, protein accumulation or folding errors, long-lived proteins, and dysfunctional organelles. Macroautophagy, Microautophagy, and CMA are the three main types of Autophagy [57].

2.2.2 The Ubiquitin–Proteasome System (UPS)

By breaking down proteins, the UPS controls a variety of biological functions. These include transcription, apoptosis, cellular division control, DNA repair, and the reaction to cellular stress [58]. The UPS breaks down proteins in two different but successive steps: (i) numerous ubiquitin molecules covalently linked to the protein substrate, and (ii) the 26S proteasome complex breaks down the tagged protein, freeing up ubiquitin for further use. Precise regulation of both ubiquitin conjugation and the degradation of ubiquitinated substrates is necessary to provide effective and targeted protein removal at the appropriate moment [59]. With 76 amino acid residues, ubiquitin is a small polypeptide that can join up with a Lys residue on a substrate protein to generate an isopeptide. A coordinated effort by several different enzymes is required for the process of ubiquitination: the E1 enzyme initiates ubiquitin, the E2 enzyme transports activated ubiquitin, and the E3 enzyme makes ubiquitin transfer happen promptly from E2 to the substrate protein. Furthermore, by accelerating the creation of isopeptide bonds between successive ubiquitin moieties, a subclass of E3 enzymes promotes the elongation of the ubiquitin chain and produces polyubiquitin chains on the substrate protein [60], [61]. Unlike the numerous E2s and E3s found in cells, only one functional E1 enzyme has been discovered in mammalian animals [62]. The E3 ubiquitin ligase enzyme is the key factor influencing selectivity in the UPS. More than a thousand different E3 ligases are present in eukaryotic cells, and they are responsible for the carefully controlled covalent interaction of proteins of interest with ubiquitin (Ub).

These E3 enzymes first create an Ub and a residue of lysine on the recipient protein forms an isopeptide bond before catalyzing the transfer of the active Ub moiety from an E2 enzyme that conjugates. The E3 ligases then aid in the successive conjugation of more Ub molecules to the developing Ub moiety, producing an extended polyubiquitin chain that is attached to the target protein substrate [63]. Seven lysine residues (Lys6, Lys11, Lys27, Lys29, Lys33, Lys48, and Lys63) are found in ubiquitin and can all be utilized to build chains via isopeptide linkages [64]. K48- and K11-linked chains serve as crucial indicators for elimination by the proteasome, while K6, K27, K33, K63, and linear chains typically do not lead to degradation[65]. The 26S proteasome, a macromolecular complex that is widely distributed in the cytoplasm and

nucleus of eukaryotic cells and makes up between 1 and 2% of the total cell mass, catalyzes the rapid destruction of ubiquitinated proteins. The 26S proteasome is a supramolecular assembly made up of one or two 19S regulatory particles connected to the extremities of the 20S core and a central 20S core particle with barrel-like design layered rings, each with seven distinct but structurally linked subunits, form the hollow tubular 20S central component. The catalytic machinery that breaks down substrate proteins by proteolysis is housed inside this complex architecture. Located at the ends of the 20S core, the 19S regulatory particles are complex multimeric complexes made up of at least 18 distinct subunits. These regulatory particles play a pivotal role in recognizing ubiquitinated substrates, facilitating their entry into the 20S core, and orchestrating the deubiquitination and unfolding processes essential for efficient proteolysis [66], [67]. De-ubiquitinating enzymes (DUB) add another layer of complexity in terms of regulation to this process [68].

2.3 USP13 Protein Structure And Function

Ubiquitin-specific protease-13 (USP13) is a DUB enzyme that makes up the cysteine-dependent protease superfamily, the human body contains large amounts of USP13, which is mostly found in the cytosol and nucleoplasm of cells (UniProt accession number: Q92995)[69]. To stop ubiquitin-mediated protein degradation, the enzyme cleaves ubiquitin off protein substrates [70]. The cytogenetic band region 3q26.2–q26.3, on the Q arm of chromosome 3 in the human genome, is where the usp13 gene is located. USP13, or isopeptidase T-3, is the protein that this gene codes for. Timms et al.'s initial identification and characterization of USP13 can be credited to their ground-breaking work. The catalytic domain of USP13 has a conserved architecture with two different structural features: the insertion of two ubiquitin-associated (UBA) domains. The motif consists of a C-box and an H-box [71]. Concerning sequence homology, USP13 and USP5 show an impressive 80% similarity. Asn, Cys, and His make up the active site triad are present in all USP family members and is responsible for cleaving the peptide link that binds ubiquitin to the substrate protein [72]. An N-terminal domain, a zinc finger (ZnF) domain spanning amino acid residues 209 to 281, and a catalytic USP domain spanning residues 336 to 861 comprise the conserved domain architecture shared by these two deubiquitinating enzymes. One unique characteristic of the catalytic USP domain is a two-ubiquitin-associated (UBA) domain insertion that is surrounded by the standard C-box and H-box motifs. Domains like UBA (Ub associated), ZnF (zinc finger), UIM (Ub interacting motif) contribute significantly to USPs.

Although USP13 has been functionally and structurally characterized, its catalytic domain has not been structurally clarified until recently, which has impeded the development of a thorough knowledge of its atomic-level catalytic processes. Fortunately, nuclear magnetic resonance (NMR) spectroscopy has been able to successfully resolve the ZnF domain and the tandem UBA domain of USP13.

Additionally, Hu et al. unveiled the solution structure of the USP13-ZnF domain (PDB: 2L80) and the solution structure of USP13-UBA (PDB: 2LBC) [73]. There are two basic reasons why the USP13-ZnF domain fails to bind ubiquitin. First off, the putative ubiquitin-binding pocket's electrostatic charge distribution differs from the ideal arrangement needed for effective binding. Second, the USP13-ZnF domain's normally conserved residues that aid in ubiquitin binding have experienced mutations or changes, which has upset the vital intermolecular interactions required for the stable complex formation of ubiquitin and the ZnF domain [74].

Given that USP13's two ubiquitin-associated (UBA) domains lack Nuclear Overhauser Effect (NOE) correlations, it is likely that the existence of an extended flexible linker region prevents direct intermolecular interactions between these domains. Both the UBA1 and UBA2 domains adopt a three-helix bundle fold and contain the conserved MGF motif, which is consistent with the canonical architecture of UBA domains. This suggests that the paired UBA regions of USP13 and its counterpart USP5 might have similar Ub-Adhering properties. There is experimental evidence that the USP13 UBA1 and UBA2 domains individually are capable of binding ubiquitin. Moreover, the regulatory function of USP13's UBA domains is greatly diminished by the introduction of mutations that interfere with ubiquitin binding. The conclusions drawn from these discoveries indicate that the enzymatic active region and tandem UBA domains play a significant role in USP13's deubiquitinating function. This implies that the Ub removal capability of USP13, coupled with the Ub-binding capability of its UBA domains, plays a pivotal role in modulating the cellular levels of protein substrates [73].

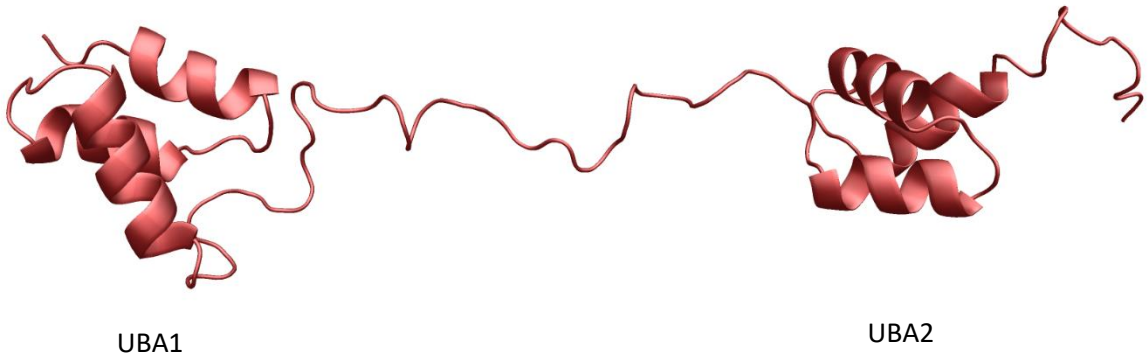


Figure 2.4. Displaying the UBA domains of USP13 Protein

2.4 **Role of USP13 In Parkinson**

Deubiquitinating enzymes (DUBs) have a critical function in the elimination of harmful proteins linked to neurodegenerative diseases [75]. Most misfolded proteins are conjugated with ubiquitin, which makes them vulnerable to proteasome destruction. Protease inhibition, a common occurrence in many clinical circumstances, prevents some misfolded protein aggregates from being broken down by proteases [76]. Initial investigations by the research group revealed significantly elevated levels of USP13 in post-mortem midbrain samples obtained from PD patients compared to healthy individuals of the same age. This finding implicated a potential role for USP13 in the pathogenesis of the disease. Additional research utilized mesencephalic neurons from mice that were either wild-type (WT) or PARK2 knockout (KO). The expression of USP13 and the production of α-synuclein were altered in these neurons [77].

The study demonstrated that USP13 has a detrimental impact on Parkin's ubiquitination, stability, and function as well as on the amount, ubiquitination, and degradation of α-synuclein. Furthermore, in animal experiments, it was seen that USP13 influences the activity of proteasomes without relying on Parkin, which has an impact on dopaminergic neuron survival and motor abilities. In live transgenic mice harboring mutant α-synuclein, USP13 reduced ubiquitination and elimination of α-synuclein were verified. Moreover, it was shown that elevated USP13 levels can offset the beneficial effects of Nilotinib, a medication that improves α-synuclein clearance in this particular animal research study [78], [79]. These findings collectively implicate USP13 as a critical regulator of protein homeostasis and clearance mechanisms, with potential implications in the pathogenesis of PD and related NDD.

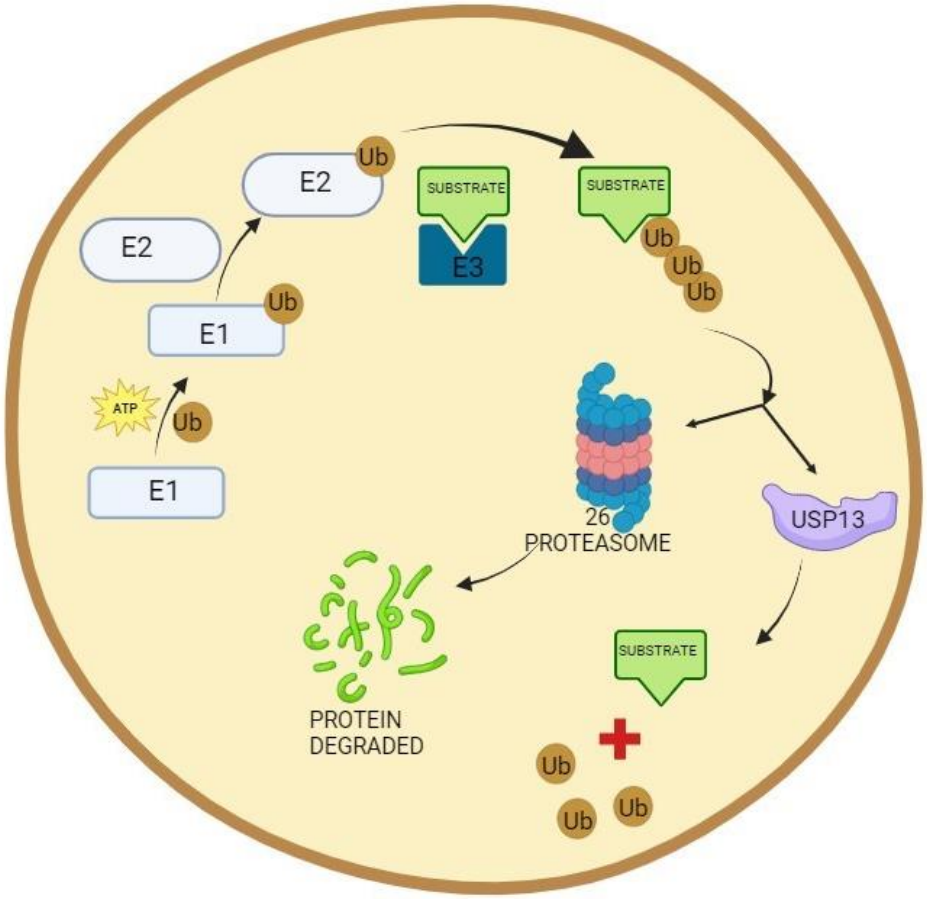


Figure 2.5. De-Ubiquitinase (DUB) activity of USP13

2.5 Therapeutic Potential of USP13

Degenerative dopaminergic brain cells in the nigrostriatal system and the formation of proteinaceous aggregates called LBs are hallmarks of a class of disorders known as alpha-synucleinopathies. It seems that USP13 could be a useful therapeutic target for these conditions. Aggregated alpha-synuclein, a protein connected to PD and DLB, is the primary constituent of these LBs. These neurodegenerative diseases are characterized by alpha-synuclein misfolding and intraneuronal accumulation within LBs. Interestingly, a particularly crucial genetic harmful factor for DLB and PD is alpha-synuclein. Further research into the functional consequences of this deubiquitinating enzyme holds potential for understanding novel therapeutic options targeting alpha-synucleinopathies, given its key role in modifying the clearance processes of alpha-synuclein. The capacity to regulate USP13 activity and the results it yields on the degradation and clearance of misfolded alpha-synuclein represents an intriguing area of research with potential therapeutic implications for these debilitating neurodegenerative disorders[14], [79]. One intriguing therapeutic approach for reducing the detrimental aftereffects of neurodegenerative diseases is the suppression

of USP13, which modifies the (de)ubiquitination cascades controlling neurotoxic proteins. With IC50 values ranging from 0.6 to 0.7 μM, spautin-1, a known small molecule inhibitor of USP10 and USP13, has minimal significance in targeting neurological diseases due to its weak blood-brain barrier (BBB) permeability[80]. It has also been demonstrated that USP13 can be pharmacologically inhibited by Spautin-1 or genetically knocked down, which can hinder the ability of different tumor types to proliferate, differentiate, and invade. This provides a possible way to get around drug resistance mechanisms in cancer cells[81]. USP13 is now a desirable therapeutic target for neurological illnesses as a result of these findings. However, a critical unmet need remains in the development of USP13 inhibitors with more BBB permeability than Spautin-1. With the ultimate goal of determining their efficacy as medications to relieve the corresponding pathogenic cascades and decelerate the advancement of the disease, the synthesis of these ligands may make it possible to investigate USP13 inhibition as a novel therapeutic approach for neurological disorders.

2.6 Computational and Bioinformatics Software And Analytical Tools

With the use of computer-aided drug design (CADD) methodologies, the drug development process has become efficacious and economical. Through the prudent guidance of experimental endeavors toward viable molecular candidates, CADD approaches have made it possible to lessen the cost and temporal constraints that come with traditional drug development pipelines. Notably, in the field of CADD, virtual screening (VS) and molecular docking techniques have become indispensable auxiliary methods to the labor- and resource-intensive high-throughput screening (HTS) experimental procedure [82]. By prioritizing the best compounds for further experimental validation, these computational methods provide a supplementary approach that streamlines the entire drug discovery process. Highly specific subsets have been successfully identified using computational screening of large compound libraries, depending on either complementarity to target structures (structure-based) or similarity to existing inhibitors (ligand-based). After that, the activity of these subgroups can be experimentally verified.

2.6.1 Molecular Docking and Drug Re-Purposing Approach

Molecular Docking

Molecular docking, a popular computational technique in structure-based drug design (SBDD) has been extensively employed in drug discovery [83]. By identifying possible binding modes and evaluating binding affinity, molecular docking aims to evaluate and determine molecular recognition at both a geometric and thermodynamic level. Initially, the interactions between target macromolecules (proteins) and small molecules (ligands) were the main focus of molecular docking. However, in the past

10 years, nucleic acid (DNA and RNA)-ligand docking, protein-protein docking, and nucleic acid-protein-ligand docking have all received more attention [82].Two interconnected steps make up docking: first, analyzing various ligand shapes inside the protein's active region; second, assessing these forms using a scoring system [84]. A scoring formula and a search technique make up docking protocols. The objective of the search method is to provide an ideal group of setups that include the binding mode that was found through experimentation.

However, because the search space is so large, a comprehensive search is computationally infeasible. Only a small portion of the entire conformational space may be sampled in real-world applications, necessitating a trade-off between computing cost and the amount of search space scanned. To minimize the problem's dimensionality and navigate such large search areas with efficiency, limitations, limits, and approximations are utilized. All of the six degrees of linear and rotary flexibility were the sole focus of early docking algorithms. The target and ligand were both seen by these algorithms as rigid entities [85]. A scoring function is required for a broad range of binding modes to result from protein-ligand interactions. It should be able to discriminate between all other modes that the search algorithm has examined and experimental binding modes. Examples of scoring techniques include knowledge-based functions [86], empirical free energy scoring functions [87], and force fields from molecular mechanics.

Currently, available docking methods use two types of scoring functions: a two-step grading function technique that ranks the resulting structures using a stricter scoring function after a reduced function directs the search approach. Complete scoring functions score a protein-ligand conformation. Many of the scoring methods now in use use snapshot solvent models that overlook solvation effects or the structural significance of bound solvent molecules and ions [85], [88]. Many uses for molecular docking exist in the field of drug discovery: structure-activity studies; lead optimization; virtual screening for lead identification; generation of binding hypotheses to support mutagenesis predictions; supporting x-ray crystallography through substrate and inhibitor fitting to electron density maps; chemical mechanism investigations; and combinatorial library design [89].

Drug-Repurposing

Drug repurposing, which includes phrases like "drug repositioning," "re-tasking," "reprofiling," "rescue," "recycling," "redirection," and "therapeutic converting," is the process of finding novel uses for currently approved pharmaceuticals. Finding novel pharmacological uses for medications that are FDA-approved, marketed, experimental, failing, or already in the process of discovery is part of this process [90]. In essence, it seeks to employ these well-known medications for ailments other than those for which they were designed. This strategy gives medications that have been authorized, halted, left on hold, and in the experimental stage a second chance at

treating various illnesses [91]. Repurposing drugs can be done computationally or by experiments. The term "in silico drug repurposing" is frequently used to describe the computer method [92]. Drug repositioning has been increasingly popular in recent years; now, one-third of newly approved pharmaceuticals are repurposed medications. These repurposed drugs currently make up around a quarter of the Pharma industry's annual income [93].

The two predominant approaches to pharmaceutical repurposing are on-target and off-target strategies. A drug's known effects are used for a new purpose in on-target repurposing, resulting in diverse therapeutic outcomes while targeting the same biological target [94]. Conversely, off-target repurposing involves using medications or drug candidates on novel targets for alternative therapeutic purposes, thereby introducing new indications and objectives [90]. Activity-based repositioning is another name for the empirical strategy, which employs empirical testing to determine whether current medications have any novel applications. This approach involves conducting experiments on illnesses to analyze proteins, without requiring prior knowledge of the target proteins' structure. Among the empirical-based repositioning methods accessible are target testing, model organisms, cell assessments, and trials in patients [95], [96]. Nevertheless, in silico repositioning makes use of computational biology and bioinformatics/cheminformatics techniques to virtually examine sizable public databases including medical and chemical information. The chemical interactions between therapeutic compounds and protein targets are examined in this method to identify putative bioactive substances. It's critical to expand our knowledge using a mix of computational and experimental techniques to increase medication repositioning success rates. Repositioning medications to be more effective will be made possible by combining these strategies [97]

2.6.2 ADME(T) Analysis and Blood-Brain-Barrier (BBB) Permeability

ADME Analysis

Absorption, Distribution, Metabolism, Excretion, and Toxicity studies, or ADMET studies for short, assess a drug's pharmacokinetics. This is a critical step in drug development since it entails forecasting the behavior and effects of the medication in the body, including the amount absorbed orally and in the gastrointestinal system. Neurotoxicity and nephrotoxicity may result from poor absorption, which can also have a detrimental effect on distribution and metabolism. A novel medication must bind to its therapeutic target efficiently, but it also has to be able to get to the target site at high enough concentrations to safely provide the intended physiological impact. Because ADMET features are now taken into account early in the drug development process, the number of compounds that fail in clinical trials owing to inadequate ADMET profiles has dramatically decreased [98], [99], [100]. Simply put, ADMET research enables us to comprehend the internal processing of drug molecules in living

things. In light of this, ADMET is essential to computational drug design [101]. Even those who are not familiar with CADD may submit data and analyze findings with ease using the user-friendly interface of the free SwissADME online application, which can be found at http://www.swissadme.ch. When it comes to sophisticated techniques like iLOGP and the BOILED-Egg model, SwissADME offers a distinct advantage over other free web-based ADME and pharmacokinetics programs like pk-CSM and admetSAR.

Its benefits include many input possibilities, multi-molecule data computation capabilities, and shared, interactive graphs and individual results display, saving, and sharing for each molecule [102]. Through experimentation assessing the ADMET characteristics of small compounds is laborious and costly, and results are rarely well-translatable from animal models to humans. The development of drug candidates from discovery leads may be sped up by developments in computer techniques for pharmacokinetic and toxicity property optimization. The intricate connection between physicochemical traits and pharmacokinetic and toxicological profiles makes it difficult to anticipate ADMET-related attributes for novel drugs. As a result, to improve compound quality and success rates, novel approaches are now required to comprehend, investigate, and forecast the ADMET features of small compounds [103].

BBB (Blood-Brain-Barrier) Permeability

The BBB is a selectively permeable membrane, which is achieved by the combined actions of astrocytes, pericytes, and endothelial cells. Maintaining brain homeostasis, this barrier shields the brain from diseases and poisons by preventing them from entering the brain's circulation. The barrier is formed mostly by endothelial cells, and its activity is regulated by astrocytes and pericytes via several signaling pathways [104], [105]. A restricted set of solutes can pass through the BBB without the aid of facilitators. Only gases, like carbon dioxide and oxygen, and tiny, lipid-soluble compounds, like ethanol and antidepressants, with a molecular weight of less than 400 Da or fewer than eight hydrogen bonds, can passively permeate over the blood-brain barrier [106]. A crucial measure for assessing the blood-brain barrier's (BBB) integrity is the barrier's permeability, which shows the degree of paracellular and transcellular movement [107].

