"*In-silico* study to repurpose DJ1 binding compounds for Alzheimer's disease and Parkinson's related dementia"

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Submitted by
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CERTIFICATE

This is to certify that the dissertation entitled: ***In-silico*** **STUDY TO REPURPOSE DJ1 BINDING COMPOUNDS FOR ALZHEIMER'S DISEASE AND PARKINSON'S RELATED DEMENTIA** in the partial fulfilment of the requirements for the reward of the degree of Masters of Engineering, Delhi Technological University (Formerly Delhi College of Engineering, University of Delhi), is an authentic record of the candidate's own work carried out by him/her under my guidance. The information and data enclosed in this thesis is original and has not been submitted elsewhere for honouring of any other degree.

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"In-silico study to repurpose DJ1 binding compounds for Alzheimer's disease and Parkinson's related dementia"

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1. ABSTRACT

Alzheimer's disease (AD) and Parkinson's disease (PD) are the most common chronic neurodegenerative disorders affecting worldwide population and is expected to increase over the years. Alzheimer's disease marked by agglomeration of beta amyloid and tau fibrillary tangles, exhibit loss of memory with time. While Parkinson's disease is characterized by progressive loss of dopaminergic neurons of the substantia nigra that results into clinical symptoms like tremor, bradykinesia, rigidity, slower movement and postural instability. However, both AD and PD are associated with cognitive impairment leading to dementia along with other canonical symptoms. This proposes a cross link between the diseases and hence toxic proteins. Studies suggest the oxidized form of DJ-1, also an oncogene; has-been observed in patients with AD and PD. Recent work on finding compounds that can modulate the protein DJ1 has brought Compound-23 and Compound-B into focus as it has been observed to exert neuroprotective effect against neurodegeneration in PD model, proposing it to be a lead compound for PD therapies. Hence the overall objective of our work is to apply *in-silico* approaches to study the gene architecture of PARK7 along with the TFBEs to explore its role in PD pathology. Further, the study involves *in-silico* screening of common protein targets of AD and PDD, followed by performing comparative molecular docking of these compounds against the targets while using conventional drugs as control. This may pave the way for developing effective preventative and therapeutic strategies to treat this devastating disease.

Keywords: Alzheimer's disease, Parkinson's disease, neuroprotective agents, therapeutics.

2. INTRODUCTION

The most common neurodegenerative disorder is Alzheimer's disease (AD) followed by Parkinson's disease (PD) affecting about 36 million people worldwide with an increasing dominance observed in the coming years[1]. AD is a progressive neurodegenerative disorder that impairs memory and cognitive functions effecting the behaviour, language, and visuospatial skills in elderly people. The neuro-pathological features of AD are the result of nucleation of certain proteins forming extracellular plaques as a result of β-amyloid (Aβ) deposition and intracellular neuro-fibrillary tangles (NFTs) due to tau hyper-phosphorylation. However, both AD and PD are associated with cognitive impairment leading to dementia along with other symptoms like sleep disorder, psychosis, aggression, hallucination, agitation, and depression[2].Although till date there is no cure available but pharmacological treatment to lower or slow down the symptoms against the disease include the use of conventional FDA approved drugs, such Memantine, Donepezil, Galantimine, Rivastigmine for AD. Interestingly, both AD and PD are considered to have a cross link as a result of their common genetic mutations, oxidative stresses, ageing, environmental stresses and other physiopathological processes that leads to neuronal damage.

In this regard, a specific group of mitochondrial proteins associated to familial forms of Parkinson's disease (PD) may be used as therapeutic targets for AD and Senile dementia. To date, proteins namely, Parkin, PINK-1, DJ-1, LRRK2 and α-synuclein have been reported to play essential roles in preventing cell death by maintaining normal mitochondrial function, protecting against oxidative stress, mediating mitophagy and preventing apoptosis. It has been reported recently that DJ1-binding compounds (Compound 23 and Compound B) has shown significant inhibition in cell death under H_2O_2 condition[3-4].Keeping the current scenario in mind and the advancement in technologies has led to the need of an alternative therapeutic way.

The overall objective of this proposal is to apply *in-silico* approaches to focus on PARK7 gene architecture and explored the transcription factors; their role in PD pathology. This will give insight on the recent advances concerning the molecular and cell biology of DJ-1 and its importance for PD physiopathology. Further, *in-silico* study of the two DJ1 binding compounds are performed against common targets of commercially available drug molecules of PD, AD and Senile dementia.

Parkinson 's disease

Parkinson's disease (PD) is the second most common chronic neurodegenerative disorder. This invalidating disease marked by progressive loss of dopamine neurons in the substantia nigra pars compacta, leading to reduced level of dopamine in the striatum and the presence of toxic aggregates of alpha synuclein along with other proteins forming intra-cytoplasmic inclusions named Lewy bodies[5]. It is characterized by canonical clinical symptoms like tremor, bradykinesia, rigidity, slower movement and postural instability. It is considered as idiopathic and several researches are going on to elucidate the root cause of it. PD is classified into two forms: Familial and Sporadic. Familial PD accounts for 10% of total cases of PD, but investigations provide support that it also play roles in the pathogenesis of sporadic PD[6].

Parkinson's disease is usually considered as an old age health challenge but it has also been diagnosed in patients under the age of 20 years and such cases are termed as juvenile-onset of Parkinson's disease. The juvenile PD is considered the recessive form, characterized by an early onset, a slow progression and shows a good response towards the levodopa replacement therapy. To date, four proteins account for the recessive familial PD-forms, namely, Parkin, PINK-1, DJ-1 and ATP13A2.

Structure, Function and Expression of DJ1

DJ-1 is a homodimer belonging to the peptidase C56 family. DJ1 comprises of 189 amino acids with seven β-strands and nine α-helices. DJ-1, however, contains an additional α-helix at the C-terminal region, which blocks the putative catalytic site of DJ-1.

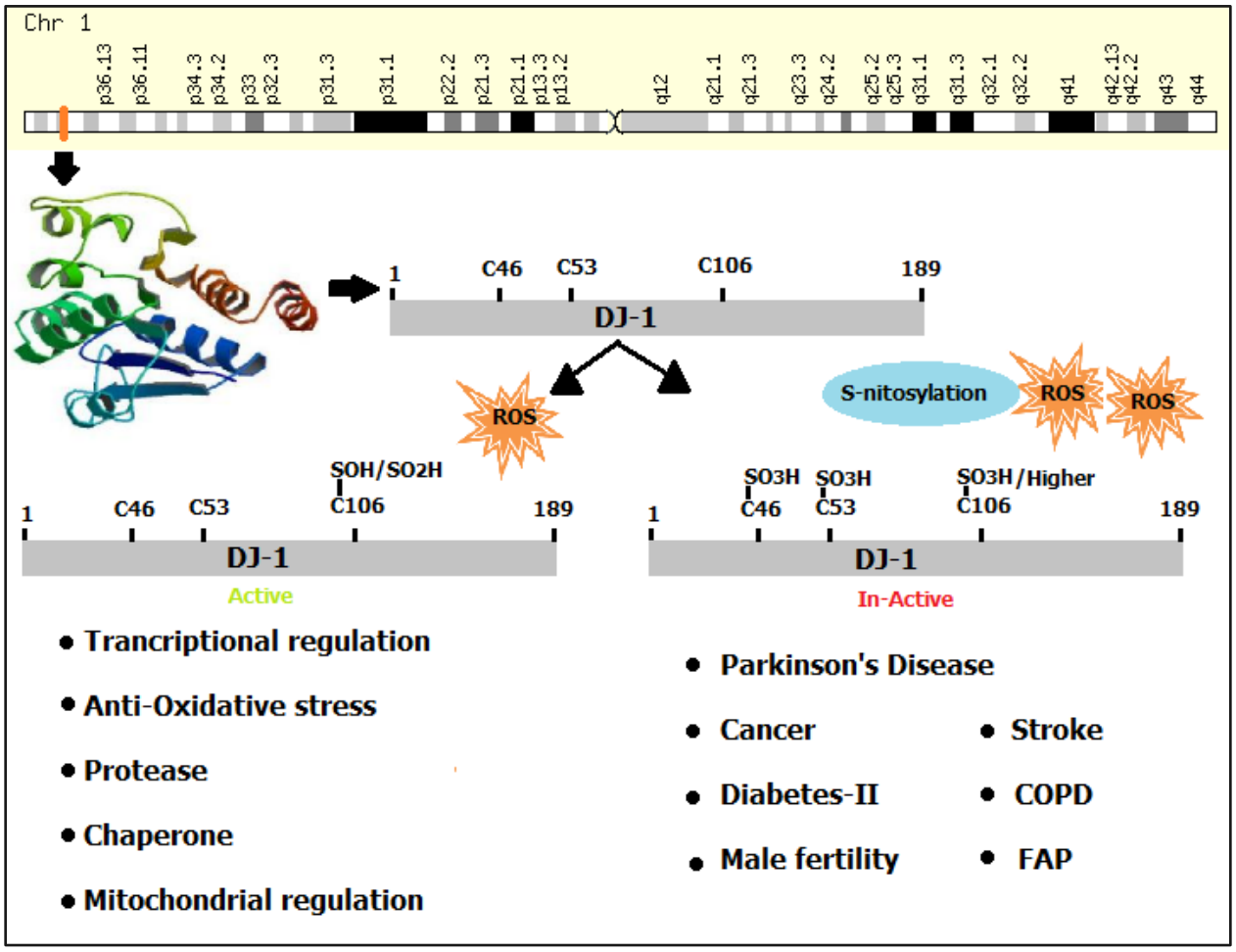


Figure 1: Causes of DJ1 Dysfunction (Source: Lev N. (2007). Role of DJ-1 in Parkinson's disease. Journal of Molecular Neuroscience)

DJ-1 is expressed in both neurons and glia cells of the brain and in the cytoplasm, nucleus, and mitochondria in cells. The over expression of DJ-1 is observed under oxidative stress condition, and in reactive astrocytes in sporadic PD and other neurodegenerative diseases. It works as a redox

sensitive chaperone and inhibits aggregation of α-synuclein under oxidative condition[7-8]. DJ-1 contains three cysteine residues, at position C46, C56, and C106. C106 being highly susceptible to oxidative stress and is easily oxidized to sulfinic acid. Oxidation to sulfinic acid and mutation causes loss of all of DJ-1's functions. An excessive oxidized DJ-1 has been observed in brains of patients with PD and Alzheimer's disease[9]. DJ-1 is also modified by sumoylation, S-nitrosylation, and phosphorylation. Sumoylated at lysine-130 in response to the oxidative stress condition in concomitant with acidic shift. Excess sumoylation is observed in an L166P pathogenic mutant of DJ-1. S-nitrosylation affects dimerization of DJ-1, which is necessary for DJ-1 to exert its function. Phosphorylation of DJ1 takes place but the effect on DJ-1 function is not known. From these points, it is thought that DJ-1 also participates in the pathogenesis of sporadic PD as well as familial PD.

Although till date there is no cure available but pharmacological treatment to lower or slow down the symptoms of these diseases include the use of conventional FDA approved drugs, such Memantine, Donepezil, Galantamine, Rivastigmine for AD; and Levodopa, Carbidopa, Bromocriptine, Pramipexole, Ropinirole for PD. However, the only treatment against PD-associated dementia is Rivastigmine, a cholinesterase inhibitor. This has led to the study of alternative therapeutic way. Computational drug repurposing is one such alternative approach that utilizes the potential *in-silico* study to repurpose the approved conventional drugs and the drugs undergoing preclinical and clinical phases that can be used as disease-modifying therapies.

Drug Repurposing or Repositioning is an alternative to de novo drug discovery process. It aims to find new clinical indications of existing drugs; these includes the FDA approved drugs or investigational drugs or the one present in the drug discovery pipeline[10]. The need for drug repurposing is to improve R&D productivity over the years in contrast to the previous de novo drug discovery process, which was time consuming costing billions of dollars.

In this approach, we focus on investigating drugs that exhibit neuroprotective activity and can be proposed to be used as a novel therapeutic compound against the known target molecules of Alzheimer's disease, Senile dementia and Parkinson's disease. Recently, Kitamura *et al* in his study suggested a new DJ1 binding compound, Compound-23 and Compound-B to exert neuroprotective activity against neurodegeneration in PD model[3-4]. Hence, here we perform comparative molecular docking analysis of these compounds against the common targets of AD and PDD and used conventional drugs as a control.

