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Repurposing of Tetracyclines: Beyond Antibacterial Activity—Neuroprotective Mechanisms and Molecular Modification Strategies for Parkinson's Disease

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Abstract

Parkinson's disease (PD) is the second most common progressive neurodegenerative disorder, characterised by the loss of dopaminergic neurons and associated with pathological processes including α-synuclein (α-Syn) aggregation, neuroinflammation and oxidative stress. Most current drugs, such as levodopa and dopamine agonists, focus on restoring dopaminergic signalling and alleviating motor symptoms rather than preventing neuronal degeneration, which creates a need for disease-modifying neuroprotective strategies. Tetracyclines (TCs), particularly doxycycline (DOX) and minocycline (MIN), are a long-established family of broad-spectrum antibiotics; however, growing preclinical evidence suggests that they possess neuroprotective activities that are independent of their antibacterial effects, including the regulation of α-Syn aggregation. Their long history as antibiotics provides established safety profiles and pharmacokinetic properties, making them attractive candidates for drug repurposing in PD. Nevertheless, their antibacterial activity remains a major limitation for long-term neurological application because of concerns regarding antimicrobial resistance and microbiome disruption. Molecular modification strategies have therefore been explored to separate antibacterial functions from neuroprotective properties. This review compares the neuroprotective effects and pharmacological properties of DOX and MIN, showing their different mechanisms of action in PD models, including MIN-mediated regulation of neuroinflammation and DOX-associated modulation of α-Syn aggregation. This review further explores molecular modification strategies for DOX, such as the generation of DDOX, designed to remove antibacterial functions while preserving neuroprotective activity. Despite positive findings in experimental models, further studies are needed to confirm whether these neuroprotective effects can benefit patients with PD.

Keywords

tetracycline repurposing, Parkinson's disease, minocycline, doxycycline, α-synuclein aggregation, neuroinflammation, blood–brain barrier

1. Introduction

PD is classified as a neurological disorder. In PD, dopamine, an inhibitory neurotransmitter that is responsible for maintaining the balance with acetylcholine to prevent excessive excitation and ensure smooth

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movements, becomes insufficient. Motor symptoms such as bradykinesia, rigidity, resting tremor and postural instability, as well as non-motor symptoms such as olfactory dysfunction, sleep disturbances and depression, are consequences of PD caused by dopamine deficiency.

Current treatments for PD, such as levodopa and dopamine agonists, primarily provide symptomatic relief by restoring dopamine levels, but they do not halt or slow the underlying neurodegeneration. Long-term use can even cause motor fluctuations owing to a disrupted homeostatic balance. Neuroprotective drugs that can prevent neuronal degeneration therefore remain an unmet need.

Among potential candidates, TCs — a well-established antibiotic family — have recently attracted attention for their neuroprotective properties. In 1953, Lloyd Conover pioneered the substitution of hydrogen for the chlorine atom in aureomycin, obtaining tetracycline (TC) with stronger activity and fewer side effects, which was followed by the development of doxycycline (DOX) and minocycline (MIN) a few decades later. All tetracycline antibiotics share a common core scaffold — four linearly fused six-membered rings, named the A, B, C and D rings in sequential order. This scaffold forms a rigid, planar, hydrophobic π-system with conformational restriction, which enables the molecule to interact with multiple biological targets and thereby determines its intermolecular interactions. TCs inhibit bacterial protein synthesis by binding to the 30S ribosomal subunit [1]. Over decades of clinical trials, TCs have acquired well-established safety profiles and favourable pharmacokinetic properties, especially the ability to cross the blood–brain barrier (BBB), which makes them attractive for repurposing in central nervous system disorders [2]. In recent years, evidence has shown that TCs possess anti-inflammatory, antioxidant and anti-apoptotic properties [2]. The chemical structure of the tetracyclic core underpins this multifunctionality: the upper region, particularly the C4 dimethylamino group, is responsible for antibacterial activity, whereas the oxygen-rich lower region, containing phenolic hydroxyls, a β-diketone and a keto–enol system, mediates metal chelation, ROS scavenging and matrix metalloproteinase (MMP) inhibition [1, 3]. This multifunctionality within a single core is a valuable property for repurposing TCs in neurodegenerative diseases such as PD, in which oxidative stress, neuroinflammation and apoptosis are core pathological mechanisms.

