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Review Article

Potential Alternative Inhibitors of Alpha-Synuclein and Effective Treatment of Parkinson's Disease

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ABSTRACT

Parkinson's disease (PD) is the second common prevalent progressive neurodegenerative disorder mainly affecting the elderly. The disease gradually exhibits symptoms from resting tremor, bradykinesia, rigidity, and postural instability. Misfolding and aggregation of alpha-synuclein is considered the main pathological hallmark of the disease. Currently, treatment options for PD are only symptom-targeted, while an effective therapeutic strategy remains a challenge. Therefore, this study aims to comprehensively review *α*-synuclein, its implications and unique structural features as an effective therapeutic target for PD treatment. The earmarked structural modifications of the protein target in the pathogenesis of the disease include aggregation, propagation, and misfolding, while inhibitions of formation and propagation of monomers, oligomers and fibrils are essentially implicated as pharmacological pathways. Through experimental studies such as enzyme-linked immunosorbent assay (ELISA), Luminex, and thioflavin T (ThT) assay, potent inhibitors such as myricetin, curcumin, crocin, nanobodies including VH14*PEST, and NbSyn87*PES are notably identified as therapeutic alternatives and can be subjected to computational study and derivatized to obtained new molecules that could be of therapeutic value. The review highlights important critical implications of α-synuclein in the pathogenesis of PD and some therapeutic potentials against PD that is amenable for further translational study.

Keywords: Neurodegeneration**,** Parkinson's disease, *α*-synuclein, Neuropharmacology, Oligomers, Therapeutic design.

Introduction

Parkinson's disease (PD) is one of the most common neurodegenerative diseases characterized by the aggregation of α-synuclein and degeneration of dopaminergic neurons in the substantia nigra region of the brain.[1] It affects 1–2% of the world population, predominantly people aged above 60 years. The disease symptoms come on gradually and manifest progressively over time as motors and non-motors. The most apparent motors include shaking, rigidity, leisurely movement, speech impairment, trouble walking, and postural instability, which appears in the later stage of the disease.[2] Patients affected with PD also exhibit non-motor symptoms such as gastrointestinal, urogenital, neuropsychiatric, and sleep disturbances.[3] Dopamine (DA) is a primary neurotransmitter released to modulate some neuro activities in the central nervous system (CNS), and its imbalance has been implicated in the onset progression of some neurological diseases, including Alzheimer's disease (AD), depression and PD.[4] The three dopaminergic pathways associated in the pathogenesis of PD are the nigrostriatal, mesocorticolimbi**c**, tuberoinfundibular. These neuropharmacological pathways regulate cognition, movement, emotion, and memory. [5] The progressive neurodegeneration of DA in the pathophysiological regions, especially the nigrostriatal part of the midbrain, contributes to PD development. Subsequently, its imbalance between the direct and indirect pathways constitutes some basic pathomechanisms of PD, including hyperactivity of the globus pallidus and hypophonia.[5, 6]

Strategies to mitigate pathomechanism of PD involve the application of dopaminergic agonists to prevent α-synuclein aggregation and subsequently neurodegeneration.[7, 8] The aggregation process involves nucleation, formation of the toxic intermediate oligomeric state (protofibrils), and fibrillar amyloid with characteristics such as neuroinflammation and cell death, especially in the secondary stage. [2] Therefore, understanding the structural features of α-synuclein and the processes leading to oligomers formation to fibrils are essential for designing potent chemotherapeutic alternatives for effective management of PD.[9] The predominant clinical PD management involves the application of DA precursors such as L-dihydroxyphenylalanine (L-DOPA and levodopa) usually synthesized from tyrosine and phenylalanine. [10] Unfortunately, this dopaminergic replacement therapy manifests effectively after a long-term medication and provokes undesirable side effects, [11] thereby making a search for alternative therapeutics imperative. Manipulations of α-synuclein aggregation are in the limelight of experimental investigations of PD treatment with several progressive reports. However, the overview of these interesting therapeutic innovations needed to advance effective PD prevention and treatment knowledge remains sporadic. Several reviews have documented the implications of α-synuclein modelling in PD therapy,[2,12,13]; however, the overview of the recent development regarding modulations such as phosphorylation, aggregation and misfolding as neuropathophysiology of the disease framework and innovative treatment approach is limited. Therefore, this study aims to comprehensively review structural features, implications of α-synuclein, and its effective therapeutic target for PD treatment. The earmarked structural features include phosphorylation, aggregation and misfolding, while the

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inhibition of formation and propagation of monomers, oligomers and fibrils were focused on as essential pharmacological pathways. The experimental methods such as enzyme-linked immunosorbent assay (ELISA), Luminex, thioflavin T (ThT) assay, mass spectrometry (MS) and Biomolecular fluorescence complementation assay (BiFC) to demonstrate potent inhibitors.

Methodology

We searched the literature using Medline through PubMed electronic database from 2009-2019. However, recent literatures were also added. The literature search included studies on the small bioactive molecules with anti-PD potency, explicitly targeting the alpha-synuclein. Other relevant studies were identified from Google Scholar and manual search of included articles.

Results

α-synuclein

The α-synuclein is a member of intrinsically disordered proteins (IDPs), which play essential roles in various biological activities, both in normal body function and in various chronic conditions such as PD. It was first reported by in 1988 as a localized protein in the presynaptic nerve terminals and nucleus, hence, referred to as synuclein, with a unique structure identified in human brain tissue in the early 1990s during the ultrastructural study of amyloid plaques in an AD patient.[14] The synucleins found in humans include alpha (α), beta (β), and gamma (γ), which are small soluble proteins primarily expressed in neurons, and at other parts of the body in lower concentrations.[15] Moreover, the significant amyloid-beta protein fragment that was already investigated, a detailed analysis of the amino acid sequence revealed a second component called the non-A-beta component (NAC). Its precursor was named NAC precursor (NACP).[16]

