The current status of pharmacotherapy for the treatment of Parkinson's disease: transition from single-target to multitarget therapy

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Parkinson's disease (PD) is a neurodegenerative disorder characterized by degeneration of dopaminergic neurons. Motor features such as tremor, rigidity, bradykinesia and postural instability are common traits of PD. Current treatment options provide symptomatic relief to the condition but are unable to reverse disease progression. The conventional single-target therapeutic approach might not always induce the desired effect owing to the multifactorial nature of PD. Hence, multitarget strategies have been proposed to simultaneously target multiple proteins involved in the development of PD. Herein, we provide an overview of the pathogenesis of PD and the current pharmacotherapies. Furthermore, rationales and examples of multitarget approaches that have been tested in preclinical trials for the treatment of PD are also discussed.

Introduction

Parkinson's disease (PD) is a progressive nervous system disorder that considerably affects the mobility of patients. It is characterized by selective loss of dopaminergic neurons in the *substantia nigra* of the human brain, resulting in depletion of dopamine production [1]. Presently, therapeutic options for PD mainly rely on the use of pharmacological agents to improve the cardinal motor symptoms such as tremor, rigidity, bradykinesia and postural instability. These motor features are often accompanied with other cognitive impairments and psychiatric symptoms in PD patients [2]. To date, there is no treatment to stop or at least slow down the progression of the disease [3,4]. Most of the current drugs for PD are selective compounds that target individual proteins (i.e., one-compound–one target), particularly the dopamine receptors. Among these pharmacotherapies, dopamine replacement therapy represents the major therapeutic approach to restore the dopamine level to alleviate motor symptoms 4]. Other adjuvant drugs are also used in clinical practice to increase the activity of the

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Giampiero Spalluto received his degree in chemistry and pharmaceutical technology in 1987 from the University of Ferrara, Italy. He obtained his PhD in organic chemistry from the University of Parma in 1992. Between 1995 and 1998, he worked at the University of Ferrara as assistant professor of medicinal chemistry. From November 1998 to December 2012 he occupied a position of associate professor at the University of Trieste. Since December 2012, he has held the position of full professor of medicinal chemistry at the same university. His scientific interests have focused on the enantioselective synthesis of natural compounds and medicinal chemistry of ligands for adenosine receptor subtypes, kinase inhibitors and

dopamine system, or to prevent the metabolism of dopamine by endogenous enzymes, thus increasing the dopamine concentration in the brain [3].

To date, various mechanisms of neuronal degeneration in PD have been proposed, which are shown to overlap and influence one another. This has challenged the dominant single-target approach in the treatment of PD in view of the multifactorial nature of the disease. In turn, multitarget strategies [5,6] have increasingly been considered as alternative options for the management of PD, especially when the disease progresses to the advanced stages. Interestingly, some of the existing drugs for PD have been found to possess activities at more than one pathophysiological pathway of PD. For example, biperiden is an acetylcholine muscarinic receptor antagonist and effective in improving the tremor and rigidity in PD; it has been shown to exhibit weak inhibitory activities on the enzyme acetylcholinesterase (AChE), which might be beneficial in alleviating the cognitive deficits associated with PD [7]. Similarly, talipexole is marketed as a dopamine D_2 receptor agonist for PD, while also demonstrating neuroprotective effects against apoptosis in the neuronal cells [8]. In fact, neuroprotective properties have been found in some symptom-relieving drugs for PD. The neuroprotective activities can prevent or slow down the neuronal degeneration via various mechanisms and therefore restrain the progressive loss of neurons [9]. Taken together, these observations substantiate the importance of multitarget approaches in the treatment of PD.