Among TCs, DOX and MIN are the most studied for PD. DOX has been shown to inhibit α-synuclein aggregation and seeding, whereas MIN demonstrates potent inhibition of microglial pro-inflammatory cytokine release. Both drugs can effectively mitigate the damage caused by oxidative stress and neuroinflammation-triggered apoptosis.

However, TCs are fundamentally antibiotics, which are unnecessary when treating PD and may even exert a negative effect: in patients who take these drugs over the long term, the antibiotic component would nurture strongly resistant bacteria, which is extremely harmful. Molecular modification strategies are therefore required to separate their neuroprotective effects from their antibiotic action.

2. Tcs as Neuroprotective Agents: Targeting PD Pathology and Symptoms

2.1 PD Pathological Mechanisms

PD pathology involves three interconnected mechanisms: α-synuclein aggregation, oxidative stress and neuroinflammation.

The pathological process begins with the misfolding of α-synuclein and eventually causes seeding-induced aggregation. As α-synuclein aggregates, oligomers are formed as the first toxic intermediate and the one that damages dopaminergic neurons the most, because they are small, soluble and highly mobile and can therefore penetrate cell membranes, damage mitochondria and disrupt calcium homeostasis. As aggregation continues, protofibrils are formed, followed by mature fibrils and finally Lewy bodies. Interestingly, as their size increases their mobility decreases, and consequently their neurotoxicity declines, since they can no longer easily penetrate the cell membrane to damage dopaminergic neurons.

Besides directly damaging the dopaminergic cell membrane, these toxic aggregates trigger oxidative stress. The core function of mitochondria is to generate ATP through the electron transport chain (ETC), transferring electrons from Complex I to Complex IV and ultimately combining them with oxygen to form water. When the mitochondrial membrane is attacked by oligomers, impairment of Complex I in the electron transport chain obstructs electron flow, causing premature reactions between electrons and oxygen that produce incomplete

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reduction products known as reactive oxygen species (ROS), including superoxide anion, hydrogen peroxide and the highly reactive hydroxyl radical [4]. ROS attack neurons through multiple mechanisms: they induce lipid peroxidation, leading to membrane destruction and increased permeability, which makes neurons more vulnerable; they cause oxidative modifications of proteins, resulting in enzyme denaturation and accelerating protein misfolding; and they inflict oxidative damage on DNA, triggering mutations and activating apoptotic signalling pathways.

In addition to oxidative stress, α-synuclein aggregates also trigger neuroinflammation. Aggregates are recognised by microglia, the immune cells of the central nervous system (CNS), which are activated to release pro-inflammatory cytokines such as TNF-α and IL-1β. These cytokines can directly activate death receptors on neurons, induce excitotoxicity that triggers neuronal apoptosis, and further influence mitochondrial function. Importantly, inflammatory signals also promote ROS production, while ROS enhance microglial activation, establishing a vicious loop between oxidative stress and neuroinflammation.

2.2 Neuroprotective Mechanisms of TCs

2.2.1 Anti-inflammatory Mechanism

TCs reduce microglial activation and alleviate the release of pro-inflammatory cytokines such as TNF-α and IL-1β, thereby protecting dopaminergic neurons [2]. At the molecular level, the tetracyclic core interferes with NF-κB signalling, a transcription factor, by blocking the phosphorylation and degradation of its inhibitor IκBα, thereby preventing the nuclear translocation of NF-κB and suppressing the transcription of pro-inflammatory genes [5]. In addition, microglia are activated when the intercellular free Ca^{2+} concentration is high; however, the β-diketone in the tetracycline structure chelates divalent cations such as Ca^{2+}, reducing the intracellular free Ca^{2+} concentration and suppressing the inflammatory response [3].

2.2.2 Antioxidant Mechanism

Tetracyclines act as strong ROS scavengers: the phenolic hydroxyl groups (–OH) donate hydrogen atoms to ROS free radicals, forming phenoxyl radicals that are stabilised by resonance delocalisation through the π-system of the ring, thereby terminating radical chain reactions [3]. Moreover, redox-active metal ions such as Fe^{2+} and Cu^{2+} are a second key messenger for microglial activation, and the β-diketone of TC at C11–C12 acts as a bidentate ligand, forming six-membered chelation rings [3]. This chelation impedes Fenton chemistry ($Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^- + \cdot OH$) at its source, preventing further generation of the hydroxyl radical [3].