The α-synuclein exists in different forms in synucleinopathies suggesting its pathological heterogeneity among synucleinopathies, which may help develop specific medications.[17] The protein is disordered in its monomeric form but can self-assemble into partially or highly oligomers and fibrils forms.[18] Although, there is a broad debate on the existence of α-synuclein in a dynamic equilibrium between unfolded monomers and α-helically folded tetramers under normal conditions. Whilst some reports indicated α-synuclein existence in the form of stable tetramers,[19] many revealed the existence of the protein in dimers and trimers, especially in those with no reported mutations; this makes it difficult to target alpha-synuclein protein by bioactive compounds.[20-22] Therefore, the α-synuclein is of significant interest due to its clinical relevance.[2,20,23,24]

Structure of α-synuclein

The α-synuclein is a short protein with one hundred and fourty (140) amino acid residues, and its aggregation is linked to pathogenesis and progression of PD.[25] Its structure is normal coil formation with three main domains *viz*:

a. The N-terminal domain or amino-terminal is responsible for forming amphipathic helices and has 11 amino acid imperfect repeats with a consensus motif of KTKEGV.
b. The central hydrophobic domain, known as the non-amyloid beta component, is responsible for increased fibril formation.
c. The primarily unfolded C-terminal domain or carboxyterminal with a high density of negatively charged amino acids (Figure 1).[25, 26]

At the amino acid sequence level (Figure 2), α-synuclein has three amino acid regions: residues 1–60 constitute the amphipathic α-helices; residues 61–95 constitute the hydrophobic and highly amyloidogenic NAC region; and the third residues 96–140 having highly acidic residues. The first two regions comprise a membrane-binding domain, whereas the C-terminal tail contains protein-protein and protein-small molecule interaction sites.[25] However, there are six-point mutations identified in the N-terminal domain of α-synuclein (A30P, E46K, A53T, A53E, H50Q and G51D). The most common mutations are three A30P, E46K and A53T. All mutations are related to autosomal dominant forms of PD. Therefore, modulation of these mutations could be an effective therapeutic pathway (Figure 2).[27]

The three mutations have tendencies to form fibrils more quickly than α-synuclein. The human A53T α-synuclein expressed in mice causes alteration of dopamine transport. The A30P mutant α-synuclein suggestively decreases the binding of the cell membrane to lipids, while E46K produces more aggregates than other mutants.[27]

The random coiling structure of α-synuclein gives a broad peak at 1650 cm^{-1} as observed using Fourier transform infrared spectroscopy. Circular dichroism spectra of α-synuclein indicated a 70% random coiling and 2% α-helical content.[12] The molecular mass of α-synuclein is not consistent due to an extended structure (C-terminal).[28] Due to its high negatively charged residues at the C-terminal, the molecular weight observed using sodium dodecyl sulfate-polyacrylamide gel electrophoresis is approximately 19 kDa.[12]

Function of α-synuclein

The α-synuclein is a highly abundant, soluble protein enriched in nerve terminals and has a central role in PD's pathogenesis. The protein plays a critical role in regulating synaptic vesicle recycling, vesicular storage, and the release of neurotransmitters.[29] In a previous study, it has been documented that α-synuclein can regulate the enzyme tyrosine hydroxylase, modulation of dopamine transporter activity, and regulation of monoamine transporter.[30] Some of the enumerated functions of α-synuclein protein are summarised in Figure 3. Notably, several studies reportedly suggest that over-expression of α-synuclein, mutations of the protein, and dopamine-modified-α-synuclein promote toxic interactions between its oligomers and lipids.[26,31] Subsequently, the aggregation, propagation, phosphorylation and misfolding of the protein have been implicated in the pathomechanisms of onset PD development.[32]

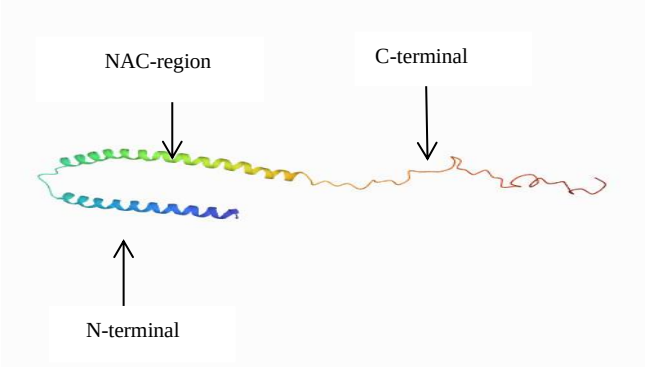

Figure 1: Structure of Human micelle-bound alpha-synuclein PDB (1X8Q) showing its various domain (DOI: 10.2210/pdb1XQ8/pdb).

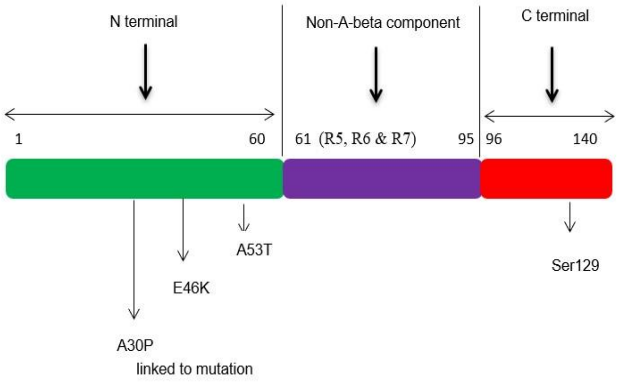

Figure 2: Structure of α-synuclein showing the NAC domain.