3. REVIEW OF LITERATURE

Parkinson's disease (PD), the second most common NDD, is a progressive multi-factorial disease caused by both genetic and environmental factors[7]. The three cardinal motor symptoms, akinesia in combination with either tremor at rest or rigidity, are still the basis of the clinical diagnosis. Up to 2016, we still have no treatment to slow down or even stop the progression of the disease. Available therapy is symptomatic[6]. To have an insight into the molecular and cellular determinants that influence the pathology of Parkinson's disease (PD) is essential for developing effective diagnostic, preventative and therapeutic strategies to treat this invalidating disease[8]. The neuropathological hallmarks of the disorder: the so-called Lewy bodies (proteinaceous intra-cytoplasmic inclusion bodies) containing aggregations of the protein alpha-synuclein and the loss of pigmented melanin containing neurons in the midbrain. The latter reflects the neurodegeneration of dopaminergic neurons in the substantia nigra (SN) leading to a marked dopamine deficit in the striatum[8]. There are two types of PD, familial and sporadic. However familial PD accounts for 10% of total cases of PD, investigations of the functions of familial PD gene products have provided great insights into the molecular mechanisms of the onset of PD, and are thought to also play roles in the pathogenesis of sporadic PD. An increase in the cellular stress affects the post-translational modifications system to break down or introduce non-specific post-translational modifications that do not occur during physiological conditions[11-13]. In 2003, Bonifati et al. observed a large deletion and missense mutation in the DJ-1 gene in Italian and Dutch PD patients that targeted DJ-1 gene as a causative gene for familial PD park7 with recessive inheritance[8]. Twenty-three pathogenic deletion and point mutations were found in patients with PD. Compared to the other causative genes Parkin and Pink1 that showed higher mutation rate, the number of mutations in the DJ-1 gene is small. It was

hypothesized that DJ-1 may be placed upstream of Pink1 and Parkin, during onset of PD[8]. Park7, DJ-1 gene, is located on chromosome 1p36 and spreads over 8 exons. Exons 1a/b are alternatively spliced and non-coding while exons 2-7 encode for a 189 amino-acid protein. DJ1 exhibit anti-oxidant activity, transcriptional regulation, chaperone activity and protein degradation. In 2004, Taira *et al.* focused on the efficacy of DJ-1 as an antioxidant, since oxidative stress is strongly associated with PD pathogenesis. Mouse embryonic fibroblast NIH3T3 cells, harbouring pathogenic mutants of DJ-1, were more vulnerable to H2O2-induced cytotoxicity than cells with wild-type (WT) DJ-1.[14]. Furthermore, treatment of recombinant WT DJ-1, but not the L166P mutant of DJ-1, reduced the H2O2-induced fluorescence of 2′,7′-dichlorodihydrofluorescein, a reactive oxygen species (ROS) specific indicator, in rat primary mesencephalic cultures and human neuroblastoma SH-SY5Y cells.[15-16]. Deficiency of DJ-1 in neurons differentiated from embryonic stem cells and SH-SY5Y cells showed vulnerability to oxidative stress.[15,17]. Another point of view is that DJ-1 activates an antioxidant pathway, such as the nuclear factor erythroid 2-related factor 2 (Nrf2)–antioxidant responsive element (ARE) pathway. The Nrf2–ARE pathway is known to be a common antioxidant pathway for the production of antioxidants (*e.g.* glutathione, thioredoxin and peroxiredoxin). Im *et al.* reported that DJ-1 was associated with the production of thioredoxin1 by the activation of the Nrf2–ARE pathway without direct interaction with Nrf2 and Kelch-like ECH-associated protein 1. By contrast, pathogenic mutants of DJ-1 (M26I and L166P) and a missense mutant of DJ-1 at cysteine 106 residue to serine (C106S) did not increase thioredoxin1 expression[18-19].

DJ1 was reported to be a transcriptional regulator in several pathways, including the Nrf2–ARE pathway mentioned above. DJ-1, but not pathogenic mutants of DJ-1, positively regulated transcriptional activation and suppressed polypyrimidine tract-binding protein-associated splicing

factor-induced apoptosis in SHSY5Y cells[20]. On the other hand, DJ-1 also acts as a negative regulator of transcription. Fan *et al.* revealed that DJ-1 interacted with p53 and inhibited p53-mediated Bax expression and the subsequent apoptosis pathway in mouse neuroblastoma neuro2a cells and human embryo kidney 293 cells[21]. Moreover, DJ-1 regulated transcription factors such as androgen receptors and sterol regulatory element-binding protein.[22-24] Transcriptional regulation by DJ-1 was also associated with the expression of tyrosine hydroxylase (TH), the rate-limiting enzyme of dopamine synthesis[25].

Abnormal protein aggregations, such as α-syn, amyloid β (Aβ), tau, and huntingtin, are observed as characteristic features in neurodegenerative disorders. In pathogenic conditions, these proteins form abnormal folding and aggregation. Shendelman *et al.* revealed that WT DJ-1, but not the L166P mutant of DJ-1, prevented the aggregation of α-syn in cell-free assay and reduced the α-syn content in a detergent-insoluble fraction from murine neuroblastoma Cath.a-differentiated (CAD) cells[26]. Huntington's disease is characterized by a mutant huntingtin (mHTT) protein with polyglutamine repeats. Sajjad *et al.* showed the chaperone activity of DJ-1 in resolving the abnormal aggregation of mHTT[27].

DJ-1 has been reported to be involved in proteolytic pathways, such as the ubiquitin proteasome system and autophagy lysosome system. Especially, DJ-1 seems to be associated with the degradation of α-syn. α-Syn is known to be taken into neurons, astrocytes and microglia and to receive clearance in these cells[28-29].

Parkin is known as the causative gene of familial PD (PARK2), and functions as a ubiquitin E3 ligase[30]. Xiong *et al.* reported that the complex of Parkin, phosphatase and tensin homolog deleted from chromosome 10 (PTEN)-induced putative kinase 1 (PINK1) and DJ-1 worked

as a ubiquitin E3 ligase complex, and promoted proteolysis[31]. Furthermore, DJ-1 is also associated with the autophagy lysosome system. In the PD brain, autophagy failure, induced by the production of ROS and reactive nitrogen species, is observed[32]. Autophagy is known as the self-degradation of cellular organelles and protein aggregates. Recent studies have revealed the autophagy pathway involved in the degradation of α-syn, implying that autophagy is strongly associated with PD pathogenesis.[33-35]. DJ-1 deficiency exhibited an impairment of autophagy in mouse embryonic fibroblasts and in M17 human dopaminergic neuroblastoma cells[36-37].

Mutations on DJ- 1 gene are very rare and account for less than 1% of the cases identified by means of familial-based linked studies. The first mutation was declared in a Dutch family and represents a homozygous deletion of exons 1-5. Another Italian kindred harbours a mutation corresponding to a substitution of a highly conserved leucine into a proline in position 166 of DJ- 1 sequence[8]. Clinically, DJ-1 pathology is associated to an early onset, good and prolonged response to levodopa, slow progression and presence of psychiatric symptoms like anxiety and/or dystonic features (blepharospasm). Later, several studies targeting samples associated to young-onset PD led to the identification of a number of heterozygous pathogenic sporadic associated DJ-1 mutations. Later studies revealed that, a role for DJ-1 in sporadic PD is supported by the data showing that the levels of DJ-1 in the cerebrospinal fluids are increased in samples derived from sporadic PD patients[8]. DJ-1 interacts physically with α-synuclein, and also with Parkin and PINK1. A family of PD patients carrying novel heterozygous missense mutations in both PINK1 (P399L) and DJ-1 (DJ-1A39S) have been identified. Interestingly, both proteins share a mitochondrial localization and interact when co-over expressed in SHSY5Y neuroblastoma cells[8]. The over-expression of DJ-1 enhances the steady-state levels of PINK1 and the two proteins synergistically protect cells from MPP+ induced

cell death. The data highlight the importance of the cross-talk between the familial PD-associated proteins to the etiology of this complex pathology.

4. MATERIALS AND METHODS

[1] Retrieval of PARK 7 gene architecture

Analyzing the gene architecture of PARK7 was done with the help of NCBI (https://www.ncbi.nlm.nih.gov/).The NCBI houses a series of databases relevant to biotechnology and biomedicine and an important resource for bioinformatics tools and services.

[2] Analysis of Transcription Factors

The comprehensive study of the various TFBEs (transcription factor binding elements) of PARK7 was done using Matinspector and Physbinder online tool. Matinspector is a large library that searches TFBEs in the promoter region of the input sequence. Physbinder (http://bioit.dmbr.ugent.be/physbinder/) is another online tool that utilizes its extensive algorithm for TFBE identification.

[3] Protein-Protein Interaction (PPI) study

The STRING database (http://string-db.org/) was mined to analyze the PPI network of PARK7. STRING database evaluates and integrates the association of proteins by scoring function based on their functional modularity and interconnectivity within the cells. PPI network of PARK7, PARK2, PINK1, SNCA, PTEN, SOD1, PRDX5, PRDX2, NIT2, MIF, LRRK2 was obtained with the help of STRING database.

[4] Data Mining

Screening of all the launched conventional drugs and its target associated to Alzheimer's disease, Parkinson's disease and Senile dementia, was done using Clue Repurposing tool (https://clue.io/repurposing-app) followed by sorting the data having common targets. The Clue Repurposing tool has a collection of 5000 drugs and compounds that provides opportunity to

repurpose drugs and improves disease treatment. The targets pdb files were retrieved from RCSB Protein Data Bank (http://www.rcsb.org/pdb/home/home.do).

[4] Retrieval of Ligand structure

The compound-23 and Compound-B was mined from Zinc database (Zinc ID: 629557 and Zinc ID: 66348516; respectively) and their sdf files to retrieve the chemical structure along with Rivastigmine, Citicoline, Memantine was downloaded from PubChem database (https://pubchem.ncbi.nlm.nih.gov/).

[5] Analysis of the Physiochemical Properties, Drug likeliness and ADMET test

The physiochemical properties, lipophilicity, water solubility, pharmacokinetics (ADMET), drug likeliness (based on Lipinski Filter and Ghose Filter) of Compound-23 and Compound-B were performed using the online tool SwissADME (http://www.swissadme.ch/index.php). The qualifying parameters of (1) Lipinski filter and (2) Ghose Filter; that attributes drug likeliness are–Lipinski: Mol Wt. <=500 Dalton, MLogP<=4.15, H-bond acceptor <=10, H-bond donor<=5, Molar Refractivity 40-130; Ghose: Mol Wt. 160-480, WLogP -0.4-5.6, Molar Refractivity 40-130, Number of Atoms 20-70.

[6] Preparation of Target Protein and Ligand Molecule

The removal of any water molecule and ligand attached to the target protein molecule was done using Discovery Studio 4.1, followed by addition of hydrogen atoms and charges to the protein using AutoDock tools 1.5.6. Further, the ligand preparation was done by addition of charges.

[7] Molecular Docking

Autodock tools 1.5.6 was used to execute molecular docking of the prepared target protein and ligand that would predict the possible protein-ligand interactions in the form of affinity. Further, the results were viewed and analyzed using PyMol Molecular Visualization Tool.

5. RESULTS

Selection of TFBE in association to Parkinson's disease

The screened TFBE along with their respective binding position, sequence and mechanism of action are depicted below in Table 1. The below Figure 2, represents the gene architecture of PARK7. PARK7 located at chromosome 1p36 comprising of 31,215 bases, with 1.1Kb in the 5' flanking sequence region along with the various TFBE in association to PD are depicted in the given figure.