Administering medications to the central nervous system (CNS) efficiently is still an enormous challenge in the therapy of neurodegenerative ailments (NDs), even with great advancements in our knowledge of the cellular and molecular mechanisms that govern underlying illnesses and their medicines [108]. As a built-in defence mechanism, the BBB constitutes one of the central nervous system's most important barriers. To protect the central nervous system from neurotoxic substances and to provide necessary nutrients and oxygen, the BBB must operate properly [109]. The BBB has a variety of cell surface sensors and carriers that allow drugs to flow across and fulfil the high energy needs of the brain. Furthermore, lipophilic compounds have

an easy time diffusing into the parenchyma of the brain. Therapeutics that can cross the blood-brain barrier can be created using these physiological traits. Despite having substantial BBB permeability, levodopa—a popular dopamine prodrug—has limited efficacy because of insufficient targeting. Thus, improving the anti-Parkinson's medication's brain-targeting effectiveness continues to be a major treatment problem [110]. Drug delivery has become much more precise and efficient because of advancements in medical nanotechnology. The possible use of "old" pharmaceuticals, such as herbal and pnictogen-based remedies, has been revitalized by this breakthrough. Numerous nanomaterials have been shown to enhance medication transport to the brain, and centred on the physiological functions of the BBB, several BBB-compliant techniques have been created.

2.6.3 PASS Analysis (Prediction of Biological Activity for Substances)

With the help of the application PASS (Prediction Of Activity Spectra For Substances), which uses a drug's structure equation to predict a large number of pharmacological effects and biochemical processes mechanisms, it is possible to effectively identify new targets (mechanisms) for certain molecules and, in turn, identify innovative ligands for certain biological substrates [111]. Pharmacological effects, biological and metabolic reactions, and particular toxicities are all included in a compound's biological activity spectrum (BAS). Based on the molecule's chemical structure, it can offer valuable information on potential medicinal uses. The V.N. Orechovich Institute of Biomedical Chemistry received funding from the Russian Foundation of Basic Research to develop the PASS online tool. It predicts 3,678 pharmacological impacts, techniques, and hazards while utilizing a vast database of more than 180,000 biologically significant chemicals to determine a compound's BAS. High-throughput screening is made easier by PASS, and its uses include quick BAS forecasting for big compounds, drug lead optimization, and therapeutic advances. The assumptions made by PASS, nevertheless, fail to hinge on 2D geometries [112]. The chemical is likely to exhibit the expected action in trials if Pa is more than 0.7, although it additionally seems likely to be akin to already available pharmacological medicines. Although the drug is not as likely to mimic well-known medications, it has a decent possibility of demonstrating the action in studies when Pa ranges from 0.5 and 0.7. The chemical is unlikely to exhibit the anticipated behavior during trials if Pa is less than 0.5. But if the substance's action is verified by experimentation, it may constitute a brand-new species of chemical [112].

2.6.4 Molecular Dynamics Simulation (MDS)

Molecular dynamics (MD) simulations employ an elaborate representation of actual rules regulating interatomic relations to predict the motion of individual atoms inside

a molecule of protein or molecular complex over time [113]. With femtosecond temporal resolution and accurate atomic dimensions, these computational models can clarify a wide range of important biomolecular events, such as polypeptide folding, conformational changes, and ligand associations. Most importantly, they are also able to predict how biomolecules would react at the microscopic level to various perturbations, including mutations, phosphorylation events, protonation states, and ligand engagement and disengagement [114]. There are two main drivers behind the growing enthusiasm for molecular dynamics (MD) simulations. To begin with, empirical architectures for particular chemical categories that are essential to neuroscience have been much more readily available in the past decade. Such chemical groups include the significant targets of many drugs used in Neuropharmacology [115], [116]. Additionally, the potential and affordability of MD simulations have substantially grown due to breakthroughs in the field. In times gone by, powerful MD simulation work demanded supercomputers to run fortunately comprehensive simulations may now be carried out locally at a reasonable cost because of recent advancements in technology for computers, notably graphics processing units (GPUs) [117], [118].

A molecular mechanics force field, a representation tuned to quantum mechanical computations, and frequent data from experiments are used to calculate forces in simulations using MD. This force domain contains components for several additional interatomic connections as well as terms for electrostatic (Coulombic) relations among elements and spring-like components for establishing equilibrium lengths of covalent bonds. Such fields of force are fundamentally approximative, notwithstanding their practicality [114]. Regarding simulations with MD, selecting the right force field is essential. notably popular ones, which come in various forms, are OPLS, AMBER, and CHARMM. Various force fields have distinct benefits and disadvantages even if their operational shapes remain identical [119], [120]. MD simulations have been becoming more prevalent and well-known in the last couple of years, especially among empirical molecular biology researchers. These computer models are widely used in research on scientific architectural biological processes, helping to guide empirical attempts and evaluate results from research. This pattern is especially noticeable in the field of neuroscience, where simulations have been used to study molecules that are necessary for neuronal signaling [121], [122], aid in the generation of neuro pharmaceuticals [123], [124], clarify the processes that govern protein accumulation associated with neurodegenerative diseases [125], [126], enhance the design of sophisticated optogenetics instruments [127].

CHAPTER 3

METHODOLOGY

3.1. Computational Resources

Databases Utilized: the following databases were referred for Literature Review, Protein Target acquisition, small molecules (ligands) Library acquisition, and data retrieval and Analysis.

- **Pubmed (https://pubmed.ncbi.nlm.nih.gov/):** Medline, scientific publications, and digital books are just a few of the medical sources that are cited extensively in PubMed. the references contain full-text information accessed via PubMed Central as well as links to author site.
- **Drugbank (https://go.drugbank.com/):** It's an indispensable tool for any biopharmaceutical research because of its comprehensive and trustworthy drug data, which is arranged for easy access or software integration.
- **Pubchem (https://pubchem.ncbi.nlm.nih.gov/):** PubChem is an open-access chemistry database that is run by the National Institutes of Health (NIH) that allows people to submit and share scientific data. Hundreds of informational entries have been regularly given by PubChem periodically since its founding, solidifying its position as an indispensable tool for scholars and the general public.
- **PDB (https://www.rcsb.org/):** It's a collection of 3D structural information for important biological molecules including proteins, genetic material, and RNA, and has the RCSB PDB (RCSB.org) as its US data center. Undertaking research and offering instruction in the domains of basic biological sciences, wellness, power, and biotechnology are the main goals of the RCSB PDB.
- **Swiss ADME (http://www.swissadme.ch/)** During the drug development process, this online application helps users predict ADME variables pharmacokinetic profiles, drug-likeness, and medicinal chemistry compatibility for one or more small molecules [102].
- **pKCSM (https://biosig.lab.uq.edu.au/pkcsm/):** More lead compounds are being discovered using high throughput drug discovery techniques than with traditional medicinal chemistry, and they are doing so faster. Nevertheless, many of these promising compounds frequently fall short due to inadequate ADMET characteristics. Strategies aid in lowering these dangers in silico screening. pkCSM

is a unique method that uses graph-based signatures to predict pharmacokinetic parameters.

- **PassWebserver(https://www.way2drug.com/passonline/predict.php):** "Prediction of Activity Spectra for Substances" is what the acronym PASS stands for, a pharmacological function profile that is close to the actual one may be generated by entering the structural equation of the chemical-based molecule that resembles a medicine [111].

Software Utilized:

- **Pymol:** In many scientific domains, such as computational chemistry and structural biology, molecular visualization software has grown to be a highly useful tool. A degree of detail and customization that would be unattainable in a laboratory setting is made possible by them when it comes to the visualization and analysis of the structures of molecules like proteins, nucleic acids, and tiny chemical compounds.
- **PYRX:** PyRx is an open-source program that operates on every main operating system and has an easy-to-use interface (Linux, Windows, and Mac OS) [128].
- **Discovery Studio:** With the help of the comprehensive set of reliable tools offered by BIOVIA Discovery Studio, computational chemists and structural biologists may create innovative biotherapeutics and small molecule medications that are stable, optimized, and have attractive safety profiles [129].
- **GROMACS:** Newtonian motion may be modeled for systems with hundreds to millions of particles using the highly configurable molecular dynamics software package GROMACS. It is a community-driven, cooperative effort [130].

3.2 Workflow

A comprehensive analysis and survey of the literature revealed a high correlation between the onset of PD pathophysiology and elevated USP13 expression and activity, indicating USP13 as a prospective target in PD. To repurpose FDA-approved medications for USP13 targeting, Drug Bank provided a library of 3,674 medications. Ligand structures were created using PubChem and Open Babel was used to convert them to the. pdbqt format. Biovia Discovery Studio Visualizer was used for interaction analysis after PyRx was used for molecular docking. The best-affinity compounds were then assessed using Swiss-ADME and pkCSM for advantageous ADMET characteristics. The best candidate's biological activity was predicted using the PASS webserver. Additionally, computational techniques known as MD simulations were employed to assess the dynamic characteristics and structural integrity of the ligand-receptor interaction, aiding in the selection of a promising candidate for experimental validation.

3.2.1 Data Extraction

From the RCSB Protein Data Bank (PDB ID: 2LBC) (https://www.rcsb.org/structure/2LBC), the 3-D structural coordinates of the USP13 protein were identified. Drug repositioning approach involved the acquisition of 3,674 FDA-approved medications from the DrugBank database. With PyRx, the OpenBabel tool was utilized to convert the ligands from SDF format to PDBQT format, while optimizing their energy states. In addition, Spautin-1, a recognized USP13 inhibitor, was obtained from PubChem as a reference or control molecule.

3.2.2 Target Receptor Preparation

The protein's visualization and editing were conducted using PyMOL, which included the elimination of water molecules bound to the structure. Subsequently, the optimization of the protein receptor's energy was conducted using the Swiss-PDB Viewer software tool. This procedure entailed the repetitive application of molecular mechanics force fields alongside optimization methods. The primary aim was to attain a thermodynamically stable conformation, thereby augmenting the overall stability of the protein structure.

3.2.3 Ligand Molecules Preparation

To facilitate the repurposing of FDA-approved drugs against USP13, a collection of 3,674 drugs was sourced from DrugBank. The three-dimensional structures of these ligands were initially generated in SDF (Structure Data File) format via PubChem. Subsequently, the Open Babel tool was employed to convert these ligands from SDF to PDBQT format.

3.2.4 Molecular Docking-Based Virtual Screening

An essential method for determining the ideal alignment and affinity for attachment of small molecules to proteins that serve as receptors is molecular docking [131]. After ligand and receptor structures were prepared, the molecular docking procedure was carefully carried out using the free GUI program PyRx. Using a blind docking 00technique, the docking site was first enlarged to its maximum size and then refined with the Vina Wizard to include the whole receptor in the grid (X-120.7809, Y-31.0808, Z-29.5051 Å). When docking was finished, the findings were methodically retrieved in.csv format, revealing the binding affinities of every ligand to the receptor protein (USP13). The distinct output file for every docked ligand was carefully maintained, offering a thorough account of the interactions and hydrogen bond forms between the ligand and the protein. For a future 2D interaction study, every docked

ligand's distinct output file was carefully saved. An in-depth description of the associations and formation of H bonds between the binding component and the receptor was provided in this file. To examine and assess these interactions, the Biovia Discovery Studio Visualizer was utilized. Substances exhibiting binding energies of -8.0 kcal/mol or below, relative to Spautin1 (-7.9 kcal/mol), were chosen for more in-depth examination.

3.2.5 Interaction analysis

Investigating the various bonds and binding forces allowed for a more thorough assessment of ligand-USP13 interactions was carried out. PyMOL and Discovery Studio Visualizer were used to generate the docking data from the selected compounds and check them for possible configurations. The various interactions between the top 10 affinity ligands and Protein receptor (USP13) were noted.

3.2.6 Physicochemical properties of compounds

The pkCSM and SwissADME servers were used to conduct a second screening stage on the chosen compounds from the docking research, based on their physicochemical and ADMET (Pharmacokinetics) characteristics. The objective of this screening was to find chemicals with both drug-like properties and advantageous ADMET profiles that were BBB permeable. Since it lowers expenses and lessens the chance that novel medications won't work out in clinical studies, assessing ADMET characteristics is essential [132].

3.2.7 Prediction of Biological Activity for Substances (PASS Analysis)

The PASS website was employed to forecast the pharmacological characteristics of certain compounds. In order to produce forecasts, this program evaluates compounds according to their structure-activity connections and compares them to known molecules. The Pa to Pi ratio indicates the probability that a molecule possesses specific biological characteristics. Drug development relies heavily on the ability to predict biological action.

3.2.8 MD Simulations

Molecular dynamics (MD) simulations are a valuable tool for investigating the atomic movements in protein and protein-ligand systems [133]. Using MD modeling tests, the docking results for the interactions between USP13 and the selected chemical Canrenone were verified. GROMACS v5.5.1 was used to do the simulations, which

included the geometric dimensions of the solvated framework that included both unbound USP13 and its Canrenone complex. The CHARMM 36 force field was applied in these simulations to create a configuration for the receptor-ligand complex, canrenone has been defined via parameterization using the CGNF platform. To facilitate solvation in the Simple Point Charge (SPC216) water model, every system was positioned inside a cubic box with a 10 Å gap between the box borders. For the simulated systems to remain neutral, appropriate concentrations of opposing ions such as sodium and chloride were added. Energy minimization of the solvated systems was carried out to address potential steric clashes between atoms using a two-step approach involving 1500 steps of the conjugate gradient algorithms after the most pronounced descent methodology. Subsequently, with the ambient conditions of 1atm pressure, progressive heating from 0-300K at a fixed volume, a periodic boundary conditions were used for a 100 ps equilibration process with two-step conditions. All systems underwent 100 ns of simulation, and the resulting data were analyzed to assess the strength of the protein-ligand associations using GROMACS tools. QtGrace [134] was used to analyze and represent data from the Data.

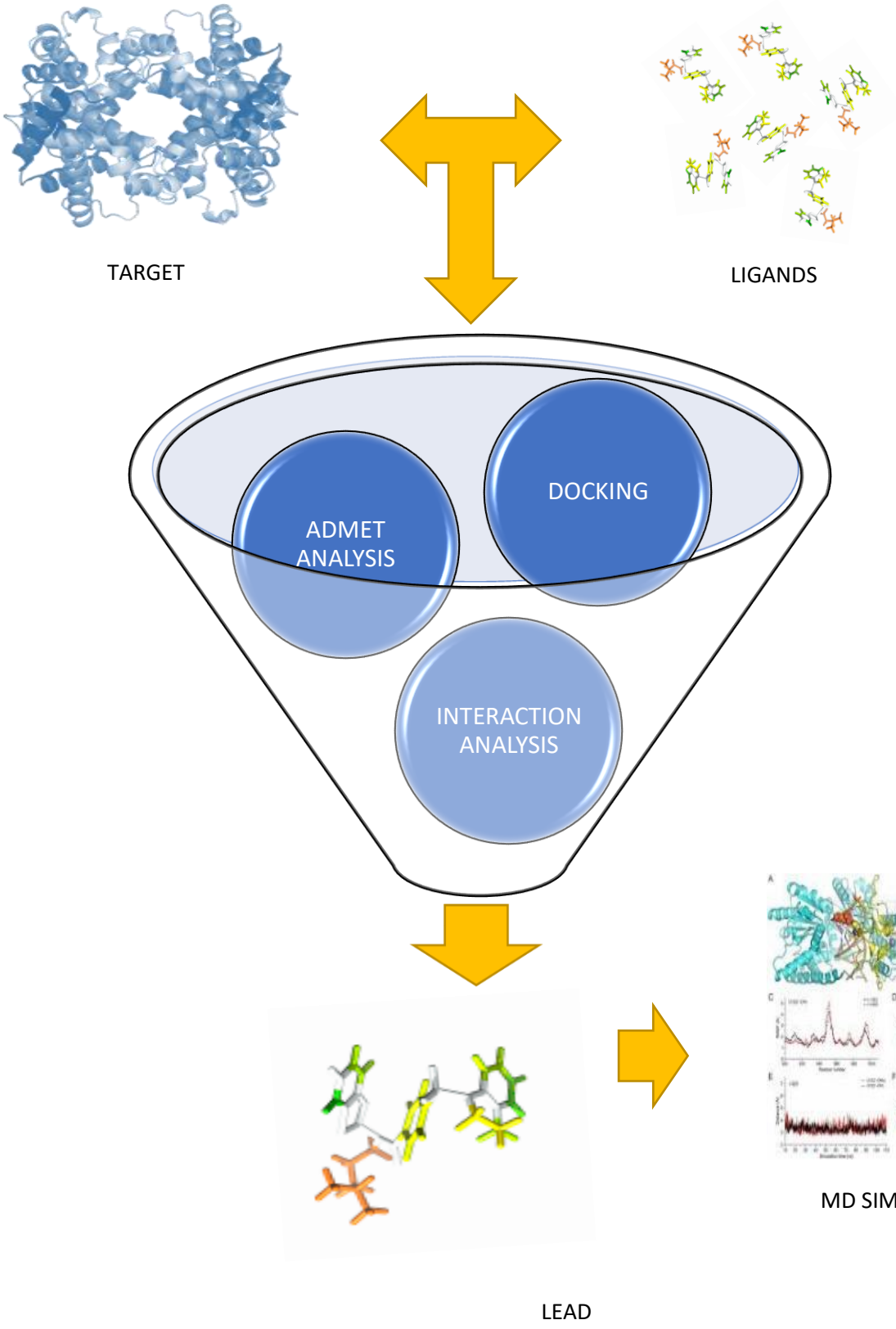


Figure 3.1. Methodology Overview

CHAPTER 4

RESULT AND DISCUSSION

4.1. Molecular docking-based virtual screening

To find possible drugs against predetermined biological targets, a computational strategy called virtual screening is used [135]. To minimize empirical effort and save time, it has become a crucial approach. Using molecular docking-based virtual screening to reduce the amount of in vitro work required, compounds with significant binding scores with USP13 were chosen and subjected to additional research. The reference medication Spautin-1 has a binding energy of -7.9 kcal/mol, but 144 of the 3647 FDA-approved medications showed a binding energy of -8 kcal/mol or lower, satisfying the requirement of having an enhanced binding affinity as shown in Table 4.1. The Molecular Docked complex of USP13 with ligands with top 3 binding affinity is shown in Fig.4.1.

