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ORIGINAL RESEARCH

Drug Repurposing Targeting Mitochondrial Dysfunction in Parkinson's Disease: FBXO7-Focused Approach Through Network Analysis and *In Silico* Molecular Docking

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ABSTRACT

FBXO7 is a promising but underexplored therapeutic target in Parkinson's disease (PD), having role in mitophagy, proteasomal degradation, and synaptic function. This study aims to investigate the therapeutic potential of targeting mitochondrial dysfunction in PD through an FBXO7-centered drug repurposing approach. A protein-protein interaction (PPI) network was constructed using the STRING database, and Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analyses were performed to identify key pathways associated with FBXO7. Additionally, *in silico* molecular docking was conducted using the AutoDock Vina algorithm in SwissDock to evaluate the binding affinities of selected clinically approved drugs to FBXO7 and identify promising candidates for potential repurposing in PD treatment. Docking analysis identified several compounds with high binding affinity to FBXO7, including fluorometholone (-6.367 kcal/mol), bendroflumethiazide (-6.354 kcal/mol), lasofoxifene (-6.173 kcal/mol), penicillin V (-6.102 kcal/mol), hydromorphone (-6.067 kcal/mol), and cefamandole (-6.036 kcal/mol). These drugs are involved in biological pathways related to mitochondrial function, neuroinflammation, and cellular stress responses, highlighting their potential as disease-modifying agents in PD. However, limitations such as the potential for exacerbating disease progression or systemic side effects may restrict their direct repurposing. This study highlights several clinically approved drugs with high binding affinities to FBXO7, suggesting their potential for targeting mitochondrial dysfunction in PD. While some compounds may present challenges for or direct use, their molecular interactions offer valuable insights for developing novel mitochondrial-targeted therapies. Further experimental validation and structural optimization are required to enhance their therapeutic potential and minimize side effects, paving the way for novel therapeutic strategies in PD.

Keywords: FBXO7. Drug repurposing. Mitochondrial dysfunction. Neurodegeneration. Parkinson's disease.

Parkinson Hastalığında Mitokondriyal Disfonksiyonu Hedefleyen İlaç Yeniden Konumlandırma: Network Analizi ve *In Silico* Moleküler Docking Aracılı FBXO7 Odaklı Bir Yaklaşım

ÖZET

FBXO7, mitofaji, proteazomal degradasyon ve sinaptik işlevlerde rol oynayan, Parkinson hastalığı (PH)'nda umut vadeden ancak yeterince araştırılmamış bir terapötik hedeftir. Parkinson hastalığında PH'de mitokondriyal disfonksiyonu hedef alan, FBXO7 merkezli bir ilaç yeniden konumlandırma yaklaşımının terapötik potansiyelini araştırmayı amaçlamaktadır. Bu amaçla, STRING veritabanı kullanılarak bir protein-protein etkileşim (PPI) ağı oluşturulmuş; ardından FBXO7 ile ilişkili temel biyolojik yolları belirlemek için Gen Ontolojisi (GO) ve KEGG zenginleştirme analizleri gerçekleştirilmiştir. Ayrıca, seçilen klinik olarak onaylı ilaçların FBXO7'ye bağlanma afinitelerini değerlendirmek ve PH tedavisi için yeniden konumlandırılmaya uygun adayları belirlemek üzere SwissDock üzerinden AutoDock Vina algoritması kullanılarak *in silico* moleküler docking çalışmaları yapılmıştır. Docking analizi sonucunda FBXO7'ye yüksek bağlanma afinitesi gösteren çeşitli bileşikler tanımlanmıştır: florometolon (–6,367 kcal/mol), bendroflumetiyazid (–6,354 kcal/mol), lasofoksifen (–6,173 kcal/mol), penisilin V (–6,102 kcal/mol), hidromorfon (–6,067 kcal/mol) ve sefamandol (–6,036 kcal/mol). Bu ilaçlar, mitokondri işlevi, nöroinflamasyon ve hücresel stres yanıtlarıyla ilişkili biyolojik yollarla bağlantılı olup, PH'de hastalık modifiye edici ajanlar olma potansiyeline sahiptir. Ancak, hastalık progresyonunu kötüleştirme veya sistemik yan etkiler gibi sınırlılıklar doğrudan yeniden kullanımını kısıtlayabilir. Bu çalışma, FBXO7 ile yüksek bağlanma afinitesi gösteren klinik olarak onaylı çeşitli ilaçları ortaya koyarak, bunların PH'de mitokondriyal disfonksiyonu hedeflemek için potansiyel taşıdığını göstermektedir. Bazı bileşiklerin doğrudan kullanımıyla ilgili zorluklar bulunsa da, elde edilen moleküler etkileşim verileri mitokondri odaklı yeni tedavi stratejileri geliştirmek için değerli bilgiler sunmaktadır. Terapötik potansiyelin artırılması ve yan etkilerin azaltılması için ileri düzey deneysel doğrulama ve yapısal optimizasyon gereklidir; bu da PPH için yeni tedavi yaklaşımlarının önünü açabilir.