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Structural modifications of α-synuclein

The structural modifications of α-synuclein as pathogenesis of PD development occur through some neurodegenerative pathomechanisms such as aggregation and propagation, misfolding and phosphorylation.[33]

Aggregation and propagation

Aggregation of α-synuclein from Lewy's body (Figure 4) mainly occurs naturally through the binding of α-synuclein to the cell membrane. The monomeric α-synuclein binds to the membrane and adopts α-helical conformation, leading to membrane-bound β-sheets.[34] Moreover, cholesterol and lipid play a significant role in the aggregation of α-synuclein.[35] Therefore, the self-association of oligomers forms the membrane-bound β-sheets and is capable of mediating α-synuclein toxicity. In an experimental model, α-synuclein aggregates were shown to consist of β-sheets formed by maintaining a high concentration of protein, increased time of incubation, maintaining the temperature at 37ºC, and the addition of appropriate metal ions, and dopamine addition or nitration.[36] The monomers, oligomers, and fibrillar forms of α-synuclein can be transferred *via* cells and extended to different PD pathogenesis regions.[37] Other transmission mechanisms include endocytosis and trans-synaptic processes, which also contribute to spreading the disease to various parts of the brain.[37] Following changes in pH and ionic strength, increase in molecular crowding, and phosphorylation, the α-synuclein misfolds into protofibrils and higher-order oligomers.[38] From experimental evidence, it was demonstrated that α-synuclein could spread by a cell-to-cell transmission mechanism. The protein is reportedly present in monomeric and oligomeric forms in both human neurons and the cell culture,[39] while its cell-to-cell transmission has also been observed in human and animal models of PD, [40] leading to the 'prion-like' hypotheses, "α-synuclein transportation occurs from one cell to another *via* extracellular space".[41] This misfolding of α-synuclein begins in the new cell with the formation of LBs.[40] The release of α-synuclein monomers and oligomers from axone terminal and reuptake to neighbouring cells has also been demonstrated using ELISA quantitative sandwich immunoassay. The conditions that lead to misfolding of the α-synuclein are responsible for an increased protein level outside the cells. Thus, an excess amount of α-synuclein earmarked induces neurotoxicity within the cytoplasm of nearby cells, causing damage to the cells in the extracellular space.[42]

Misfolding of α-synuclein

The processes leading to misfolding of α-synuclein, fibrils formation, to LBs formation are poorly understood.[1] The amount of α-synuclein aggregation outside the LBs and Lewy neutrals (LNs) are hypothetically much higher than those within the same components. Subsequently, synaptic dysfunction and neuronal cell death occur due to aggregation of α-synuclein, [43], thereby inducing PD development. A study recently proved that added preformed fibrils (PFFs) initiate misfolding and aggregation of internal α-synuclein in both cell and animal PD models.[44] Previous findings indicated that excess α-synuclein and its mutations promote toxic interactions between α-synuclein oligomers and lipids cell membrane. [29] A typical partially and misfolded α-synuclein is presented in Figure 5 A and B.

The α-synuclein primarily exists as a random coil (Figure 6A). The hydrophobic domain residue (66-95 amino acid), especially 71-82, is essential for the oligomerization of α-synuclein into an anti-parallel β-sheet (Figure 6B) due to hydrophobic interactions and covalent modifications leading to the formation of fibrils, which are the main biomarkers in PD.[45] Thangavel et al. (2020)[46] reported amino acid residues essential for the interaction between some synthesized 7,8-dihydroxyflavone derivatives at Ala 90, Val 95, Gly 93, Lys 97, and Leu 100 as compared to L-Dopa having interactions with amino residues Asp 98, Phe 94, Gln 99, Leu 100, Val 95, and Lys 97. The synthesized compounds inhibited aggregation more than the standard, and this might be due to broader interactions of compounds with essential amino acid residues responsible for aggregation located at the NAC and C-terminal regions on α-synuclein. Other residues with which the compounds and L-Dopa standard reportedly exhibit similar interactions are Leu 100, Val 95, and Lys 97, primarily located in the

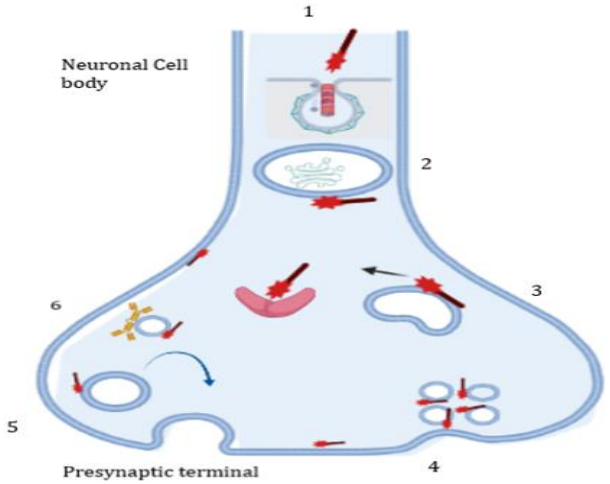

Figure 3: Functions of α-synuclein. α-synuclein has many functions.

1, the free disordered synuclein may localize to the nucleus or inner membrane and interact with DNA or histones in either a functional or pathological manner. **2**, the α-synuclein binds to the mitochondrial membrane where oxidized α-synuclein binds to the mitochondrion and forms a membrane-bound extended helix; some of the oxidized synuclein also binds to the neuronal walls to form the extended helix too, and these influence its morphology and fragmentation. 3. It interacts with calmodulin to moderate membrane interactions. **4**, at the presynaptic terminal α-synuclein cluster vesicles and inhibit reclustering after exocytosis from the cell. **5**, It bridges between plasma and synaptic membrane and, therefore, stabilizes vesicles docking or fusion. **6**, it acts as a nonclassical chaperon facilitating SNARE complex assembly through putative interaction with synaptobrevin, and this helps in membrane fusion and exocytosis of neurotransmitters from the cell, maintaining signal transmission at the synapse. **7**, it facilitates the redox shuttle, keeping the membranes reduced.