Table 4.1. Binding Affinity of Top 10 Ligands with

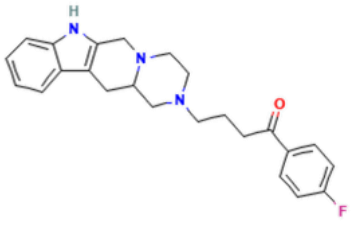
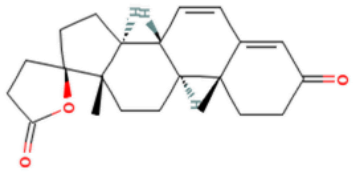
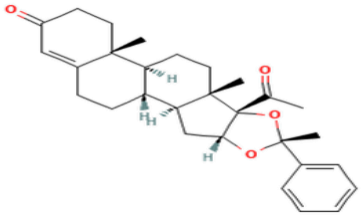
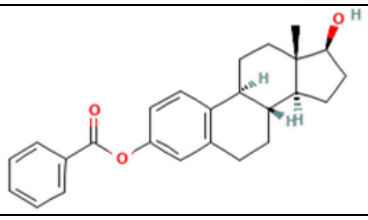
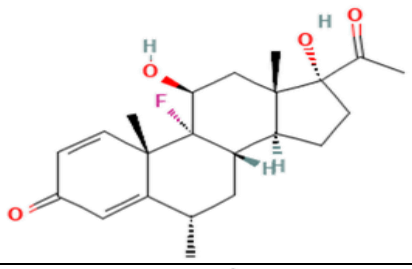
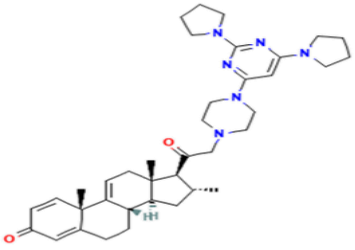
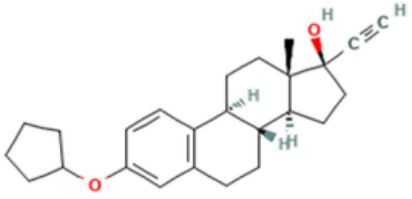
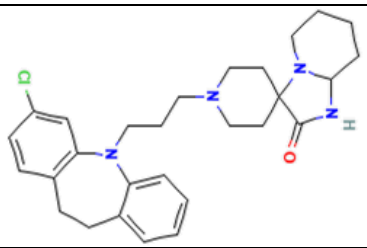
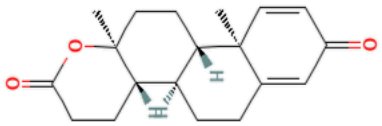
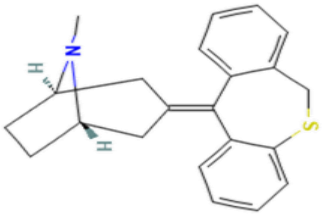
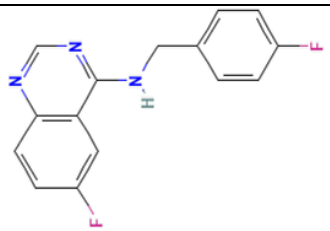
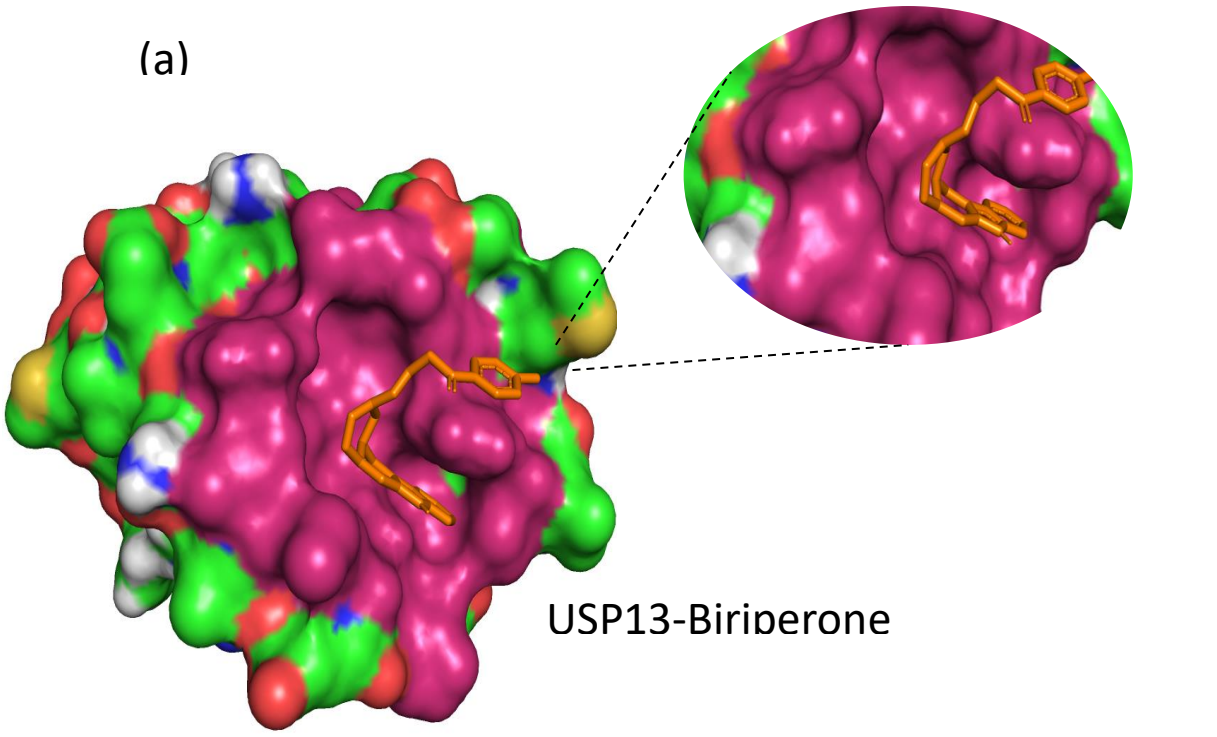
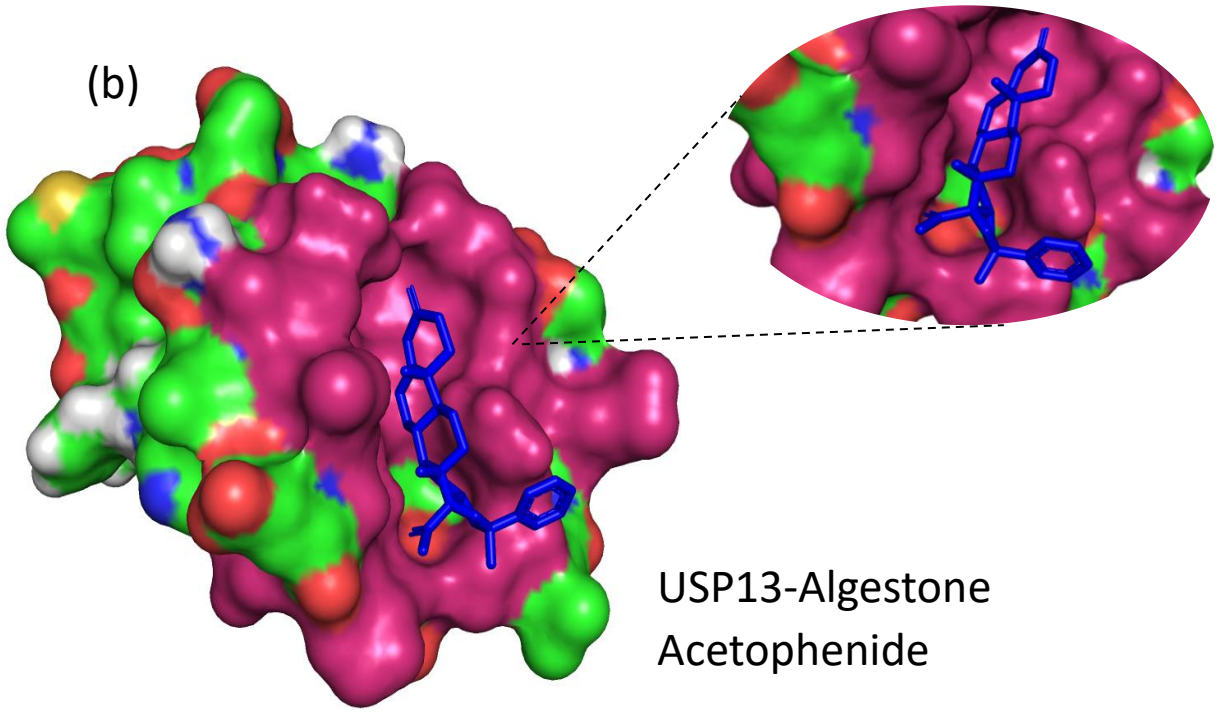
S.No.	Pubchem Cid	Ligands	Structure	Binding Affinity (Kcal/Mol)
1	68663	Biriperone		**-9.4**
2	13789	Canrenone		**-9.2**
3	5284538	Algestone Acetophenide		**-9.1**
4	222757	Estradiol Benzoate		**-8.7**
5	9878	Fluoromethol one		**-8.7**
6	104903	Tirilazad		**-8.7**

Table 4.1 (Continued)				
7	9046	Quinestrol		**-8.6**
8	4257	Mosapramine		**-8.5**
9	13769	Testolactone		**-8.50**
10	3034047	Tropatepine		**-8.5**
11	51037431	Spautin-1 (Reference drug)		**-7.9**


(a)
USP13-Biripерone


(b)
USP13-Algestone
Acetophenide

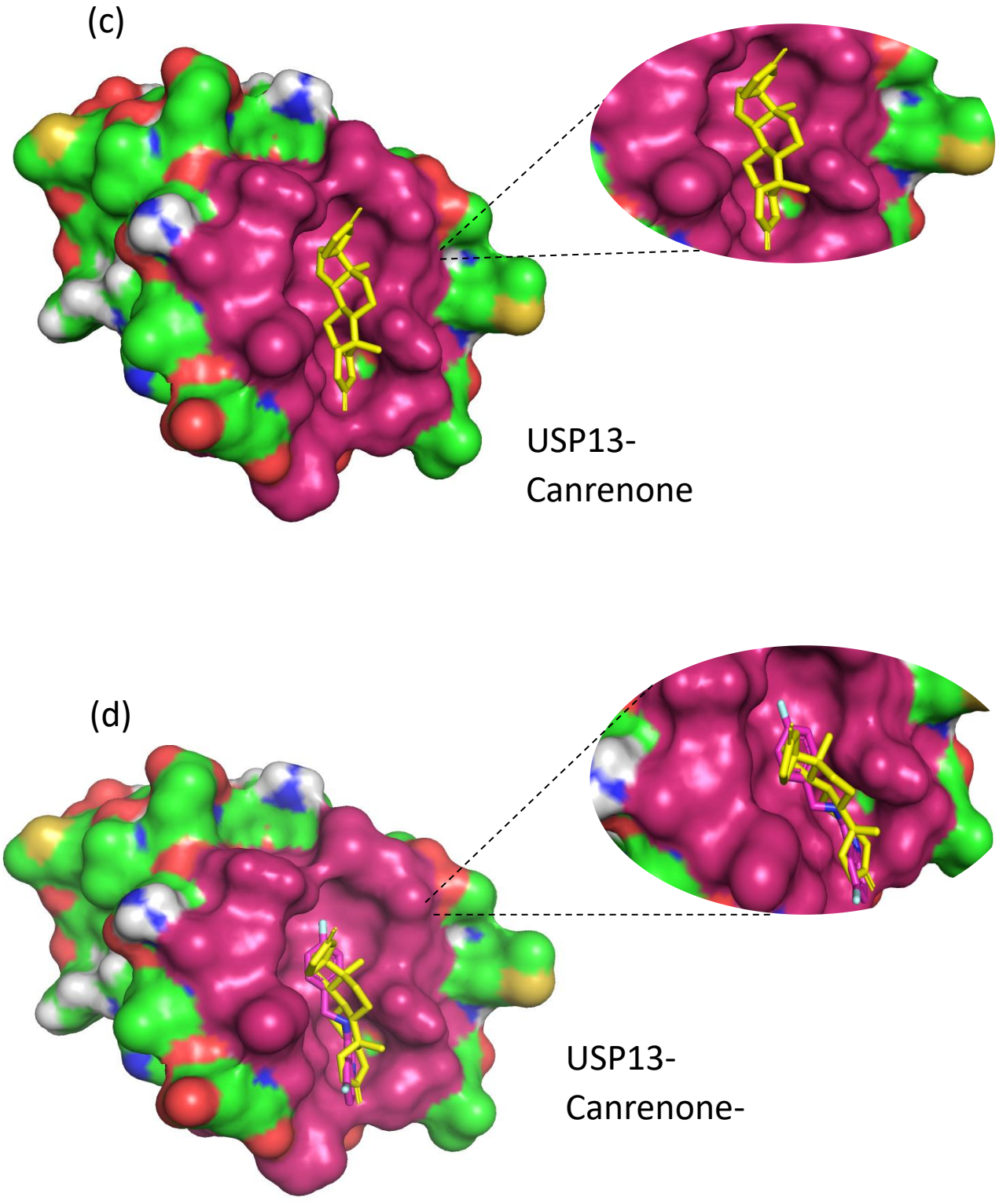


Figure 4.1. Molecular Docked Complexes: (a)USP13 bound to Biriperone (b) USP13 bound to Acetophenide Algestone (c) USP13 bound to Carenone (d) Canrenone and Spautin1 Paired at the binding site of USP13

4.2 Interaction Analysis

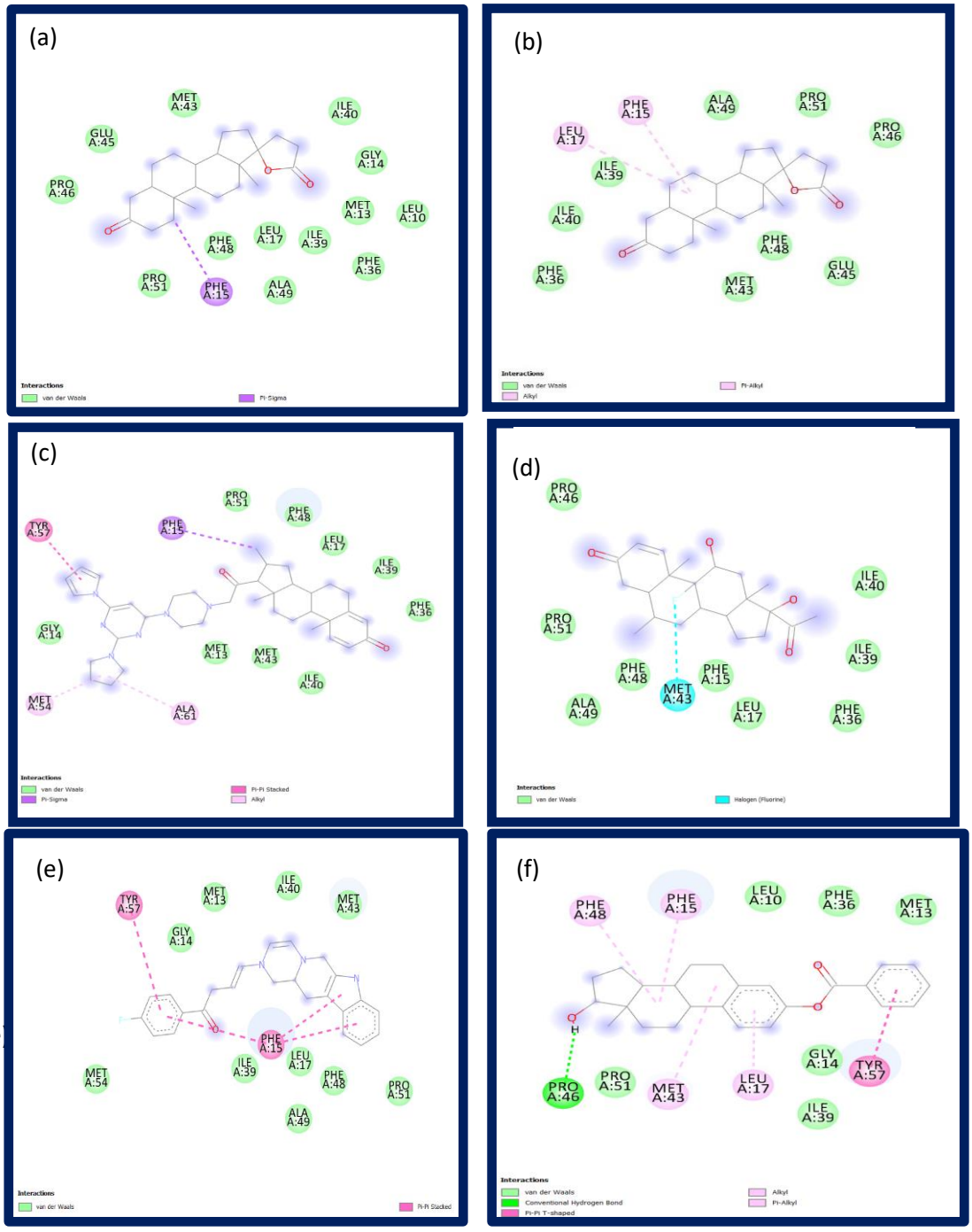
A thorough identification and documentation of the different intermolecular interactions between the ligands and the receptor were done after the interaction analysis of the top 10 ligands. Table 4.2 provides an extensive analysis of these interactions, which include π-π stacking, hydrophobic interactions, electrostatic interactions, and hydrogen bonds. The comprehensive explanation offered here offers a significant understanding of the binding affinities and particular interaction processes that support the general stability and effectiveness of the ligand-receptor complexes. Furthermore, it was observed that the interactions of Reference Drug with specific amino acids of the binding site of protein which included Pro46, Met24, Glu45, Phe48, Ile40, Phe36, Leu17, Ile39, Phe15, Pro51 were also found in the top 10 ligands depicting the coherency of the binding site of the ligands.

Table 4.2. Interaction Analysis

S. No.	PUBCHEM CID	Ligands	Amino Acids Are Involved In Different Types Of Binding Interactions
1	68663	Biriperone	Tyr57, Gly14, Met13, Ile40, Met43, Pro51, Phe48, Ala49, Leu17, Phe15, Ile39, Met54
2	13789	Canrenone	Phe36, Ile40, Ile39, Leu17, Phe15, Ala49, Pro51, Pro46, Glu45, Phe48, Met43
3	5284538	Algestone Acetophenide	Pro46, Glu45, Met43, Ile40, Gly14, Met13, Leu10, Phe36, Ile39, Leu17, Ala49, Phe48, Phe15, Pro51
4	222757	Estradiol Benzoate	Pro46, Met24, Glu45, Phe48, Ile40, Phe36, Leu17, Ile39, Phe15, Pro51

Table 4.2 (Continued)			
5	9878	Fluorometholone	Pro46, Ile40, Ile39, Phe36, Leu17, Phe15, Met43, Phe48, Ala49, Pro51
6	104903	Tirilazad	Tyr57, Phe15, Pro51, Phe48, Leu17, Ile39, Phe36, Ile40, Met 43, Met13, Ala61, Met54, Gly14
7	9046	Quinestrol	Phe36, Leu17, Gly14, Phe48, Pro46, Pro51, Met43, Phe15, Ile39, Ile40, Met13, Leu10
8	4257	Mosapramine	Pro51, Ile39, Ile40, Met43, Leu10, Phe36, Met13, Gly14, Leu17, Phe15, Phe48, Ala49
9	13769	Testolactone	Phe114, Phe90, Leu110, Met88, Glu107, Asp111
10	3034047	Tropatepine	Phe48, Met43, Phe15, Leu17, Ala49, Pro51, Pro46
11	51037431	Spautin-1 (Reference drug)	Pro46, Pro51, Phe48, Glu50, Ala49, Phe15, Gly14, Leu17, Met13, Leu10, Phe36, Ile40, Ile39, Met43

2-D Interaction Diagrams



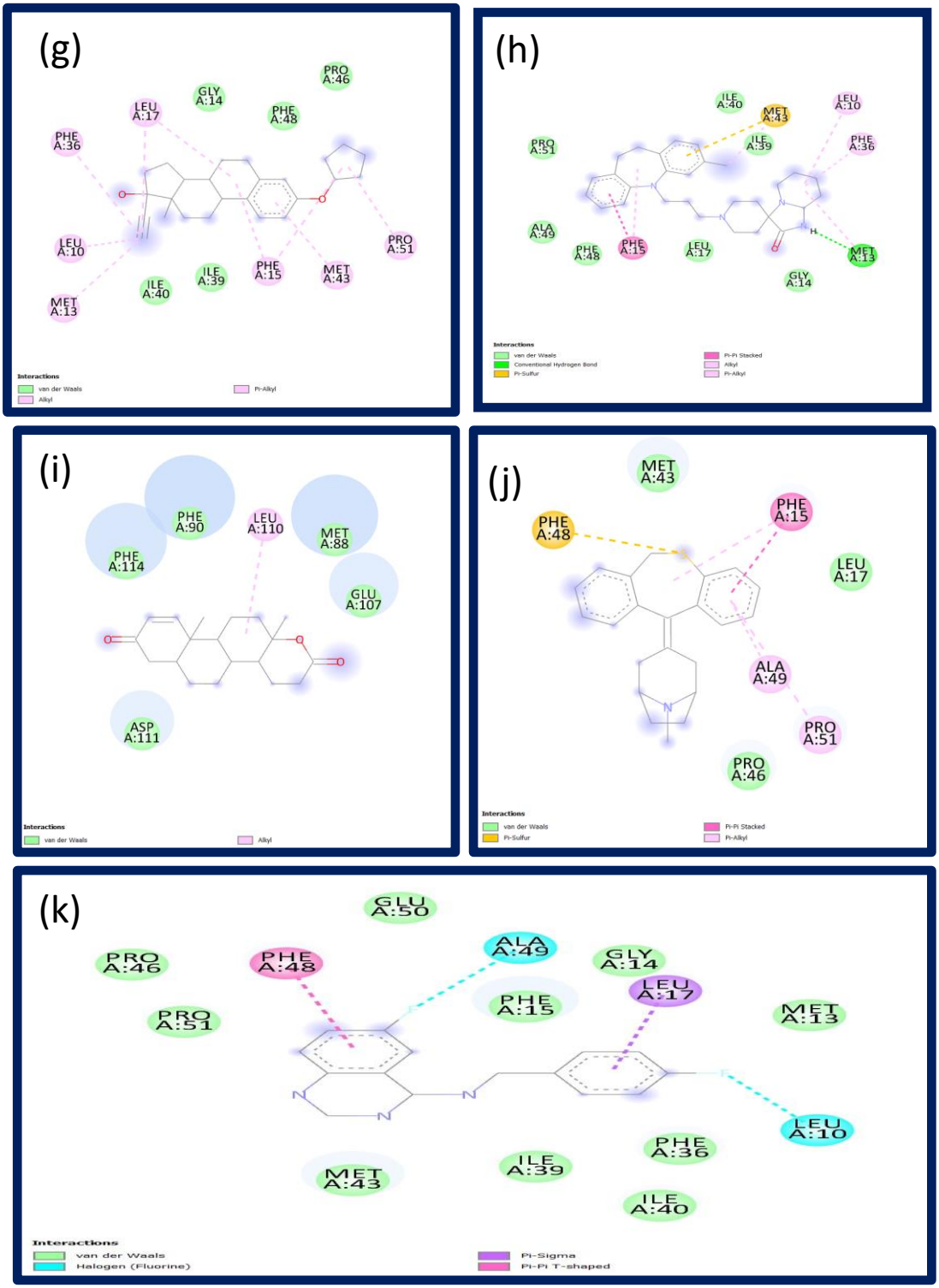


Figure 4.2. Two-dimensional (2D) structural representation of USP13 residues interacting to the compound (a) Biriperone (b) Canrenone (c) Algestone Acetophenide (d) Estradiol Benzoate (e) Fluorometholone(f) Tirilazad (g) Quinestrol (h) Mosapramine (i) Testolactone (j) Tropatepine (k) Spautin-1 (Reference)

4.3 Physicochemical properties of compounds

The strongest affinity ligands were subjected to pharmacokinetic study and evaluation of blood-brain barrier (BBB) permeability in order to find possible targets for Parkinson's disease. The ligands' BBB permeability and pharmacokinetic characteristics were assessed using software programs like SwissADME and pkCSM. Sixty ligands out of the 140 that showed an affinity higher than the benchmark medication spautin1 (-7.9 kcal/mol) were able to penetrate the blood-brain barrier. Table 4.3 lists the top 10 ligands with verified BBB permeability and the greatest negative binding energies. Together with pertinent ADMET (Absorption, Distribution, Metabolism, Excretion, and Toxicity) features that were taken into consideration while determining drug-likeness, this table offers a thorough summary of their potential as Parkinson's disease treatment agents.

Following a thorough examination of a variety of pharmacokinetic and pharmacodynamic parameters, such as blood-brain barrier (BBB) permeability, solubility, gastrointestinal absorption, adherence to drug-likeness criteria (as outlined by Lipinski, Ghose, and Muegge), adherence to lead-likeness criteria, hepatotoxicity, and AMES toxicity, for the top 10 ligands, it was determined that only Canrenone displayed notably high binding affinity while adhering to drug-likeness and lead-likeness criteria. Additionally, Canrenone exhibited no signs of toxicity. Conversely, the other ligands, while having BBB permeability unlike the reference drug, exhibited multiple criteria violations and/or tested positively for toxicity. Furthermore, several ligands showed binding energy disparities in comparison to the reference drug that was less significant than Canrenone's, thus rendering them unsuitable as lead candidates for further investigation.