Anahtar Kelimeler: FBXO7. İlaç yeniden konumlandırma. Mitokondriyal disfonksiyon. Nörodejenerasyon. Parkinson hastalığı.

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Parkinson's disease (PD) is a progressive neurodegenerative disorder characterized by the loss of dopaminergic neurons in the substantia nigra pars compacta (SNpc) and the presence of Lewy bodies that leads to motor and non-motor symptoms[1]. Although the etiology of PD is multifactorial, mitochondrial dysfunction has emerged as a central pathological mechanism. Mitochondria are critical regulators of cellular energy production, redox balance, and apoptosis. Any dysfunction in these organelles is linked to increased neuronal susceptibility in PD[2]. Since mitochondria are highly multifunctional organelles, their proper functioning is critical for the health and longevity of neurons[3]. Therefore, targeting mitochondrial dysfunction is increasingly recognized as a promising strategy for therapeutic intervention in PD.

Genetic studies on monogenic forms of PD have highlighted the significant role of mitochondrial dysfunction in the disease pathogenesis[4]. Although only about 10% of PD cases are linked to specific genetic mutations, investigations into familial PD (PARK) genes have significantly enhanced our knowledge of the causes and mechanisms of PD. Familial PD cases often involve mutations in genes such as Parkin (PRKN), glucocerebrosidase (GBA1), leucine-rich repeat kinase 2 (LRRK2), vacuolar protein sorting-associated protein 35 (VPS35), PTEN-induced putative kinase 1 (PINK1), and F-box only protein 7 (FBXO7), many of which are directly associated with mitochondrial dysfunction and compromised cellular integrity [3,4]. FBXO7 has gained attention in recent years due to its significant role in maintaining mitochondrial homeostasis. It is a part of the Skp-Cullin-F-box (SCF) E3 ubiquitin ligase, playing a crucial role in mitophagy, a process disrupted in PD[5]. Mutations of PARK15, which encodes FBXO7 protein, cause early-onset autosomal recessive PD, highlighting its importance in neurodegenerative disease mechanisms[6]. In addition to its role in mitophagy, FBXO7 is involved in mitophagy, proteasome assembly, synaptic function, and motor control, processes that are less emphasized in other PARK genes[7,8]. It also causes a unique clinical syndrome combining parkinsonian and pyramidal signs, suggesting a distinct pathogenic pathway[8]. These features make FBXO7 an important target for drug repurposing efforts. Despite its critical involvement in pathways related to mitochondrial quality control and protein degradation, FBXO7 remains underexplored as a therapeutic target. To our knowledge, this study represents the first structure-based repurposing approaches focused specifically on FBXO7, highlighting its novelty and potential impact.

Drug repurposing is an appealing strategy for speeding up the development of new therapies, particularly in complex diseases like PD, where traditional drug discovery demands much time and money[9]. This approach allows for identifying new targets for drugs that are already approved for the treatment of a disease. In this context, *in silico* methodologies, including molecular docking and network analysis, are effective tools for facilitating the identification of drug-target interactions quickly and cost-effectively[10].

This study aims to investigate the therapeutic potential of addressing mitochondrial dysfunction in PD through an FBXO7-focused approach. We performed a comprehensive network analysis to identify key protein-protein interactions involving FBXO7 and its related pathways. Furthermore, using *in silico* molecular docking studies, we assessed the binding affinities of selected drugs to FBXO7 to identify promising candidates for repurposing. The integration of network pharmacology and molecular modelling comprehensive perspective on the potential mechanisms through which repurposed drugs could modulate FBXO7-related pathways, offering insights into their suitability for PD therapy.

Material and Method

Drugbank Database Search and Selection of Mitochondria-Related Drugs

This study focused on identifying drugs that influence mitochondrial function and have the potential to be repurposed for PD based on the FBXO7 protein, which is a significant contributor to both mitochondrial dysfunction and PD pathology.