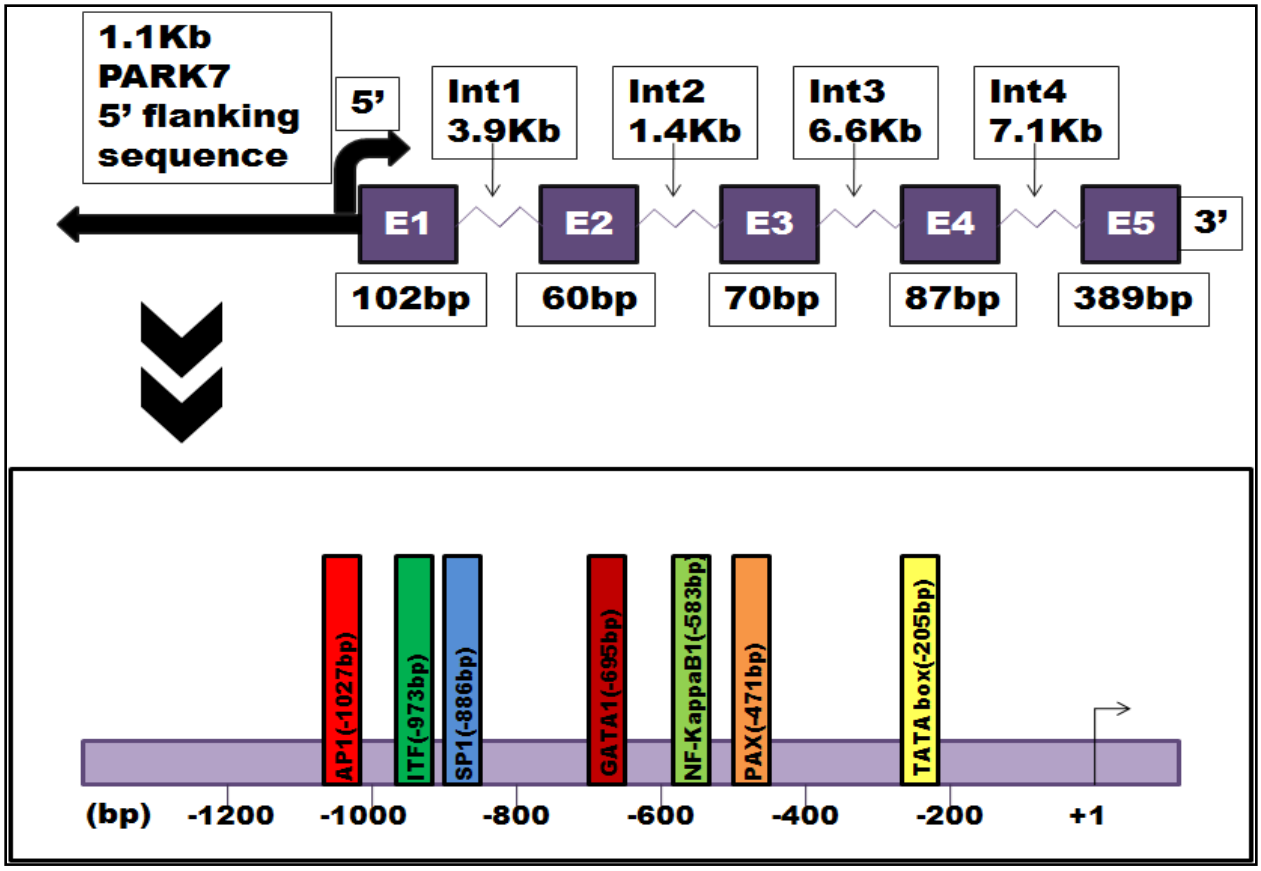


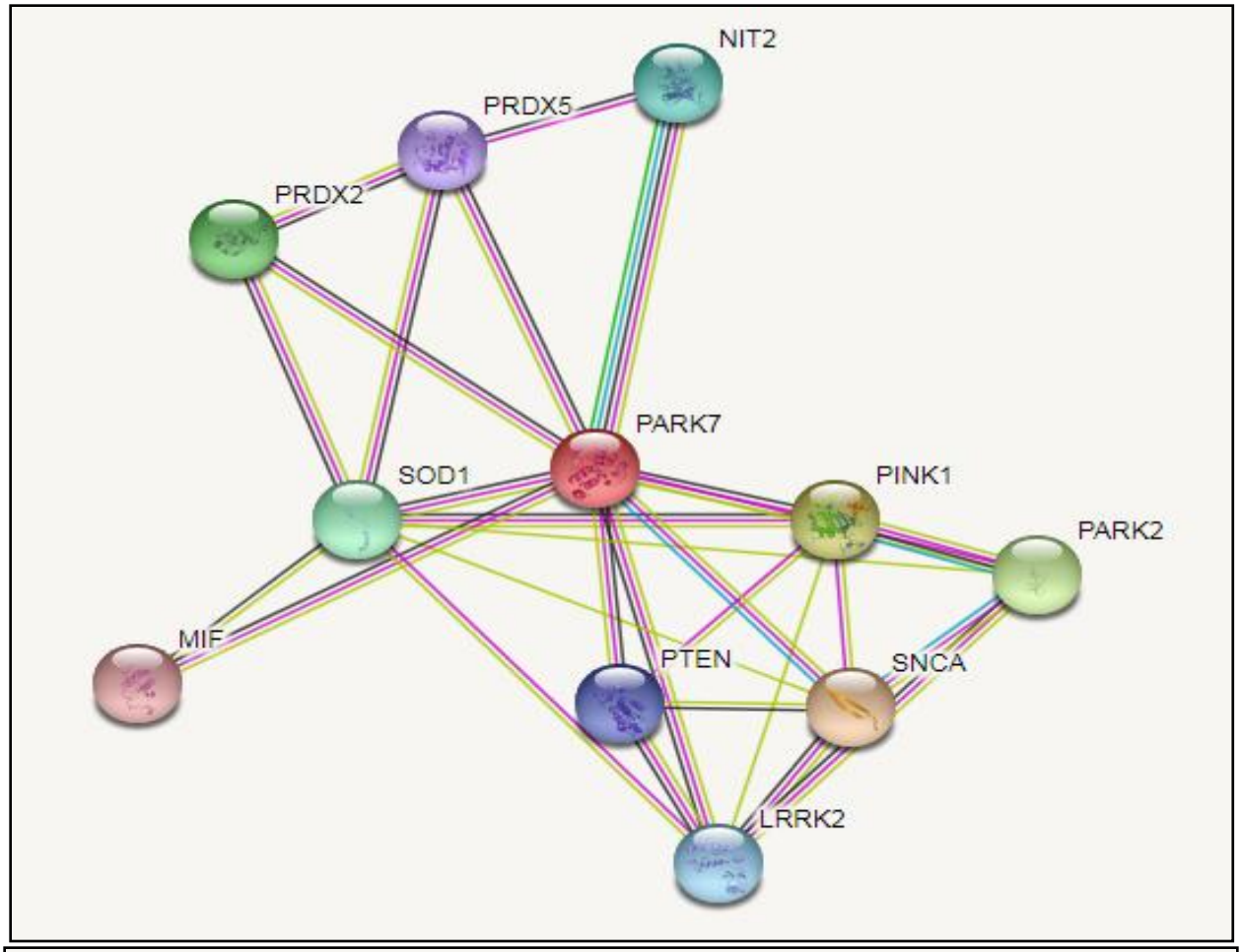
Figure 2: Gene Architecture and Transcription Factors of Park7 Gene associated with Parkinson's disease

TFBE	BINDING POSITION	STRAND	SEQUENCE	ROLE
Sp1	chr1: 8011747-8011756	+	GGGGCAGGGA	Sp1 is up-regulated by oxidative stress, have downstream effects on NMDA receptor subunit expression that leads to neuronal degeneration.
p53	chr1: 8006850-8006868	+	CACCATGCCCGGCCCTGTA	DJ-1 is a target of p53 and it plays a role in the p53-regulated AKT pathway and p53-driven oxidative-stress response.
NFkappaB	chr1: 8004856-8004868	+	GAGGGAATCTCTT	NF-KappaB pathway plays a key role in the activation and regulation of inflammatory mediator production during inflammation.
NFkappaB1	chr1: 8011718-8011727	+	AGGGATCTCC	Unknown
GATA1	chr1: 8005204-8005213	-	AAGGCTTATC	It directly regulate Parkinson's disease linked gene and induces an increase in alpha-synuclein.
GATA2	chr1:	-	CTCAGAATAG	SNCA expression is

	8007620-8007629			controlled by GATA2 that has a role in CNS development and erythroid cell differentiation.
GATA3	chr1: 8011000-8011009	-	ACGGGAATAG	Unknown
NF-Y	chr1: 8014316-8014332	+	ACTCGGCCAATCCCGGG	Unknown
Pax-4a	chr1: 8022914-8022944	-	AGATGTCATGAGGCGAGCTGGGGTAAGTCCC	It is a well-accepted neurogenic determinant, and its expression may be protective against dopaminergic cell loss in Parkinson's disease.
SEF-1	chr1: 8014489-8014508	-	CCCTCACAGAACTCGGTGTC	Unknown
STAT5A	chr1: 8011460-8011472	-	AAGTTCCAGGAAG	JAK2-STAT5 signalling pathway mediates IL3 induced activation of microglia that is associated to neurodegenerative disorders.
c-Rel	chr1: 8002790-8002800	+	GGGGGGTTACC	Late onset of Parkinsonism is observed in c-Rel

				deficit mice.
c-Ets-1	chr1: 8014386-8014396	-	AGGTTTCCGGC	Unknown
SREBP-1	chr1: 8009447-8009458	-	GAGCACGTAATC	Components of SREBP pathway regulates Parkin mediated microphagy
POU2F1	chr1: 8015839-8015852	+	GTGCATAAAGAGTT	Unknown
CUTL1	chr1: 8015947-8015962	+	ACAGCAGTATTTTTTG	Unknown
CBF(2)	chr1: 8014316-8014332	+	ACTCGGCCAATCCCGGG	Unknown
C/EBP alpha	chr1: 8017409-8017422	-	TTCCTTTGCAAATA	Unknown

Table 1: Transcription Factor Binding Elements in Parkinson's disease.



Your Input:

		Neighborhood	Gene Fusion	Cooccurence	Coexpression	Experiments	Databases	Textmining	[Homology]	Score
PARK7	*Parkinson protein 7; Protein deglycase that repairs methylglyoxal- and glyoxal-glycated amino acids and proteins, and releases repaired proteins and lactate or glycolate, respectively. Deglycates cysteines, arginines and lysines residues in proteins, and thus reactivates these proteins by reversing glycation by glyoxals. Acts on early glycation intermediates (hemithioacetals and aminocarbinols), preventing the formation of advanced glycation endproducts (AGE) (PubMed-25416785). Plays an important role in cell protection against oxidative stress and cell death acting as oxidative stress [...] (189 aa)*									

Predicted Functional Partners:

SNCA	*Synuclein, alpha (non A4 component of amyloid precursor); May be involved in the regulation of dopamine release and transpor...*									0.982
PINK1	*PTEN induced putative kinase 1; Protects against mitochondrial dysfunction during cellular stress by phosphorylating mitochon...*									0.970
PARK2	*Parkinson protein 2, E3 ubiquitin protein ligase (parkin); Functions within a multiprotein E3 ubiquitin ligase complex, catalyzing t...*									0.939
PRDX2	*Peroxiredoxin 2; Involved in redox regulation of the cell. Reduces peroxides with reducing equivalents provided through the thior...*									0.904
SOD1	*Superoxide dismutase 1, soluble; Destroys radicals which are normally produced within the cells and which are toxic to biologic...*									0.884
NIT2	*Nitrilase family, member 2; Has a omega-amidase activity. The role of omega-amidase is to remove potentially toxic intermediat...*									0.858
LRRK2	*Leucine-rich repeat kinase 2; Positively regulates autophagy through a calcium- dependent activation of the CaMKK/AMPK sign...*									0.855
PTEN	*Phosphatase and tensin homolog; Tumor suppressor. Acts as a dual-specificity protein phosphatase, dephosphorylating tyrosin...*									0.845
PRDX5	*Peroxiredoxin 5; Reduces hydrogen peroxide and alkyl hydroperoxides with reducing equivalents provided through the thioredoxi...*									0.842
MIF	*Macrophage migration inhibitory factor (glycosylation-inhibiting factor); Pro-inflammatory cytokine. Involved in the innate immu...*									0.828

Figure 3: Protein-Protein interaction network of PARK7, PARK2, PINK1, SNCA, PTEN, SOD1, PRDX5, PRDX2, NIT2, MIF, LRRK2 using STRING database.

STRING database provides the functional protein association network. It predicts the interaction network by retrieving data from sources such as genomic context prediction, high-throughput lab experiments, co-expression, automated text-mining and previously available knowledge in databases. The PPI network is generated by providing either an input protein name or sequence and selecting the organism of interest. The output is a network of protein-protein interaction with score values that depicts the strength of interaction. The Figure 3 depicts PPI network of PARK-7 that provides various interaction with PARK2, PINK1, SNCA, PTEN, SOD1, PRDX5, PRDX2, NIT2, MIF, LRRK2 proteins that would assist in developing effective therapeutic mode of action (Parkinson`s disease).

On the basis of data retrieved from experiments, databases, text-mining, co-expression; the networks of proteins are allotted with scoring function. Among all the associated proteins, PARK7-SNCA was found to have the best score of 0.982, depicting the highest interaction. The other network were of PINK1, PARK2, PRDX2, SOD1, NIT2, LRRK2, PTEN, PRDX5, MIF with scores 0.970, 0.939, 0.904, 0.884, 0.858, 0.855, 0.845, 0.842, 0.828 respectively.

Selection of Target Proteins

The screened drugs along with their respective targets proteins, mechanism of action and indications are depicted below in Table 2. Among the available conventional drugs of Alzheimer's Disease (AD) and Senile Dementia (SD) only the common drugs between AD, SD or PD were considered. Therefore, targets of the selected drugs namely, Citicoline, Memantine and Rivastigmine were chosen for further studies.

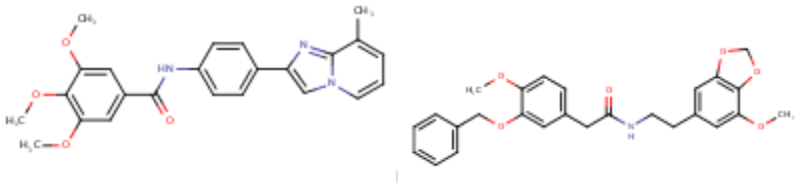
DRUG NAME	INDICATION	TARGET	MECHANISM OF ACTION
Citicoline	Stroke, AD, SD, PD, ADHD, Glaucoma	ACHE, SLC1A2	Membrane permeability enhancer, Glutathione transferase stimulant
Donepezil	AD	ACHE, HTR2A	Acetyl cholinesterase inhibitor
Memantine	AD,SD	CHRFAM7A, DRD2, GRIN1, GRIN2A, GRIN2B, GRIN3A, HTR3A	Glutamate receptor antagonist
Physostigmine	Hypotension, AD, Glaucoma, Gastroparesis	ACHE, BCHE	Cholinesterase inhibitor
Rivastigmine	AD, PD, SD	ACHE, BCHE	Acetyl cholinesterase inhibitor
Tacrine	AD	ACHW, BCHE	Acetyl cholinesterase inhibitor
Bifemelane	SD, Glaucoma	MAOA, MAOB	Acetylcholine release enhancer

Galantamine	SD	ACHE, BCHE, CHRNA1,CHRNA10, CHRNA2, CHRNA3, CHRNA4, CHRNA5, CHRNA6, CHRNA7, CHRNA9, CHRNB1, CHRNB2, CHRNB3, CHRNB4, CHRND, CHRNE, CHRNG	Acetyl cholinesterase inhibitor
L-Arginine	Congestive heart failure, Hypertension, Coronary artery disease, Claudication, SD, Erectile dysfunction, Infertility, Common	ARG2, ASL, ASS1, AZIN2, GPRC6A, NOS2, NOS3, SLC7A1, SLC7A3, SLC7A4	Nitric oxide precursor
Piracetam	SD	GRIA1, GRIA2, GRIA3, GRIA4	Acetylcholine receptor agonist

Table 2: Common drugs of Alzheimer's disease (AD), Senile dementia (SD) and their respective target molecules.

Analysis of SwissADME Results

Below are the chemical structures of the two compounds and in Table 3 is the summarized result of SwissADME that assess the ligand based on physiochemical properties, lipohilicity, water solubility, pharmacokinetics (ADMET), drug likeliness.