Table 4.3. Pharmacokinetic (Admet) Analysis

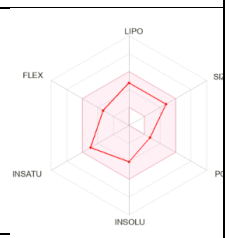
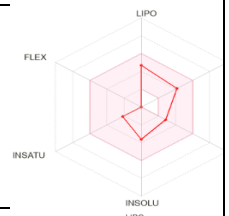
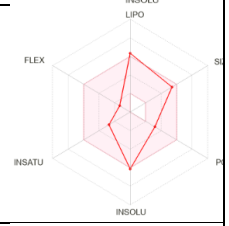
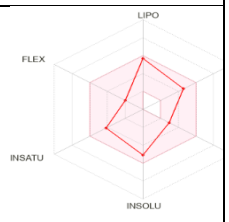
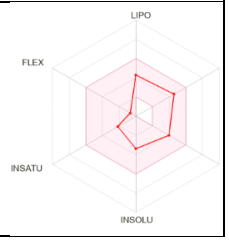
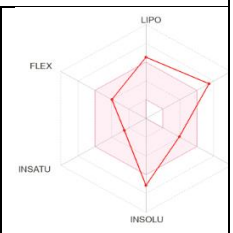
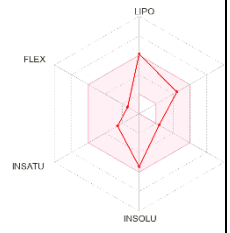
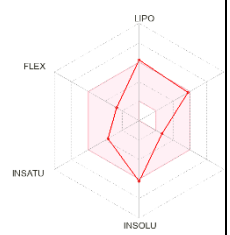
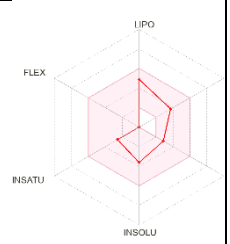
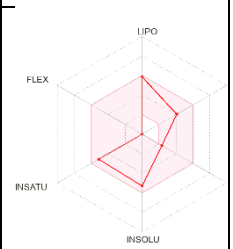
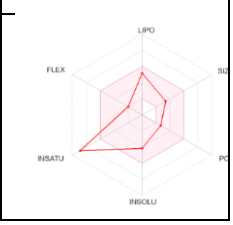
S. No.	Ligand	ADMET Properties							Bioavailabiliy Radar Illustration
		BBB Permeability	Solubility	GI Absorption	Drug-likeness Violations (Lipinski, Ghose and Muegge)	Lead likeness violation	Hepa. Tox.	AMES Tox.	
1	Biriperone	YES	MODERATE	HIGH	NO	YES	YES	NO	
2	Canrenone	YES	SOLUBLE	HIGH	NO	NO	NO	NO	
3	Algestone Acetophenide	YES	POOR	HIGH	YES	YES	NO	NO	
4	Estradiol Benzoate	YES	MODERATE	HIGH	YES	YES	NO	NO	
5	Fluorometholone	YES	SOLUBLE	HIGH	NO	YES	NO	NO	

Table 4.3 (Continued)

6	Tirilazad	YES	POOR	HIGH	YES	YES	YES	NO	
7	Quinestrol	YES	MODERATE	HIGH	YES	YES	NO	YES	
8	Mosapramine	YES	POOR	HIGH	YES	YES	YES	NO	
9	Testolactone	YES	SOLUBLE	HIGH	NO	NO	NO	NO	
10	Tropatepine	YES	POOR	HIGH	YES	YES	YES	NO	
11	Spautin-1 (Reference Drug)	NO	POOR	HIGH	NO	YES	YES	NO	

4.4 PASS Analysis

The biological effects of natural substances are diverse and can lead to either antagonistic or synergistic consequences. Evaluating a compound's biological activity and possible targets is a necessary step in developing safe and effective pharmaceuticals. By using a machine learning approach that takes structure-activity correlations into account, PASS analysis provides a way to anticipate these actions. The PASS webserver was employed to examine the biological activity properties of the chemical (Canrenone) selected for investigation.

Canrenone has shown promising predictions for Anti-Inflammatory activity, JAK2 inhibition, Neurotrophic Factor Enhancer, and potential treatment for Dementia, with Pa (probability of activity) values ranging from 0.183 to 0.579, where Pa > Pi (probability of inactivity). These values indicate a higher likelihood of the compound being active in these therapeutic areas, further supporting its potential as a lead candidate for further analysis and development.

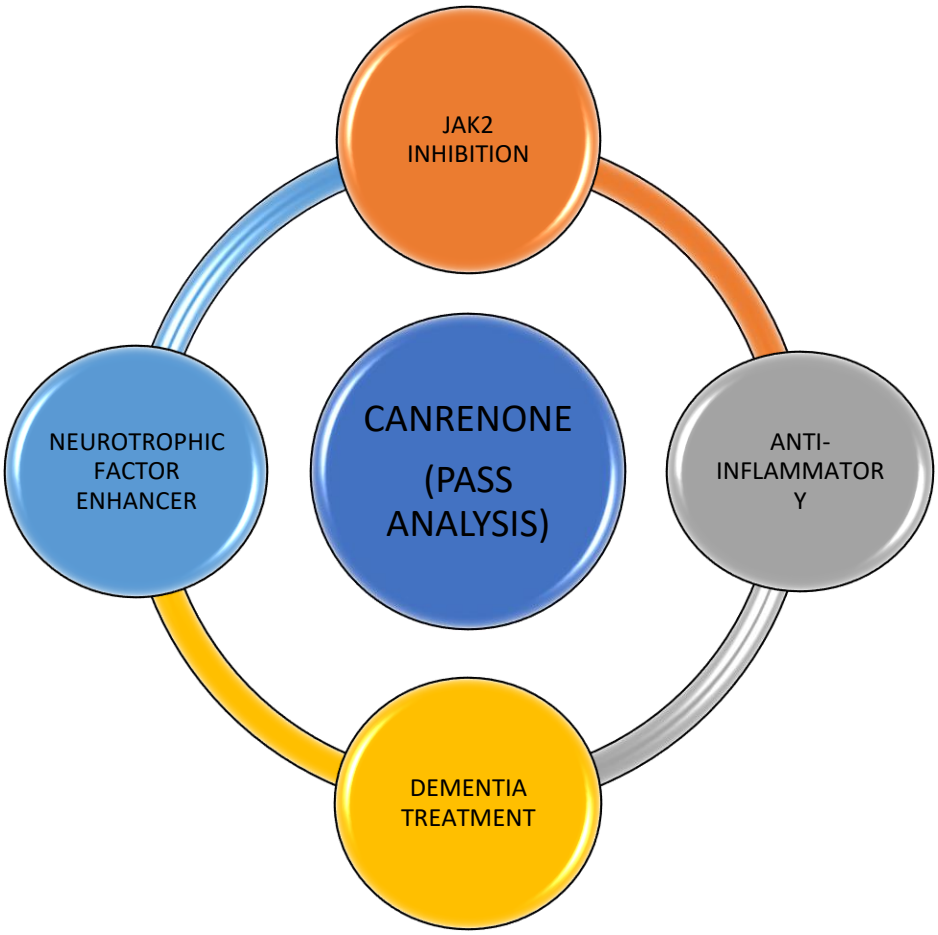


Figure 4.3. Predicted Biological Properties of Canrenone Using PASS Analysis

Table 4.4. Pass Analysis

Drug	Pass predictions	Literature
Canrenone	JAK2 INHIBITOR	JAK2 inhibition can hinder the function of the JAK/STAT system, resulting in a decrease in neuroinflammation and neurodegeneration seen in individuals with PD. This process is substantiated by data regarding genetics, biology, and physiology [136], [137], [138].
	NEUROTROPHIC ENHANCER	These factors play a crucial role in supporting the protection and regeneration of neurons. In experimental models of PD, these factors have been shown to increase the longevity of dopaminergic neurons, enhance dopaminergic neurotransmission, and improve motor skills [139], [140].
	DEMENTIA TREATMENT	Dementia is a common occurrence in individuals with PD, with an estimated yearly occurrence of approximately 10% among those with PD. The presence of dementia has a significant impact on the well-being of both patients and their caregivers and is linked to unfavorable results. Rivastigmine, the sole medication endorsed for addressing dementia linked with PD, emphasizes the significance of addressing dementia in the treatment of PD and underscores the urgent necessity for additional investigation in this realm [141], [142].
	ANTI-INFLAMMATORY	Experimental findings demonstrate a link between the suppression of the inflammatory response and a decrease in neuronal damage, suggesting that inflammation may contribute negatively to the neurodegeneration observed in PD. This implies that strategies targeting inflammation could present a viable therapeutic option for PD. Therefore, therapies designed to reduce inflammation have considerable potential in terms of halting disease advancement and enhancing outcomes for individuals with PD [143], [144], [145].

Canrenone was chosen for MD simulation studies with USP13 based on extensive interaction studies, pharmacokinetic properties, specific interactions with USP13, and PASS analysis. The selection of Canrenone was guided by its substantial binding affinity, favorable pharmacokinetic profile, absence of drug-likeness and lead-likeness violations, lack of toxicity, and promising forecasts for anti-inflammatory activity, JAK2 inhibition, and dementia treatment. The MD simulation studies aim to enhance understanding of the stability and dynamics of the Canrenone-USP13 complex, providing deeper insights into its potential as a therapeutic agent.

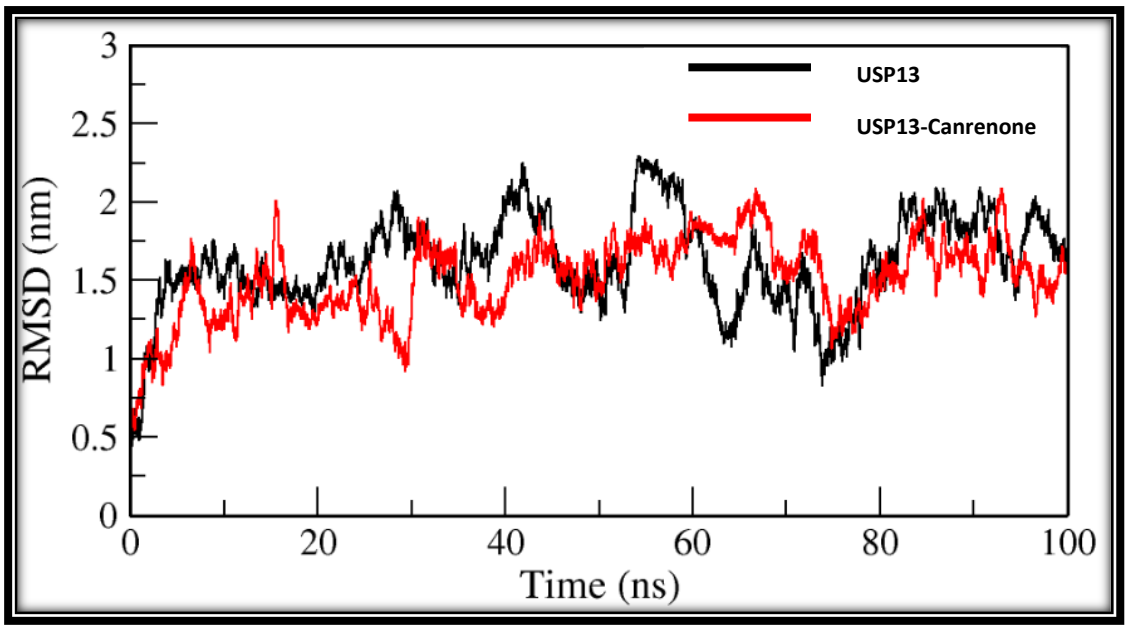
4.5 MD simulation

The realization that deportation and movement of biomolecular systems at this atomic plus molecular scale are one of the most important tasks, supporting the simulation of molecular activities (MD). This unique ability of MD simulation to provide comprehensive perceptions within this kinetic, thermodynamic, as well as architectural feature concerning natural macromolecules is what makes it highly valued. The above-mentioned computational techniques are used extensively to enhance and improve the docked frameworks. While addressing this intricate architectural complexity, which may be beyond traditional experimental methods, this computational technique emerges as a valuable resource. To retroflex the physiological situation and to assess the architectural robustness and vibrant properties of the structures under investigation, the MD simulation was performed in a solvated ecosystem for a specified duration. In particular, the widening 100 ns MD simulation of the docking complex, including USP13 and Canrenone, makes it possible to carry out comprehensive studies on the various organizational and active parameters above the simulated trajectory.

The structural variation in addition to protein activity is investigated using the root-mean-square deviation (RMSD) research. To quantify the residual flexibility of USP13 in their unbound assert plus post-binding with Canrenone, a computation was performed in conjunction with a graphic representation of the root-mean-square fluctuation (RMSF) supporting each residue. To evaluate the conformational robustness of USP13 before and after Canrenone interactions, this radius of rotation (Rg) held steady under both circumstances. By observing the development of hydrogen bonds (H-bonds) within a range of 0.35 nanometers throughout this model, the stability of USP13 was further assessed. Moreover, a probability distribution function (PDF) for the corresponding parameter was generated to obtain a thorough comprehension of the framework's operations.

USP13 has notable intrinsically disordered regions (IDRs), which are distinguished by a high degree of conformational flexibility and the lack of a well-defined three-dimensional structure. The sequence and structure of the protein naturally contain these IDRs, which support the protein's dynamic activity and conformational diversity. When analyzing the RMSD and root-mean-square fluctuation (RMSF) of major fluctuations and departures from the reference structure in MD simulations, one may detect the presence of IDRs. Consequently, when interpreting the MD simulation results for this protein, careful consideration of the intrinsic disorder and flexibility associated with the IDRs is necessary, as they might be a possible cause of the observed random fluctuations and deviations. These variations might not always be attributed to specific protein-ligand interactions, but rather to the inherent conformational diversity and dynamics of the disordered regions within the protein itself.

(a)



(b)

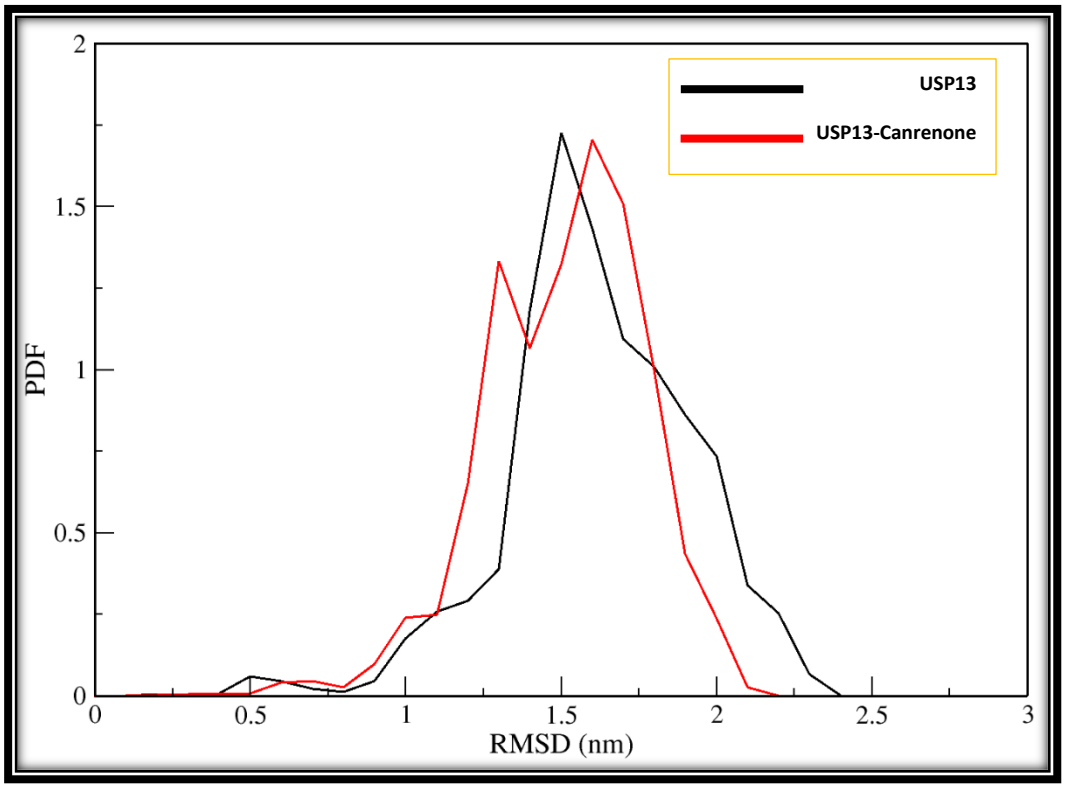
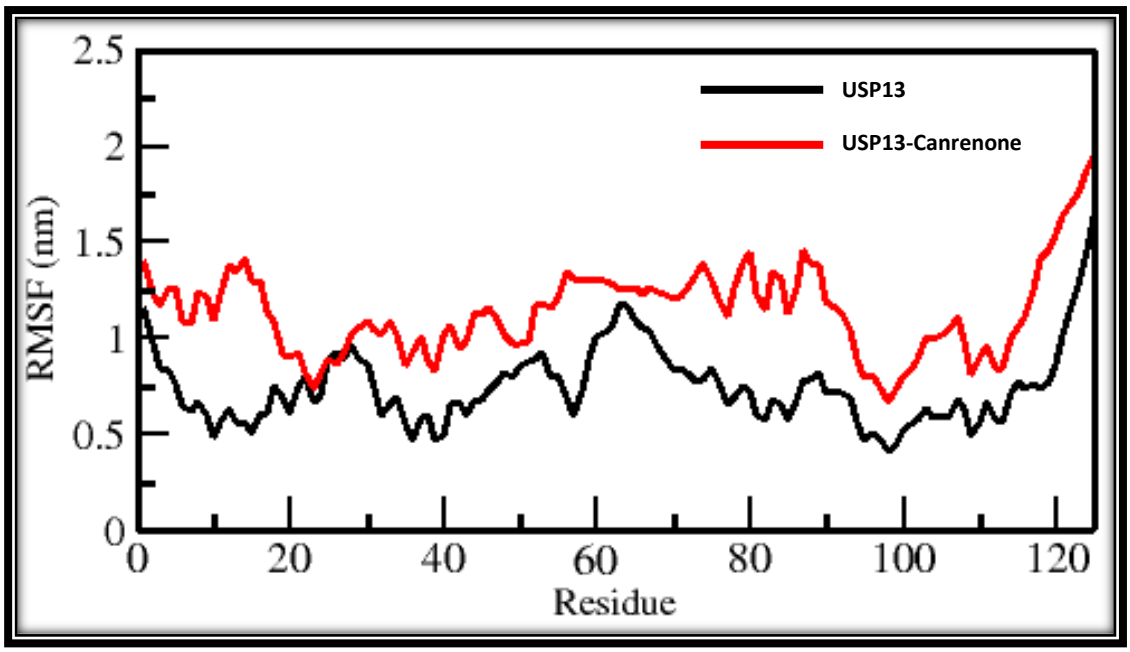


Figure 4.4. Canrenone binding-induced conformational changes in USP13 as a function of time. (A) The RMSD plot of USP13 in the Canrenone complex. (b)The PDF Curve is displayed on the graph.

(a)



(b)

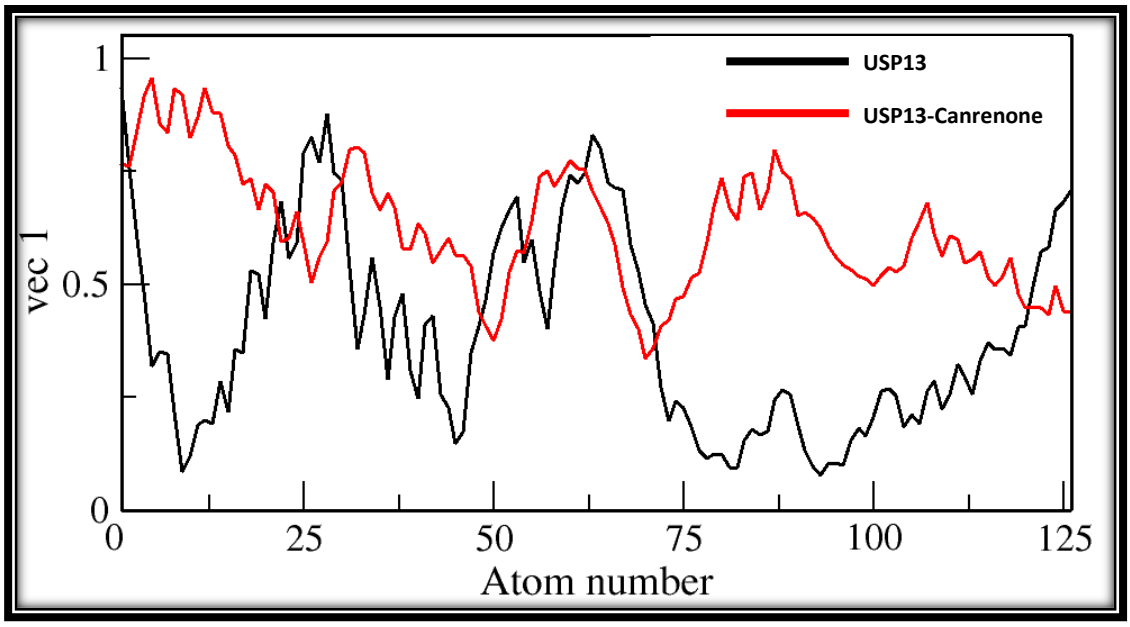


Figure 4.5. Canrenone binding-induced conformational changes in USP13 as a function of time. (A) The RMSF plot of USP13 in the Canrenone complex. (b)The PDF is displayed on the graph.