An initial query was performed in the DrugBank database (https://www.drugbank.com) (accessed on 09 September 2024), specifically targeting drugs associated with FBXO7. Following the search, the initial list of drug candidates was generated. The criteria for selection were:

- Drugs are known to interact with or modulate FBXO7 or related mitochondrial pathways.
- Only drugs approved for clinical use to prioritize safety and feasibility for repurposing.

Python programming scripts were utilized to further process the data. The list of drugs obtained from DrugBank was subjected to a literature review to identify their known effects on mitochondrial function.

Automated Literature Screening Using Python

To ensure comprehensive screening, automated searches were performed on PubMed using the following two queries for each drug:

- *Mitochondria-related research:* The search term "(Drug Name[Title/Abstract]) AND (mitochondria

[Title/Abstract])" was used to identify research focused on the drug's impact on mitochondrial function, including energy production, oxidative stress regulation, and apoptosis.

- *Parkinson-related research*: The search term "(Drug Name [Title/Abstract]) AND (Parkinson [Title/Abstract])" was used to filter out drugs that had been previously studied in the context of Parkinson's disease.

Python's requests library was used to automate the interaction with PubMed's Application Programming Interface (API), and pandas were utilized for data management and processing. The API enabled automated retrieval of article metadata for each compound, while pandas facilitated the filtering, organization, and comparison of the resulting datasets.

Filtering and Final Drug Selection

Following the retrieving the PubMed data, the subsequent step was to filter the drugs based on the following criteria:

- *Inclusion Criteria:* Drugs with at least one study documenting mitochondrial function effects but no studies linking them to Parkinson's disease were selected for further analysis.
- *Exclusion Criteria:* Drugs that had documented neurotoxic effects, exacerbated PD symptoms, or negatively impacted mitochondrial function in neurodegenerative conditions were excluded through a manual literature review.

This filtering process resulted in a final list of 53 drugs, all of which were previously studied for their effects on mitochondrial processes but had no known research linking them to PD. These drugs were selected as candidates for further in silico analysis and potential repurposing.

Network Pharmacology Study

Protein-Protein Interaction Network

To investigate the protein-protein interactions (PPIs) involving FBXO7 and its associated proteins, we utilized the STRING database (version 11.5; https://string-db.org) (accessed on 17 October 2024) to construct the PPI network. STRING aggregates known and predicted PPIs from multiple sources, including direct (physical) and indirect (functional) associations. For this study, we set the minimum required interaction score to high confidence (0.7). Disconnected nodes were hidden to focus on the core network. The PPI network was visualized and further analyzed using Cytoscape software (version 3.9.1). Nodes represent proteins, while edges indicate the interactions between them. No additional filtering for hub genes was applied. This analysis aimed to clarify the role of FBXO7 in pathways related to PD, especially through its interactions with proteins involved in mitochondrial function and cellular stress responses.

GO and KEGG Pathway Analysis

Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analyses were performed using the STRING database (https://string-db.org/) (accessed on 17 October 2024). The interaction network for FBXO7 and its associated proteins was constructed using a high confidence interaction score of 0.7. GO enrichment analysis was conducted to assess the biological processes, cellular components, and molecular functions relevant to PD pathology. Additionally, KEGG pathway enrichment analysis was used to explore key pathways associated with the interactome of FBXO7. The top enriched terms were visualized as bubble charts, generated using the STRING platform, where the size and color of the bubbles represent the significance and gene count for each enriched term, respectively. Only terms with a False Discovery Rate (FDR) < 0.05 were considered statistically significant.

In Silico Studies

Molecular Docking

The *Autodock Vina* algorithm provided by SwissDock was used for evaluating the binding affinities of ligands targeting the FBXO7 protein. This platform is widely utilized for predicting potential binding conformations and energies between target proteins and ligands, allowing for the identification of optimal binding sites. The crystal structure of the target protein, FBXO7, was obtained from the Protein Data Bank (PDB). The binding region of the protein was identified, unnecessary water molecules were removed, and only amino acids crucial for the binding site were retained. The prepared protein structure was uploaded to the SwissDock platform. The canonical SMILES of the selected compounds were obtained from the PubChem (https://pubchem.ncbi.nlm.nih.gov/) (accessed on 9 October 2024) and uploaded to the SwissDock platform. These ligands were included in the docking analysis to investigate their potential interactions with FBXO7. The *Autodock Vina* algorithm provided by SwissDock utilizes a specific energy function to estimate the binding energies between the ligand and the protein. Default parameters were used for docking, considering a rigid target protein and flexible ligand conformations. The minimum binding score was evaluated based on the reference ligand BC1464 (-5.870), a small molecule that has been experimentally shown to bind directly to the FBXO7[11].