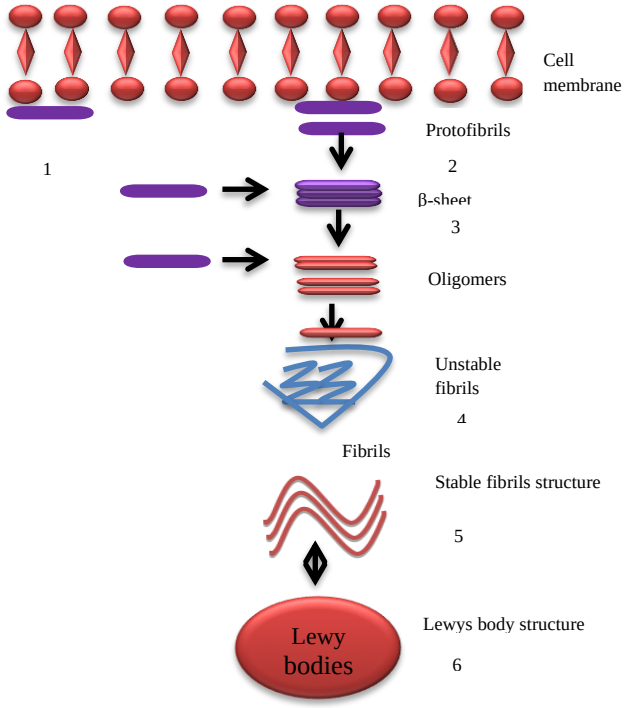

Figure 4: Aggregation and formation of Lewy bodies

(1) The α-synuclein monomers bind to cell membranes and adopt α-helical to form an intermediate structure (protofibrils). **(2)** Self-aggregation of protofibrils and monomers resulted in β-sheet structure. **(3)** The bound membrane β-sheets self-assemble and sometimes with

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monomers to form oligomers. **(4)** The oligomers aggregate to form unstable small fibrils through self-association. **(5)** The unstable small fibrils self-associate to form highly toxic stable fibrils. (**6**) Aggregation of stable fibrils present in Lewy bodies. [33]

hydrophobic region (blue colour Figure 6B).[46] Importantly, some experimental reports indicate a potent inhibition of α-synuclein by some natural neuroprotective compounds such as baicalein, curcumin, nordihydroguaiaretic acid, metformin and myricetin due to their ability to bind to N and C-terminals of the α-synuclein with a high number of hydrogen bonding while neurotoxic compounds such as rotenone, amphetamine, cocaine and paraquat bind to N-terminal only.[47]

Phosphorylation and toxicity of α-synuclein

The commonly reported post-translational modifications (PTMs) of α-synuclein include phosphorylation at the C-terminal, ubiquitination, cross-linking, truncations of protein chain and nitration. The variations play a vital role in the management of α-synuclein accumulation and toxicity.[48] Phosphorylation is a reversible addition of phosphate group (PO4) to the polar group R of amino acids with the aid of protein kinases.[49] Phosphorylation of α-synuclein at serine residue (S129) has attracted growing interest from scientists due to its considerable involvement in neurodegeneration and neurotoxicity, specifically through the interaction of the protein with lipid molecules on the mitochondrial cell membrane's surface.[11,49,50] Several studies reportedly demonstrate the phosphorylation of α-synuclein protein using experimental models including *in vitro* cell cultures, *in vivo* zebrafish, Maurine and fruit flies.[11,48,51]

Phosphorylation of α-synuclein was demonstrated *via* induction by polo-like kinase 2, which does not affect the accumulation and regulation of α-synuclein clearance through the lysosomal autophagy pathway.[52] The inhibition of ubiquitin-proteasome system and autophagy-lysosomal pathway were also documented as processes leading to a significant increase in phosphorylated α-synuclein on neuroblastoma, indicating that an α-synuclein degradation *via* phosphorylation.[53] Although only a small fraction of human α-synuclein immunized in rabbits *in vivo* is reported to be phosphorylated at S129 under normal conditions.[54-56] However, in patients suffering from synucleinopathies, revealing aggregation-dependent phosphorylation (>90%) of S129 has been observed, [54] More so, there exists a scientific debate on the active role of phosphorylation in the accumulation and toxic effects of α-synuclein or a response mechanism of cells to eliminate poisonous species of α-synuclein.[13] this evidence strongly supports the hypothesis that phosphorylation at S129 plays an essential role in regulating α-synuclein normal functions, control of its aggregation, fibrillation, LBs formation, and neurotoxicity.[2]

In addition to aggregation, propagation, misfolding and phosphorylation, other factors contributing to the pathogenesis of neurodegenerative diseases, especially PD, through the pathomechanisms of α-synuclein modifications are also worthy of investigation.[57]

Measurement of total α-synuclein using experimental assays and computational models

The significance of α-synuclein measurement is that the pathological form of α-synuclein is seen in solid tissues, such as Lewy bodies and Lewy neurites. It has also been identified in other parts of the body, including cerebrospinal fluid, plasma and saliva, and thus is readily secreted into extracellular spaces leading to neurodegeneration.[58] Many analytical techniques can be employed to measure the total amount of α-synuclein, its oligomers and phosphorylated α-synuclein *in vivo*, and such methods include western blot, ELISA, Luminex assay, mass spectrometry, ThT assay and biomolecular fluorescence complementation assay, each having its advantages and disadvantages (Table 1).[58-62] Others include transmission electron microscopy and circular dichroism (CD) spectroscopy.[61,63]

Specifically, ELISA for oligomers while ThT assay for Fibrils is employed. Using a fluorescent dye, the ThT fluorescence assay is often used to detect α-synuclein, especially the fibrils.

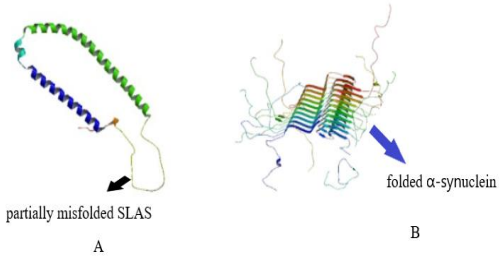

Figure 5: Misfolded α-synuclein.
(A) Partially folded sodium lauroyl sarcosinate (SLAS) α-synuclein from (PDB 2KKW). (B) Misfolded α-synuclein (PDB 2N0A) (retrieved from **DOI**: 10.2210/pdb2kkw/pdb and **DOI:** 10.2210/pdb2NOA/pdb)