[A] **[B]**

Figure 4: Chemical Structure of Compound-23 and Compound-B

(A)Compound-23:3,4,5-trimethoxy-N-[4-(8-methylimidazo[1,2-a]pyridin-2-yl)phenyl]benzamide;
(B)Compound-B:2-(3-benzyloxy-4-methoxy-phenyl)-N-[2-(7-methoxy-1,3-benzodioxol-5-yl)ethyl]acetamide

Parameters		Compound 23	Compound B
Physiochemical Properties	**Mol Wt. (g/mol)**	417.46	449.50
	Mol Formula	C24H23N3O4	C26H27NO6
Lipophilicity	**XLogP3**	4.54	4.31
Water Solubility	**LogS (ESOL)**	-5.33	-5.020
Pharmacokinetics	**BBB Permeant**	Yes	Yes
Drug Likeliness	**Lipinski Filter**	Yes	Yes
	Ghose Filter	Yes	Yes

Table 3: Physiochemical Properties, Drug Likeliness and ADMET analysis

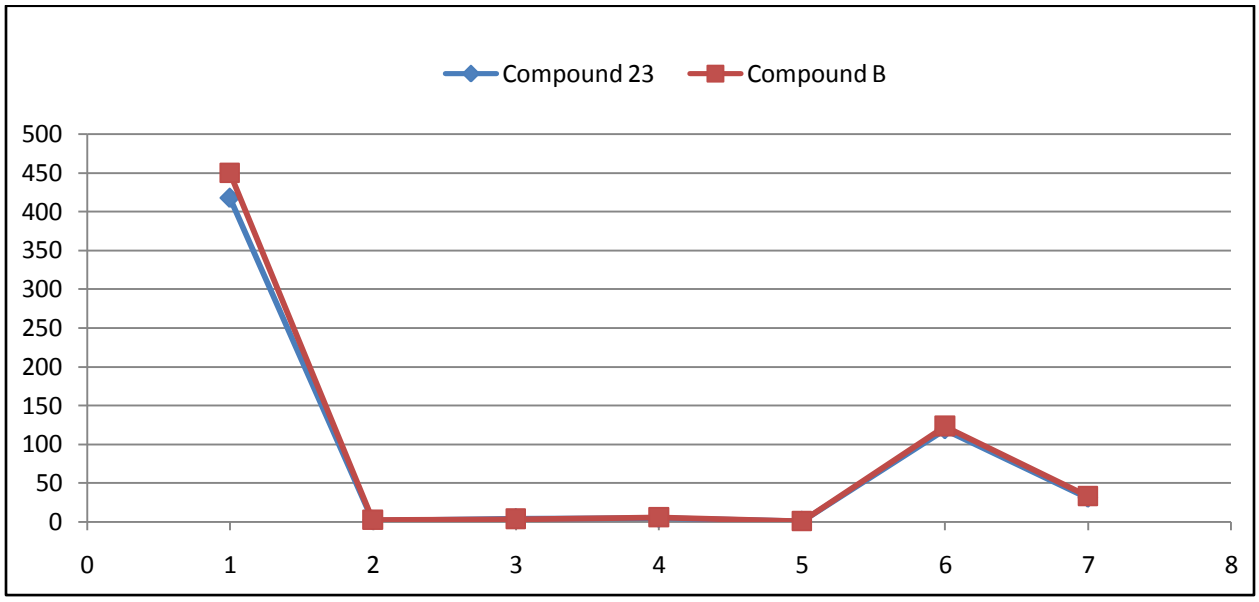
Ligand drugability profiling based on Lipinski Rule of Five and Ghose filter

The drugability of both the ligands were tested on the basis of Lipinski filter and Ghose filter. The qualifying parameters of the filters applied are as follows:

(1) Lipinski filter – Mol Wt. <=500 Dalton, MLogP<=4.15, H-bond acceptor <=10, H-bond donor<=5, Molar Refractivity 40-130

(2) Ghose Filter – Mol Wt. 160-480, WLogP -0.4-5.6, Molar Refractivity 40-130, Number of Atoms 20-70.

Both the ligands, Compound 23 and Compound B pass the drug likeliness parameters.



Molecules	Molecular Weight	MLogP	WLogP	H-bond acceptors	H-bond donors	Molar Refractivity	Number of Atoms
Compound 23	417.46	2.13	4.4	5	1	119.28	31
Compound B	449.5	2.42	3.76	6	1	123.34	33

Figure 5: Drug likeliness prediction using Lipinski and Ghose filter

Molecular Docking

Results of molecular docking using AutoDock are listed in Table 4. The binding affinity of ligand molecules comprising of Compound 23, Compound B and Conventional drugs (control) with respective shortlisted target proteins are depicted.

Ligand	Binding Affinity (kcal/mol)					
	ACHE	BCHE	SLC1A2	DRD2	GRIN1/ GRIN2A	GRIN2B
Compound 23	-6.9	-8.2	-7.3	-6.5	-6.9	-6.6
Compound B	-6.9	-7.2	-7.4	-7.5	-7.0	-7.2
Rivastigmine	-8.0	-6.7	--	--	--	--
Citicoline	-8.4	--	-7.2	--	--	--
Memantine	--	--	--	-5.3	-7.5	-6.0

Table 4: Comparative Molecular docking simulation results of Compound 23 and Compound B with the conventional drugs (as control) against the selected target proteins.

Rivastigmine on docking with its target proteins ACHE and BCHE, had a binding affinity of -8.0 and -6.7 kcal/mol, respectively. Citicoline docked to ACHE and SLC1A2 had -8.4 and -7.2 kcal/mol respectively; as their binding affinity. Further, Memantine generated a binding affinity of -5.3 kcal/mol to DRD2, -7.5 kcal/mol to GRIN1, and -6.0 to GRIN2B protein molecules. Conversely, Compound 23 and Compound B were docked to all the target proteins and the generated results were close to the docked results of the conventional drugs, suggesting it to have potential to be used as therapeutic agent. The docking study of all the target proteins to the ligand compounds with their interacting residues are depicted in the following figures (6-17).

Molecular Docking of the target proteins with Compound 23

Compound 23 and ACHE:

The binding affinity of Compound 23 and ACHE (PDB: 4EY7) was -6.9 kcal/mol. Compound 23 formed hydrogen bonds with GLN527 of segment 159 and also with HIS381 of segment 166 of chain B. The hydrogen bond formation between the protein ACHE and ligand molecule Compound 23 is shown in figure 6.

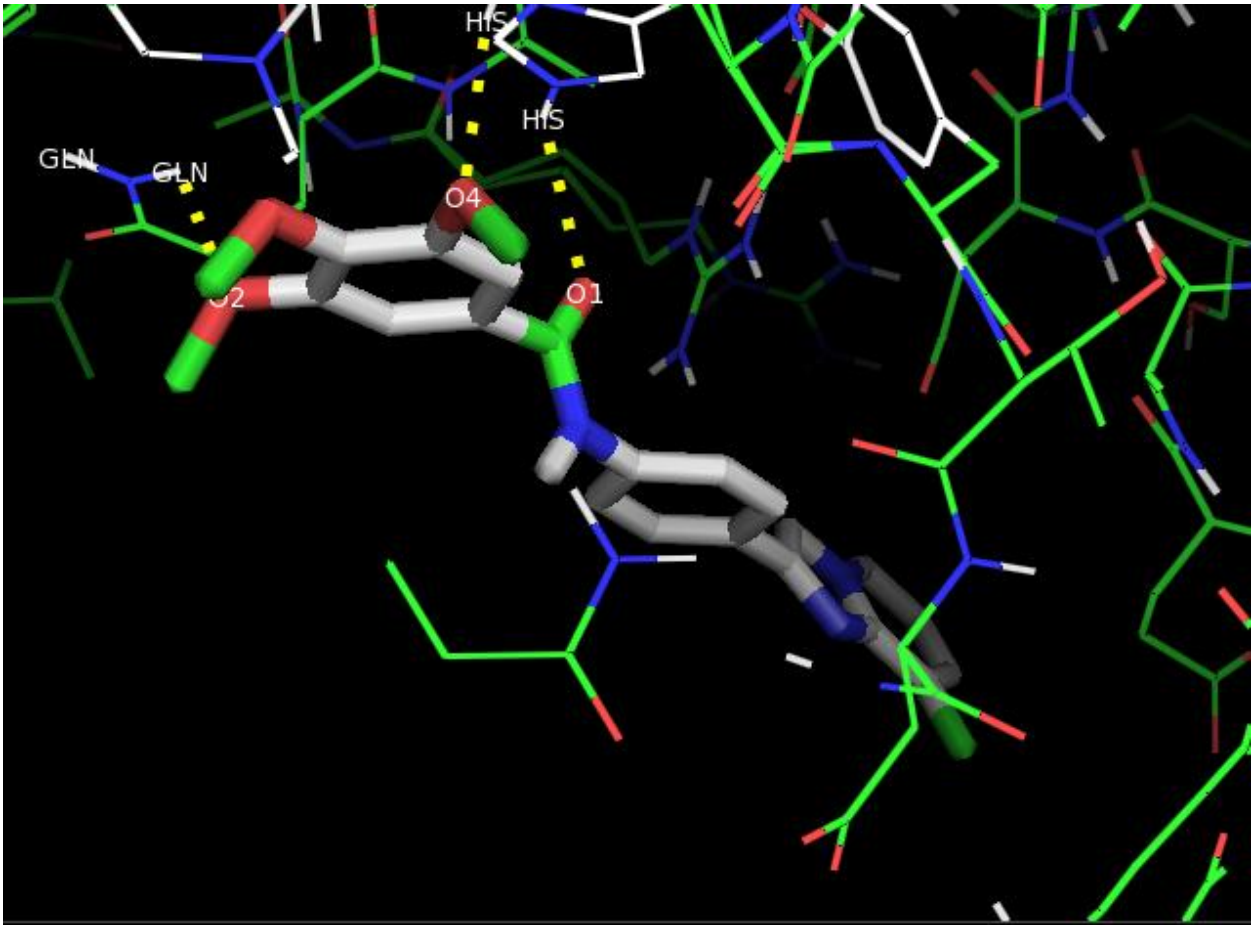


Figure 6: Docking of Compound 23 to ACHE (PDB: 4EY7)

Compound 23 and BCHE:

The binding affinity of Compound 23 and BCHE (PDB: 5DYY) was -8.2 kcal/mol. Compound 23 formed hydrogen bonds with ASN68 of segment 274 of chain B. The hydrogen bond formation between the protein BCHE and ligand molecule Compound 23 is shown in figure 7.

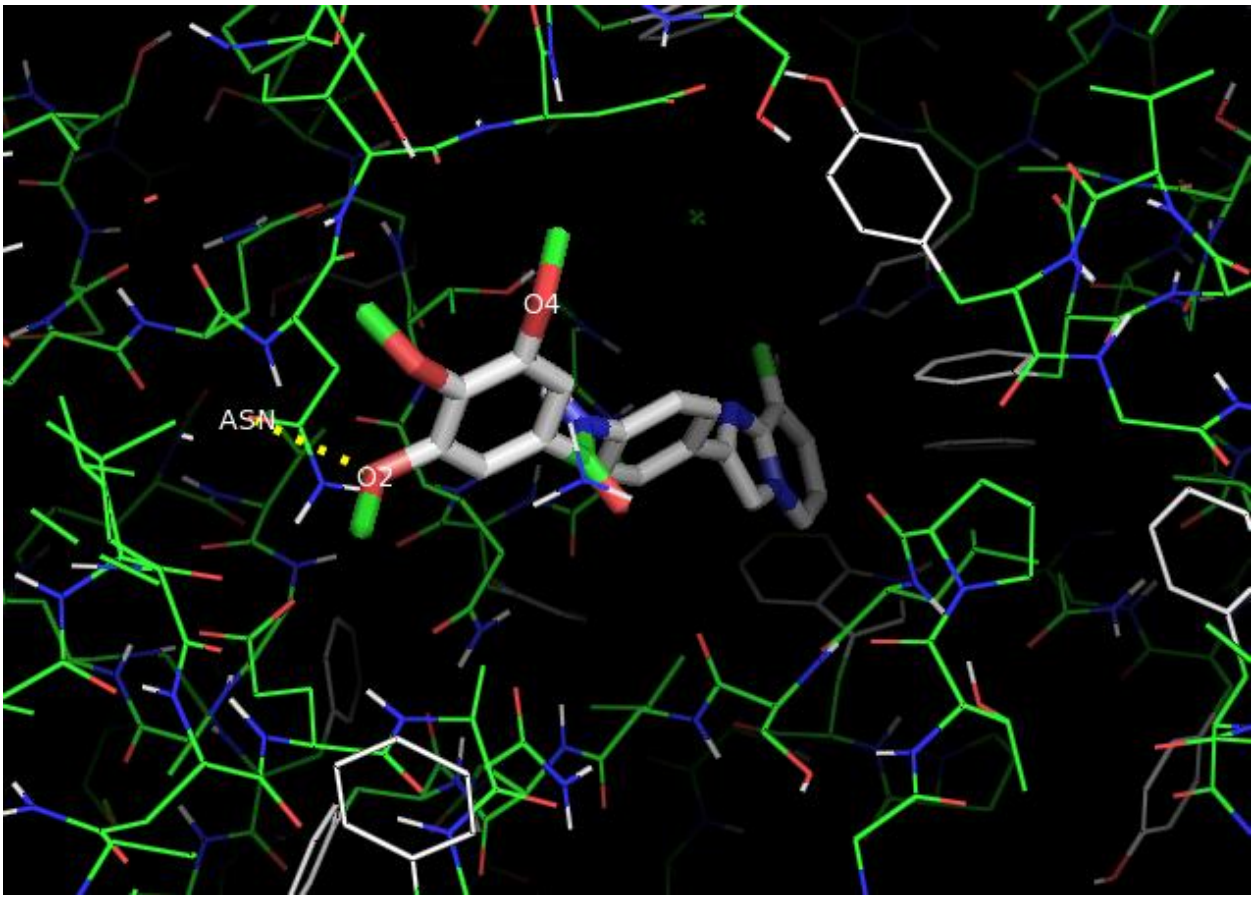


Figure 7: Docking of Compound 23 to BCHE (PDB: 5DYY)

Compound 23 and SLC1A2:

The binding affinity of Compound 23 and SLC1A2 (PDB: 5LM4) was -7.3 kcal/mol. Compound 23 formed hydrogen bonds with SER195 of segment 209 and with ASN182 of segment 159 of chain A. The hydrogen bond formation between the protein SLC1A2 and ligand molecule Compound 23 is shown in figure 8.