Data Analysis

Docking scores obtained from the SwissDock platform were analyzed. These scores represent the binding energies, with lower (more negative) energy values indicating stronger binding. The lowest energy score for each ligand was compared to the reference molecules, BC1464. The binding affinity of the drugs was determined by comparing its score to that of the reference molecules. The ligand with the most negative binding energy was considered to have the strongest potential interaction with FBXO7.

Visualization of Results

Output files and three-dimensional binding positions provided by SwissDock were visualized using ChimeraX v1.9 for detailed structural analysis. The binding affinities of the selected drugs to FBXO7 were assessed using results from docking studies, which allowed for an evaluation of their potential interactions. By using BC1464 as a reference ligand known for its strong binding affinity, other candidate molecules were compared to it in terms of their binding strength to FBXO7. This comparative approach, visualized using ChimeraX, provided a reliable method for determining the relative binding affinities of each drug to the target protein. Additionally, it offered insights into the potential therapeutic efficacy of the drugs based on these binding interactions.

Statistical Analysis

The docking scores of selected compounds were qualitatively compared with the reference ligand (BC1464) to identify potential candidates for FBXO7 inhibition. No statistical tests were applied to compare docking scores quantitatively. Statistical significance in enrichment analyses was determined using an FDR threshold of <0.05, corrected by the Benjamini-Hochberg method to account for multiple comparisons. The significance of PPI networks was assessed using STRING's PPI enrichment p-value, ensuring that observed interactions were not due to random chance.

Results

Protein-Protein Interaction Network Analysis

The STRING analysis demonstrated a well-structured protein-protein interaction (PPI) network, comprising 21 nodes and 93 edges, resulting in an average node degree of 8.86 (Figure 1). This network showed significant PPI enrichment with a p-value of <1.0e-16, which suggests that these proteins interact more than would be expected by random chance, further supporting their functional relationships. The clustering coefficient of 0.892 also supports that the proteins in this network are highly connected, proposing a common functional role, particularly in the context of PD. FBXO7 interactors PRKN, PINK1, and PARK7 are known players in the regulation of mitochondria, further implicating FBXO7 in key PD-related mechanisms.

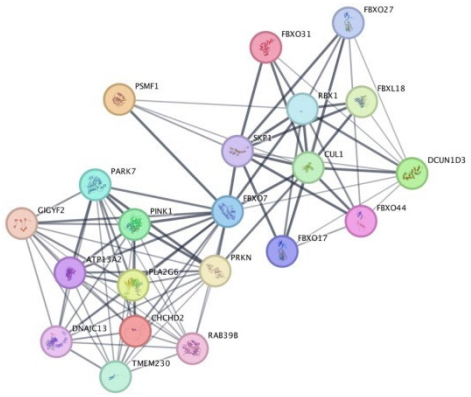

Figure 1.
Protein-protein interaction network of FBXO7 and its functional partners. This network illustrates the interaction between FBXO7 and its associated proteins. Nodes represent individual proteins, while edges indicate the predicted or known interactions based on experimental data, databases, gene neighbourhood, gene co-occurrence, and text mining. The node color distinguishes different protein groups, and the node size is proportional to the degree of interaction. The network shows a densely interconnected cluster, highlighting the key proteins involved in ubiquitination and mitochondrial regulation, which are critical processes in Parkinson's disease pathology.

GO and KEGG Enrichment Analyses

GO and KEGG Enrichment analyses covered KEGG pathways and GO terms, including biological process, molecular function, and cellular component. Bubble charts were used to visualize the top 10 enriched terms in each category (Figure 2). GO biological process enrichment analysis identified key pathways related to ubiquitin-proteasome system dysfunction, mitochondrial homeostasis, and oxidative stress response, all of which are central to PD pathology (Fig 2a). The most significantly enriched process was SCF-dependent proteasomal ubiquitin-dependent protein catabolic process (FDR = 7.78e-12), followed by Proteasome-mediated ubiquitin-dependent protein catabolic process (FDR = 2.21e-09) and Ubiquitin-dependent protein catabolic process (FDR = 5.54e-10). These findings reinforce the role of FBXO7 in protein degradation, highlighting its contribution to proteostasis imbalance in PD. Additionally, pathways directly related to mitochondrial function were enriched, including Regulation of autophagy of

mitochondria (FDR = 5.22e-07) and Positive regulation of mitochondrion organization (FDR = 1.37e-05). Negative regulation of oxidative stress-induced neuron death (FDR = 8.85e-06) was also significantly enriched, emphasizing the neuroprotective mechanisms modulated by FBXO7.