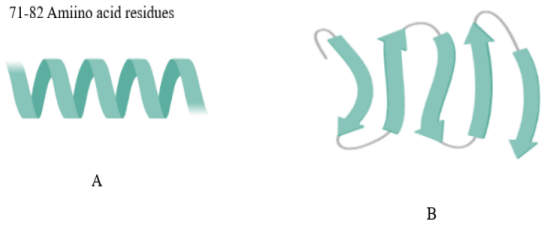

Figure 6: Misfolded (A) and standard (B) random coil α-synuclein.[45]

The dye has a weak fluorescent in the presence of α-synuclein monomers or fibrils. [64] ThT fluorescence intensity increases due to α-synuclein fibrils by several immensity at a high wavelength of emission (λmax = 483 nM).[7,25] In most cases, the maximum wavelength during the ThT fluorescent is λmax = 485 nM and excitation wavelength of λmax = 440 nM. However, when Th T is incubated in the presence of monomers, a negligible increase in fluorescent emission is observed, indicating the selectivity of α-synuclein fibrils to the Th T assay.[7] The intensity of the Th T fluorescent ratio is about 30-fold higher compared to a single component.[65] Therefore, the Th T fluorescence intensity of monomers is small and is unlikely to interfere with measuring the binding affinities of potential compounds against α-synuclein inhibition. [66] A variety of techniques are used to provide a divergent structural understanding of IDPs when assayed using either ELISA or ThT.[67] Specifically, techniques such as circular dichroism and infrared spectroscopic give a divergent view of IDP residues present in some secondary structures of α-synuclein.[68] Nuclear magnetic resonance provides information on the chemical environment around the residues, while electron paramagnetic resonance gives comprehensive information on the relative orientations of residues.[68] Another vital technique is fluorescence spectroscopy which highlights similar inter-residue distances and fluctuation of conformational changes within a compound.

Molecular simulation is an essential technique in drug discovery due to its unique application in molecular modelling and comprehensive view of compounds. Therefore, it is a scale where the atomic motion of compounds is analyzed. Significantly, molecular simulations predict experimental results using theoretical data and techniques to avoid waste and costly resources.[69]

Therefore, the application of simulation techniques could eventually lead to the identification of molecules that could target various pathophysiological conditions in PD, mainly targetting Human Tau protein, NAC and the C-terminal region of α-synuclein for effective treatment of the disease.

A study by Kakish *et al*. (2016) revealed how small active compounds could bind to either N- and C-terminus of α-synuclein and the related activities of the binding mode of small molecule compounds.[47] Using molecular modelling techniques such as mass spectrometry and radioligand binding studies, small bioactive molecules can bind to α-synuclein fibrils at three different sites with moderate affinities.[7] Fullerenols have been explored as potential nano-medicinal candidates to inhibit such aggregates, which can be easily experimented.[70]

Even though there is optimism about developing potential therapeutic agents targeting the α-synuclein, several fundamental challenges remain a concern. Oligomers, protofibrils and fibrils grown under *in vitro* experimental conditions cannot accurately mimic the physiological conditions leading to their development in the human brain, where there may be various competing and dynamic changes that may induce the production of different forms of oligomers. These conditions variably make the interpretation of α-synuclein properties in native and disease states inadequate. Therefore, further research is needed, especially by selectively choosing reported small molecules inhibitors of α-synuclein to optimize the understanding of the clinical-pathological relationships between the various species of the protein and the development of PD. [71] Indication thatMisfolding of α-synuclein occurs early in the disease process supporting the concept that it is necessary to target α-synuclein at the initial disease process before extensive neurodegeneration, and this will require developing an effective and high degree of sensitive biomarkers to allow diagnosis with a high degree of accuracy even in the absence of some vital motor symptoms.[72]

There is a concern that excessive therapy-induced downregulation of the levels of α-synuclein monomers could be detrimental and cause toxicity. Further research into the basic pathobiology of α-synuclein will undoubtedly provide new insights that could guide developing strategies and new agents for PD.[71,72]

Therapeutic alternatives of α-synuclein

The α-synuclein is becoming an attractive critical target for inhibitory design, owing to its synucleinopathies. Thus, its inhibitors in this study were selectively chosen due to their high ability to target both one off-pathway mechanisms and toxic species of the protein that can be efficiently designed to target the monomers, intermediate oligomeric aggregates to convert them into nontoxic, off-pathway aggregates, and fibrillar forms. [12] The Fibrilization pathway (Figure 7) of α-synuclein illustrates how monomers become oligomers to proto-fibrils (toxic species) and a final fibrillar state so that the compounds of interest can be designed by understanding the pathophysiology of the disease and having a specific target on monomers to fibrils for development of alternative therapeutics against PD.[33]

Table 1: Advantages and disadvantages of various analytical techniques for measuring α-synuclein

Technique	Advantages	Disadvantages	References
Enzyme-linked immunosorbent assay	Quick and convenient. Highly specific and sensitive. Reagents are relatively affordable with a long shelf life. Equipment is relatively inexpensive and available.	Monoclonal antibodies are preferred to achieve high specificity. False positives/negatives are possible. Enzyme/substrate reaction is short-term, so wells must be read as soon as possible.	59
Mass Spectrometry	Small sample size is required. Fast turn around time. Broad applicability. Sensitive and specific.	Equipment is expensive. Cannot distinguish between optical and geometric isomers.	58
Western blot	Migration is proportional to molecular weight.	Background can result from cross-reactivity of antibodies. Gel preparation is time-consuming. A large amount of protein is required for detection.	62
Luminex	Highly sensitive and specific. Can analyze up to 100 analytes in a single assay. sample in small quantity is sufficient Equipment is widely available.	Cross-reactivity and non-specific binding. Costly. Monoclonal antibodies are preferred to achieve high specificity. False positives/negatives are possible.	58
Thioflavin T assay (Th T)	Highly sensitive to measure binding affinities of all compounds.	Fibrils are detected only at maximum wavelength emission.	61
Biomolecular fluorescence complementation assay (BiFC)	BiFC can identify signals that regulate the formation and localization of protein complexes.	The fluorescent protein fragments must be able to associate with each other when tethered to the protein complex.	60