2.2.3 Anti-apoptotic Mechanism

Oxidative stress and inflammatory signals act together to trigger the opening of the mitochondrial permeability transition pore (mPTP), a channel in the inner mitochondrial membrane [6]. When mPTP opens, the resulting full permeability abolishes the membrane's function and causes the leakage of cytochrome c into the cytosol, where caspase-9 and subsequently caspase-3 are activated, initiating the apoptotic signal that leads to neuronal death [6]. By preventing mPTP opening, tetracyclines consolidate mitochondrial membrane integrity and halt the apoptotic cascade [6]. To stabilise the packed conformation of the pore, the hydrophobic planar structure of the tetracyclic core engages in π–π stacking and hydrophobic interactions with the hydrophobic grooves of mPTP proteins (e.g., adenine nucleotide translocase and cyclophilin D) [6]. Simultaneously, the phenolic hydroxyl groups form hydrogen bonds with polar residues on mPTP proteins, preventing pore opening under oxidative stress [6].

3. Comparative Neuroprotective Mechanisms of DOX and MIN in PD Models

3.1 MIN-mediated Regulation of Neuroinflammation

In addition to the progressive loss of dopaminergic neurons, neuroinflammation has increasingly been recognised as an important factor in the pathogenesis and progression of PD [7]. Microglia, the resident immune cells of the CNS, normally participate in maintaining neural homeostasis and providing immune defence in the neural environment. Nevertheless, excessive activation of microglia transforms this protective response into a detrimental inflammatory process.

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According to Kim and Joh [8], activated microglia can induce neuronal toxicity through the release of inflammatory mediators, including IL-1β, TNF-α, IL-6, nitric oxide (NO), prostaglandin E2 (PGE2) and superoxide. In PD models, pathological α-Syn accumulation can activate microglial responses and promote the release of pro-inflammatory mediators, resulting in a chronic neuroinflammatory environment associated with progressive dopaminergic neuronal degeneration [9].

Strategies that target neuroinflammation have attracted increasing attention because microglial activation is important in PD progression. MIN is a second-generation TC antibiotic that exhibits neuroprotective properties beyond its antimicrobial activity, including anti-inflammatory, antioxidant and anti-apoptotic effects [10]. MIN modulates the activation state of microglia rather than broadly suppressing the immune response, thereby limiting excessive neuroinflammation while preserving neuronal viability [11].

This mechanism was demonstrated in an adeno-associated virus (AAV)-mediated human mutant α-synuclein (hmαSyn) mouse model established by Wang et al. [12]. Twelve weeks after α-Syn overexpression, the number of tyrosine hydroxylase (TH)-positive neurons in the substantia nigra decreased significantly, from 11,045 ± 534 cells in control mice to 5,503 ± 451 cells ($P < 0.01$). Following MIN treatment, TH-positive neurons increased to 8,750 ± 412 cells ($P < 0.05$). Similarly, striatal TH-positive fibre density had declined to approximately 30% of the control level and increased 2.5-fold after treatment. MIN also restored striatal dopamine and 3,4-dihydroxyphenylacetic acid (DOPAC) concentrations, which had been reduced to approximately 50% and 44% of control values, respectively. These findings suggest that modulation of microglial activation alleviates α-Syn-induced neuroinflammation and preserves the integrity of the nigrostriatal dopaminergic pathway.

The neuroprotective effects of MIN are further supported by studies exploring the molecular pathways behind microglia-mediated neuronal damage. Shah et al. [13] found that MIN can suppress inflammatory and oxidative signalling pathways, thereby reducing the production of neurotoxic mediators released from activated microglia. To support these mechanistic observations, Wu et al. [14] evaluated MIN in an MPTP-induced mouse model of PD, in which mice received four injections of MPTP followed by MIN treatment (45 mg/kg, twice daily). MPTP exposure triggered greater than 55% depletion of TH-positive substantia nigra neurons. In contrast, MIN robustly alleviated dopaminergic neuron degeneration in a dose-dependent manner.