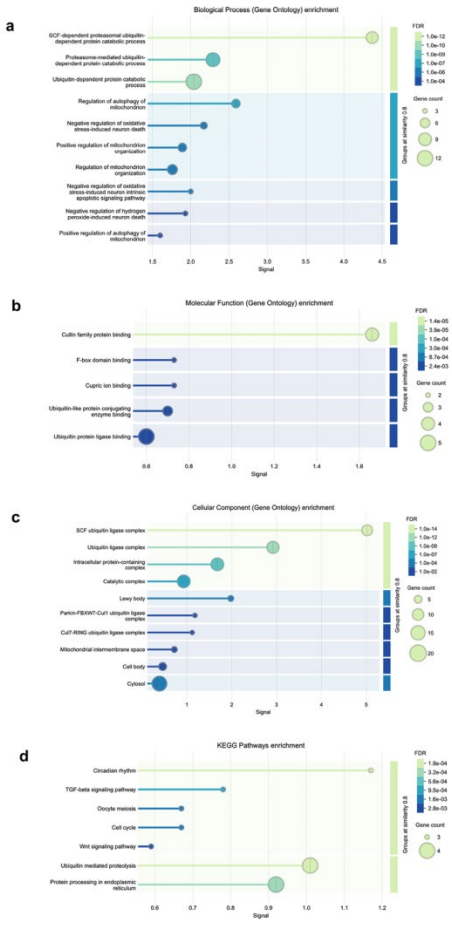

Figure 2.
GO and KEGG Pathway Enrichment Analysis of FBXO7-associated Proteins. The bubble chart displays the enriched Gene Ontology (GO) terms and KEGG pathways related to FBXO7 and its interacting proteins. The size of each bubble represents the gene count, while the color gradient indicates the False Discovery Rate (FDR), reflecting the significance of enrichment.

GO molecular function enrichment analysis further confirmed the involvement of FBXO7 in ubiquitin-related interactions (Fig 2b). The most significant terms were Ubiquitin protein ligase binding (FDR = 2.40e-03) and Ubiquitin-like protein conjugating enzyme binding (FDR = 8.70e-04). The identification of Cullin family protein binding (FDR = 1.40e-05) and F-box domain binding (FDR = 3.60e-05) suggests that FBXO7 participates in Cullin-RING ubiquitin ligase (CRL) complexes, which are critical for protein turnover and mitochondrial quality control.

GO cellular component analysis highlighted key subcellular structures involved in protein degradation and mitochondrial function (Fig 2c). The most significantly enriched terms included SCF ubiquitin ligase complex (FDR = 1.00e-14) and Ubiquitin ligase complex (FDR = 1.00e-12), confirming the importance of FBXO7 in ubiquitin-mediated proteolysis. Notably, Lewy bodies (FDR = 1.00e-06), a hallmark of Parkinson's disease pathology, were also significantly enriched. Additionally, the Parkin-PINK1 ubiquitin ligase complex (FDR = 1.00e-06) was among the top-ranked terms, reinforcing the well-established role of these proteins in mitochondrial autophagy (mitophagy) and neuroprotection.

KEGG pathway enrichment analysis identified Ubiquitin-mediated proteolysis (FDR = 3.20e-04) and Protein processing in the endoplasmic reticulum (FDR = 2.80e-03) as the most enriched pathways, aligning with the role of FBXO7 in maintaining protein homeostasis. Additionally, TGF-beta signaling pathway (FDR = 3.20e-04) and Wnt signaling pathway (FDR = 9.80e-04) were significantly enriched, suggesting potential regulatory mechanisms that could influence neurodegeneration and neuronal survival (Fig 2d).