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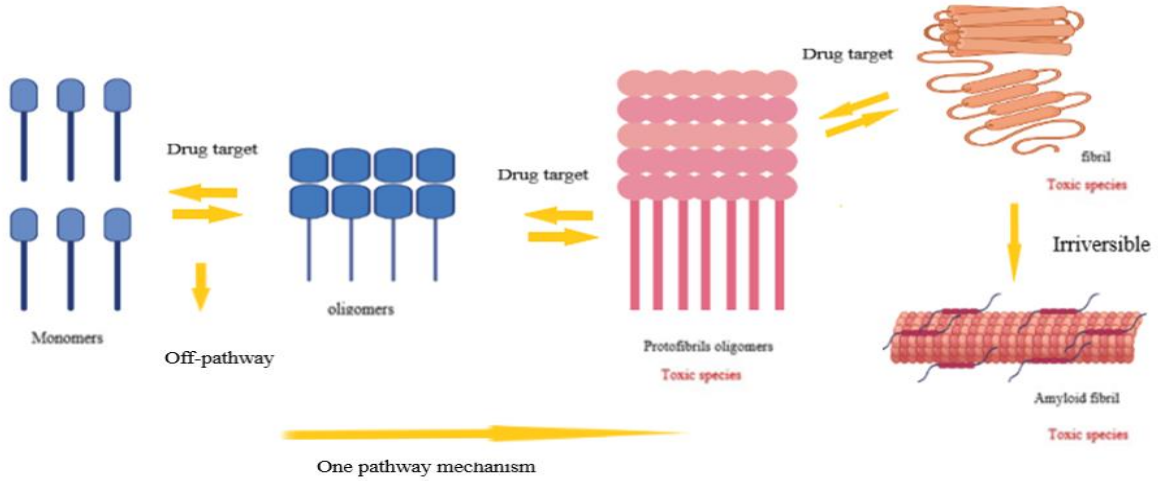

Figure 7: Schematic diagram of the fibrilization pathway showing oligomerization and fibrils formation of α-synuclein.[33]

A number of potential candidates have been investigated *in vitro* and *in vivo*, targeting some specific components of the fibrillization pathway, especially the monomers, oligomers and fibrils forms of α-synuclein.[73,74] In a study by Chatterjee et al., (2018),[75] nanobodies such as VH14*PEST and NbSyn87*PEST target the non-amyloid component and C-terminal region, respectively, remarkably reducing the level of S-129 phosphorylation, observed *in vivo* Sprague–Dawley rat model. [75] The nanobodies demonstrate a promising efficacy for PD, worthy of further consideration. Recently, an oligomer modulator (anle138b) was reported with the potential to inhibit α-synuclein and subsequently help in restoring dopamine release, prevention of cell death, and improvement of gait impairment observed in a transgenic mouse line. [76]

Small molecular compounds such as unsubstituted phenothiazine (PTZ), a parent molecule for many drugs and its derivatives (Figure 8), have gained a lot of scientific interest. This could be related to its unique metabolic transformation through either oxidation or conjugation processes, producing an active metabolite with potential inhibitory properties against many disease conditions, including PD. Other advantages of the compound include the ability to cross the blood-brain barrier, high lipophilicity, protective effect against cell death, high binding affinity to DA receptors, strong antioxidant effects to prevent neuronal damages, and a low expression for toxicity, CNS depression, and carcinogenicity. [77] Other PTZ derivatives with reported potentials for anti-PD through affinities to α-synuclein include 3-methoxy-7-nitro-10H-phenothiazine, 3-Bromo-7-nitro-10H-phenothiazine, 3-(2-Fluoroethoxy)-7-nitro-10H-phenothiazine, and (E)-3-(3-Iodoallyloxy)-7-nitro-10H-phenothiazine.[65,78] Considering these unique properties, the drug deserves further translational design into PD therapeutic. Some natural products reportedly retard the progression of the disease and show promising anti-oligomerization and anti-fibrillation against the α-synuclein protein. These include epigallocatechin-3-gallate (EGCG), salvianolic acid B, [79] and curcumin [80] (Table 2).

Other important alternative ligands that target α-synuclein aggregation include crocin, baicalein and its analogues (Figure 9), all with promising anti-fibrils or anti-oligomeric effects. Baicalein is an important flavonoid obtained from *Scutellaria baicalensis* Georgi, commonly called "Huang qin" in Chinese. All the compounds have structural similarities, such as benzene rings, ketones, hydroxyl and other functional groups critical for inhibitory effects against α-synuclein in a structure-activity relationship. [81] Crocin is another important α-synuclein inhibitor of interest. It is a carotenoid compound obtained from saffron and has shown promising antioxidant and neuroprotective properties. [82]

Crocin acts as a molecular chaperone to prevent amyloid fibril formation, especially the E46K α-synuclein mutant, in a concentration-dependent manner.

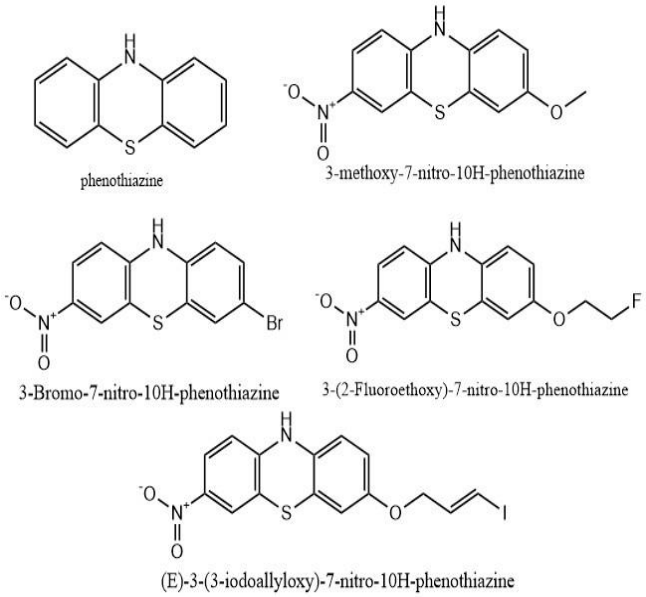

Figure 8: Chemical structure of phenothiazine and its derivatives

Moreover, crocin can change the E46K α-synuclein mutant pathway from a fibril-formation to an amorphous aggregation pathway, thereby reducing the aggregation tendencies of α-synuclein (Figure 10).[82]