In a milder model induced by four injections of16 mg/kg MPTP, MIN provided approximately 90% neuroprotection against dopaminergic neuronal loss. At the mechanistic level, MIN inhibited microglial activation, suppressed the formation of mature interleukin-1β (IL-1β), reduced inducible nitric oxide synthase (iNOS) activity and NADPH oxidase activation, and significantly decreased nitrotyrosine levels in the ventral midbrain from 32.2 ± 6.0 to 21.8 ± 1.8 pg/μg protein, approaching the control level of 16.2 ± 1.3 pg/μg protein ($P < 0.05$). Evidence from α-Syn and MPTP models demonstrates that MIN provides neuroprotection by suppressing microglia-driven inflammatory and oxidative pathways, thus limiting progressive dopaminergic neurodegeneration in PD.

3.2 DOX-associated Modulation of α-Syn Aggregation

Unlike MIN, DOX mainly targets the abnormal aggregation of α-Syn, a key pathological hallmark of PD. Soluble α-Syn oligomers are considered the most neurotoxic species because they disrupt mitochondrial function and propagate pathology through seeding mechanisms [15]. Attention has therefore increasingly shifted towards remodelling toxic aggregation intermediates rather than simply preventing protein accumulation.

Transmission electron microscopy (TEM) analysis revealed that untreated samples developed abundant mature fibrils after 96 h, whereas DOX-treated samples predominantly contained non-fibrillar oligomeric aggregates. Solid-state nuclear magnetic resonance (NMR) further showed that DOX preferentially interacted with early aggregation intermediates, redirecting them into off-pathway oligomers that failed to mature into amyloid fibrils. These remodelled oligomers also exhibited markedly reduced cytotoxicity and seeding capacity, suggesting that DOX decreases the pathogenicity of α-Syn aggregates rather than simply reducing their abundance [16]. The relationship between α-Syn aggregation remodelling and long-term neuronal survival requires further investigation, since reducing aggregation does not necessarily mean completely preventing disease progression.

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The ability of DOX to remodel α-Syn aggregates was further confirmed by Dominguez-Meijide et al. [17]. Real-time quaking-induced conversion (RT-QuIC) assays showed a dose-dependent reduction in ThT fluorescence, while electron microscopy showed a transition from fibrillar structures to predominantly non-fibrillar aggregates at 10 μM DOX. DOX also reduced the seeding activity of preformed α-Syn aggregates and lowered mitochondrial ROS production in differentiated SH-SY5Y cells, indicating that remodelling protein aggregation can alleviate downstream oxidative stress. Importantly, differences between experimental concentrations and achievable drug exposure in humans should be considered when evaluating the therapeutic potential of DOX.

In addition to directly targeting α-Syn aggregation, DOX also modulates neuroinflammatory pathways. Cho et al. [18] reported that 10 μM DOX completely abolished lipopolysaccharide (LPS)-induced matrix metalloproteinase-3 (MMP-3) expression in BV-2 microglia. Furthermore, NO production, which increased to 8.7 ± 0.7-fold following LPS stimulation, was reduced to 58% and 35% of the LPS-treated level after treatment with 5 μM and 10 μM DOX, respectively. DOX therefore provides complementary neuroprotection by simultaneously remodelling toxic α-Syn species, suppressing oxidative stress and alleviating neuroinflammation.

3.3 Pharmacological Comparison Between DOX and MIN

Although DOX and MIN share a similar TC scaffold, differences in their pharmacological properties contribute to distinct therapeutic characteristics. Comparing these derivatives provides insight into how structural variation within the same drug family influences pharmacological behaviour and drug development potential [19, 20].

Table 1: Pharmacological Comparison of Doxycycline and Minocycline

Pharmacological parameter	Doxycycline (DOX)	Minocycline (MIN)	Pharmacological significance
Molecular formula	$C_{22}H_{24}N_2O_8$; tetracycline scaffold containing a 6-deoxy modification	$C_{23}H_{27}N_3O_7$; tetracycline scaffold with an additional dimethylamino modification	Structural differences alter molecular properties and contribute to different pharmacological profiles
Lipophilicity	LogP = −0.2	LogP = 0.5	Higher lipophilicity of MIN may influence membrane interaction and tissue distribution
Pharmacokinetic profile	Half-life: 15 h; protein binding: 85%	Half-life: 20 h; protein binding: 90%	Differences affect drug exposure and duration of pharmacological action
Drug development relevance	Provides a well-characterised scaffold for further optimisation	Represents a structurally modified tetracycline derivative with distinct pharmacological properties	Comparison helps identify desirable characteristics for next-generation derivatives

Note: Physicochemical data adapted from [19].