Results of Docking Analysis

Table I provides a summary of the docking scores, ranking the tested compounds based on their binding strength relative to BC1464. Among the tested compounds, fluorometholone (-6.367), bendroflumethiazide (-6.354 kcal/mol), lasofoxifene (-6.173 kcal/mol), penicillin V (-6.102 kcal/mol), cefamandole (-6.036 kcal/mol) and hydromorphone (-6.067) exhibited the strongest binding affinities, surpassing the reference ligand.

Table I. Molecular Docking Scores and Binding Affinities of Selected Compounds for FBXO7

Drug	Docking Score (kcal/mol)	Binding Affinity Category
Fluorometholone	-6.367	High
Bendroflumethiazide	-6.354	High
Lasofoxifene	-6.173	High
Penicillin V	-6.102	High
Cefamandole	-6.036	High
Hydromorphone	-6.067	High
BC1464 (Reference)	-5.870	Reference
Bimatoprost	-5.651	Moderate
Cilastatin	-5.542	Moderate
Doconexent	-5.354	Moderate
Elvitegravir	-4.97	Low
Amlexanox	-4.89	Low
Gadobutrol	-3.808	Low
Lumefantrine	-2.832	Low

A subset of compounds exhibited docking scores close to the reference ligand but did not surpass it. Bimatoprost (-5.651 kcal/mol), cilastatin (-5.542 kcal/mol), and doconexent (-5.354 kcal/mol) fell into this category, indicating moderate binding affinity. These compounds showed potential for FBXO7 interaction, albeit with weaker binding strength compared to the high-affinity group.

Several compounds demonstrated weaker binding affinities relative to the reference ligand. Amlexanox (-4.89 kcal/mol) and elvitegravir (-4.97 kcal/mol) exhibited moderate-to-low binding potential. Lumefantrine (-2.832 kcal/mol) and gadobutrol (-3.808 kcal/mol) showed the least favorable docking scores, suggesting minimal interaction with FBXO7.

The docking analysis revealed that the top-binding compounds exhibited stable interactions with critical residues within the FBXO7 binding pocket. The structural alignment of these drugs within the active site of FBXO7, as visualized in Figure 3, supports their potential role in modulating mitochondrial dysfunction in PD.

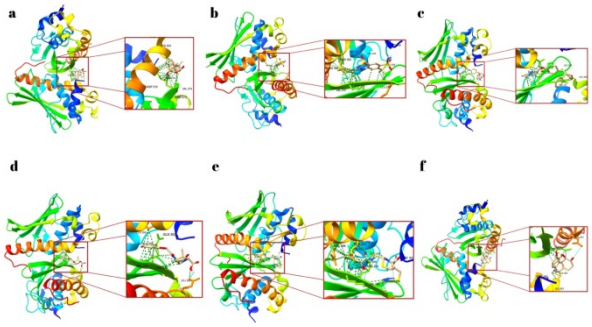

Figure 3.

Best docking poses for the top-binding drugs with FBXO7. (a) Fluorometholone interacts with key residues VAL274, LEU307, ASP310, ARG276 and ARG272, (b) bendroflumethiazide interacts with key residues GLN302, ARG306, VAL274, LYS275, LEU277, LEU255 and MET207, (c) lasofoxifene interacts with key residues GLN302, ARG276, ASN196, VAL274, and LEU280, (d) penicillin V interacts with key residues LYS275, VAL274, LEU280, GLU209 and GLN278, (e) cefamandole interacts with key residues ARG276, ARG306, SER182, GLN278, GLN302, LEU208, and GLU209. (f) hydromorphone interacts with key residues LYS275, VAL274, GLN302, LEU301, and GLN311. Hydrogen bonds are shown in blue and van der Waals interactions are shown in green.

Discussion and Conclusion

PD is a complex neurodegenerative disorder characterized by dopaminergic neuronal loss, mitochondrial dysfunction, oxidative stress, and disruption of proteostasis[12]. Despite extensive research, disease-modifying treatments remain unavailable, necessitating the exploration of novel therapeutic targets. In this study, we identified FBXO7 as a central player in PD-associated mitochondrial dysfunction and conducted *in silico* molecular docking to assess the potential of clinically approved drugs for repurposing. Our findings indicate that several high-affinity binders to FBXO7 could serve as candidates for modulating key PD-related pathways, particularly those involved in mitophagy, ubiquitin-proteasome degradation, and oxidative stress regulation.