The mutant E46K α-synuclein aggregates to form β-sheet oligomers, which on self-association aggregates to form fibrils, which, due to self-association with monomers, form stable fibrils. Crocin decreases the self-association of oligomers by forming unstable oligomers. This instability makes them from smaller fibrils and, therefore, the lesser form of the fibrils in the Lewy bodies, which will decrease degradation of the protein and, subsequently, possible therapeutic alternative in the management of PD.[82]

Flavonoids are polyphenols present in vegetables, fruit, and beverages of plant origin which have antioxidant, antimutagenic, and antiproliferative properties.[83] Thus, they may have a potential protective role in various chronic diseases, including common cancers and neurodegenerative diseases.[84] Extracts containing these bioactive products possess some beneficial activities, such as strong antioxidant effects, potentially changing PD's biochemical and physiological characteristics.[85] Intake of the flavonoids reduces the risk of PD development in men, potentiating therapeutic alternatives.[86] Polyphenols are natural compounds abundant in plants with numerous reported pharmacological activities, including neuroprotection.[87]

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Polyphenols with common scaffolds such as myricetin, EGCG, curcumin, rosmarinic acid, and nordihydroguaiaretic acid (Figure 11), present in green tea reportedly display neuroprotective effects, especially the catechins, (EGCG and myricetin).[84] The bioactive molecules inclusively demonstrate the ability to inhibit the formation of α-synuclein fibrils, destabilize the preformed fibrils (oligomers), protect neurons against injury and promote memory, learning and cognitive function.[88] For instance, myricetin (25 µg) in ration 1:1 with α-synuclein was found to block fibrilization. Interestingly, at a higher concentration (250µg), it blocked the oligomerization of α-synuclein, making it a potential therapeutic compound for further development as an effective anti-PD.[87] It has been demonstrated that myricetin can reduce oxidative stress by decreasing monoamine oxidase, glutathione-S-transferase activity, protein carbonyl content, reduction in lipid peroxidation, and decreased LBs formation.[89] Thus, focusing on these compounds could be an avenue to find new therapeutic compounds against PD. [87, 90]

Another crucial alternative pathway for inhibition of α-synuclein is inhibiting a highly conserved enzyme called prolyl oligopeptidase (PREP). PREP is a serine protease family member that selectively hydrolyzes oligopeptides with less than three kDa. [91]

Table 2: Inhibitory effects of phenothiazine compounds against either fibrils or oligomers of α-synuclein protein

Anti-fibrillin agents	Concentration (µM)	Anti-oligomers agents	Concentration (µM)
EGCG		Baicalein (α-syn)	100
Salvianolic acid B (Aβ)	1-5	Curcumin	2.6
Baicalein (Aβ)	DPM, 1-30		
Ferulic acid (Aβ)	NR		
Curcumin	2.05		

Note: Aβ = Alpha beta-amyloid found in AD; α-syn = α-synuclein found in PD; DP = dose-dependent manner, NR = not reported.

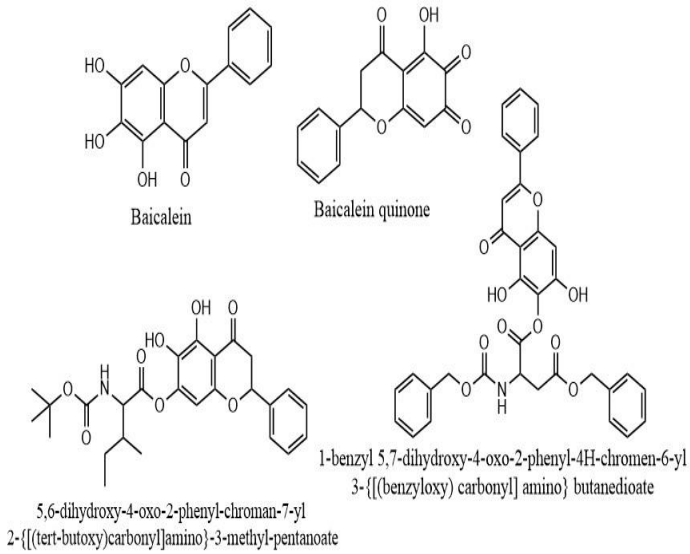

Figure 9: Chemical structures of baicalein and its derivatives

Recently, PREP has been implicated in the α-synuclein aggregation process, and the presence of PREP was sufficient to increase the aggregation rate of *α*-synuclein in a cell-free model. [92] PREP action can be blocked by small-molecule inhibitors such as KYP-2047[92] or the active site.[93,94] PREP inhibitors reportedly improve memory in some animal models that exhibited a cognitive decline. Therefore, PREP inhibition can be proposed as a therapeutic approach to counteract some neuropeptides and α-synuclein oligomers' low levels to find a better therapeutic alternative for anti-PD.[95]

Mounting evidence suggests that PD's pathogenesis is associated with soluble oligomeric complexes of *α*-synuclein rather than fibrils observed in the Lewi's body deposit.[96] Among various proteopathic proteins, human tau protein is considered key in the pathogenesis of PD. It has a high tendency to aggregate fast and forms insoluble fibrillar structures within the brain.[97]

Limitations and Future perspectives

The present review has the following limitations; first, the search for the literature was limited to two electronic databases. This approach may have excluded some eligible studies from the review. Secondly, we have not assessed the qualities of the included studies in this review. We summarise their findings within this limitation and hope that readers would be aware of such shortcomings and be cautious in concluding them.