Based on Table 1, TC derivatives with the same main structure can have different pharmacological profiles. Differences in molecular properties and pharmacokinetic behaviour influence their suitability as therapeutic candidates. Understanding the relationship between TC structure and pharmacological characteristics is therefore important for optimising derivatives for neurological applications.

4. Limitations of TCs for Long-term Neurological Application

4.1 Antibacterial Activity and Antimicrobial Resistance

Despite the neuroprotective properties of MIN and DOX, their antibacterial activity remains a major limitation for their long-term application in PD. TCs exert antibacterial effects by inhibiting bacterial protein synthesis through binding to the 30S ribosomal subunit and preventing aminoacyl-tRNA entry into the ribosomal A site [21]. This activity is associated with their chemical structure. The C4 dimethylamino group is important for antibacterial activity, and modifications at this position can reduce antimicrobial potency [19]. However, some structural features involved in antibacterial activity also contribute to other biological effects,

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creating a challenge in selectively removing antibacterial function while preserving neuroprotective properties. This creates a pharmacological challenge, because the same molecular structure may contribute to both therapeutic effects and undesirable antibacterial activity.

Long-term application of TCs can promote the development of antimicrobial resistance (AMR) by creating continuous selective pressure on bacterial populations. Historical analysis has shown that TC resistance was initially uncommon, with only approximately 2% of 433 Enterobacteriaceae isolates collected between 1917 and 1954 showing resistance, but resistance became increasingly prevalent after clinical use expanded [21]. At the molecular level, AMR occurs mainly through two mechanisms. Efflux proteins encoded by tet genes reduce intracellular TC concentrations by exporting TC–cation complexes; ribosomal protection proteins, including Tet(M) and Tet(O), interact with bacterial ribosomes and induce conformational changes that reduce tetracycline binding while allowing protein synthesis to continue [21, 22]. These resistance mechanisms show that maintaining antibacterial activity may limit the application of conventional TCs in long-term neurological treatment.

4.2 Microbiome Disruption and Clinical Translation Challenges

In addition to AMR, long-term TC application affects the gut microbiome. Because TCs are broad-spectrum, they can influence both pathogenic bacteria and commensal microorganisms, leading to changes in microbial diversity and in the abundance of antimicrobial resistance genes (ARGs). According to Fishbein et al. [23], antibiotic exposure can cause persistent alterations in microbial communities, with recovery remaining incomplete after treatment.

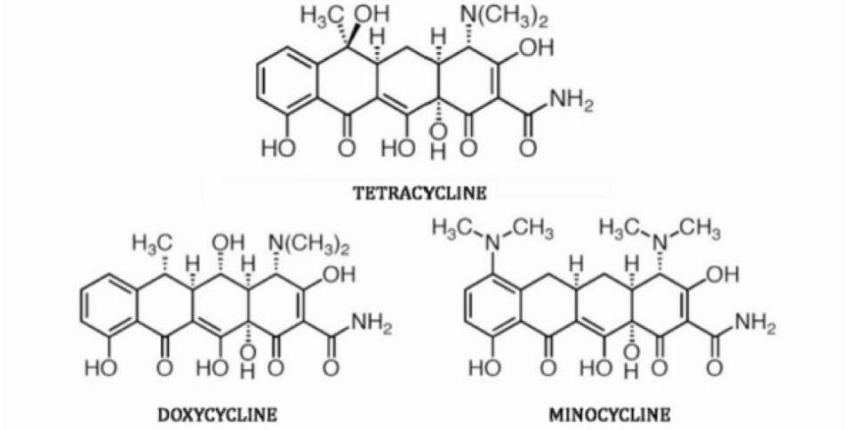
Experimental evidence also supports the impact of tetracyclines on microbial ecosystems. Yin et al. [24] reported that TC treatment changed the composition of the gut microbiota in mice, reducing the number of detected bacterial genera from 194 to 89. It also increased Firmicutes abundance from 24.9% to 39.8% while decreasing Bacteroidetes abundance from 69.8% to 51.2%. The overall abundance of ARGs increased from approximately 307.3 ppm to 1492.7 ppm, suggesting that TC exposure can expand resistance reservoirs within the gut microbiome.