Among the multitarget strategies, combination therapies (i.e., cocktail-drug–multiple-targets) that combine several drugs acting independently on different pathophysiological targets have indeed been gradually adopted in clinical practice to improve the motor symptoms of PD [4,10,11]. These drugs could act on additional targets of the same pathway, or on different pathways that are involved in the pathogenesis of PD. Nevertheless, they are often associated with side effects arising from drug–drug interactions and varying pharmacokinetic or pharmacodynamic profiles of each component drug [6]. In recent years, another multitarget approach (i.e., one-compound–multiple-targets) has been introduced and regarded as a potential polypharmacological therapy for PD [5,12]. In this approach, a single drug compound is designed to simultaneously target two or more specific proteins involved in the development of PD. The single chemical entity can beneficially eliminate side effects derived from interactions among drugs in the combination therapies and could have a more predictable pharmacokinetic profile compared with multiple drugs administered in combination. It can also improve patient compliance with simple dosing schedules, which is especially advantageous in the elderly who are commonly prescribed multiple medications to control motor symptoms [13].

Overall, the multitarget drugs could represent a valuable alternative to the therapeutic regimens based on the cocktail drug combinations. In the light of the merits of this approach, many scientists have since embarked on the design and discovery of various multitarget ligands with antiparkinsonian activities. In this review, pathogenesis of PD and its current drug therapies will be summarized. In addition, the rationale of multitarget approaches and examples of multitarget drugs that have been tested so far in preclinical trials for PD will also be illustrated in detail.

Pathogenesis of PD

There are four major dopaminergic pathways, namely the nigrostriatal, mesolimbic, mesocortical and tuberoinfundibular pathways. These pathways extend onto other areas of the brain and use dopamine as the neurotransmitter. Among them, the nigrostriatal pathway originates from the *substantia nigra pars compacta* (SNpc) of the midbrain and projects diffusely into the dorsal striatum [14]. The neurodegeneration of dopaminergic neurons in the SNpc leading to depletion of striatal dopamine has been known as the main factor responsible for PD pathogenesis and the consequent sensory–motor symptoms. When the motor symptoms start to appear at the onset of PD, patients have often already lost 60% of their dopaminergic neurons from the SNpc, and striatal dopamine has been depleted by 80% [15]. Such disease has been suggested to manifest in different stages; it begins with alterations in anterior olfactory structures and loss of smell, followed by changes in *substantia nigra* and other nuclei of the basal midbrain and forebrain, resulting in psychiatric symptoms and other neurological deficits associated with PD. In the final stages, lesions occur in the prefrontal cortex with increasing cholinergic neuronal degeneration and onset of dementia [16,17].

Even though the pathophysiology of neuronal degeneration remains to be fully elucidated, many studies have proposed that the neuronal death is a multifactorial process with intertwined

antitumor agents.

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molecular events involving dopaminergic neurons, nondopaminergic neurons (e.g., cholinergic and GABAergic neurons) and non-neuronal cells (e.g., microglia, astrocytes) [18]. It has been increasingly recognized that the PD pathogenesis could be caused by a combination of cell-autonomous and non-cell-autonomous mechanisms (Fig. 1) [18]. The cell-autonomous mechanisms occur within the degenerative neurons and mainly involve mitochondrial dysfunction. Such dysfunction results in production of reactive oxygen species (ROS), which has been implicated in ageing, the development of various neurodegenerative diseases, and ischemia–reperfusion injury. This mitochondrial oxidative stress could be caused by underexpression of peroxisome proliferator-activated receptor gamma coactivator-1α (PGC-1α), which accounts for stimulation of mitochondrial electron transport and suppression of ROS. Previous studies have found that such underexpression of PGC-1α is linked to the mutation of the *parkin* gene (*PARK2*), which brings about early-onset hereditary parkinsonism [19]. By contrast, the overexpression of PGC-1α protects the neurons from oxidative damage. Notably, several genes associated with the development of PD are shown to play a part in mitochondrial function. The idea of a functional connection between derailed mitochondrial operation and the development of PD is corroborated by observations that mutations in other PD-related genes like *PARK6* (coding for PINK1, a putative mitochondrial protein kinase) and *PARK7* (coding for DJ1, a *parkin*-associated protein) also affect the mitochondrial function [20]. Leucin-rich repeat kinase 2 (LRRK2), encoded by *PARK8*, is another PD-associated protein where mutation might contribute toward mitochondrial dysfunction [21,22].