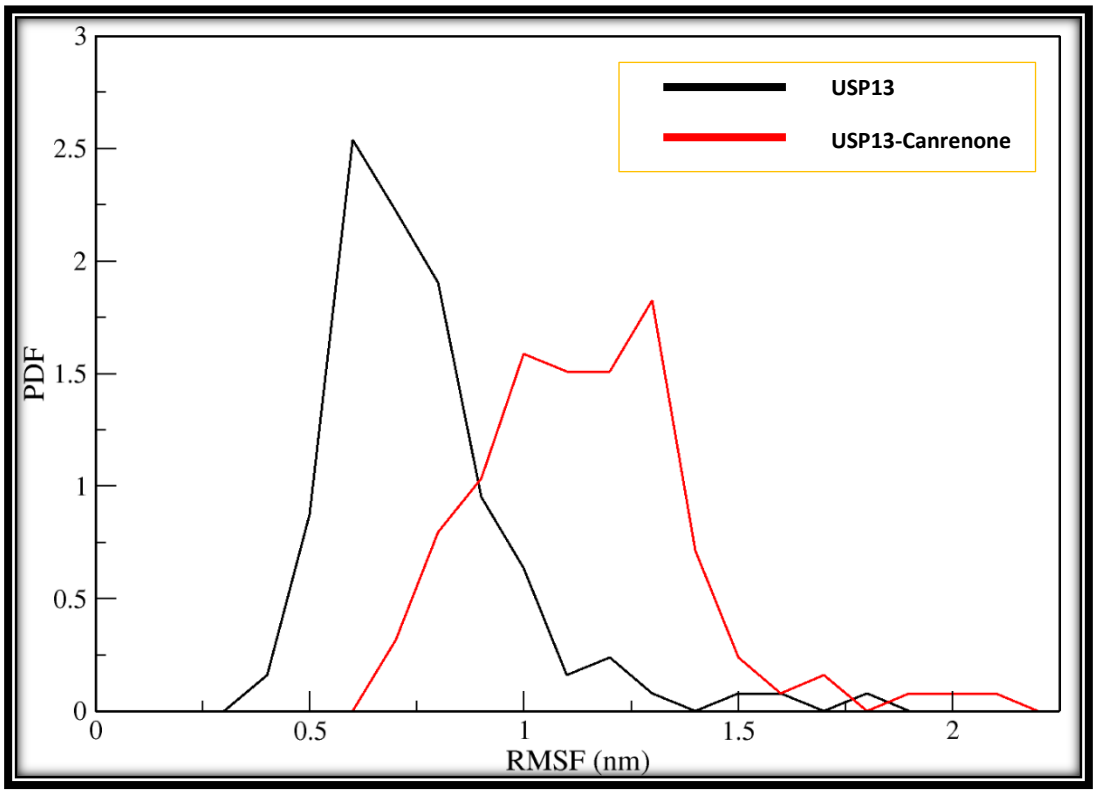


Figure 4.6. PDF, or average residual fluctuations, is a graph that displays the probability distribution function.

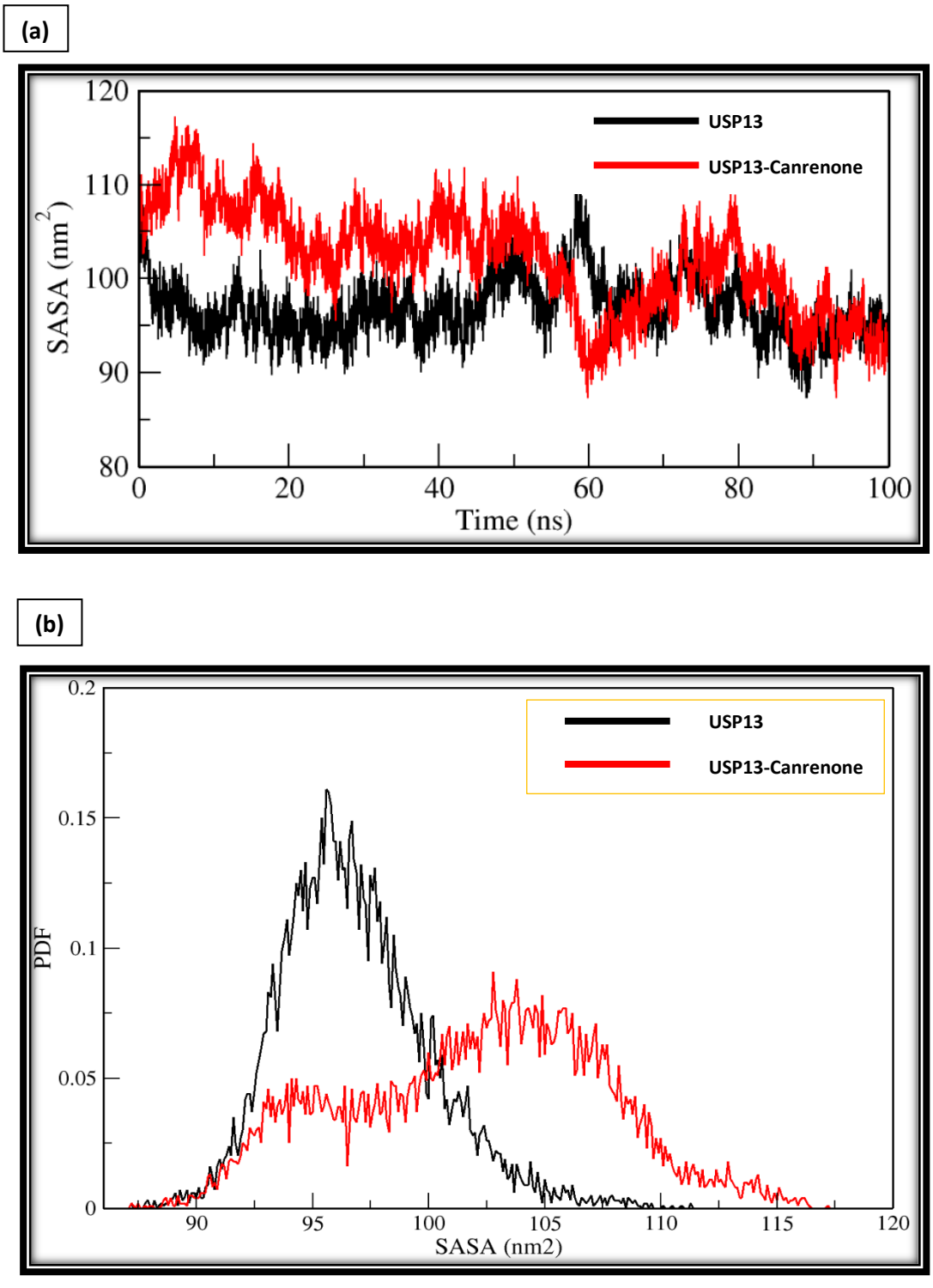
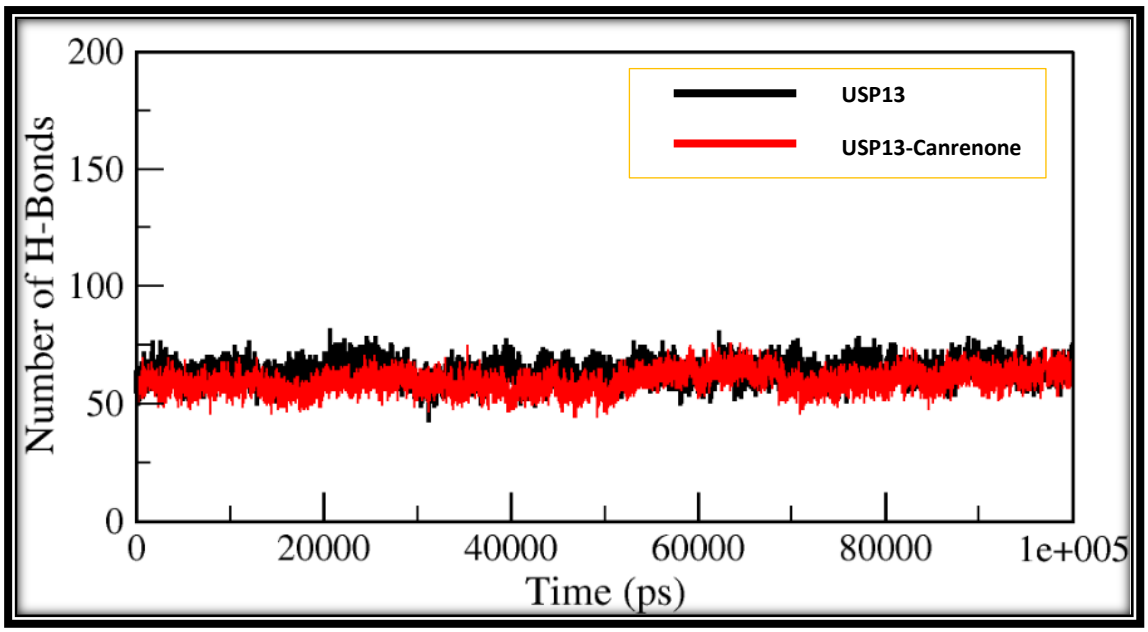


Figure 4.7. USP13's structural tightness and bending upon Canrenone adhesion as a function of time. (a) The USP13 SASA plot plotted against the period before and after Canrenone interaction. (b) The graph displays the PDF values for the estimated dispersion curve.

(a)



(b)

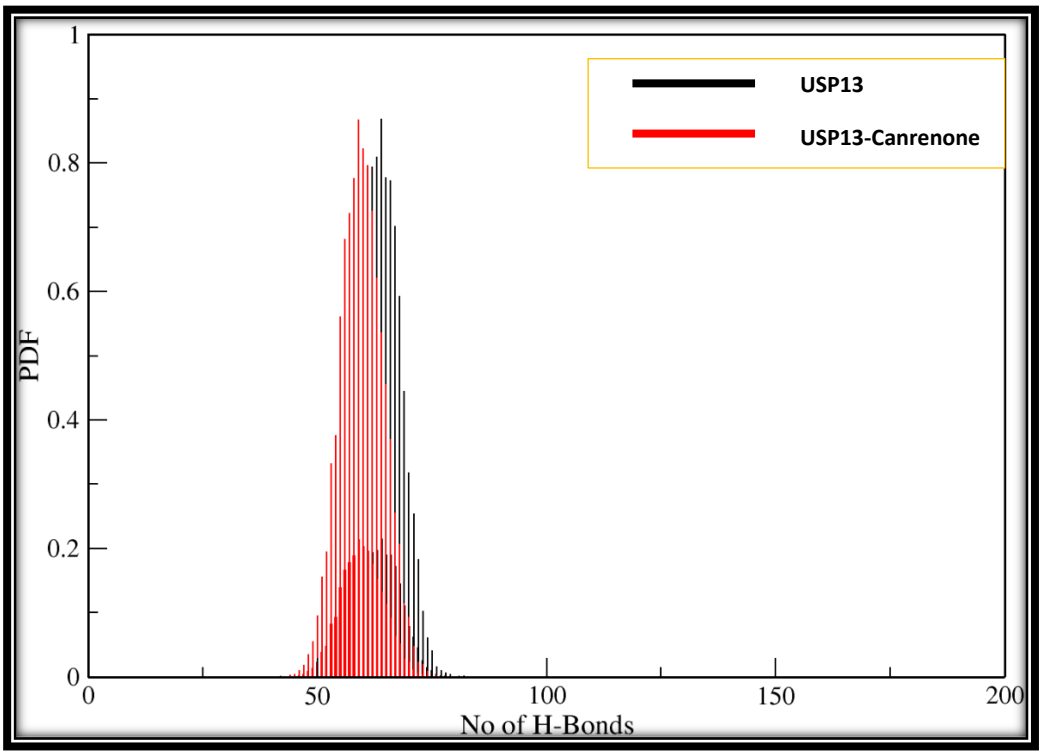
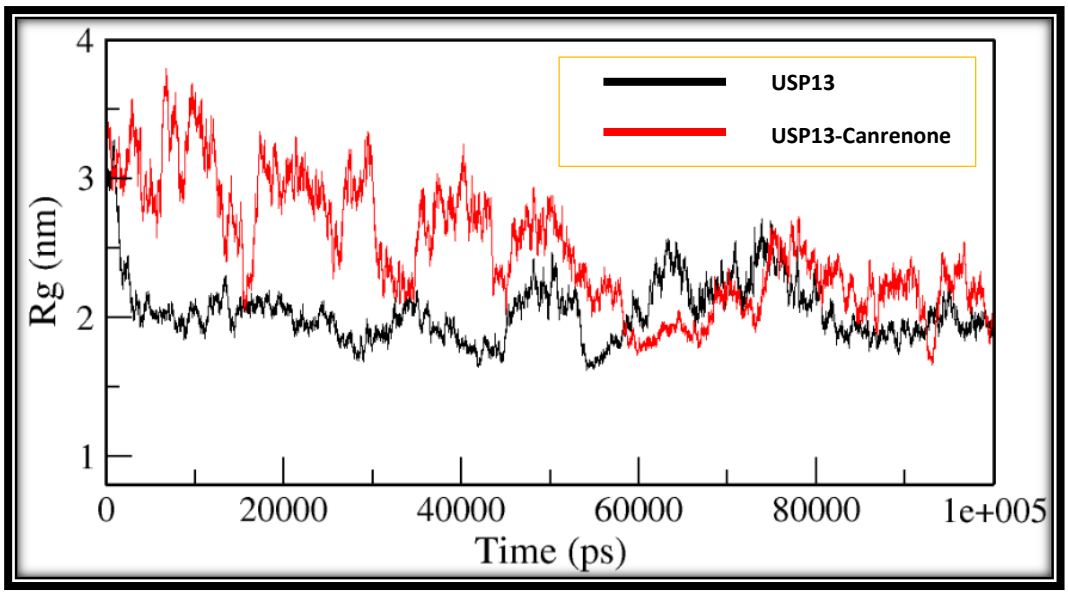


Figure 4.8. Observation of H-bonds. (a) The intramolecular H-bonds that form within 0.35 nm and their time progression (b) The graph displays the H-bond distribution's PDF.

(a)



(b)

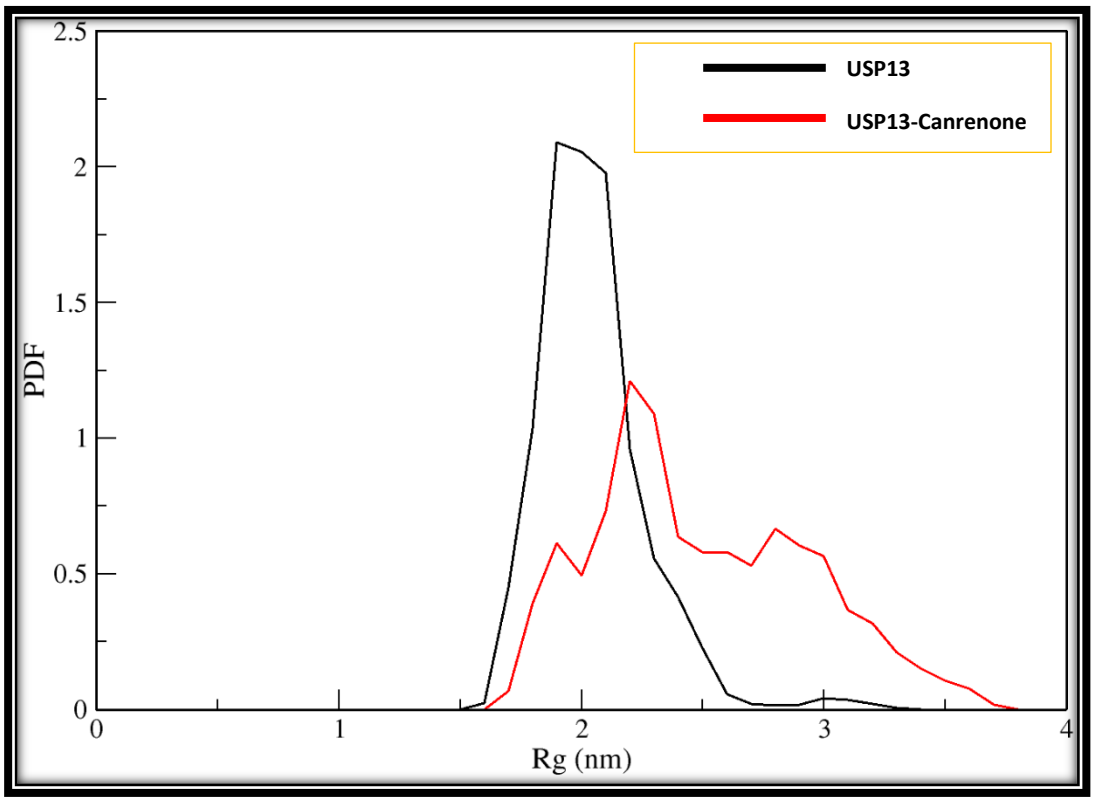


Figure 4.9. Folding and structural integrity of USP13 upon Canrenone interaction about time. (A) Gyrational radius to time. (B) The graph displays the values of the expected distribution curve for the radius of gyration as PDF.

Structural Dynamics Analysis

When linked to USP13, canrenone promotes stability, according to the RMSD analysis. In comparison to the USP13-ligand complex, ApoUSP13 is less stable. Ligand binding lessens structural alterations and increases stability. Over time, canrenone contributes to the stability of USP13. Compared to ApoUSP13, the ligand resulted in decreased RMSD values. Certain residues in USP13 are more flexible when canrenone is bound to them (Fig. 4.4a and Fig. 4.4b). In comparison to its dynamic activity when it interacts with canrenone, USP13 is less flexible when acting alone. Protein flexibility is increased by canrenone binding, enabling essential conformational modifications. Canrenone binding affects USP13's flexibility, demonstrating its function in regulating protein flexibility. By examining the PDF of RMSF, it was determined that the average values of ApoUSP13 and USP13-Canrenone complex were, respectively, 1.15 and 1.35. (Fig. 4.5a and Fig. 4.5b).

SASA quantifies interactions between solvent and protein surfaces. Information on protein dynamics is provided by solvent behavior at the SASA interface. USP13 stability and folding utilizing SASA were examined in the study. SASA plot somewhat changed during the simulation. Using canrenone, USP13 remained steady. Protein with ligand adjustment, displaying some preliminary SASA plot alterations. Overall, even after binding to canrenone, USP13 did not change. The ApoUSP13 and USP13-Canrenone complex mean SASA values were 99.2 and 102.25, respectively. (Fig. 4.7a and Fig. 4.7b). The radius of gyration (Rg) is used to assess the structure and compactness of proteins. High Rg values indicate a partly packed structure, while low Rg values indicate a tightly packed structure. The Canrenone-bound protein has larger and more variable Rg values compared to the apoprotein, indicating dynamic stability but less rigidity and significant conformational changes. This suggests that Canrenone induces structural variability and flexibility in the protein, which may be important for its functional activity. (Fig. 4.9a and Fig. 4.9b)

Molecular Analysis of H-Bonds

H-bonds inside molecules are essential for the stability of proteins. The development of H-bonds with a 3.5 Å distance limit provides information on the interactions between proteins and ligands as well as structural integrity. It was determined that there were, on average, 63 intramolecular H-bonds formed in usp13 both before and after canrenone binding. According to the present investigation, the internal hydrogen bonding system of the protein is not considerably disrupted by the interaction between the ligand and the protein. This implies that the protein sustains its stability in the face of minute structural changes to protect its systematic honor. This demonstrates how well the protein and ligand cooperate to provide stability and dependability. Strong hydrogen bond formation was seen in the results, improving protein structure. The existence of hydrogen bonds throughout the simulation was verified using PDF analysis. (Fig.4.8b).

CHAPTER 5

CONCLUSION AND FUTURE PROSPECTS

NDDs, such as PD, are becoming an increasingly critical and global epidemic and require intensive research to improve effective curative interventions. The present study has made significant progress in its examination of the pharmacologic impediment of USP13, and its involvement in the pathogenesis of Parkinson's disease. Through repurposing an FDA-approved drug that is a promising USP13 inhibitor, Canrenone was selected because it is a promising inhibitor for USP13 and has high affinity and durability in its interactions with the target protein. It's currently being used for the management of initial hyperaldosteronism and diseases (such as heart failure) when there is excessive fluid retention as a result of subsequent hyperaldosteronism. This analysis used computational approaches to examine the various pharmacokinetics as well as the pharmacodynamic parameters, including BBB permeability, solubility, gastrointestinal absorption, and attachment to establish the standard of pharmacokinetic similarity, lead-likeness standard, hepatotoxicity, and AMES toxicity.

Canrenone has emerged as a potential candidate because it exhibits extraordinary adhesion affinity while complying with the drug-likeness and lead-likeness standards and showing no signs of toxicity. The results of the MD simulation confirm this remarkable stability and the advantageous trades in the USP13-Canrenone complex. The RMSD evaluation of the protein binding to Canrenone revealed increased stability, with low RMSD standards and minimal fluctuations detected throughout the replica. Moreover, our data suggest Canrenone's conformity to USP13 promotes conformational changes promoting energetic behavior, thus increasing its flexibility. The study established the basis for further investigation into PD treatment. More research on Canrenone's effect on PD is necessary to fully understand its capability value. In order to continue testing in vivo using animal models, Canrenone as a Parkinson's disease treatment has to. Understanding the interaction between canrenone and USP13 may be assisted by sophisticated computational methods such as metadynamics. In order to increase the efficacy of canrenone, precise inhibitors may be added to the design as well as the optimization process alongside the use of the current understanding.

References

[1] T. A. Yacoubian, “Neurodegenerative Disorders,” in *Drug Discovery Approaches for the Treatment of Neurodegenerative Disorders*, Elsevier, 2017, pp. 1–16. doi: 10.1016/B978-0-12-802810-0.00001-5.

[2] I. Solanki, P. Parihar, and M. S. Parihar, “Neurodegenerative diseases: From available treatments to prospective herbal therapy,” *Neurochem Int*, vol. 95, pp. 100–108, May 2016, doi: 10.1016/j.neuint.2015.11.001.