Although DOX and MIN have shown promising neuroprotective effects in PD models, their clinical translation remains limited because current evidence is mainly obtained from cellular and animal studies [20]. Further modification of TC structures is therefore required to reduce antibacterial activity while maintaining their neuroprotective effects, providing the basis for the development of modified DOX derivatives.

5. Molecular Modification Strategies for Tetracyclines

5.1 Functional Region Division

Figure 1: Mechanisms of tetracycline resistance. Reproduced from [3] (Figure 1, p. 29)



The tetracycline molecule can be divided into two functionally distinct halves, and the upper and lower halves handle different biological functions.

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The upper half carries the structural determinant of antibacterial activity: the dimethylamino group, $–N(CH_3)_2$, a tertiary amine with a basic nitrogen centre. The nitrogen atom carries a lone pair of electrons. At physiological pH (7.4), this lone pair on the nitrogen accepts a proton from water, converting the neutral amine into a protonated ammonium ion ($–NH^+(CH_3)_2$). This converts the entire tetracycline molecule into a zwitterionic form. The resulting ammonium cation then associates with bacterial rRNA through three synergistic interactions.

First, the phosphodiester backbone of rRNA carries one negative charge per three nucleotides within the ribosome binding pocket. These closely packed anions form a negatively charged patch, and the protonated ammonium group is captured by non-specific electrostatic attraction to this patch. In addition, the binding is further stabilised by a magnesium ion that mediates salt bridge formation between the drug and the rRNA phosphate backbone [25, 26]. This electrostatic interaction is followed by a second, more specific interaction: a hydrogen bond network. The electrostatic interaction and the hydrogen bond network exert synergistic effects, resulting in a negative binding free energy of the tetracycline–ribosome complex and rendering the complex thermodynamically stable.

The hydrogen bond network is highly directional. Three hydrogen bond donors on the tetracycline molecule — the protonated ammonium at C4, the hydroxyl group on the A-ring, and the keto–enol tautomer — pair with the phosphoric acid or the ketonic oxygen, nitrogen and oxygen atoms of the 16S rRNA bases, respectively. A third type of interaction arises from the physical properties of the tetracycline skeleton itself: π–π stacking. The four fused rings form a rigid, planar aromatic π-system, generating delocalised electron density above and below the ring plane. The nucleotide bases of 16S rRNA, especially adenine and guanine, are also planar aromatic heterocycles with delocalised π-electron clouds. When the two aromatic systems approach each other in a face-to-face orientation, their π-electron clouds overlap, giving rise to a hydrophobic effect. The vertical distance between the two aromatic rings is typically 3.3–3.8 Å, and the superposition of multiple stacking interactions yields a significant stabilising effect. Importantly, π–π stacking depends on the planar conformation and π-electron density of the tetracycline core rather than on the C4 substituent. As a result, neuroprotective activity can be retained even if C4 is modified in a later step. Taken together, these non-covalent interactions stabilise ribosomal binding, block the binding of tRNA to mRNA codons and inhibit protein synthesis.

The dimethylamino group is the structural determinant of antibacterial activity: it protrudes from the A-ring with a specific stereochemical orientation and precisely matches the steric complementarity of the ribosome binding pocket. If the group is removed or modified, antibacterial activity will be significantly attenuated or even completely abolished.

The lower half of the molecule, focused on the C- and D-rings, mediates neuroprotective activity primarily through metal chelation. At C11–C12, a β-diketone structure is present. Two carbonyl groups are separated by a methylene group, and the hydrogen atoms on this methylene group are acidic. In the presence of metal ions, these protons can be removed, followed by electron delocalisation and equalisation of the two oxygen atoms, forming a keto–enol tautomer. The β-diketone at C11–C12 therefore exists in its enolate form, in which the two oxygen atoms become equivalent coordinating atoms. The spatial distance between them (approximately 2.5–2.7 Å) matches the coordination geometry of various divalent metal ions. The alternation of single and double bonds between C11 and C12 forms a conjugated system that acts as a bidentate ligand, coordinating with metal ions through both oxygen atoms simultaneously to form a six-membered chelation ring. The chelation targets include iron, copper and zinc ions. This inhibits the Fenton reaction, reducing hydroxyl radical generation; attenuates oxidative catalysis; and suppresses MMP activity.