While most cases of PD occur sporadically, approximately 10% have a hereditary origin. In recent years, multiple mutations linked to an increased risk of PD have been discovered, providing a deeper understanding of the disease's pathogenic mechanisms[13]. Genetic mutations in PARK15, the gene encoding FBXO7, have been linked to early-onset autosomal recessive PD, supporting its involvement in neurodegeneration[14,15]. Consistent with previous findings, our network analysis confirmed FBXO7's strong interaction with well-known PD-related proteins, including PRKN (Parkin), PINK1, and PARK7, which are key regulators of mitophagy [16]. Disruptions in these pathways contribute to the accumulation of damaged mitochondria, increased oxidative stress, and neuronal loss, reinforcing the significance of FBXO7 as a potential therapeutic target. GO and KEGG enrichment analyses further corroborated the role of ubiquitin-mediated proteolysis and mitochondrial quality control pathways in PD. The enrichment of SCF-dependent proteasomal ubiquitin-dependent protein catabolic process and regulation of autophagy of mitochondria suggests that targeting proteasomal degradation and mitochondrial homeostasis could be key to developing neuroprotective strategies. Notably, the enrichment of Lewy body-related processes aligns with the histopathological hallmark of PD, indicating a possible link between FBXO7 and abnormal protein aggregation.

The *in silico* screening process identified several drugs with high binding affinity to FBXO7, positioning them as potential candidates for disease-modifying interventions in PD. Among these, fluorometholone, bendroflumethiazide, lasofoxifene, penicillin V, hydromorphone, and cefamandole demonstrated stronger interactions with FBXO7 than the reference ligand. Given the role of FBXO7 in mitophagy regulation, protein degradation, and oxidative stress response, compounds that effectively bind to this target may have therapeutic implications in slowing or modifying disease progression in PD.

Fluorometholone is a synthetic glucocorticoid with well-established anti-inflammatory effects[17]. Glucocorticoids have been implicated in modulating mitochondrial biogenesis, reducing oxidative stress, and regulating the Nuclear Factor kappa B signaling pathway, which is a key driver of neuroinflammation in PD[18]. Given the chronic neuroinflammatory component of PD, glucocorticoids have been hypothesized to offer therapeutic benefits[19]. However, the long-term use of systemic glucocorticoids in PD remains controversial, as these drugs may both alleviate neuroinflammation and exacerbate neurodegeneration by affecting mitochondrial function and synaptic plasticity[20,21]. Fluorometholone has a high anti-inflammatory potency but is exclusively available as a topical formulation, primarily in the form of eye drops or ointments[17]. It is not used systemically due to its design and efficacy as a topical treatment, which provides significant local anti-inflammatory effects with minimal systemic absorption[22]. Given its strong docking affinity for FBXO7, fluorometholone could theoretically play a role in mitigating mitochondrial dysfunction in PD. However, the lack of systemic formulations and blood-brain barrier (BBB) permeability significantly limits its applicability in treating neurodegenerative diseases. Although fluorometholone itself is not an ideal candidate for systemic PD therapy, its structure and pharmacodynamics provide insights for developing safer glucocorticoid-based treatments with neuroprotective potential.

Bendroflumethiazide is a thiazide diuretic primarily used for hypertension and fluid retention management. However, recent studies suggest that thiazides may exert neuroprotective effects through their ability to modulate oxidative stress[23]. Although bendroflumethiazide's current clinical use is limited to its diuretic effects, and there is no direct evidence supporting its efficacy in neurodegenerative diseases, given its high binding affinity to FBXO7, future studies should investigate whether bendroflumethiazide can modulate mitochondrial ion homeostasis and reduce oxidative stress in PD models.

Lasofoxifene is a third-generation selective estrogen receptor modulator (SERM) used for osteoporosis prevention and estrogen-related disorders[24]. Current research has highlighted estrogen's neuroprotective actions, particularly in mitochondrial regulation, oxidative stress reduction, and inflammatory modulation, all of which are relevant to PD pathophysiology[25,26]. Estrogen signaling supports mitochondrial biogenesis and improves mitophagy efficiency, which is in line with FBXO7's role in modulating mitochondrial clearance[27]. In addition, epidemiological evidence indicates that women are less likely to develop PD prior to menopause, likely due to the protective effects of estrogen on dopaminergic neurons [28]. Lasofoxifene's high affinity for FBXO7 suggests a possible synergistic role in mitochondrial repair and proteostasis, that needs further investigation. However, SERMs have limited BBB penetration[29], and their long-term systemic use may pose cardiovascular risks and thromboembolic complications[30]. Therefore, nanoparticle-based formulations of lasofoxifene or structural modifications may be necessary to optimize its neuroprotective potential in PD.