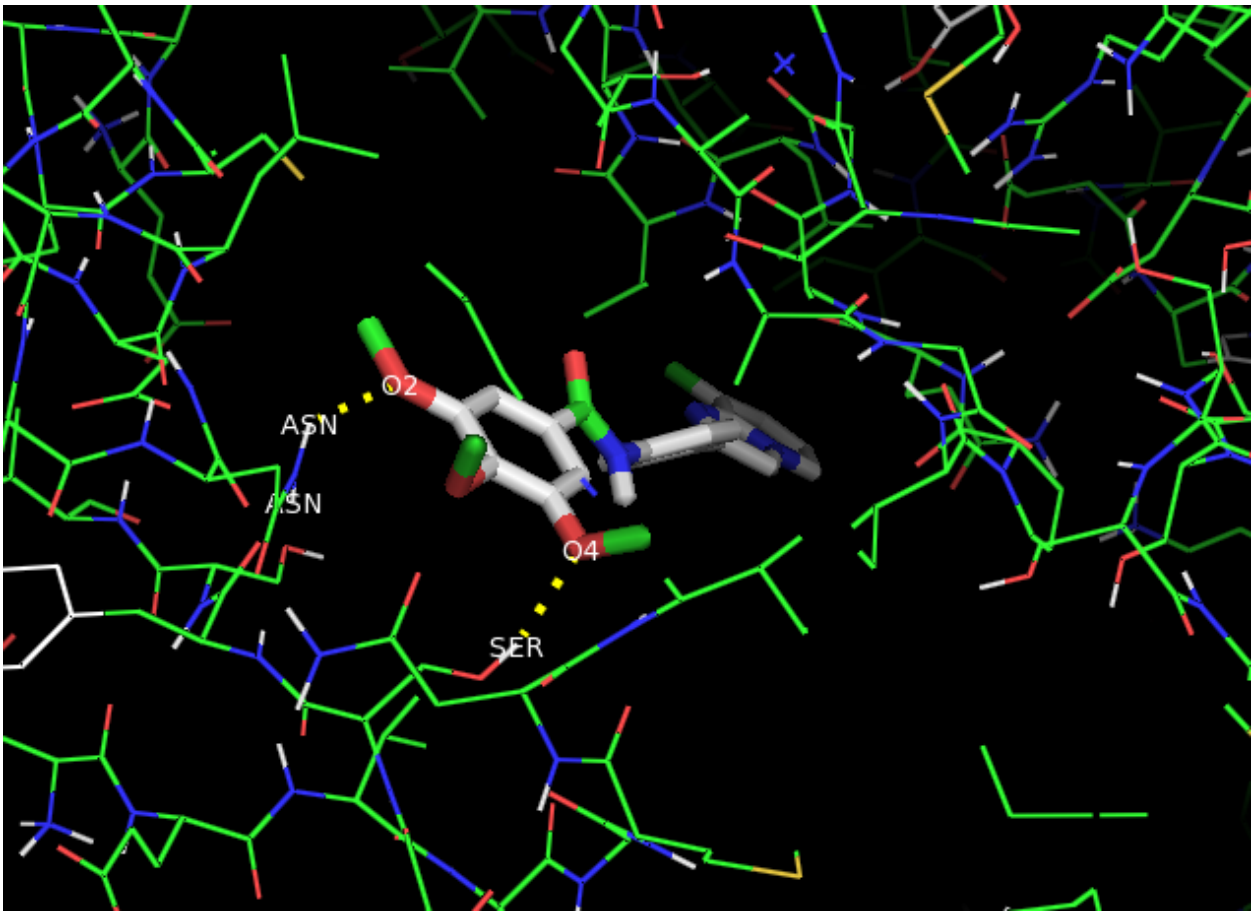


Figure 8: Docking of Compound 23 to SLC1A2 (PDB: 5LM4)

Compound 23 and DRD2:

The binding affinity of Compound 23 and DRD2 (PDB: 5AER)was -6.5 kcal/mol. Compound 23 formed hydrogen bonds with ASN143 and also with GLY133 of segment 271 of Chain A. The hydrogen bond formation between the protein DRD2 and ligand molecule Compound 23 is shown in figure 9.

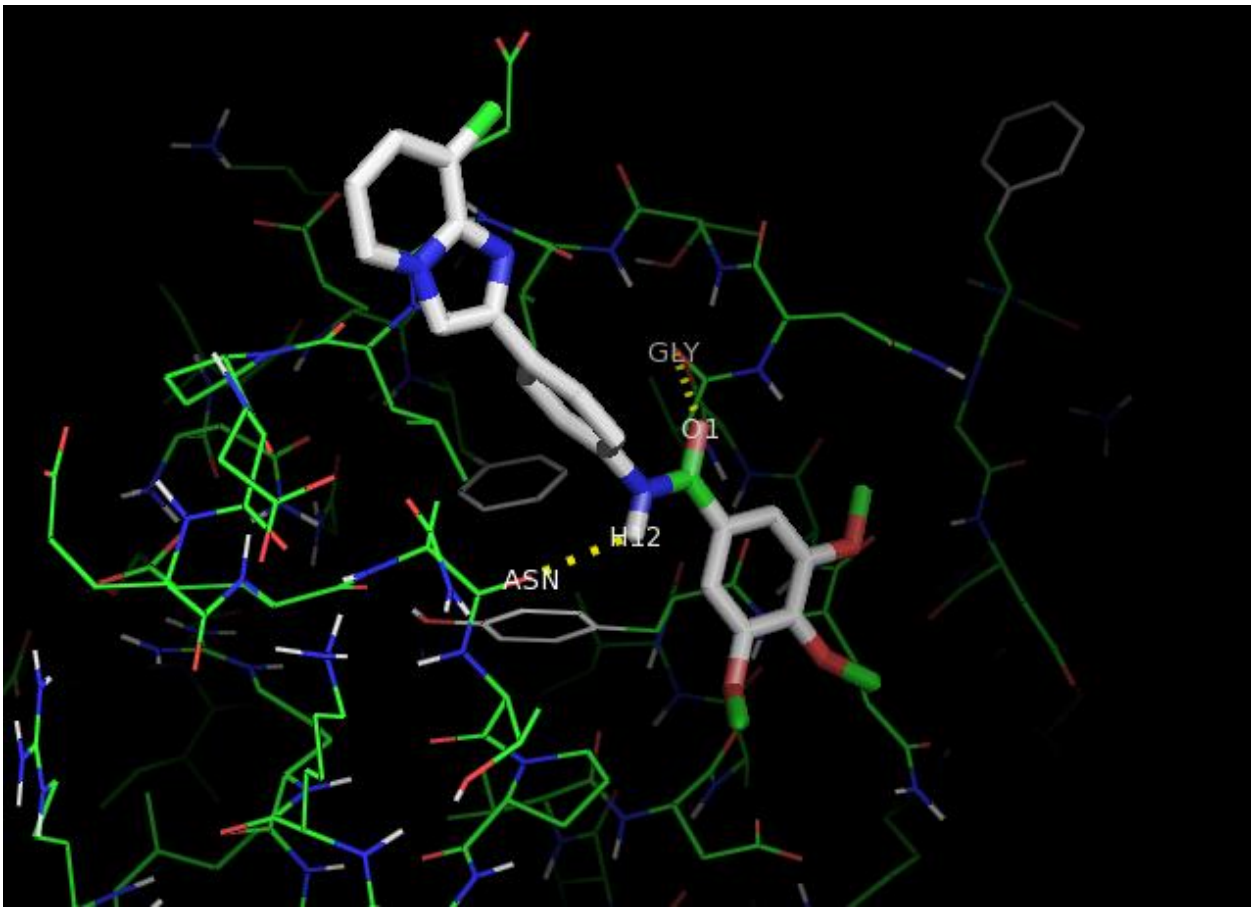


Figure 9: Docking of Compound 23 to DRD2 (PDB: 5AER)

Compound 23 and GRIN1:

The binding affinity of Compound 23 and GRIN1 (PDB: 5TP9) was -6.9 kcal/mol. Compound 23 formed hydrogen bonds with GLN62 of segment 274 of chain B. The hydrogen bond formation between the protein GRIN1 and ligand molecule Compound 23 is shown in figure 10.

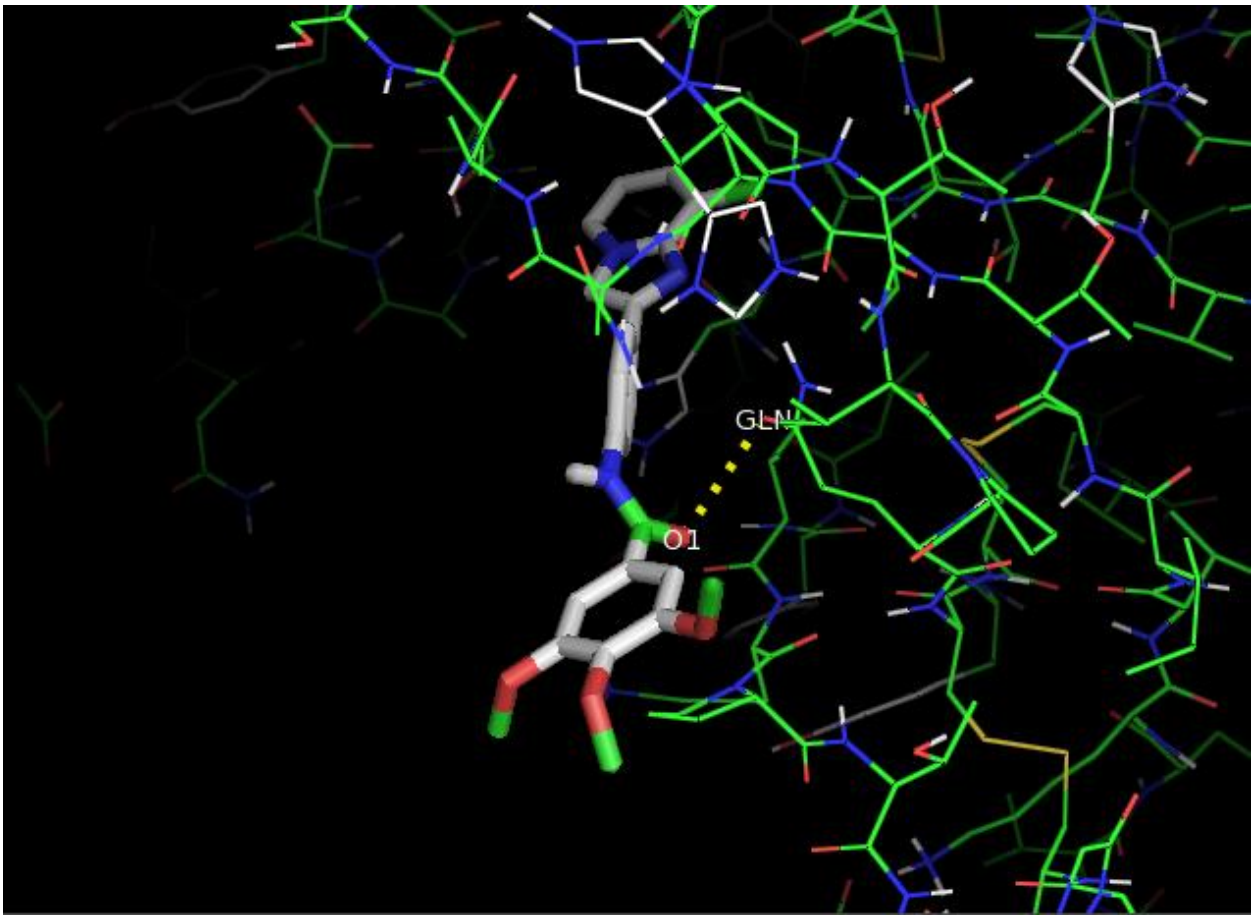


Figure 10: Docking of Compound 23 to GRIN1 (PDB: 5TP9)

Compound 23 and GRIN2B:

The binding affinity of Compound 23 and GRIN2B (PDB: 5EWJ) was -6.6 kcal/mol. Compound 23 formed hydrogen bonds with SER140 of segment 209, ARG328 of segment 174 and also with ARG328 of segment 271 of chain B. The hydrogen bond formation between the protein GRIN2B and ligand molecule Compound 23 is shown in figure 11.