[3] E. R. Dorsey *et al.*, “Global, regional, and national burden of Parkinson’s disease, 1990–2016: a systematic analysis for the Global Burden of Disease Study 2016,” *Lancet Neurol*, vol. 17, no. 11, pp. 939–953, Nov. 2018, doi: 10.1016/S1474-4422(18)30295-3.

[4] R. Donev, M. Kolev, B. Millet, and J. Thome, “Neuronal death in Alzheimer’s disease and therapeutic opportunities,” *J Cell Mol Med*, vol. 13, no. 11–12, pp. 4329–4348, Nov. 2009, doi: 10.1111/j.1582-4934.2009.00889.x.

[5] D. V. Telegina, G. K. Suvorov, O. S. Kozhevnikova, and N. G. Kolosova, “Mechanisms of Neuronal Death in the Cerebral Cortex during Aging and Development of Alzheimer’s Disease-Like Pathology in Rats,” *Int J Mol Sci*, vol. 20, no. 22, p. 5632, Nov. 2019, doi: 10.3390/ijms20225632.

[6] A. Xie, J. Gao, L. Xu, and D. Meng, “Shared Mechanisms of Neurodegeneration in Alzheimer’s Disease and Parkinson’s Disease,” *Biomed Res Int*, vol. 2014, pp. 1–8, 2014, doi: 10.1155/2014/648740.

[7] K. Chou, H. Hurtig, and A. Eichler, “Clinical manifestations of Parkinson disease - UpToDate,” *Uptodate*, vol. 83, no. 2, 2023.

[8] S. Fahn, “The 200-year journey of Parkinson disease: Reflecting on the past and looking towards the future,” *Parkinsonism Relat Disord*, vol. 46, pp. S1–S5, Jan. 2018, doi: 10.1016/j.parkreldis.2017.07.020.

[9] T. Capriotti and K. Terzakis, “Parkinson Disease,” *Home Healthc Now*, vol. 34, no. 6, pp. 300–307, Jun. 2016, doi: 10.1097/NHH.0000000000000398.

[10] Q. Almeida and H. Hyson, “The Evolution of Pharmacological Treatment for Parkinsons Disease,” *Recent Pat CNS Drug Discov*, vol. 3, no. 1, pp. 50–54, Jan. 2008, doi: 10.2174/157488908783421500.

[11] J. W. Scannell, A. Blanckley, H. Boldon, and B. Warrington, “Diagnosing the decline in pharmaceutical R&D efficiency,” *Nat Rev Drug Discov*, vol. 11, no. 3, pp. 191–200, Mar. 2012, doi: 10.1038/nrd3681.

[12] R. M. Plenge, E. M. Scolnick, and D. Altshuler, “Validating therapeutic targets through human genetics,” *Nat Rev Drug Discov*, vol. 12, no. 8, pp. 581–594, Aug. 2013, doi: 10.1038/nrd4051.

[13] N. Novac, “Challenges and opportunities of drug repositioning,” *Trends Pharmacol Sci*, vol. 34, no. 5, pp. 267–272, May 2013, doi: 10.1016/j.tips.2013.03.004.

[14] X. Liu, M. Hebron, W. Shi, I. Lonskaya, and C. E.-H. Moussa, “Ubiquitin specific protease-13 independently regulates parkin ubiquitination and alpha-synuclein clearance in alpha-synucleinopathies,” *Hum Mol Genet*, vol. 28, no. 4, pp. 548–560, Feb. 2019, doi: 10.1093/hmg/ddy365.

[15] C. B. Joseph, “Parkinson Disease,” *J Consum Health Internet*, vol. 27, no. 2, pp. 221–224, Apr. 2023, doi: 10.1080/15398285.2023.2212529.

[16] T. Pringsheim, N. Jette, A. Frolkis, and T. D. L. Steeves, “The prevalence of Parkinson’s disease: a systematic review and meta-analysis.,” *Mov Disord*, vol. 29, no. 13, pp. 1583–90, Nov. 2014, doi: 10.1002/mds.25945.

[17] N.-T. Yao *et al.*, “Positron emission computed tomography/single photon emission computed tomography in Parkinson disease,” *Chin Med J (Engl)*, vol. 133, no. 12, pp. 1448–1455, Jun. 2020, doi: 10.1097/CM9.0000000000000836.

[18] A. H. Rajput, B. Rozdilsky, and A. Rajput, “Accuracy of Clinical Diagnosis in Parkinsonism — A Prospective Study,” *Canadian Journal of Neurological Sciences / Journal Canadien des Sciences Neurologiques*, vol. 18, no. 3, 1991, doi: 10.1017/S0317167100031814.

[19] J. C. Dreher and L. Tremblay, *Handbook of Reward and Decision Making*. 2009. doi: 10.1016/B978-0-12-374620-7.X0001-5.

[20] D. Athauda and T. Foltynie, “Drug Repurposing in Parkinson’s Disease,” *CNS Drugs*, vol. 32, no. 8, pp. 747–761, Aug. 2018, doi: 10.1007/s40263-018-0548-y.

[21] J. Jankovic, “Parkinson’s disease: Clinical features and diagnosis,” *Journal of Neurology, Neurosurgery and Psychiatry*, vol. 79, no. 4. 2008. doi: 10.1136/jnnp.2007.131045.

[22] K. R. Chaudhuri and A. H. Schapira, “Non-motor symptoms of Parkinson’s disease: dopaminergic pathophysiology and treatment,” *The Lancet Neurology*, vol. 8, no. 5. 2009. doi: 10.1016/S1474-4422(09)70068-7.

[23] S. Muzerengi, D. Contrafatto, and K. R. Chaudhuri, “Non-motor symptoms: Identification and management,” *Parkinsonism Relat Disord*, vol. 13, no. SUPPL. 3, 2007, doi: 10.1016/S1353-8020(08)70048-8.

[24] D. Berg, K. Marek, G. W. Ross, and W. Poewe, “Defining at-risk populations for Parkinson’s disease: Lessons from ongoing studies,” *Movement Disorders*, vol. 27, no. 5, 2012, doi: 10.1002/mds.24985.

[25] T. Gasser, T. Wichmann, and M. R. DeLong, “Parkinson Disease and Other Synucleinopathies,” in *Neurobiology of Brain Disorders*, Elsevier, 2015, pp. 281–302. doi: 10.1016/B978-0-12-398270-4.00019-7.

[26] J. W. Langston and I. Irwin, “MPTP: Current concepts and controversies,” *Clinical Neuropharmacology*, vol. 9, no. 6. 1986. doi: 10.1097/00002826-198612000-00001.

[27] N. Simola, M. Morelli, and A. R. Carta, “The 6-Hydroxydopamine model of parkinson’s disease,” *Neurotox Res*, vol. 11, no. 3–4, pp. 151–167, Sep. 2007, doi: 10.1007/BF03033565.

[28] S. Duty and P. Jenner, “Animal models of Parkinson’s disease: a source of novel treatments and clues to the cause of the disease.,” *Br J Pharmacol*, vol. 164, no. 4, pp. 1357–91, Oct. 2011, doi: 10.1111/j.1476-5381.2011.01426.x.

[29] A. Schrag and J. M. Schott, “Epidemiological, clinical, and genetic characteristics of early-onset parkinsonism,” *Lancet Neurol*, vol. 5, no. 4, pp. 355–363, Apr. 2006, doi: 10.1016/S1474-4422(06)70411-2.

[30] J. M. Beitz, “Parkinson s disease a review,” *Frontiers in Bioscience*, vol. S6, no. 1, p. S415, 2014, doi: 10.2741/S415.

[31] S. K. Van Den Eeden, “Incidence of Parkinson’s Disease: Variation by Age, Gender, and Race/Ethnicity,” *Am J Epidemiol*, vol. 157, no. >11, pp. 1015–1022, Jun. 2003, doi: 10.1093/aje/kwg068.

[32] D. Radhakrishnan and V. Goyal, “Parkinson’s disease: A review,” *Neurol India*, vol. 66, no. 7, p. 26, 2018, doi: 10.4103/0028-3886.226451.

[33] E. R. Dorsey *et al.*, “Projected number of people with Parkinson disease in the most populous nations, 2005 through 2030,” *Neurology*, vol. 68, no. 5, 2007, doi: 10.1212/01.wnl.0000247740.47667.03.

[34] D. W. Dickson *et al.*, “Neuropathological assessment of Parkinson’s disease: refining the diagnostic criteria,” *Lancet Neurol*, vol. 8, no. 12, pp. 1150–1157, Dec. 2009, doi: 10.1016/S1474-4422(09)70238-8.

[35] K. Del Tredici and H. Braak, “Lewy pathology and neurodegeneration in premotor Parkinson’s disease,” *Movement Disorders*, vol. 27, no. 5, 2012, doi: 10.1002/mds.24921.

[36] R. B. Postuma, J. F. Gagnon, and J. Montplaisir, “Clinical prediction of Parkinson’s disease: planning for the age of neuroprotection,” *J Neurol*

Neurosurg Psychiatry, vol. 81, no. 9, pp. 1008–1013, Sep. 2010, doi: 10.1136/jnnp.2009.174748.

[37] G. M. Halliday and H. McCann, "The progression of pathology in Parkinson's disease," *Ann N Y Acad Sci*, vol. 1184, no. 1, pp. 188–195, Jan. 2010, doi: 10.1111/j.1749-6632.2009.05118.x.

[38] P. Damier, E. C. Hirsch, Y. Agid, and A. M. Graybiel, "The substantia nigra of the human brain," *Brain*, vol. 122, no. 8, pp. 1437–1448, Aug. 1999, doi: 10.1093/brain/122.8.1437.

[39] A. H. V. Schapira, "Etiology and Pathogenesis of Parkinson Disease," *Neurol Clin*, vol. 27, no. 3, pp. 583–603, Aug. 2009, doi: 10.1016/j.ncl.2009.04.004.

[40] M. H. Polymeropoulos *et al.*, "Mutation in the α-Synuclein Gene Identified in Families with Parkinson's Disease," *Science (1979)*, vol. 276, no. 5321, pp. 2045–2047, Jun. 1997, doi: 10.1126/science.276.5321.2045.

[41] M. Kasten and C. Klein, "The many faces of alpha-synuclein mutations," *Movement Disorders*, vol. 28, no. 6, pp. 697–701, Jun. 2013, doi: 10.1002/mds.25499.

[42] H. Houlden and A. B. Singleton, "The genetics and neuropathology of Parkinson's disease," *Acta Neuropathologica*, vol. 124, no. 3. 2012. doi: 10.1007/s00401-012-1013-5.

[43] T. T. Warner and A. H. V. Schapira, "Genetic and environmental factors in the cause of Parkinson's disease," *Ann Neurol*, vol. 53, no. S3, pp. S16–S25, 2003, doi: 10.1002/ana.10487.

[44] M. R. Cookson, G. Xiromerisiou, and A. Singleton, "How genetics research in Parkinson's disease is enhancing understanding of the common idiopathic forms of the disease," *Curr Opin Neurol*, vol. 18, no. 6, pp. 706–711, Dec. 2005, doi: 10.1097/01.wco.0000186841.43505.e6.

[45] S. Lesage and A. Brice, "Parkinson's disease: from monogenic forms to genetic susceptibility factors," *Hum Mol Genet*, vol. 18, no. R1, pp. R48–R59, Apr. 2009, doi: 10.1093/hmg/ddp012.

[46] D. T. Dextera and P. Jenner, "Parkinson disease: From pathology to molecular disease mechanisms," *Free Radical Biology and Medicine*, vol. 62. 2013. doi: 10.1016/j.freeradbiomed.2013.01.018.

[47] G. DeMaagd and A. Philip, "Parkinson's disease and its management part 1: Disease entity, risk factors, pathophysiology, clinical presentation, and diagnosis," *P and T*, vol. 40, no. 8, 2015.

[48] D. G. Graham, "Oxidative pathways for catecholamines in the genesis of neuromelanin and cytotoxic quinones," *Mol Pharmacol*, vol. 14, no. 4, 1978.

[49] K. H. Chang and C. M. Chen, “The role of oxidative stress in Parkinson’s disease,” *Antioxidants*, vol. 9, no. 7. 2020. doi: 10.3390/antiox9070597.

[50] P. Jenner *et al.*, “Oxidative stress in Parkinson’s disease,” *Annals of Neurology*, vol. 53, no. SUPPL. 3. 2003. doi: 10.1002/ana.10483.

[51] Y. Glinka, M. Gassen, and M. B. H. Youdim, “Mechanism of 6-hydroxydopamine neurotoxicity,” *Journal of Neural Transmission, Supplement*, no. 50. 1997. doi: 10.1007/978-3-7091-6842-4_7.

[52] A. Lannuzel *et al.*, “The mitochondrial complex I inhibitor annonacin is toxic to mesencephalic dopaminergic neurons by impairment of energy metabolism,” *Neuroscience*, vol. 121, no. 2, 2003, doi: 10.1016/S0306-4522(03)00441-X.

[53] V. M. Mann, J. M. Cooper, D. Krige, S. E. Daniel, A. H. V. Schapira, and C. D. Marsden, “Brain, skeletal muscle and platelet homogenate mitochondrial function in parkinson’s disease,” *Brain*, vol. 115, no. 2, 1992, doi: 10.1093/brain/115.2.333.

[54] T. B. Sherer, S. Chowdhury, K. Peabody, and D. W. Brooks, “Overcoming obstacles in Parkinson’s disease,” *Movement Disorders*, vol. 27, no. 13. 2012. doi: 10.1002/mds.25260.

[55] L. BenMoyal-Segal and H. Soreq, “Gene-environment interactions in sporadic Parkinson’s disease,” *Journal of Neurochemistry*, vol. 97, no. 6. 2006. doi: 10.1111/j.1471-4159.2006.03937.x.

[56] P. C. Trivedi, J. J. Bartlett, and T. Pulinilkunnil, “Lysosomal Biology and Function: Modern View of Cellular Debris Bin,” *Cells*, vol. 9, no. 5, p. 1131, May 2020, doi: 10.3390/cells9051131.

[57] N. Mizushima, B. Levine, A. M. Cuervo, and D. J. Klionsky, “Autophagy fights disease through cellular self-digestion,” *Nature*, vol. 451, no. 7182, pp. 1069–1075, Feb. 2008, doi: 10.1038/nature06639.

[58] N. M. Kocaturk and D. Gozuacik, “Crosstalk Between Mammalian Autophagy and the Ubiquitin-Proteasome System,” *Front Cell Dev Biol*, vol. 6, Oct. 2018, doi: 10.3389/fcell.2018.00128.

[59] A. Ciechanover and A. L. Schwartz, “The ubiquitin-proteasome pathway: The complexity and myriad functions of proteins death,” *Proceedings of the National Academy of Sciences*, vol. 95, no. 6, pp. 2727–2730, Mar. 1998, doi: 10.1073/pnas.95.6.2727.

[60] H.-C. Tai and E. M. Schuman, “Ubiquitin, the proteasome and protein degradation in neuronal function and dysfunction,” *Nat Rev Neurosci*, vol. 9, no. 11, pp. 826–838, Nov. 2008, doi: 10.1038/nrn2499.

[61] O. Coux, K. Tanaka, and A. L. Goldberg, “STRUCTURE AND FUNCTIONS OF THE 20S AND 26S PROTEASOMES,” *Annu Rev Biochem*, vol. 65, no. 1, pp. 801–847, Jun. 1996, doi: 10.1146/annurev.bi.65.070196.004101.

[62] P. M. Handley, M. Mueckler, N. R. Siegel, A. Ciechanover, and A. L. Schwartz, “Molecular cloning, sequence, and tissue distribution of the human ubiquitin-activating enzyme E1,” *Proc Natl Acad Sci U S A*, vol. 88, no. 1, 1991, doi: 10.1073/pnas.88.1.258.

[63] S. H. Lecker, A. L. Goldberg, and W. E. Mitch, “Protein Degradation by the Ubiquitin–Proteasome Pathway in Normal and Disease States,” *Journal of the American Society of Nephrology*, vol. 17, no. 7, pp. 1807–1819, Jul. 2006, doi: 10.1681/ASN.2006010083.

[64] G. L. Grice, I. T. Lobb, M. P. Weekes, S. P. Gygi, R. Antrobus, and J. A. Nathan, “The proteasome distinguishes between heterotypic and homotypic lysine-11-Linked polyubiquitin chains,” *Cell Rep*, vol. 12, no. 4, 2015, doi: 10.1016/j.celrep.2015.06.061.

[65] C. Behrends and J. W. Harper, “Constructing and decoding unconventional ubiquitin chains,” *Nature Structural and Molecular Biology*, vol. 18, no. 5. 2011. doi: 10.1038/nsmb.2066.

[66] D. Voges, P. Zwickl, and W. Baumeister, “The 26S proteasome: A molecular machine designed for controlled proteolysis,” *Annual Review of Biochemistry*, vol. 68. 1999. doi: 10.1146/annurev.biochem.68.1.1015.

[67] A. F. Kisselev and A. L. Goldberg, “Proteasome inhibitors: From research tools to drug candidates,” *Chemistry and Biology*, vol. 8, no. 8. 2001. doi: 10.1016/S1074-5521(01)00056-4.

[68] R. H. Chen, Y. H. Chen, and T. Y. Huang, “Ubiquitin-mediated regulation of autophagy,” *Journal of Biomedical Science*, vol. 26, no. 1. 2019. doi: 10.1186/s12929-019-0569-y.

[69] F. E. Reyes-Turcu, J. R. Horton, J. E. Mullally, A. Heroux, X. Cheng, and K. D. Wilkinson, “The Ubiquitin Binding Domain ZnF UBP Recognizes the C-Terminal Diglycine Motif of Unanchored Ubiquitin,” *Cell*, vol. 124, no. 6, pp. 1197–1208, Mar. 2006, doi: 10.1016/j.cell.2006.02.038.

[70] M. J. Clague and S. Urbé, “Ubiquitin: Same Molecule, Different Degradation Pathways,” *Cell*, vol. 143, no. 5, pp. 682–685, Nov. 2010, doi: 10.1016/j.cell.2010.11.012.

[71] K. M. Timms, M. A. Ansari-Lari, W. Morris, S. N. Brown, and R. A. Gibbs, “The genomic organization of Isopeptidase T-3 (ISOT-3), a new member of the ubiquitin specific protease family (UBP),” *Gene*, vol. 217, no. 1–2, 1998, doi: 10.1016/S0378-1119(98)00341-2.

[72] S. M. B. Nijman *et al.*, "A Genomic and Functional Inventory of Deubiquitinating Enzymes," *Cell*, vol. 123, no. 5, pp. 773–786, Dec. 2005, doi: 10.1016/j.cell.2005.11.007.

[73] Y. H. Zhang, C. J. Zhou, Z. R. Zhou, A. X. Song, and H. Y. Hu, "Domain analysis reveals that a deubiquitinating enzyme usp13 performs non-activating catalysis for lys63-linked polyubiquitin," *PLoS One*, vol. 6, no. 12, 2011, doi: 10.1371/journal.pone.0029362.

[74] X. Li *et al.*, "USP13: Multiple Functions and Target Inhibition," *Front Cell Dev Biol*, vol. 10, Apr. 2022, doi: 10.3389/fcell.2022.875124.

[75] A. C. Jacomin, E. Taillebourg, and M. O. Fauvarque, "Deubiquitinating enzymes related to autophagy: New therapeutic opportunities?," *Cells*, vol. 7, no. 8. 2018. doi: 10.3390/cells7080112.

[76] M. Galves, R. Rathi, G. Prag, and A. Ashkenazi, "Ubiquitin Signaling and Degradation of Aggregate-Prone Proteins," *Trends Biochem Sci*, vol. 44, no. 10, pp. 872–884, Oct. 2019, doi: 10.1016/j.tibs.2019.04.007.

[77] X. Liu, M. Hebron, W. Shi, I. Lonskaya, and C. E. H. Moussa, "Ubiquitin specific protease-13 independently regulates parkin ubiquitination and alpha-synuclein clearance in alpha-synucleinopathies," *Hum Mol Genet*, vol. 28, no. 4, 2019, doi: 10.1093/hmg/ddy365.

[78] X. Liu *et al.*, "Inhibition of Ubiquitin-Specific Protease-13 Improves Behavioral Performance in Alpha-Synuclein Expressing Mice," *Int J Mol Sci*, vol. 23, no. 15, p. 8131, Jul. 2022, doi: 10.3390/ijms23158131.

[79] X. Liu, K. Balaraman, C. C. Lynch, M. Hebron, C. Wolf, and C. Moussa, "Novel Ubiquitin Specific Protease-13 Inhibitors Alleviate Neurodegenerative Pathology," *Metabolites*, vol. 11, no. 9, p. 622, Sep. 2021, doi: 10.3390/metabo11090622.

[80] J. Liu *et al.*, "Beclin1 Controls the Levels of p53 by Regulating the Deubiquitination Activity of USP10 and USP13," *Cell*, vol. 147, no. 1, pp. 223–234, Sep. 2011, doi: 10.1016/j.cell.2011.08.037.

[81] S. SHAO *et al.*, "Spautin-1, a novel autophagy inhibitor, enhances imatinib-induced apoptosis in chronic myeloid leukemia," *Int J Oncol*, vol. 44, no. 5, pp. 1661–1668, May 2014, doi: 10.3892/ijo.2014.2313.

[82] F. Stanzione, I. Giangreco, and J. C. Cole, "Use of molecular docking computational tools in drug discovery," 2021, pp. 273–343. doi: 10.1016/bs.pmch.2021.01.004.

[83] I. D. Kuntz, J. M. Blaney, S. J. Oatley, R. Langridge, and T. E. Ferrin, "A geometric approach to macromolecule-ligand interactions," *J Mol Biol*, vol. 161, no. 2, 1982, doi: 10.1016/0022-2836(82)90153-X.