Beta-lactam antibiotics such as penicillin V and cefamandole also demonstrated strong interactions with FBXO7. Beta-lactam antibiotics have been shown to reduce neuroinflammation by modulating microglial activation, which aligns with FBXO7's role in cellular stress responses and mitophagy regulation[31]. For example, ceftriaxone has been shown to upregulate glutamate transporters, reducing glutamate excitotoxicity, a key contributor to PD neurodegeneration[32]. Although there is no evidence from these abstracts to support its neuroprotective properties, cefamandole, a cephalosporin antibiotic, showed high binding affinity to FBXO7, suggesting a possible role in mitochondrial quality control and proteostasis regulation. However, some β-lactams, including penicillin V, have the propensity to cause neurologic problems in a subpopulation of geriatric patients[33]. Moreover, in a study using a penicillin-induced epilepsy model in rats, penicillin was found to significantly increase the levels of proinflammatory cytokines, indicating a potential to promote neuroinflammation[34]. Given these limitations, direct repurposing of Penicillin V for PD appears unfeasible. However, its strong binding to FBXO7 suggests that beta-lactam scaffolds could serve as a basis for novel drug development.

Hydromorphone is a potent opioid receptor agonist primarily used for pain management[35]. Opioid receptor activation has been reported to protect against PD-related mitochondrial dysfunction by enhancing mitophagy[12]. Although the most prominent tag of these opioid receptors is their modulation of pain signaling, the analgesic effects are primarily through activation of mu opioid receptors[36]. Interestingly, delta opioid receptors, while less significant in pain management than mu opioid receptors with a lower risk of abuse[36], exhibit a distinct potential in neuroprotection and inflammatory regulation[37,38]. These studies suggest that there is insufficient evidence to support hydromorphone as neuroprotective, with some studies indicating potential neurotoxic effects in renal impairment[39]. Given its high binding affinity to FBXO7, Hydromorphone may influence mitochondrial autophagy and oxidative stress pathways, but its systemic opioid effects raise concerns regarding addiction, tolerance, and potential neurotoxicity[40-42]. Thus, while hydromorphone's

interaction with FBXO7 is compelling, its clinical utility as a disease-modifying agent in PD remains uncertain. Future studies must target opioid derivatives that have less liability for addiction and better mitochondrial targeting action.

Although the primary focus of this study was on high-affinity binders, moderate-affinity compounds such as bimatoprost, cilastatin, and doconexent may still hold biological relevance. These drugs may interact with FBXO7 in an allosteric manner or influence secondary pathways involved in mitochondrial function and protein degradation. While their therapeutic implications are less direct than high-affinity binders, their moderate interaction strength suggests a potential role in combinatorial therapeutic approaches. In contrast, low-affinity compounds such as amlexanox and elvitegravir demonstrated minimal interaction with FBXO7, suggesting limited therapeutic potential in PD.

While this study provides compelling evidence for FBXO7 as a druggable target in PD, several limitations must be acknowledged. As an *in silico* analysis, this study predicts binding interactions but does not account for pharmacokinetics, bioavailability, or *in vivo* efficacy. Experimental verification using molecular dynamics simulations, enzymatic assays, and cell-based assays is required to confirm the stability and biological relevance of these interactions. Additionally, BBB permeability remains a major challenge for most compounds revealed herein, and therefore, structural optimization or novel delivery strategies will be required to maximize central nervous system bioavailability. Off-target effects and toxicity must also be thoroughly evaluated in preclinical models before advancing these candidates to clinical translation.

The present study indicates FBXO7 as a promising therapeutic target for modulating mitochondrial dysfunction in PD and identifies a number of clinically approved drugs with strong binding affinity. The findings pave the way for exploring repurposed drugs as an approach to treat PD with considerations of optimal pharmacokinetics, enhancing central nervous system penetration, and validating neuroprotective effects in experimental models. Future studies should prioritize the most promising candidates, such as Bendroflumethiazide, lasofoxifene and cefamandole, for in-depth preclinical evaluation, with the ultimate goal of developing effective disease-modifying therapies for PD.

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Idea and design: D.N.S., Data collection and processing: D.N.S., Analysis and interpretation of data: D.N.S.; Writing of significant parts of the article: D.N.S.
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