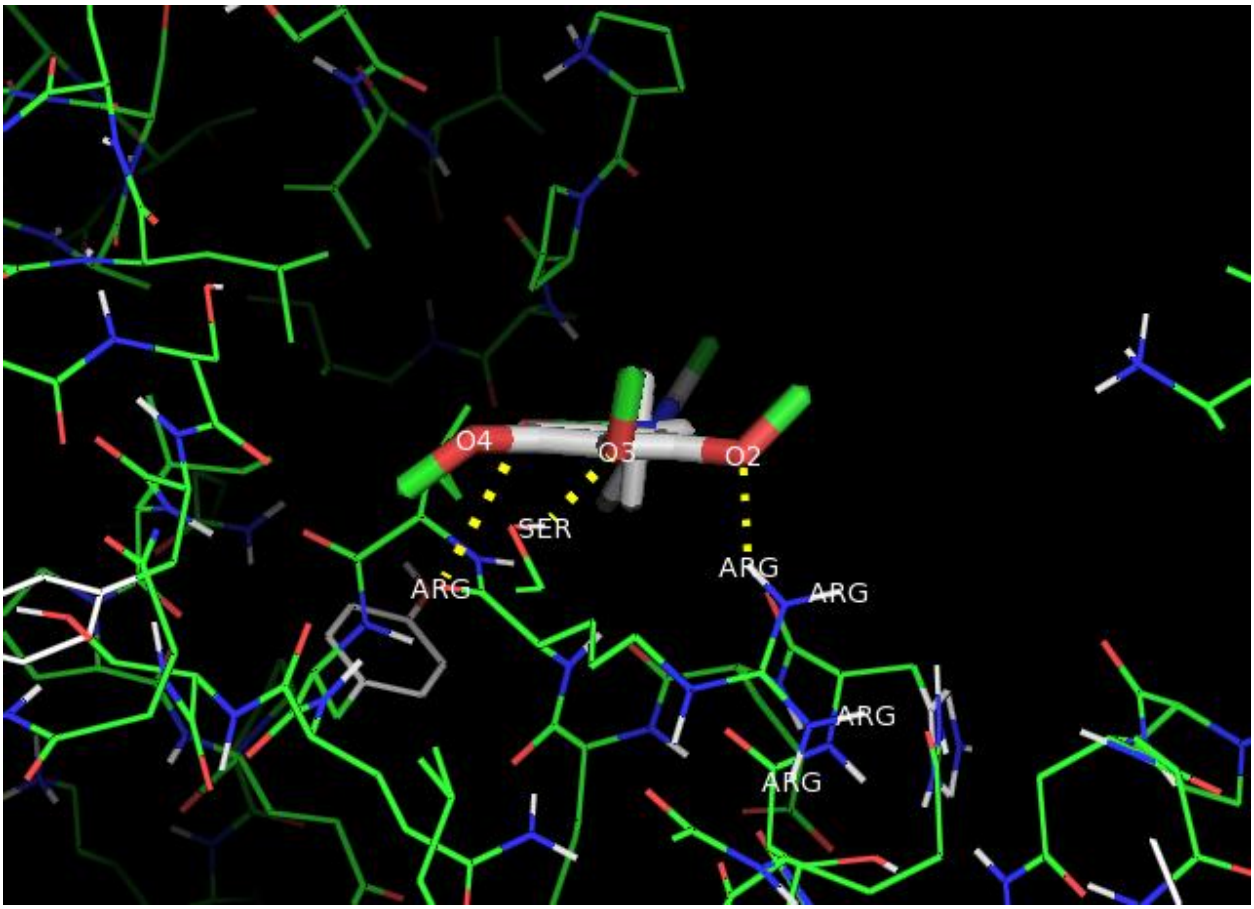


Figure 11: Docking of Compound 23 to GRIN2B (PDB: 5EWJ)

Molecular Docking of the target proteins with Compound B

Compound B and ACHE:

The binding affinity of Compound B and ACHE (PDB: 4EY7) was -6.9 kcal/mol. Compound B formed hydrogen bonds with SER293 of segment 271 of chain B. The hydrogen bond formation between the protein ACHE and ligand molecule Compound B is shown in figure 12.

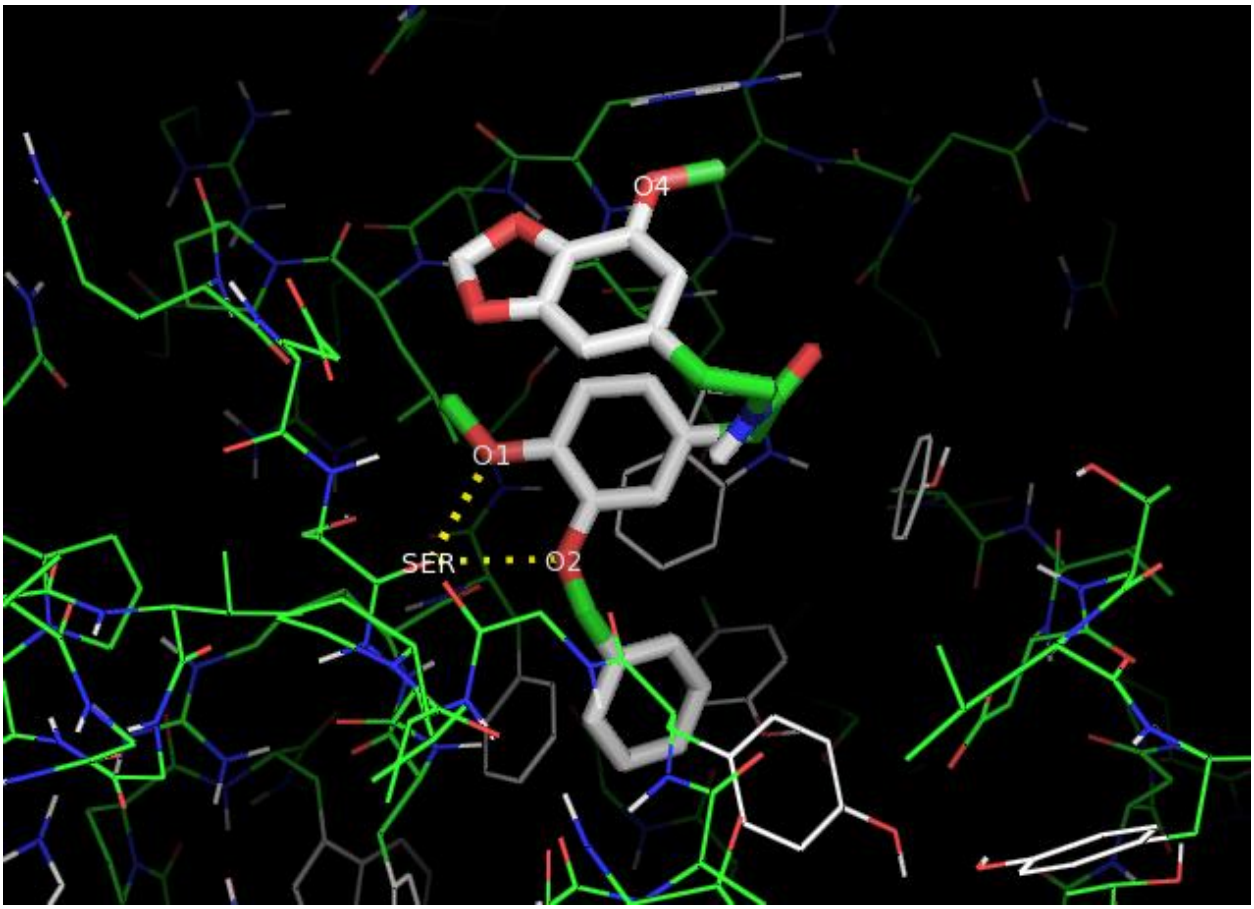


Figure 12: Docking of Compound B to ACHE (PDB: 4EY7)

Compound B and BCHE:

The binding affinity of Compound B and BCHE (PDB: 5DYY) was -7.2 kcal/mol. Compound B formed hydrogen bonds with VAL288 of segment 163, VAL288, TRP231, VAL233 of segment 271 and also with GLU238 of segment 648 of chain B. The hydrogen bond formation between the protein BCHE and ligand molecule Compound B is shown in figure 13.

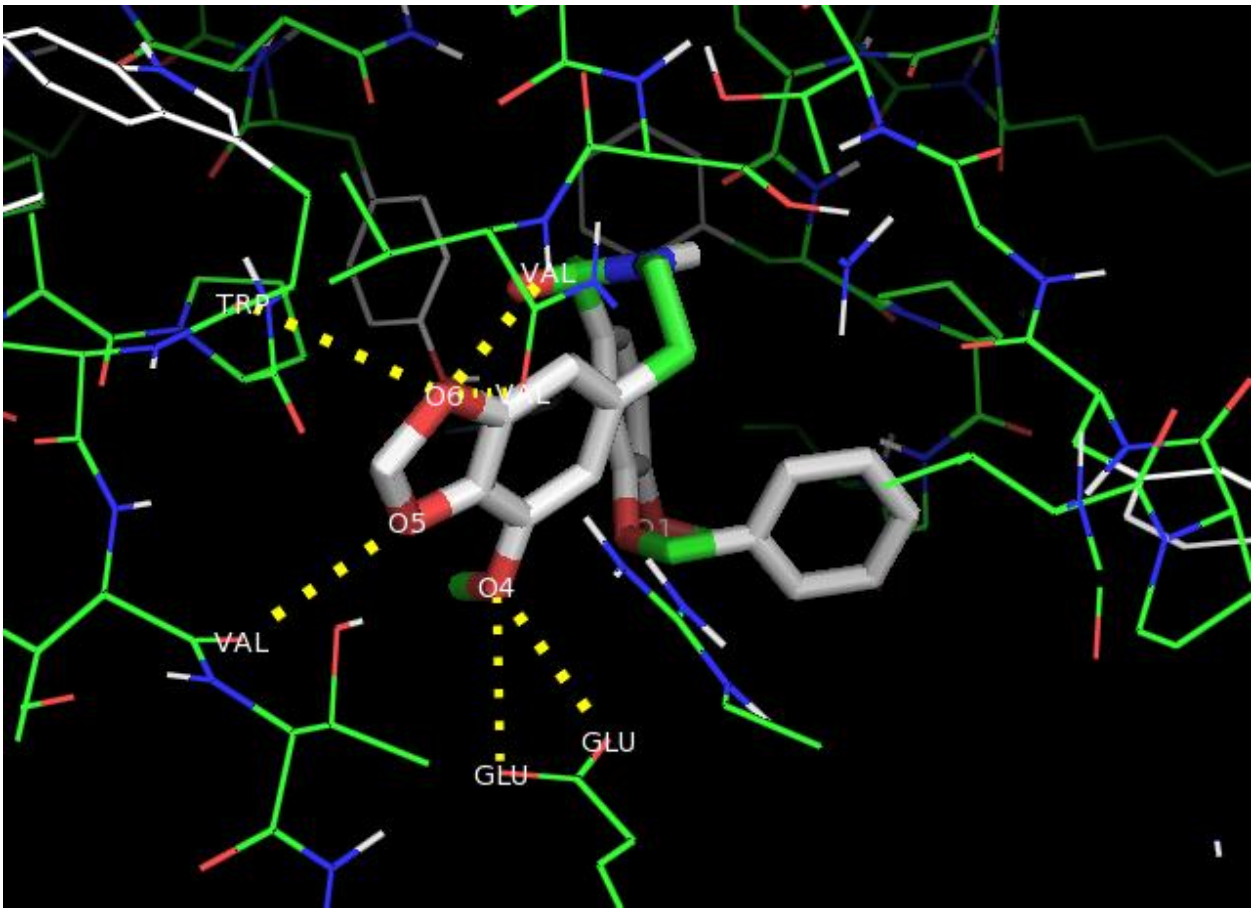


Figure 13: Docking of Compound B to BCHE (PDB: 5DYY)

Compound B and SLC1A2:

The binding affinity of Compound B and SLC1A2 (PDB: 5LM4) was -7.4 kcal/mol. Compound B formed hydrogen bonds with MET221 of segment 271 and also with SER195 of segment 398 of chain A. The hydrogen bond formation between the protein SLC1A2 and ligand molecule Compound B is shown in figure 14.

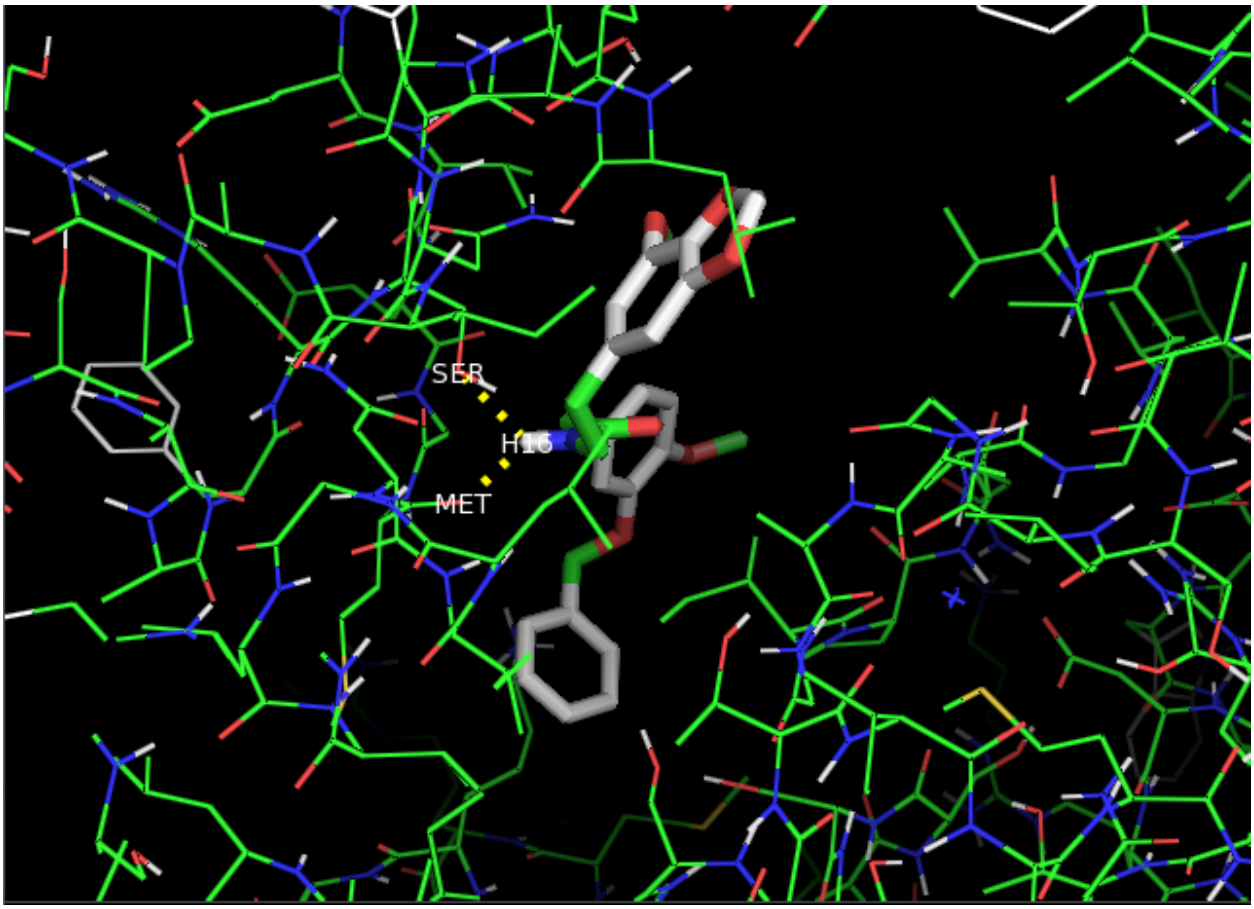


Figure 14: Docking of Compound B to SLC1A2 (PDB: 5LM4)

Compound B and DRD2:

The binding affinity of Compound B and DRD2 (PDB: 5AER) was -7.5 kcal/mol. Compound B formed hydrogen bonds with TYR31 of segment 271 and also with SER83 of segment 398 of chain A. The hydrogen bond formation between the protein DRD2 and ligand molecule Compound B is shown in figure 15.