Mitochondrial oxidative stress in the SNpc dopaminergic neurons is also related to the increase in cytosolic calcium levels [23]. Distinct L-type calcium channels located on the plasma membrane of these neurons have been shown to regulate the pace-making cycle [23]. It was found that the influx of calcium ions is not necessary for pace-making, because treatment with L-type channel antagonists leaves pace-making unaffected. However, the elevation of calcium ions has led to generation of ROS and superoxide, which creates mitochondrial oxidative stress with eventual neu-

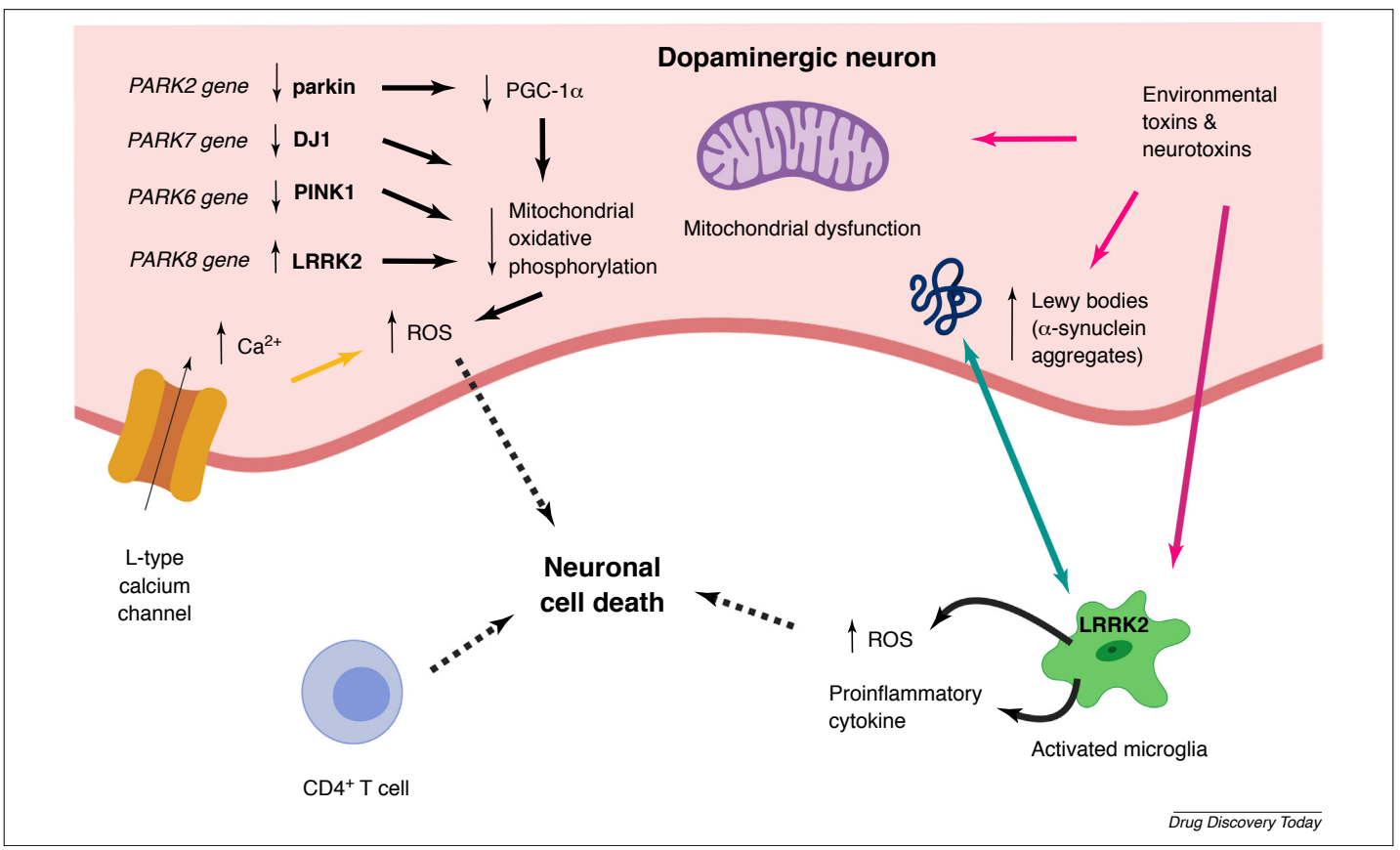

FIGURE 1

Molecular events involved in pathogenesis of Parkinson's disease (PD). Mitochondrial dysfunction with resultant production of reactive oxygen species (ROS) has been one of the possible mechanisms responsible for neuronal degeneration. The mitochondrial oxidative stress is found associated with underexpression of proliferator-activated receptor gamma coactivator-1α (PGC-1α), leading to increase of cellular ROS level and eventual cell death. The underexpression of PGC-1α is related to the mutation of the *parkin* gene (*PARK2*). Mutation of other proteins, such as PINK1 (coded by *PARK6*), DJ1 (coded by *PARK7*) and LRRK2 have also been associated with mitochondrial dysfunction. Elevation of cytosolic calcium ion levels and exposure to environmental factors, such as insecticides and neurotoxins, also results in the production of ROS. Other mechanisms include α-synuclein transfer and neuroinflammation. The soluble α-synuclein oligomers increase membrane permeability for calcium ions, leading to neurotoxicity and cell death. The immune cells, such as microglia cells surrounding the neuronal cells, are activated and