The second function of the lower half is radical scavenging. The phenolic hydroxyl group at C10 serves as the active centre. It donates a hydrogen atom to free radicals such as the hydroxyl radical, converting them into stable closed-shell molecules. The phenolic compound itself forms a phenoxyl radical, which is stabilised by π-conjugation delocalisation through the substituents on the A-ring, thereby terminating the radical chain reaction. This mechanism underlies the antioxidant activity of tetracyclines.

The third function is stabilisation of the mitochondrial membrane. The hydrophobic planar scaffold of tetracycline undergoes hydrophobic interactions and π–π stacking with the hydrophobic grooves of mitochondrial membrane proteins. This stabilises the closed conformation of the membrane permeability transition pore, inhibits cytochrome c release and suppresses apoptosis.

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In summary, the phenolic hydroxyl groups and the β-diketone in the lower half are the functional effectors of neuroprotective activity, and this region must be retained during molecular modification.

5.2 The SAR Insight: Functional Decoupling

The two functional regions of the tetracycline molecule are relatively independent. There is no direct covalent linkage or strong electronic coupling between the C4 dimethylamino group on the A-ring and the β-diketone or phenolic hydroxyl group in the lower half. They are separated by sp^3-hybridised carbon atoms of the B- and C-rings, resulting in low electron transfer efficiency between the two regions. This structural separation indicates that selective chemical modification can be performed on the upper region without disturbing the active centres in the lower region. This is the chemical basis for functional decoupling.

Based on this reasoning, the proposed modification strategy is designed to selectively convert the dimethylamino group at C4 into a hydrogen atom via reductive deamination, while leaving the rest of the molecule intact, especially the functional groups in the lower half. In this reaction, the C–N bond at C4 is cleaved, the dimethylamino group leaves as dimethylamine and the C4 site is replaced by hydrogen. This transformation is regioselective, which suggests that it occurs only at the C4 position of the A-ring, because the C–N bond at this site has a relatively low bond dissociation energy and is cleaved preferentially under reductive conditions. In contrast, the phenolic hydroxyl groups and β-diketone structures on the C- and D-rings in the lower half lack similar reactive functional groups and remain chemically inert under the same mild reductive conditions. This reaction proceeds under mild conditions with neutral pH and low temperature, which does not affect the β-diketone structure, allowing it to remain intact. The selective modification of the C4 position is therefore highly chemically feasible.

5.3 Historical Precedent: The Chemically Modified Tetracyclines (CMTs)

As early as the 1980s, researchers recognised that the antibacterial and non-antibacterial activities of tetracyclines are likely regulated by distinct structural positions of the molecule. Based on this hypothesis, they synthesised a series of tetracycline derivatives with the dimethylamino group removed at the C4 position, defined as chemically modified tetracyclines (CMTs). Among these derivatives, CMT-3 is the most well-known member [3].

Table 2: Comparison of Tetracycline and the Chemically Modified Analogue CMT-3

Properties	Tetracycline	CMT-3	Alteration
C4-position group	$–N(CH_3)_2$	–H	The dimethylamino group is substituted by hydrogen
Antibacterial activity	~1–2 μg/mL	>100 μg/mL	↓ 99%
MMP inhibitory activity	Present	Retained	Almost unchanged
Anti-inflammatory activity	Present	Retained	Almost unchanged

Note: MMP: matrix metalloproteinase.