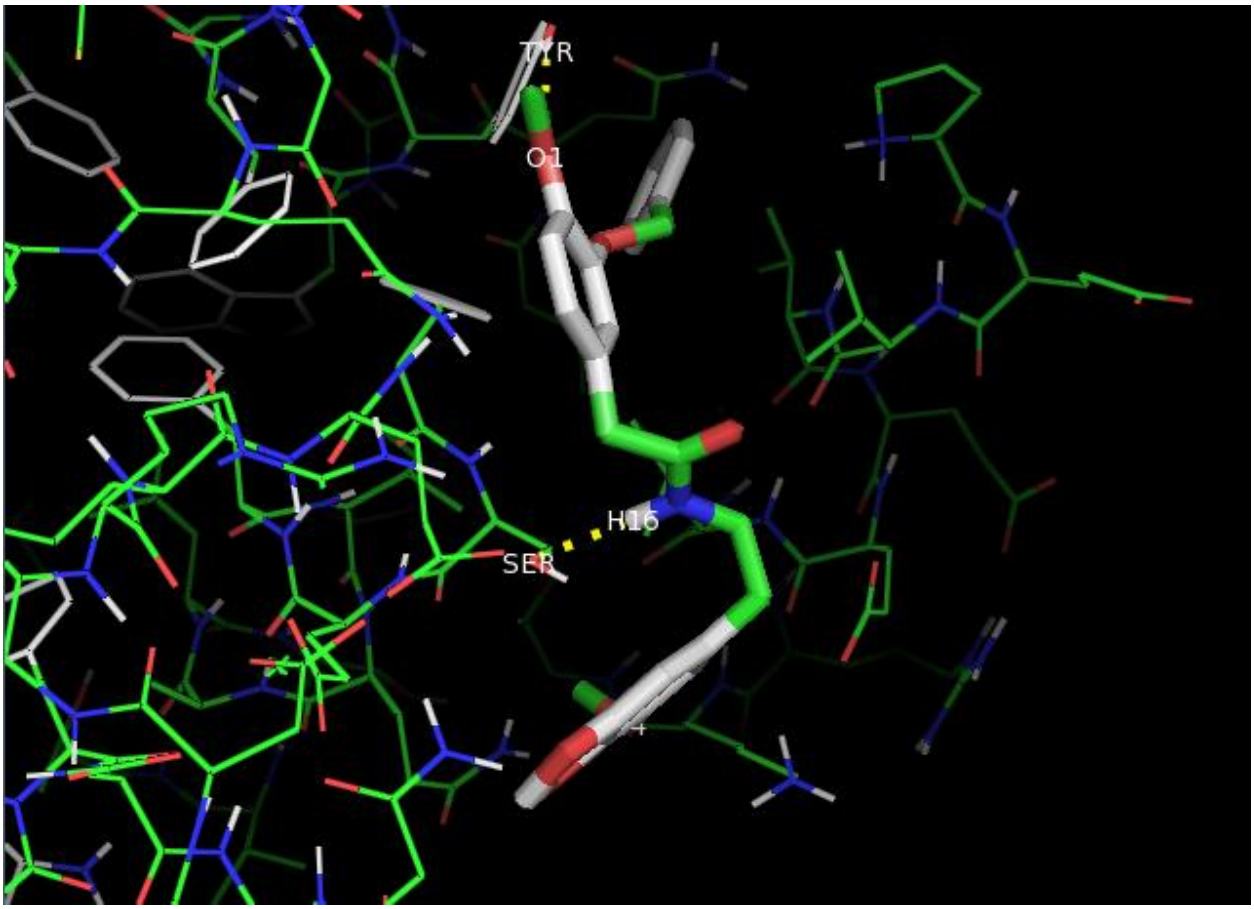


Figure 15: Docking of Compound B to DRD2 (PDB: 5AER)

Compound B and GRIN1:

The binding affinity of Compound B and GRIN1 (PDB: 5TP9) was -7.0 kcal/mol. Compound B formed hydrogen bonds with ASP90 of segment 648 and also with ASN108 of segment 159 of chain B. The hydrogen bond formation between the protein GRIN1 and ligand molecule Compound B is shown in figure 16.

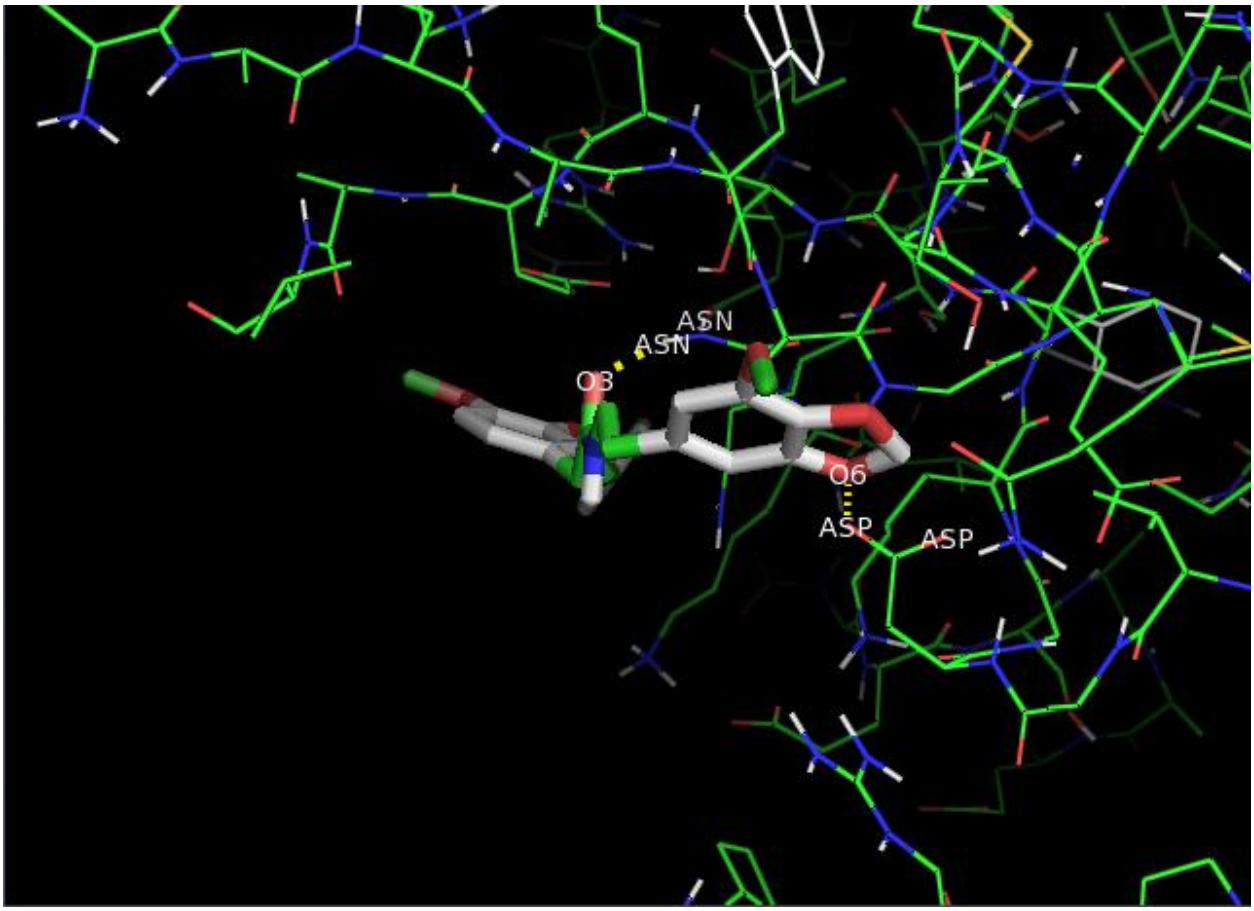


Figure 16: Docking of Compound B to GRIN1 (PDB: 5TP9)

Compound B and GRIN2B:

The binding affinity of Compound B and GRIN2B (PDB: 5EWJ) was -7.2 kcal/mol. Compound B formed hydrogen bonds with TYR282 of segment 361, HIS359 of segment 271 and ASP348 of segment 275 of chain D. The hydrogen bond formation between the protein GRIN2B and ligand molecule Compound B is shown in figure 17.

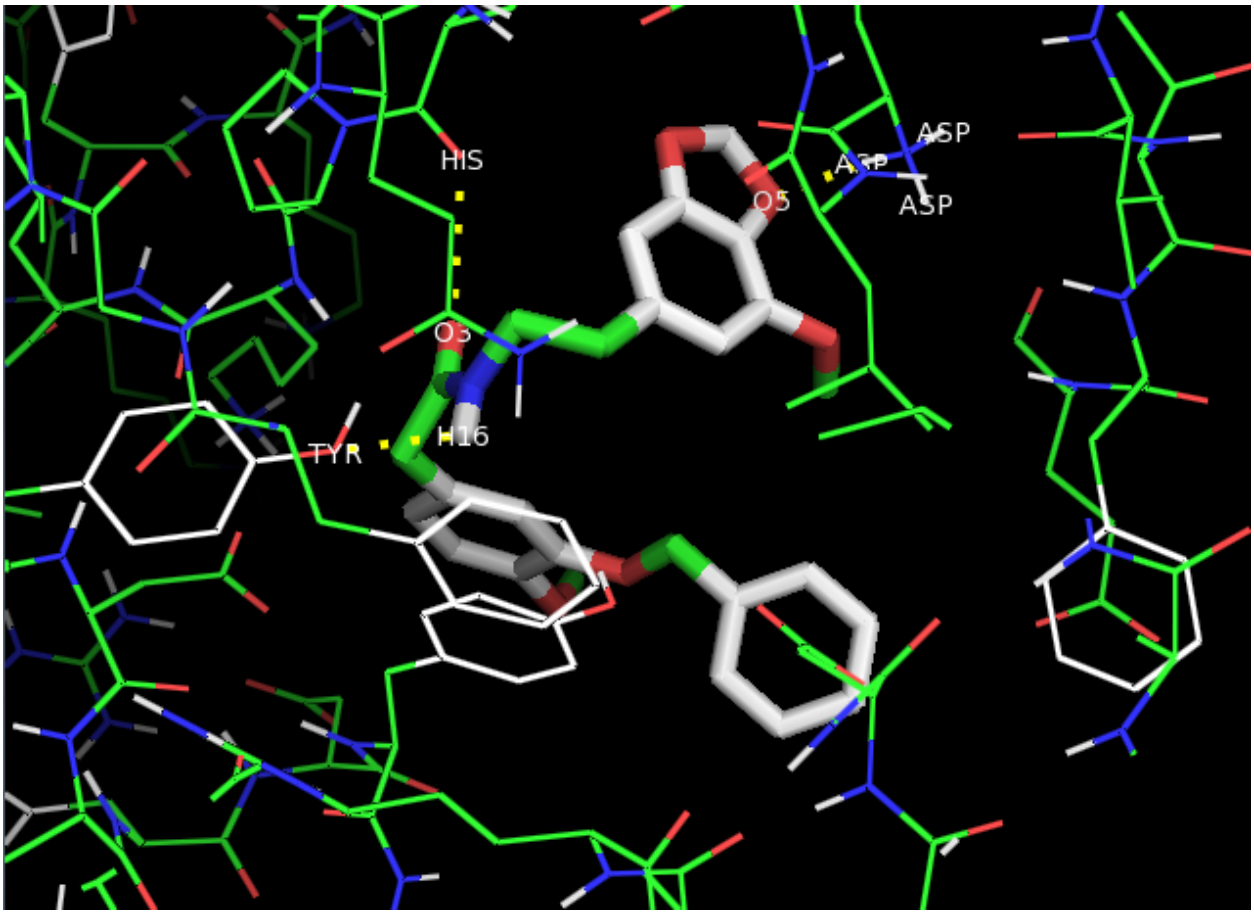


Figure 17: Docking of Compound B to GRIN2B (PDB: 5EWJ)

Target Protein	Compound 23	Compound B
ACHE (PDB:4EY7)	Chain B (segment 159)-GLN^{527}, Chain B(segment 166)-HIS^{381}	Chain B (segment 271)-SER^{293}
BCHE (PDB:5DYY)	Chain B (segment 274)-ASN^{68}	Chain B (segment 163)-VAL^{288}, Chain B (segment 271)-VAL^{288},TRP^{231},VAL^{233}, Chain B (segment 648)-GLU^{238}
SLC1A2 (PDB:5LM4)	Chain A(segment 209)-SER^{195} Chain A(segment 159)-ASN^{182}	Chain A (segment 271)-MET^{221}, Chain A (segment 398)-SER^{195}
DRD2 (PDB:5AER)	Chain A (segment 271)-ASN^{143}, GLY^{133}	Chain A (segment 271)-TYR^{31}, Chain A (segment 398)-SER^{83}
GRIN1 (PDB:5TP9)	Chain B (segment 274)-GLN^{62}	Chain B (segment 648)-ASP^{90}, Chain B (segment 159)-ASN^{108}
GRIN2B (PDB:5EWJ)	Chain B(segment 209)-SER^{140}, Chain B (segment 174)-ARG^{328}, Chain B(segment 271)-ARG^{328}	Chain D(segment 361)-TYR^{282}, Chain D (segment 271)-HIS^{359}, Chain D(segment 275)-ASP^{348}

Table 5: Interacting residues of the protein molecules with Compound 23 and Compound B

In the above table 5, as a result of the molecular docking studies, the interacting residues of the protein-ligand molecules are presented. In the first column, the target proteins along with their PDB

ID are shown. The second and third column comprises of all the interacting residues of proteins when docked to Compound 23 and Compound B respectively. The interacting residues are presented in the format of the type of chain with the segment number depicted within braces and then the residue name followed by the residue position identifier, for example Chain B (segment 159)-GLN^{527} Chain B(segment 166)-HIS^{381} shown as a result of protein ACHE docked to Compound 23. Therefore, Compound 23 interacts with residue GLN527 of segment 159 and residue HIS381 of segment 166 of chain B of protein ACHE.