[84] X.-Y. Meng, H.-X. Zhang, M. Mezei, and M. Cui, “Molecular Docking: A Powerful Approach for Structure-Based Drug Discovery,” *Current Computer Aided-Drug Design*, vol. 7, no. 2, 2012, doi: 10.2174/157340911795677602.

[85] R. D. Taylor, P. J. Jewsbury, and J. W. Essex, “A review of protein-small molecule docking methods,” *J Comput Aided Mol Des*, vol. 16, no. 3, 2002, doi: 10.1023/A:1020155510718.

[86] I. Muegge and Y. C. Martin, “A general and fast scoring function for protein-ligand interactions: A simplified potential approach,” *J Med Chem*, vol. 42, no. 5, 1999, doi: 10.1021/jm980536j.

[87] M. D. Eldridge, C. W. Murray, T. R. Auton, G. V. Paolini, and R. P. Mee, “Empirical scoring functions: I. The development of a fast empirical scoring function to estimate the binding affinity of ligands in receptor complexes,” *J Comput Aided Mol Des*, vol. 11, no. 5, pp. 425–445, 1997, doi: 10.1023/A:1007996124545.

[88] W. Jorgensen and J. Tirado-Rives, “The OPLS [optimized potentials for liquid simulations] potential functions for proteins, energy minimizations for crystals of cyclic peptides and crambin,” *J Am Chem Soc*, vol. 110, no. 6, 1988.

[89] G. M. Morris and M. Lim-Wilby, “Molecular Docking,” 2008, pp. 365–382. doi: 10.1007/978-1-59745-177-2_19.

[90] T. T. Ashburn and K. B. Thor, “Drug repositioning: identifying and developing new uses for existing drugs,” *Nat Rev Drug Discov*, vol. 3, no. 8, pp. 673–683, Aug. 2004, doi: 10.1038/nrd1468.

[91] G. Dey, “An Overview of Drug Repurposing: Review Article,” *Journal of Medical Science And clinical Research*, vol. 7, no. 2, Feb. 2019, doi: 10.18535/jmscr/v7i2.12.

[92] J. S. Shim and J. O. Liu, “Recent Advances in Drug Repositioning for the Discovery of New Anticancer Drugs,” *Int J Biol Sci*, vol. 10, no. 7, pp. 654–663, 2014, doi: 10.7150/ijbs.9224.

[93] Y. Cha *et al.*, “Drug repurposing from the perspective of pharmaceutical companies,” *Br J Pharmacol*, vol. 175, no. 2, pp. 168–180, Jan. 2018, doi: 10.1111/bph.13798.

[94] L. G. Ferreira and A. D. Andricopulo, “Drug repositioning approaches to parasitic diseases: a medicinal chemistry perspective,” *Drug Discov Today*, vol. 21, no. 10, pp. 1699–1710, Oct. 2016, doi: 10.1016/j.drudis.2016.06.021.

[95] T. I. Oprea and J. P. Overington, “Computational and Practical Aspects of Drug Repositioning,” *Assay Drug Dev Technol*, vol. 13, no. 6, pp. 299–306, Jul. 2015, doi: 10.1089/adt.2015.29011.tiodrrr.

[96] E. Lionta, G. Spyrou, D. Vassilatis, and Z. Cournia, “Structure-Based Virtual Screening for Drug Discovery: Principles, Applications and Recent Advances,” *Curr Top Med Chem*, vol. 14, no. 16, pp. 1923–1938, Oct. 2014, doi: 10.2174/1568026614666140929124445.

[97] A. Talevi, “Drug repositioning: current approaches and their implications in the precision medicine era,” *Expert Rev Precis Med Drug Dev*, vol. 3, no. 1, pp. 49–61, Jan. 2018, doi: 10.1080/23808993.2018.1424535.

[98] A. P. Li, “Screening for human ADME/Tox drug properties in drug discovery,” *Drug Discov Today*, vol. 6, no. 7, pp. 357–366, Apr. 2001, doi: 10.1016/S1359-6446(01)01712-3.

[99] C. Merlot, “Computational toxicology—a tool for early safety evaluation,” *Drug Discov Today*, vol. 15, no. 1–2, pp. 16–22, Jan. 2010, doi: 10.1016/j.drudis.2009.09.010.

[100] T. Thompson, “Early ADME in Support of Drug Discovery: The Role of Metabolic Stability Studies,” *Curr Drug Metab*, vol. 1, no. 3, pp. 215–241, Nov. 2000, doi: 10.2174/1389200003339018.

[101] N. Flores-Holguín, J. Frau, and D. Glossman-Mitnik, “Computational Pharmacokinetics Report, ADMET Study and Conceptual DFT-Based Estimation of the Chemical Reactivity Properties of Marine Cyclopeptides,” *ChemistryOpen*, vol. 10, no. 11, pp. 1142–1149, Nov. 2021, doi: 10.1002/open.202100178.

[102] A. Daina, O. Michielin, and V. Zoete, “SwissADME: a free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules,” *Sci Rep*, vol. 7, no. 1, p. 42717, Mar. 2017, doi: 10.1038/srep42717.

[103] J. G. Cumming, A. M. Davis, S. Muresan, M. Haeberlein, and H. Chen, “Chemical predictive modelling to improve compound quality,” *Nature Reviews Drug Discovery*, vol. 12, no. 12. 2013. doi: 10.1038/nrd4128.

[104] N. J. Abbott, A. A. K. Patabendige, D. E. M. Dolman, S. R. Yusof, and D. J. Begley, “Structure and function of the blood–brain barrier,” *Neurobiol Dis*, vol. 37, no. 1, pp. 13–25, Jan. 2010, doi: 10.1016/j.nbd.2009.07.030.

[105] R. Daneman, “The blood–brain barrier in health and disease,” *Ann Neurol*, vol. 72, no. 5, pp. 648–672, Nov. 2012, doi: 10.1002/ana.23648.

[106] W. M. Pardridge, “Drug transport across the blood-brain barrier,” *Journal of Cerebral Blood Flow and Metabolism*, vol. 32, no. 11. 2012. doi: 10.1038/jcbfm.2012.126.

[107] C. Saraiva, C. Praça, R. Ferreira, T. Santos, L. Ferreira, and L. Bernardino, “Nanoparticle-mediated brain drug delivery: Overcoming blood-brain barrier

to treat neurodegenerative diseases," *Journal of Controlled Release*, vol. 235. 2016. doi: 10.1016/j.jconrel.2016.05.044.

[108] J. F. Deeken and W. Löscher, "The blood-brain barrier and cancer: Transporters, treatment, and trojan horses," *Clinical Cancer Research*, vol. 13, no. 6. 2007. doi: 10.1158/1078-0432.CCR-06-2854.

[109] O. Van Tellingen, B. Yetkin-Arik, M. C. De Gooijer, P. Wesseling, T. Wurdinger, and H. E. De Vries, "Overcoming the blood-brain tumor barrier for effective glioblastoma treatment," *Drug Resistance Updates*, vol. 19. 2015. doi: 10.1016/j.drup.2015.02.002.

[110] G. Cheng *et al.*, "Anti-Parkinsonian Therapy: Strategies for Crossing the Blood–Brain Barrier and Nano-Biological Effects of Nanomaterials," *Nanomicro Lett*, vol. 14, no. 1, p. 105, Dec. 2022, doi: 10.1007/s40820-022-00847-z.

[111] A. Lagunin, A. Stepanchikova, D. Filimonov, and V. Poroikov, "PASS: Prediction of activity spectra for biologically active substances," *Bioinformatics*, vol. 16, no. 8, 2000, doi: 10.1093/bioinformatics/16.8.747.

[112] P. S., "Prediction of activity spectra for substances," *J Pharmacol Pharmacother*, vol. 2, no. 1, pp. 52–53, Mar. 2011, doi: 10.4103/0976-500X.77119.

[113] M. Karplus and J. A. McCammon, "Molecular dynamics simulations of biomolecules," *Nat Struct Biol*, vol. 9, no. 9, pp. 646–652, Sep. 2002, doi: 10.1038/nsb0902-646.

[114] S. A. Hollingsworth and R. O. Dror, "Molecular Dynamics Simulation for All," *Neuron*, vol. 99, no. 6, pp. 1129–1143, Sep. 2018, doi: 10.1016/j.neuron.2018.08.011.

[115] J. A. Coleman, E. M. Green, and E. Gouaux, "X-ray structures and mechanism of the human serotonin transporter," *Nature*, vol. 532, no. 7599, 2016, doi: 10.1038/nature17629.

[116] D. Hilger, M. Masureel, and B. K. Kobilka, "Structure and dynamics of GPCR signaling complexes," *Nat Struct Mol Biol*, vol. 25, no. 1, 2018, doi: 10.1038/s41594-017-0011-7.

[117] A. W. Götz, M. J. Williamson, D. Xu, D. Poole, S. Le Grand, and R. C. Walker, "Routine microsecond molecular dynamics simulations with AMBER on GPUs. 1. generalized born," *J Chem Theory Comput*, vol. 8, no. 5, 2012, doi: 10.1021/ct200909j.

[118] J. E. Stone, M. J. Hallock, J. C. Phillips, J. R. Peterson, Z. Luthey-Schulten, and K. Schulten, "Evaluation of emerging energy-efficient heterogeneous computing platforms for biomolecular and cellular simulation workloads," in

Proceedings - 2016 IEEE 30th International Parallel and Distributed Processing Symposium, IPDPS 2016, 2016. doi: 10.1109/IPDPSW.2016.130.

[119] E. Harder *et al.*, "OPLS3: A Force Field Providing Broad Coverage of Drug-like Small Molecules and Proteins," *J Chem Theory Comput*, vol. 12, no. 1, 2016, doi: 10.1021/acs.jctc.5b00864.

[120] J. Huang *et al.*, "CHARMM36m: An improved force field for folded and intrinsically disordered proteins," *Nat Methods*, vol. 14, no. 1, 2016, doi: 10.1038/nmeth.4067.

[121] G. B. Dawe *et al.*, "Distinct Structural Pathways Coordinate the Activation of AMPA Receptor-Auxiliary Subunit Complexes," *Neuron*, vol. 89, no. 6, 2016, doi: 10.1016/j.neuron.2016.01.038.

[122] L. Delemotte, M. Tarek, M. L. Klein, C. Amaral, and W. Treptow, "Intermediate states of the Kv1.2 voltage sensor from atomistic molecular dynamics simulations," *Proc Natl Acad Sci U S A*, vol. 108, no. 15, 2011, doi: 10.1073/pnas.1102724108.

[123] A. Manglik *et al.*, "Structure-based discovery of opioid analgesics with reduced side effects," *Nature*, vol. 537, no. 7619, 2016, doi: 10.1038/nature19112.

[124] J. D. McCorvy *et al.*, "Structure-inspired design of β-arrestin-biased ligands for aminergic GPCRs," *Nat Chem Biol*, vol. 14, no. 2, 2018, doi: 10.1038/nchembio.2527.

[125] J. Khandogin and C. L. Brooks, "Linking folding with aggregation in Alzheimer's β-amyloid peptides," *Proc Natl Acad Sci U S A*, vol. 104, no. 43, 2007, doi: 10.1073/pnas.0703832104.

[126] C. Wu and J. E. Shea, "Structural Similarities and Differences between Amyloidogenic and Non-Amyloidogenic Islet Amyloid Polypeptide (IAPP) Sequences and Implications for the Dual Physiological and Pathological Activities of These Peptides," *PLoS Comput Biol*, vol. 9, no. 8, 2013, doi: 10.1371/journal.pcbi.1003211.

[127] M. Takemoto *et al.*, "Molecular dynamics of channelrhodopsin at the early stages of channel opening," *PLoS One*, vol. 10, no. 6, 2015, doi: 10.1371/journal.pone.0131094.

[128] S. Dallakyan and A. J. Olson, "Small-Molecule Library Screening by Docking with PyRx," 2015, pp. 243–250. doi: 10.1007/978-1-4939-2269-7_19.

[129] T. Miyata, "Discovery studio modeling environment.," *Ensemble*, vol. 17, no. 2, 2015.

[130] M. J. Abraham *et al.*, "GROMACS: High performance molecular simulations through multi-level parallelism from laptops to supercomputers," *SoftwareX*, vol. 1–2, pp. 19–25, Sep. 2015, doi: 10.1016/j.softx.2015.06.001.

[131] H. Naz *et al.*, "Binding studies and biological evaluation of β-carotene as a potential inhibitor of human calcium/calmodulin-dependent protein kinase IV," *Int J Biol Macromol*, vol. 96, pp. 161–170, Mar. 2017, doi: 10.1016/j.ijbiomac.2016.12.024.

[132] R. Arunkumar *et al.*, "Effect of diallyl disulfide on insulin-like growth factor signaling molecules involved in cell survival and proliferation of human prostate cancer cells in vitro and in silico approach through docking analysis," *Phytomedicine*, vol. 19, no. 10, 2012, doi: 10.1016/j.phymed.2012.04.009.

[133] M. Gulzar *et al.*, "Elucidation of interaction mechanism of ellagic acid to the integrin linked kinase," *Int J Biol Macromol*, vol. 122, pp. 1297–1304, Feb. 2019, doi: 10.1016/j.ijbiomac.2018.09.089.

[134] P. Turner, "XMGRACE, Version 5.1. 19.," *Center for Coastal and Land-Margin Research, Oregon Graduate Institute of Science and Technology, Beaverton*, 2005.

[135] A. Beg, F. I. Khan, K. A. Lobb, A. Islam, F. Ahmad, and Md. I. Hassan, "High throughput screening, docking, and molecular dynamics studies to identify potential inhibitors of human calcium/calmodulin-dependent protein kinase IV," *J Biomol Struct Dyn*, vol. 37, no. 8, pp. 2179–2192, May 2019, doi: 10.1080/07391102.2018.1479310.

[136] C. Zhao, D. Bikram Khadka, and W.-J. Cho, "Insights into the Structural Features Essential for JAK2 Inhibition and Selectivity," *Curr Med Chem*, vol. 23, no. 13, pp. 1331–1355, May 2016, doi: 10.2174/0929867323666160405112615.

[137] H. Qin *et al.*, "Inhibition of the JAK/STAT Pathway Protects Against α-Synuclein-Induced Neuroinflammation and Dopaminergic Neurodegeneration," *The Journal of Neuroscience*, vol. 36, no. 18, pp. 5144–5159, May 2016, doi: 10.1523/JNEUROSCI.4658-15.2016.

[138] N.-A. Lashgari, N. M. Roudsari, S. Momtaz, T. Sathyapalan, A. H. Abdolghaffari, and A. Sahebkar, "The involvement of JAK/STAT signaling pathway in the treatment of Parkinson's disease," *J Neuroimmunol*, vol. 361, p. 577758, Dec. 2021, doi: 10.1016/j.jneuroim.2021.577758.

[139] E. Palasz, A. Wysocka, A. Gasiorowska, M. Chalimoniuk, W. Niewiadomski, and G. Niewiadomska, "BDNF as a Promising Therapeutic Agent in Parkinson's Disease," *Int J Mol Sci*, vol. 21, no. 3, p. 1170, Feb. 2020, doi: 10.3390/ijms21031170.

[140] P. Chmielarz and M. Saarma, “Neurotrophic factors for disease-modifying treatments of Parkinson’s disease: gaps between basic science and clinical studies,” *Pharmacological Reports*, vol. 72, no. 5, pp. 1195–1217, Oct. 2020, doi: 10.1007/s43440-020-00120-3.

[141] S. Garcia-Ptacek and M. G. Kramberger, “Parkinson Disease and Dementia,” *J Geriatr Psychiatry Neurol*, vol. 29, no. 5, pp. 261–270, Sep. 2016, doi: 10.1177/0891988716654985.

[142] J. G. Goldman and S. Holden, “Treatment of Psychosis and Dementia in Parkinson’s Disease,” *Curr Treat Options Neurol*, vol. 16, no. 3, p. 281, Mar. 2014, doi: 10.1007/s11940-013-0281-2.

[143] H.-M. Gao, B. Liu, W. Zhang, and J.-S. Hong, “Novel anti-inflammatory therapy for Parkinson’s disease,” *Trends Pharmacol Sci*, vol. 24, no. 8, pp. 395–401, Aug. 2003, doi: 10.1016/S0165-6147(03)00176-7.

[144] Q. Wang, Y. Liu, and J. Zhou, “Neuroinflammation in Parkinson’s disease and its potential as therapeutic target,” *Transl Neurodegener*, vol. 4, no. 1, p. 19, Dec. 2015, doi: 10.1186/s40035-015-0042-0.

[145] E. Kip and L. C. Parr-Brownlie, “Reducing neuroinflammation via therapeutic compounds and lifestyle to prevent or delay progression of Parkinson’s disease,” *Ageing Res Rev*, vol. 78, p. 101618, Jun. 2022, doi: 10.1016/j.arr.2022.101618.

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Greetings from **SJC Institute of Technology, Chikkaballapur!**

Congratulations....!!!

We are glad to inform you that your research article entitled:" **Pharmacoinformatic-guided Drug Reprofiling for Unveiling of Novel Bioactive Inhibitors Targeting USP13 in the Treatment of Parkinson's Disease** " has been accepted for presentation and subsequent publication at "**2nd IEEE International Conference on Knowledge Engineering and Communication Systems (ICKECS)**".

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Pharmacoinformatic-guided Drug Reprofiling for Unveiling of Novel Bioactive Inhibitors Targeting USP13 in the Treatment of Parkinson's Disease

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***Abstract*- Parkinson's disease (PD) is an incapacitating neurodegenerative condition caused by the deterioration of dopaminergic neurons and the build-up of α-synuclein, which is most common in the aged population and is influenced by an intricate relationship between environmental and genetic components. This research attempts to address the lacuna in definitive treatment strategies for PD by exploring innovative therapeutic targets. The focus centers on Ubiquitin-Specific Protease 13 (USP13), widely distributed in human cells and displaying heightened levels in postmortem PD midbrains, suggesting a pivotal role in disease etiology. Experimental findings underscore USP13's intricate involvement, negatively regulating Parkin and α-synuclein, thereby intricately influencing motor function and dopaminergic neuron survival. Employing advanced molecular docking analyses through PyRx, this research seeks to uncover novel USP13 inhibitors surpassing the efficacy of Spautin-1. An added layer of practicality is introduced through the repurposing of FDA-approved drugs, accentuating the potential for therapeutic intervention. Docking-based virtual screening of 3674 FDA-approved drugs against USP13 identified 144 compounds that met the defined criteria of Binding Energy. Of these, 60 exhibited BBB permeability. The top 10 ligands with the highest negative binding energies and BBB permeability were shortlisted for further analysis. Detailed scrutiny of the top three ligands illuminates Algestone Acetophenide's solubility challenges, while the dopamine antagonist properties of Biriperone raise prudent concerns regarding its suitability for PD intervention. In stark contrast, Canrenone, a clinically established agent for hyperaldosteronism and heart failure, emerges as a compelling candidate with the potential to inhibit USP13 and exert a transformative impact on PD pathology.**

Keywords— Parkinson's Disease, Ubiquitin-Specific Protease 13, Molecular Docking, BBB permeability, Canrenone, Neurodegeneration

I. Introduction

Parkinson's disease (PD) is a common neurological illness that primarily affects people 65 years of age and older. The state is distinguished by bradykinesia, a subdued tremor at rest, and inflexibility, attributes ostensibly ascribed to the gradual deterioration of dopaminergic neurons positioned within the midbrain's compact part of the substantia nigra (SNpc). Furthermore, PD exhibits associations with non-motor irregularities encompassing cognitive inadequacies, sensory discomfort, weariness, psychological distress, cognitive decline, and challenges in achieving restful repose [1]. Although growing older is the main risk factor for the condition, there is a complicated interaction between hereditary and environmental factors. About 5–10% of cases are caused by genetic factors and are characterized by rare monogenetic forms involving genes such as DJ-1, SNCA (α-synuclein), PARK2 (Parkin), LRRK2, VPS35, and GBA, among others. Interestingly, most of the cases—referred to as sporadic—do not have a clear single etiology [2]. Lewy bodies (LBs), α-synuclein-containing inclusions discovered in postmortem brains, are a typical neuropathological characteristic of PD. Increased expression of α-synuclein can be caused by genetic alteration in the SNCA gene, which can lead to the creation of fibrils and oligomers. Aggregation can also be caused by a malfunction in the mitochondria and proteasome. These alterations give α-synuclein prion-like qualities, facilitating its spread between neurons and affecting cellular activities such as neurotransmitter release and mitochondrial functioning [3]. Therapeutic approaches for PD center around symptom mitigation, with a dearth of methodologies aimed at halting or mitigating disease advancement. The deficiency of a definitive PD remedy instigates additional exploration into the malady's pathogenesis and the unveiling of innovative targets for therapeutic intervention.

A. USP13 and its Role in Parkinson's Pathology

Ubiquitin-Specific Protease 13 (USP13), a Deubiquitinase (DUB) abundantly distributed throughout the human body, with conspicuous presence in the cytosol and nucleoplasm of cells (UniProt accession number: Q92995). According to numerous academic publications, Liu et al.'s research has thoroughly examined the correlation between USP13 and neurodegenerative illnesses, such as PD and Alzheimer's disease (AD) [4]. In comparison to healthy controls, the study revealed higher amounts of USP13 in the postmortem midbrains of PD patients, suggesting a possible involvement of the protein in the illness. Mouse neuron experiments showed that USP13 adversely governs Parkin (E3 ubiquitin ligase), affecting its solubility, ubiquitination, and activity. USP13 also negatively impacts the amount, ubiquitination, and removal of α-synuclein, while concurrently regulating proteasome activity. In animal models, these effects impact both motor function and dopaminergic neuron survival. Experimental confirmation in living organisms illustrated that USP13 diminishes the removal of α-synuclein, and its heightened expression annulled the positive outcomes of the drug nilotinib, recognized here for its capacity to enhance the purging of α-synuclein . USP13 suppression might impede the deubiquitination process of alpha-synuclein and constrain its

accumulation by fostering autophagy and facilitating proteasome elimination. Consequently, molecules inhibiting USP13 could potentially serve as therapeutic interventions in alpha-synucleinopathies [5].