Experimental evidence has confirmed that CMT-3 can effectively inhibit matrix metalloproteinase-2 (MMP-2) gelatinolytic activity and reduce MMP-9 release in vitro, demonstrating that its non-antibiotic therapeutic potential is retained [27]. Among all the tetracyclines and their chemically modified non-antibiotic analogues tested, CMT-3 has repeatedly been shown to be the most potent inhibitor of MMP activity and cytokine production [28]. The loss of antibacterial activity can be attributed to the substitution of the C4 dimethylamino group with hydrogen, which removes a key hydrogen bond donor from the molecule. The therapeutic potential of CMT-3 extends beyond enzyme inhibition: it suppresses tumour cell invasion and metastasis in vivo, effects attributed directly to its MMP inhibitory activity rather than to antimicrobial action [29]. The electrostatic complementarity required for ribosomal binding is therefore disrupted, and the binding free energy (ΔG) shifts from negative to positive, meaning that the drug can no longer bind stably to the ribosomal pocket. At the same time, neuroprotective activity is retained because the phenolic hydroxyl group and the β-diketone in the lower half remain unaffected by the C4 modification. The metal-chelating ability is preserved: the chelation constant of CMT-3 for Zn^{2+} is essentially identical to that of the parent compound. Radical scavenging capacity is also retained, since the bond dissociation energy of the phenolic hydroxyl group is unchanged. The success of CMT-3 demonstrates that C4 deamination is a feasible chemical modification pathway, and this derivative retains therapeutic potential while eliminating antibacterial side effects.

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5.4 Defining DDOX: A C4-dedimethylamino Doxycycline Derivative

DDOX (4-demethylamino-12a-deoxydoxycycline) has been proposed as a chemically modified doxycycline derivative in which the C4-dimethylamino group of doxycycline is replaced with a hydrogen atom via selective reductive deamination at the C4 position. It has been experimentally investigated as a non-antibiotic tetracycline analogue for Parkinson's disease.

Although the feasibility of this strategy has been verified by CMT-3, doxycycline (DOX) offers three chemical advantages as a parent structure. First, it has a higher Zn^{2+} affinity. Owing to the deoxygenation modification at C5 and C6, DOX shows a higher chelating affinity for Zn^{2+} than tetracycline (TC), suggesting that DDOX may outperform CMT-3 in MMP inhibition. Second, it is more lipophilic. The Log P of DOX (approximately 0.6) is higher than that of TC (approximately 0.2), and the Log P of DDOX is further elevated after C4 deamination. This indicates higher lipophilicity and facilitates blood–brain barrier (BBB) penetration [30]. Third, it has fewer oxidation sites. DOX lacks the C5-OH and C6-OH groups present in TC, which reduces potential oxidative metabolism sites and may endow DDOX with superior metabolic stability. DOX was therefore selected as the parent structure for modification rather than TC.

Notably, a recent study by Teran et al. [31] directly investigated DDOX and demonstrated that it inhibits α-Syn aggregation with an IC_{50} of 13.4 μM, prevents the cellular uptake of α-Syn preformed fibrils and mitigates lysosomal stress in cellular models. Furthermore, a recent preclinical study by Borges et al. [32] demonstrated that DDOX confers neuroprotection and alleviates motor deficits in a rat model of Parkinson's disease, providing in vivo evidence that C4-demethylamino modification retains therapeutic efficacy. These findings provide experimental support for the chemical rationale proposed in this review, confirming that C4 deamination of DOX yields a derivative with retained neuroprotective activity and without antibacterial function.

6. Conclusion

Drug repurposing is a meaningful approach to addressing unmet medical needs by discovering new therapeutic possibilities within existing medicines. The study of TCs in PD reflects how a well-established antibiotic can be reconsidered and redesigned to provide more treatment options for neurodegenerative diseases. Although DOX and MIN share a similar TC structure, their distinct pharmacological properties give them different neuroprotective mechanisms: MIN mainly regulates neuroinflammation, whereas DOX influences α-Syn aggregation. These findings reveal an important connection between molecular structure and biological function that can be exploited for more effective treatments. The development of TCs for neurological applications also reveals a broader challenge in modern drug design: therapeutic benefits and unwanted effects can coexist within the same molecular structure. The antibacterial activity that originally defined TCs also creates limitations through AMR and microbiome disruption. Molecular modification thus provides a pathway to refine existing molecules by preserving beneficial properties while reducing undesirable effects. Ultimately, combining drug repurposing with rational molecular design can transform previously established medicines into safer and more targeted therapies, creating new possibilities for patients affected by complex neurodegenerative disorders such as PD.

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Conflicts of Interest

The authors declare no conflict of interest.

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