6. DISCUSSION

The association of DJ1 with several neurodegenerative disorders has led to the analysis of its therapeutic potency. DJ1 has multiple functions, it works as a stress sensor and its expression elevates upon various stresses, including oxidative stress. Loss of function and reduced function of DJ-1 trigger the onset of oxidative stress-related diseases, including Parkinson's disease, stroke, familial amyloidotic polyneuropathy, chronic obstructive pulmonary disease (COPD), and type II diabetes. Therefore, the gene architecture of PARK7 along with various TFBEs were identified and analyzed that suggests TFBEs can be taken as therapeutic targets against NDDs. Furthermore, the association of oxidized DJ1 to AD and PD; and other common factors linking the diseases has led to our research to consider repurposing therapeutic agents of PD. The *in silico* analysis of the two compounds; Compound-23 and Compound-B against the common targets of Alzheimer's disease and Senile Dementia depicts that they have the potential to be used as neuroprotective drugable compounds. Both the ligands cleared the drugability test and ADME analysis. The molecular docking results illustrates that the two compounds have binding affinity similar to the conventional drugs used. The best molecular docking results of Compound 23 are against BCHE, DRD2, and Grin2B with binding affinity -8.2,-6.5,-6.6 (kcal/mol) respectively. Moreover, Compound B binding affinity against BCHE, DRD2, and GRIN2B were -7.2, -7.5, and 7.2 (kcal/mol) respectively. However, in the study the most effective binding affinity of both the compounds were showed against BCHE, suggesting that the compounds can be considered to work as an Acetyl cholinesterase inhibitor.

7. CONCLUSION

Over the past years there has been significant advancement in identifying new therapeutic agents to have neuroprotective properties and can modulate the proteins involved in neurodegenerative diseases. Moreover, identifying novel therapeutic properties of pre-existing drugs or compounds are also gaining recognition. Hence, the study has evaluated the potential of these compounds to be used as therapeutic agents against Alzheimer's disease and Senile Dementia and may have the ability to modulate other neurodegenerative proteins associated with other neurodegenerative disorders.

8. REFERENCES

1. World Health Organization Report on Neurological Disorders (http://www.who.int/mental_health/neurology/neurological_disorders_report_web.pdf)
2. Szeto. Y.Y. Jennifer (2016) Current Treatment Options for Alzheimer's Disease and Parkinson's Disease Dementia. *Current Neuropharmacology*, 14: 326-338.
3. Y. Kitamura (2011) Neuroprotective effect of a new DJ-1-binding compound against neurodegeneration in Parkinson's disease and stroke model rats. *Molecular Neurodegeneration*, 6: 48.
4. Kazuko Takahashi-Niki (2015) DJ-1-dependent protective activity of DJ-1-binding compound no. 23 against neuronal cell death in MPTP-treated mouse model of Parkinson's disease. *Journal of pharmacological Sciences*, 127: 305-310.
5. Wolfgang H. Oertel (2017) Recent advances in treating Parkinson's disease. *F1000Research*, 6, 260.
6. Masanori Hijioka (2017) DJ-1/PARK7:A New Therapeutic Target for Neurodegenerative Disorders. *Biol.Pharm.Bull*, 40: 548-552.
7. Lev N. (2007) Role of DJ-1 in Parkinson's disease. *Journal of Molecular Neuroscience*.
8. Hiroyoshi Ariga (2013) Neuroprotective Function of DJ-1 in Parkinson's Disease. *Oxidative Medicine and Cellular Longevity*.
9. Choi J., Bostwick D.E. (2006) Oxidative damage of DJ-1 is linked to sporadic Parkinson and Alzheimer diseases. *Journal of BiolChem*, 281: 10816-10824.
10. Oprea T.I. (2012) Drug Repurposing: Far Beyond New Targets for Old Drugs. *The AAPS Journal*, 14 (4).

11. WaniYousufWillayat, Michaël Boyer-GuittautMichaël, Dodson Matthew , Chatham John, Usmar Victor Darley and Zhang Jianhua (2015) Regulation of autophagy by protein post-translational modification. *Laboratory Investigation*.

12. Brik Ashraf (2010) Post-translational Modification in PD. *Laboratory of Molecular and Chemical Biology of Neurodegenerartion.*

13. Oueslati A, Fournier M, and Lashuel HA. (2010) Role of post-translational modifications in modulating alpha-synuclein structure, aggregation and toxicity: implications for Parkinson's disease pathogenesis and therapies. *Prog Brain Res.*, 183: 115-145.

14. Takahiro Taira, Yoshiro Saito, Takeshi Niki, Sanae M M IguchiAriga, Kazuhiko Takahashi, Hiroyoshi Ariga (2004) DJ1 has a role in antioxidative stress to prevent cell death. *EMBO Reports*, 5: 213-218.

15. Daijiro Yanagisawa (2007) DJ-1 Protects against Neurodegeneration Caused by Focal Cerebral Ischemia and Reperfusion in Rats. *Journal of Cerebral Blood Flow and Metabolism*, 28: 563-578.

16. Masatoshi Inden, Takahiro Taira (2006) PARK7 DJ-1 protects against degeneration of nigral dopaminergic neurons in Parkinson's disease rat model. *Neurobiology of Disease*, 24: 144-158.

17. Martinat C, Shendelman S, Jonason A, Leete T, Beal MF, Yang L, et al. (2004) Sensitivity to Oxidative Stress in DJ-1-Deficient Dopamine Neurons: An ES- Derived Cell Model of Primary Parkinsonism. *PLoS Biol*, 2(11): e327.

18. Joo-Young Im, Kang-Woo Lee (2010) DJ-1 protects against oxidative damage by regulating the thioredoxin/ASK1 complex. *Neuroscience Research*, 67: 203-208.

19. Joo-Young Im, Kang-Woo Lee, Jong-Min Woo, Eunsung Junn, M. Maral Mouradian (2012) DJ-1 induces thioredoxin 1 expression through the Nrf2 pathway. *Hum Mol Genet,* 21 (13): 3013-3024.

20. Jin Xu, Nan Zhong, Haoyong Wang, Joshua E. Elias, Christina Y. Kim, Irina Woldman, Christian Pifl, Steven P. Gygi, Changiz Geula, Bruce A. Yankner (2005) The Parkinson's disease-associated DJ-1 protein is a transcriptional co-activator that protects against neuronal apoptosis. *Hum Mol Genet,* 14 (9): 1231-1241.

21. Jun Fan, Haigang Ren (2008) DJ-1 Decreases Bax Expression through Repressing p53 Transcriptional Activity. *The Journal of Biological Chemistry*, 283: 4022-4030.

22. Niki T, Takahashi-Niki K, Taira T, Iguchi-Ariga SM, Ariga H. (2003) DJBP: a novel DJ-1-binding protein negatively regulates the androgen receptor by recruiting histone deacetylase complex, and DJ-1 antagonizes this inhibition by abrogation of this complex. *Mol. Cancer Res.*, **1**: 247-261.

23. Kazuko Takahashi, Takahiro Taira (2001) DJ-1 Positively Regulates the Androgen Receptor by Impairing the Binding of PIASxα to the Receptor. *The Journal of Biological Chemistry*, 276: 37556-37563.

24. Yamaguchi S, Yamane T, Takahashi-Niki K, Kato I, Niki T, Goldberg MS, et al. (2012) Transcriptional Activation of Low-Density Lipoprotein Receptor Gene by DJ-1 and Effect of DJ-1 on Cholesterol Homeostasis. *PLoS ONE*, 7(5): e38144.

25. Shizuma Ishikawa (2010) Human DJ-1-specific Transcriptional Activation of Tyrosine Hydroxylase Gene. *The Journal of Biological Chemistry*, 285: 39718-39731.

26. Shendelman S, Jonason A, Martinat C, Leete T, Abeliovich A. (2004) DJ-1 Is a Redox-Dependent Molecular Chaperone That Inhibits α-Synuclein Aggregate Formation. *PLoS Biol*, 2(11): e362.

27. Muhammad U. Sajjad, Edward W. Green, Leonor Miller-Fleming, Sarah Hands, Federico Herrera, Susanna Campesan, Ali Khoshnan, Tiago F. Outeiro, Flaviano Giorgini, Andreas Wyttenbach (2014) DJ-1 modulates aggregation and pathogenesis in models of Huntington's disease. *Hum Mol Genet*, 23 (3): 755-766.

28. He-Jin Lee (2008) Assembly-dependent endocytosis and clearance of extracellular a-synuclein. *The International Journal of Biochemistry & Cell Biology*, 40 (9): 1835-1849.

29. He-Jin Lee (2008) Clearance and deposition of extracellular α-synuclein aggregates in microglia. *Biochemical and Biophysical Research Communications*, 372 (3): 423-428.

30. Shimura H, Hattori N (2000) Familial Parkinson's disease gene product, parkin, is a ubiquitin-protein ligase. *Nature Genetics*, 25: 302-305.

31. Xiong H., Wang D. (2009) Parkin, PINK1 and DJ1 form a ubiquitin E3 ligase complex promoting unfolded protein degradation. *The Journal of Clinical Investigation*; 119: 650-660

32. Janda E., Isidoro C. (2012) Defective Autophagy in Parkinson's Disease: Role of Oxidative Stress. *Molecular Neurobiology*, 46: 639-661.

33. Webb JL., Ravikumar B. (2003) α-Synuclein Is Degraded by Both Autophagy and the Proteasome. *The Journal of Biologivcal Chemistry*, 278: 25009-25013.

34. Cuervo AM. (2004) Impaired Degradation of Mutant α-Synuclein by Chaperone-Mediated Autophagy. *Science*, 305: 1292-1925.

35. Watanabe Y, Tatebe H, Taguchi K, Endo Y, Tokuda T, Mizuno T, et al. (2012) p62/SQSTM1-Dependent Autophagy of Lewy Body-Like α-Synuclein Inclusions. *PLoS ONE,* 7(12): e52868.

36. Kelly Jean Thomas, Melissa K. McCoy, Jeff Blackinton, Alexandra Beilina, Marcel van der Brug, Anna Sandebring, David Miller, Dragan Maric, Angel Cedazo-Minguez, Mark R. Cookson (2011) DJ-1 acts in parallel to the PINK1/parkin pathway to control mitochondrial function and autophagy. *Hum Mol Genet* , 20 (1): 40-50.

37. Krebiehl G, Ruckerbauer S, Burbulla LF, Kieper N, Maurer B, Waak J, et al. (2010) Reduced Basal Autophagy and Impaired Mitochondrial Dynamics Due to Loss of Parkinson's Disease-Associated Protein DJ-1. *PLoS ONE,* 5(2): e9367.

38. Corsello SM, Bittker JA, Liu Z, Gould J, McCarren P, Hirschman JE, Johnston SE, Vrcic A, Wong B, Khan M, Asiedu J, Narayan R, Mader CC, Subramanian A, Golub TR. The Drug Repurposing Hub: a next-generation drug library and information resource.

39. H.M. Berman, J. Westbrook, Z. Feng, G. Gilliland, T.N. Bhat, H. Weissig, I.N. Shindyalov, P.E. Bourne. (2000) The Protein Data Bank Nucleic Acids Research, 28: 235-242.

40. SwissADME: a free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules. Sci. Rep. 2017: 7:42717.

41. CA Lipinski; F Lombardo; BW Dominy; PJ Feeney. (2001) *Adv Drug Deliv Rev*, 46 (1-3): 3-26.

42. AK Ghose (1999) VN Viswanadhan; JJ Wendoloski. *J Com Chem*, 1: 55-68.

43. Sanner Michel F. (1999) Python: A Programming Language for Software Integration and Development. *J. Mol. Graphics Mod.*, 17: 57-61.

44. The PyMOL Molecular Graphics System, Version 1.7.4.5 Schrödinger, LLC.