release inflammatory cytokines and ROS, resulting in inflammation and oxidative stress to the neurons. Such activation can also be triggered by the release of α-synuclein oligomers, LRRK2 protein, as well as environmental toxins. $CD4^+$ T cells were also shown to be involved in neurodegeneration, and removal reduces neuronal death.

ronal death. Other environmental factors, such as exposure to neurotoxins, pesticides and insecticides, have also been associated with mitochondrial dysfunction that results in subsequent ROS formation [20].

The non-cell-autonomous mechanisms occur outside the degenerative neurons involving cellular interactions, and mainly encompass α-synuclein transfer and neuroinflammation [18,24]. α-Synuclein is a cellular protein abundantly found in the Lewy bodies (LBs). The LBs are often observed in the surviving dopaminergic and nondopaminergic neurons of affected PD brains [25,26]. They were reported to originate in the dorsal motor nucleus in the brainstem and the olfactory bulb, which was described as the initial stage of the disease by Braak *et al.* [16,27]. It is evident that aggregation of α-synuclein is a hallmark of PD. The unfolded native monomers form soluble oligomers after assuming an at least partially misfolded structure [21]. From these, β-sheet oligomers can form, and can transform into fibrils that result in intracellular aggregates (LBs). These fibrils could enter primary neurons via endocytosis and promote recruitment of soluble α-synuclein into insoluble inclusions. Studies have reported that the soluble α-synuclein oligomers can interact with membranes making them more permeable for Ca^{2+} ions [28]. The dysregulation of Ca^{2+} homeostasis ultimately leads to neurotoxicity and cell death.