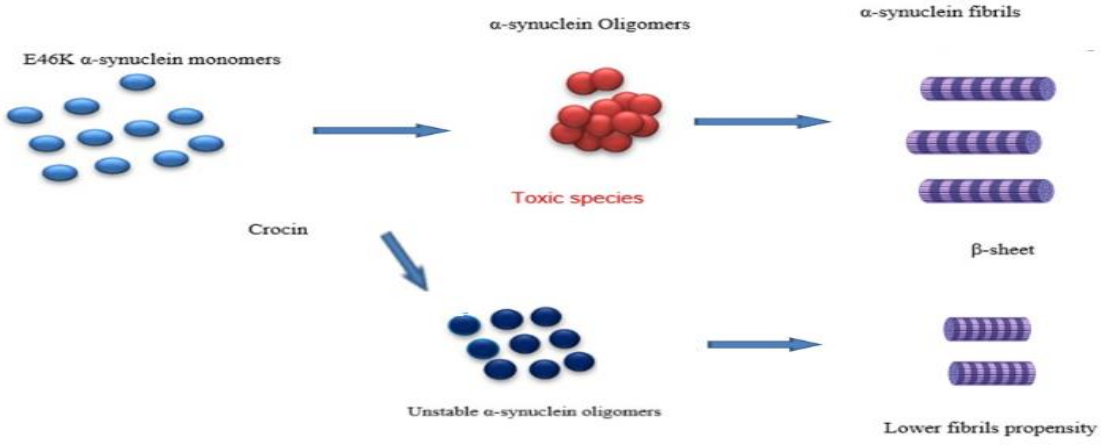

Figure 10: Illustration of how crocin prevents amyloid fibril formation of E46K

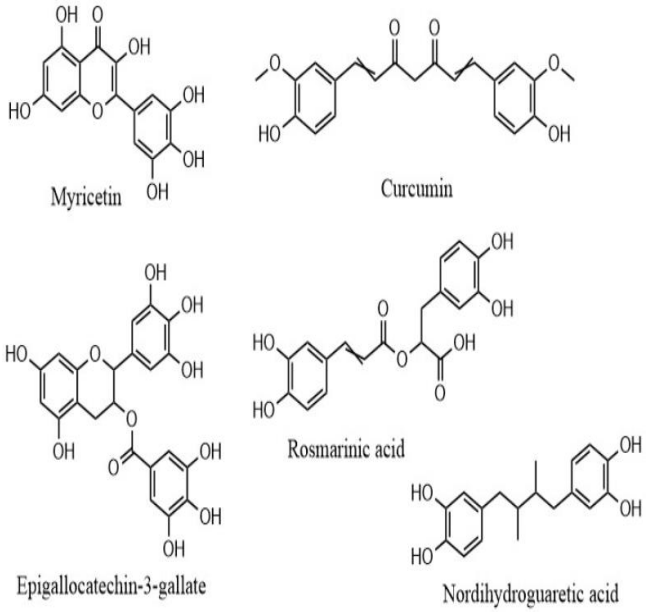

Figure 11: Chemical structures of some polyphenol compounds: myricetin, curcumin, epigallocatechin 3 gallate, rosmarinic acid and nordihydroguairetic acid.

The feature perspective is that developing anti-PD drugs is likely to be costly and time-consuming. It is essential to utilize theoretical prediction techniques (molecular simulations) and clinical investigations to reduce the cost and time of developing new agents. Individual differences and genes component makes the approach to PD treatment difficult and has failed modification of the disease pathophysiological pathways for effective treatment; therefore, the precision medicine concept will be a viable method to detect the disease at earlier onset for proper prevention rather than proper prevention one approach treatment way. [98] Although few synthetic compounds are indicated in deep brain stimulation targeting leucine-rich repeat kinase for PD treatment, it is recommended to search and develop natural compounds that could help in deep brain stimulation in the future will be essential for PD treatment [98, 99]. Treatment of Parkinson's disease needs specific compounds targeting the neurotoxic component of Lewy bodies (α-synuclein) or the matured fibrils. However, computational modelling has a lot of roles to play in modelling natural compounds. The compounds are numerous and not exhaustive; below are some recommendations that might be applied to address the growing ineffective treatment of natural compounds in Parkinson's disease treatment:

i. Computational modelling can be employed on known natural compounds against Parkinson's disease to get other possible compounds with better activity and specificity. This can be achieved through careful selection of compounds through molecular docking.
ii. Search for other plants that are not exploited, especially the newly bioactive compounds isolated from plants, so that the unpleasant side effects of the synthetic compounds can be reduced.
iii. A combination of different compounds, either natural or synthetic, to inhibit the neurotoxic oligomers will help in the curative treatment of Parkinson's disease.

Conclusion

Although α-synuclein toxicity has been implicated in PD development for a long time, understanding its functions, structural modifications such as aggregation, propagation, misfolding, and phosphorylation are essential for developing effective therapeutic alternatives for PD. Out of the six highlighted mutations in α-synuclein, three (A30P, E46K, and A53T) are commonly identified with the capability of forming fibrils more quickly than the wild-type α-synuclein protein, providing insights into an alternative therapeutic target for PD. It is important to note that neuroprotective compounds bind to C-terminals while neurotoxic compounds bind to the N-terminal of α-synuclein. Therefore, compounds targetting C-terminals are more effective.
Interestingly, natural products such as myricetin, curcumin, rosmarinic acid, NDGA, ferulic acid, crocin, baicalein, anle138b and EGCG strongly inhibit the formation of α-synuclein fibrils as well as the oligomers by binding to the C-terminal chain. Interestingly, most of the reported inhibitors are present in vast natural resources, and therefore, the application of simulation techniques reduces cost and aids cheap accessibility of potential compounds. Most of the reported experiments progressed through *in vitro* models where the physiological side effects cannot be succinctly observed. Thus, it is important to evaluate these compounds further using various *in vivo* studies for incorporation into basic and clinical experiments.
This review highlights the implications of α-synuclein in the pathogenesis of PD and some bioactive molecules as therapeutic options for effective prevention and treatment of the disease amenable for further translational study.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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