B. Mechanism

The USP13 gene's genomic locus is located on chromosome 3 of humans, specifically in the area known as 3q26.2–q26.3. Encoding the USP13 protein, also called isopeptidase T-3, is the function of this gene. According to a bioinformatics study, Ubiquitin-Specific Proteases (USPs) consist of a catalytic core domain surrounded by other functional domains that are located inside or surrounding the core domain. Notable domains that are important to the operations of USPs include UBA (ubiquitin-associated), ZnF (zinc finger), and UIM (ubiquitin interacting motif). There are two UBA insertions in the conserved C- and H-boxes of the USP13 catalytic domain. The distribution of charge within the binding pocket and the modification of conserved residues crucial for ubiquitin-binding are the two reasons why USP13-ZnF is unable to bind ubiquitin.

When looking at USP13-UBA's (PDB 2LBC) solution structure. According to a structural study, UBA is made up of three α-helices. Crucially, the two UBA units were linked by an extended loop; no direct interaction was seen between them. The classical relationship between USP13 and Ub is further supported by NMR titration experiments, which show that the tandem UBA domains function as Ub receptors for catalytic role in as USP enzyme. The active site and the tandem UBA domains are both important in this process, as demonstrated by mutations in USP13's UBA domains that block Ub binding. This implies that the regulation of cellular protein levels is significantly influenced by USP13's deubiquitinating activity and the Ub-binding ability of its UBA domains [6].

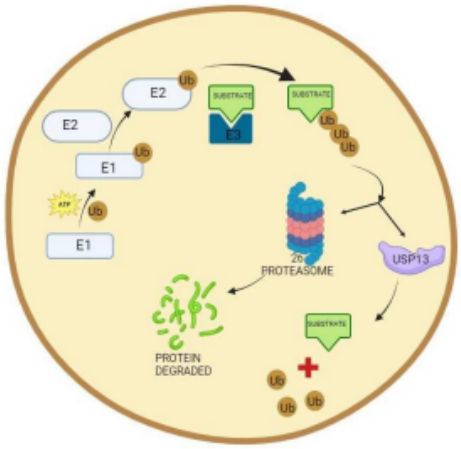


Fig. 1. Illustration depicting proteasome degradation and the role of usp13 as a Deubiquitinase (DUB) enzyme.

C. Therapeutic Potential

It has been ascertained that spautin-1 is a specific suppressor of USP10 and USP13, displaying IC50 values ranging from 0.6 to 0.7 µM. Nevertheless, it is unable to cross the blood–brain barrier (BBB) and non-selectively inhibits USP10 and USP13, which affects the autophagy and proteasome processes [7]. Through molecular docking analyses, this study tries to discover novel inhibitors that surpass Spautin-1 in terms of binding affinity and exhibit more robust molecular interactions. One of the main objectives is to create ligands that are more blood-brain permeable than Spautin-1, with the ultimate goal of investigating their potential as medicinal agents. This strategy, which is based on repurposing FDA-approved medications, aims to progress the development of USP13 inhibitors for possible therapeutic uses, especially in neurological conditions like Parkinson's disease.

D. PyRx

PyRx, an open-source program with an easy-to-use interface that works with most major operating systems, is used in the research (Linux, Windows, and Mac OS). Python-based PyRx is adaptable and works on a range of computing systems, including supercomputers and home computers. The method uses docking-based virtual screening (DBVS) to investigate the behavior of small molecules in target molecules' binding pockets. By using this technique, molecules with the best interactions can be found and subsequently investigated through experimentation. Many software solutions have been created for effective Docking-Based Virtual Screening of large molecular datasets, such as PyRx. It's a well-known tool for cross-platform inter-operability and user-friendly design. It easily incorporates open-source software components such as Open Babel, AutoDock, and AutoDock Vina.

II. Methodology

A. Data collection

The protein structure of USP13 was downloaded from the database PDB under the ID (PDB ID:2LBC) (https://www.rcsb.org/structure/2LBC). For the Repurposing of drugs against UPS13, a library of 3674 FDA-approved drugs was retrieved from DrugBank. The ligands were initially in SDF format and were transformed into PDBQT format using the OpenBabel platform integrated into PyRx. Here, the energy of ligands was also minimized.

B. Receptor preparation

Pymol was used for the representation and modification of the protein such as removal of water molecules associated with the structure.
The Swiss-PDB Viewer program was further used to minimize the energy of the protein receptor. This required using optimization methods and force fields from molecular mechanics through iterative procedures. The objective was to refine the overall stability of the protein structure by achieving a thermodynamically stable conformation.

C. Molecular docking using PyRx

The computational procedure initiated with the loading of the protein into software (PyRx), followed by its conversion into a macromolecular structure. Ligands, transformed into .PDBQT format underwent energy minimization and was subsequently chosen for the docking process. A blind docking approach was adopted, expanding the docking site to its maximum dimensions and further adjusted to include the

entire receptor within the grid (X-120.7809, Y-31.0808, Z-29.5051 Å) using the Vina Wizard. Post-docking, an Excel sheet was generated, capturing the results, specifically the binding affinity of each ligand for the macromolecule. Individual output files for each docked ligand were stored for subsequent 2-D interaction analysis, illustrating every interaction and hydrogen bond formation between the ligand and the macromolecule.

Compounds with binding energy equal to -8.0 kcal/mol or less, with reference to Spautin1 (-7.9 Kcal/mol) were considered and further analyzed.

D. BBB Permeability

Ligands meeting the defined criteria were subsequently subjected to evaluation for blood-brain barrier (BBB) permeability using SWISSADME. This assessment also provided insights into various pharmacokinetic properties of the ligands, along with an evaluation of their drug-likeness characteristics.

III. Discussions and Results

Out of the 3647 FDA Approved Drugs (Ligands), 144 ligands showed a binding energy of -8 kcal/mol or less and satisfied the predefined requirement that ligands having binding affinity higher than reference drug Spautin-1 (-7.9Kcal/mol). Out of this subset, 60 ligands indicated permeability across the blood-brain barrier (BBB). The top 10 ligands with the highest negative binding energy which are BBB permeable are shown in Table I, together with relevant information about their unique binding interactions. It also shows the above information of the Reference drug (Spautin-1).

Using a thorough ADME Analysis, the characteristics of the top three ligands—which were identified using their remarkable binding affinities—were examined in further detail. The emergence of Algestone Acetophenide demonstrated a tendency toward Poor solubility along with increased absorption through the gastrointestinal tract. On the other hand, there was a harmonious balance between Biriperone and Canrenone, with enhanced gastrointestinal absorption and moderate solubility. However, the complex nature of Biriperone, also recognized as Centbutindole, reveals that it is a dopamine antagonist that operates within the class of antipsychotic medications [8]. This characteristic, while compelling, raises a cautious eyebrow, suggesting that Biriperone might not be the optimal contender for addressing the complexities of PD. On the other hand, Canrenone's medical expertise is highlighted by its proven effectiveness in treating primary hyperaldosteronism and edematous disorders resulting from secondary hyperaldosteronism, such as heart failure. Canrenone, poised as a more auspicious candidate, invites consideration for further exploration in the therapeutic landscape.

Utilizing computational techniques, our novel approach repurposes FDA-approved medications to target Ubiquitin-Specific Protease 13 (USP13), an essential enzyme in Parkinson's disease. Our approach circumvents the necessity for de novo drug development by utilizing pre-existing pharmacological information, in contrast with traditional procedures. One well-known USP13 inhibitor, Spautin-1, has shortcomings, including a low binding affinity and poor blood-brain barrier permeability. Canrenone, a medication that has been clinically shown to treat certain medical conditions, showed promise as a USP13 inhibitor in our investigation, outperforming Spautin-1 in terms of binding affinity and BBB permeability. This finding not only solves current constraints but also emphasizes how much drug repurposing may accelerate the development of therapies for Parkinson's disease

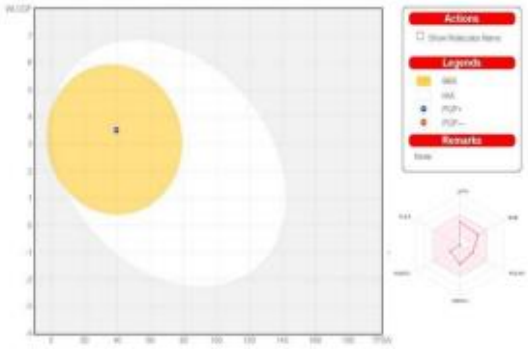
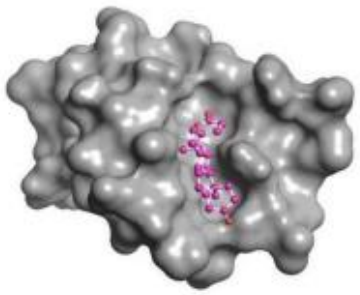
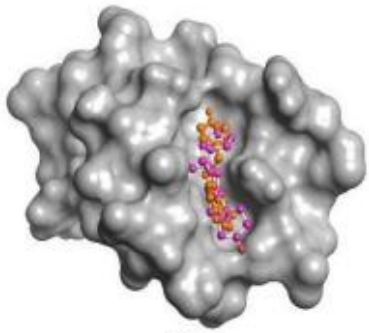


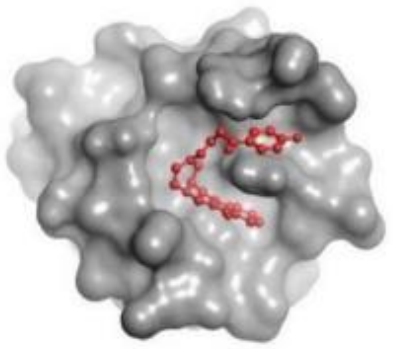
Fig. 2. Representative Visual Depiction of Canrenone Employed in ADME Analysis, Exhibiting a Graphical Illustration Analogous to the Morphology of a Boiled Egg.



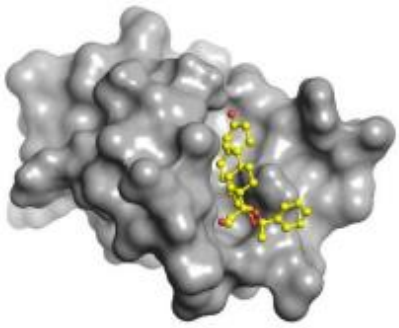
(a)



(b)



(c)



(d)

Fig. 3.
(a) Canrenone in the binding pocket of USP13 protein
(b) Canrenone and Spautin-1 overlapping in
the binding pocket of USP13 protein
(c) Biriperone in the binding pocket of USP13 protein
(d) Algestone Acetophenide in the binding pocket of USP 13 protein

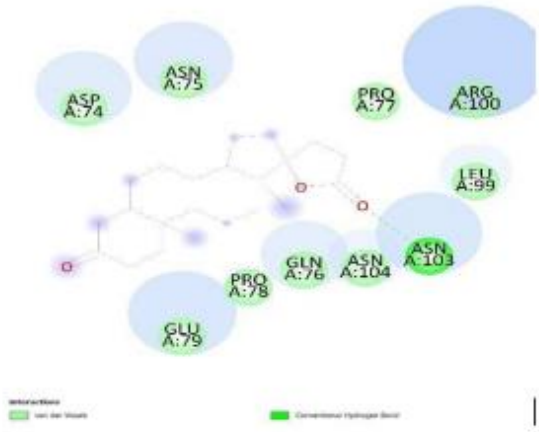


Fig. 4. Illustrates a two-dimensional graphical depiction portraying the diverse interactions between Canrenone and the USP13 protein.

IV. Conclusion

From the extensive comprehension obtained from the ADME study of the principal 3 ligands. Algestone Acetophenide showed restricted solubility and increased gastrointestinal absorption. On the other hand, Biriperone and Canrenone showed a well-balanced combination of high gastrointestinal absorption and moderate solubility. However, there are concerns regarding the suitability of Biriperone for PD due to its inherent dopamine antagonist properties.

Consequently, Canrenone, with its favorable pharmacokinetic and pharmacodynamic characteristics, can be a promising candidate as a repurposed drug for the treatment of PD. Considering the multifaceted nature of PD treatment, the proposition of Canrenone as a potential therapeutic agent warrants further experimental validation. The search for novel inhibitors is essential in the ever-changing field of PD treatment. A new frontier in therapeutic approaches is heralded by the potential involvement of Canrenone in USP13 inhibition. Since there is currently no cure for PD, research into new targets—such as the inhibition of USP13—is essential to the development of therapy approaches that could improve the quality of life for those who are coping with this severe neurological condition.

Furthermore, computational research like Molecular Dynamics (MD) simulations can offer important insights into Canrenone's conformational dynamics and binding kinetics during interactions with USP13. These silico-predictions of the pharmacokinetic characteristics of the canrenone-USP13 complex can aid in clarifying its stability. Adding experimental validation will be essential in the future to improve the translational potential and robustness of our findings. To confirm Canrenone's inhibitory action on USP13 and clarify its impact on PD-related cellular pathways, experimental research is necessary. This includes preclinical studies in animal models of PD and in vitro experiments. These studies will yield vital empirical data in favour of Canrenone's safety and effectiveness as a possible PD treatment.

By integrating experimental and computational approaches, we can comprehensively validate the therapeutic potential of Canrenone and pave the way for its translation into clinical applications. Additionally, ongoing research efforts should focus on optimizing Canrenone derivatives to enhance their potency, selectivity, and pharmacokinetic profiles, further advancing the development of USP13 inhibitors for PD treatment.

TABLE I. BINDING AFFINITY AND INTERACTIONS OF LIGANDS AND REFERENCE DRUG

Ligands (Drug molecules)	Binding affinity (Kcal/mol)	Amino acids Involved in binding Interactions
Spautin-1 (Reference drug)	-7.9	PRO(46),PRO(51),PHE(48),GLU(50),ALA(49),PHE(15),GLY(14),LEU(17),MET(13),LEU(10) ,PHE(36),ILE(40),ILE(39),MET(43)
Biriperone	-9.4	TYR(57),GLY(14),MET(13),ILE(40),MET(43),PRO(51),PHE(48),ALA(49),LEU(17), PHE(15),ILE(39), MET(54)
Canrenone	-9.2	ASP(74),ASN(75),PRO(77),ARG(100),LEU(99),ASN(103),ASN (104),GLN(76),PRO(78),GLU(79)
Algestone Acetophenide	-9.1	PRO(46),GLU(45),MET(43),ILE(40),GLY(14),MET(13),LEU(10),PHE(36),ILE(39),LEU(17),ALA(49),PHE(48),PHE(15),PRO(51)
Estradiol Benzoate	-8.7	PRO(46),MET(24),GLU(45),PHE(48),ILE(40),PHE(36), LEU(17),ILE(39),PHE(15),PRO(51)
Fluorometholone	-8.7	PRO(46),ILE(40),ILE(39),PHE(36),LEU(17),PHE(15),MET(43), PHE(48),ALA(49),PRO(51)
Tirilazad	-8.7	TYR(57),PHE(15),PRO(51),PHE(48),LEU(17),ILE(39),PHE(36),ILE(40),MET(43),MET(13),ALA(61),MET(54),GLY(14),
Quinestrol	-8.6	PHE(36),LEU(17),GLY(14),PHE(48),PRO(46),PRO(51),MET(43),PHE(15),ILE(39),ILE(40),MET(13),LEU(10),
Mosapramine	-8.5	PRO(51),ILE(39),ILE(40),MET(43),LEU(10),PHE(36),MET(13),GLY(14),LEU(17),PHE(15),PHE(48),ALA(49),
Testolactone	-8.5	PHE (114),PHE(90),LEU (110),MET(88),GLU (107),ASP (111)
Tropatepine	-8.5	PHE (48),MET (43),PHE (15),LEU(17),ALA (49),PRO(51),PRO(46),

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REFERENCES

[1] V. W. Sung and A. P. Nicholas, "Nonmotor symptoms in Parkinson's disease. Expanding the view of Parkinson's disease beyond a pure motor, pure dopaminergic problem," Neurologic Clinics, vol. 31, no. 3 S. 2013.

[2] R. Balestrino and A. H. V. Schapira, "Parkinson disease," Eur J Neurol, vol. 27, no. 1, pp. 27–42, Jan. 2020,

[3] D. A. Madsen, S. I. Schmidt, M. Blaabjerg, and M. Meyer, "Interaction between parkin and α-synuclein in park2-mediated Parkinson's disease," Cells, vol. 10, no. 2. 2021.

[4] P. Y. Ø. Nielsen, J. Okarmus, and M. Meyer, "Role of Deubiquitinases in Parkinson's Disease—Therapeutic Perspectives," Cells, vol. 12, no. 4. 2023.

[5] X. Liu, M. Hebron, W. Shi, I. Lonskaya, and C. E. H. Moussa, "Ubiquitin specific protease-13 independently regulates parkin ubiquitination and alpha-synuclein clearance in alpha-synucleinopathies," Hum Mol Genet, vol. 28, no. 4, 2019.

[6] Y. H. Zhang, C. J. Zhou, Z. R. Zhou, A. X. Song, and H. Y. Hu, "Domain analysis reveals that a deubiquitinating enzyme usp13 performs non-activating catalysis for lys63-linked polyubiquitin," PLoS One, vol. 6, no. 12, 2011, doi: 10.1371/journal.pone.0029362.

[7] J. Liu et al., "Beclin1 Controls the Levels of p53 by Regulating the Deubiquitination Activity of USP10 and USP13," Cell, vol. 147, no. 1, pp. 223–234, Sep. 2011.

[8] R. Chandra, H. Singh, P. K. Dalal, O. P. Asthana, and J. S. Srivastava, "Comparative efficacy of centbutindole & risperidone in schizophrenia.," Indian J Psychiatry, vol. 44, no. 4, pp. 365–71, Oct. 2002.

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- MOUNT CARMEL SCHOOL- 91.6%

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Post - Graduation (M.Sc. BIOTECHNOLOGY) -2022 -2024

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INTERNSHIP AND WORKSHOPS

INTERNSHIP

- **OFFLINE CADD INTERNSHIP AT JAMIA MILLIA ISLAMIA (JMI) 2months**

Understanding how to put my drug design talents to use and to refine them. I had the chance to work under the direction of prominent researchers and learn about the development of an LRRK2 inhibitor for Parkinson Disease.

WORKSHOP

- "**Computer Aided Drug Design (CADD)**" by Dollar Education
- "**Bioinformatics for Vaccine Design**" by Dollar Education
- Attended "**Genetics and Evolution: Intertwined Strands**" mini symposium by Indian Academy Sciences
- Attended Offline Hands-on workshop on "**Techniques in Molecular Microbiology**" by ANDC and DDUC (Delhi University)
- Attended Offline Summer Vacation Hobby Camp on "**Biotechnology**" at National Science Centre.
- Attended Online webinar on "**Strategies Behind Covid-19 Vaccines**" by Snapses Zoological Society (DDUC)
- Participated in a 3 day International Workshop on **NGS And Transcriptomic Data Analysis**
- Participated In TRYST-IITD Edutech Workshop on **Techniques in DNA and Proteins.**
- Participated in One-Week STUTI Workshop on "**Translational Neuroscience: Bridging Gap Between Bench and Bedside"** at Jamia Hamdard

ACHIEVEMENTS

- Awarded **Super Achiever Certificate** for Securing 10 CGPA in ClassX
- **Shri Ghasi Ram Mittal Memorial Award** (2019-2020) for securing highest marks in B.Sc.(H) Zoology 1st year.

- **Pramerica Spirit of Community Awards -** Bronze medallion for being one of the top student volunteer in community service of teaching underprivileged
- **CSIR NET (LIFESCIENCE) LS QUALIFIED - TWICE (RANK 21 AND RANK 65)**

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- **Class Representative (CR)** in Bachelors for **all 3 Conscecutive Years**

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- Secured 3rd position in Inter House Football Competition
- Secured 2nd position in Geography Quiz
- Secured 124 State Rank in French Language Olympiad
- Received Certificate of Participation in Interschool Maths Olympiad
- Received Certificate of Participation in The National Wildlife Quiz by WWF-India
- Received Certificate of Excellence in Think and learn Challenge conducted by The Times Of India & BYJU'S
- Received Certificate of Participation in the Musical Bonanza "Fiddler on the Roof".

SKILLS

Tech Skills (BASICS)

- C-Language (Programming)
- Python (Programming)
- Computational Biology (CADD)
- MS Excel
- MS Powerpoint
- MS Word
- Google Forms

Scientific Skills

- Research Methodology
- Agarose Gel Electrophoresis
- Pipetting
- DNA Extraction
- Immunodiffusion
- Microbial Inoculation Techniques and Culturing
- Basic Bioinformatic Tools
- Biostatistics
- Spectrophotometry
- Chromatography
- Centrifugation
- Autoclaving
- Incubation

Soft Skills

- Communication skills
- Leadership qualities as well as a good team player
- Critical Thinker
- Problem Solver
- Pressure-Manager

PUBLICATIONS AND CONFERENCES

- **"Synergizing drug repurposing and target identification for neurodegenerative diseases"**- Published in **Progress in Molecular Biology and Translational Science** (IF -3.6) https://doi.org/10.1016/bs.pmbts.2024.03.023
- Presented Paper in the **2nd International Conference on Knowledge Engineering and Communication Systems** held at SJC Institute of Technology in association with IEEE Bangalore on **" Pharmacoinformatic-guided Drug Reprofiling for Unveiling of Novel Bioactive Inhibitors Targeting USP13 in the Treatment of Parkinson's Disease"**
- Attended and presented Poster in 3rd International Conference on **" Antimicrobial Resistance, Novel Drug Discovery and Vaccine Development: Challenges and opportunities"**
- Participated in Poster Presentation as Delegate in the Two Days International Symposium on **"Recent Advances in Neurochemistry and Neurosciences"** held at Jamia Hamdard
- Life Member of **Society For Neurochemistry, India (SNCI).**

HOBBIES

- **Playing Guitar, Reading Books (Non-Fiction) , Bodybuilding, Listening to Music and Motorcycling**