For the innate immune system, activated microglia cells have been found in the *substantia nigra* of PD patients, of which secretion of proinflammatory cytokines and ROS results in inflammation and oxidative stress to the SNpc neurons. Such inflammatory responses are also activated by α-synuclein aggregates released by neurons and taken up by astroglial cells. Similarly, the release of α-synuclein oligomers can also trigger inflammation in the microglia [21]. Other PD-associated proteins such as LRRK2 have also been reported to activate microglia and increase proinflammatory cytokine release from the activated microglia cells, leading to neurotoxicity [29–31]. Besides, upon exposure to environmental toxins, microglia can shift to the overactivated state and release ROS [29,32]. These oxidative stress and exogenous toxins can in turn trigger the misfolding and oligomerization cascade of α-synuclein, thereby resulting in a neurotoxic effect. In the adaptive immune system, $CD4^+$ T cells are also involved in neurodegeneration, whereby neuronal death in the animal model of PD has been shown to be attenuated by removal of the $CD4^+$ T cells [33].

As a whole, the pathogenesis of PD represents a complex network of molecular events involving dopaminergic and nondopaminergic neurons as well as non-neuronal cells. Genetic susceptibility and biochemical abnormalities are believed to play a major part in the development of neurodegeneration in PD [34]. A deeper understanding of pathogenesis of the disease through biochemical and genetic characterization is essential for the development of model systems toward the study of potential pharmacotherapies for PD.

Current drug therapies for PD

One of the most common approaches utilized for treating PD is focused on increasing the dopamine (**1**) (Fig. 2) signals at the CNS [35,36], such as the administration of blood–brain barrier (BBB)-permeable dopamine precursor levodopa (L-DOPA) (**2**) (Fig. 2), which is then converted into dopamine by DOPA decarboxylase. DOPA decarboxylase is present in SNpc and the periphery; upon oral administration, most of the levodopa dose is converted into dopamine at the peripheral level leading to side effects such as vomiting, nausea, arrhythmia and postural hypotension [37,38]. Hence, L-DOPA is mostly coadministered with a peripheral DOPA-decarboxylase inhibitor: carbidopa (**3**) (Fig. 2) to increase the prodrug level in SNpc and reduce the peripheral side effects as mentioned 38]. Nevertheless, the chronic administration of L-DOPA has been associated with side effects such as dyskinesia, end-of-dose deterioration of function and a switch between mobility and immobility (on/off phenomenon) in the treated patients [39,40]. A suggested reason for these motor symptoms is the pulsatile dopaminergic stimulation given by the short half-life of dopamine (or L-DOPA). As such, dopamine agonists with longer duration of action (Fig. 2) were developed and preferentially used to treat the preliminary symptoms of PD [39,41,42].

At present, there are five subtypes of dopamine receptors, classified as D_1-like (D_1 and D_5 subtypes) and D_2-like (D_2, D_3 and D_4 subtypes), which can increase (D_1-like) or decrease (D_2-like) the adenylyl cyclase activity. Alternative splicing generates two different isoforms of D_2 receptors: D_2 long (D_{2L}) and D_2 short (D_{2S}), which are mainly found in the postsynaptic and presynaptic neurons, respectively [39,43]. In the treatment of PD, dopamine agonists stimulate postsynaptic dopamine receptors in the striatum to increase the activity of the dopamine system; the most common ones are apomorphine (**4**), bromocriptine (**5**), pramipexole (**6**), ropinirole (**7**) and rotigotine (**8**) (Fig. 2). In general, these agonists displayed better affinity toward D_2-like dopamine receptors than against D_1-like receptor subtypes (Table 1) [39]. D_2-like receptors are abundantly found in the striatum and their selective activation has been shown to be beneficial for the treatment of PD [43]. Moreover, these compounds also show multitarget properties toward other monoaminergic receptors [44–47], which could contribute to the therapeutic effect or be responsible for side effects such as psychiatric and sleep disorders [44]. Monoaminergic receptors include adrenergic receptors (α_{1A}, α_{1B}, α_{1D}, α_{2A}, α_{2B}, α_{2C}, β_1, β_2, β_3) and serotonin receptors [5-hydroxytryptamine (5-HT)$_{1A}$, 5-HT$_{1B}$, 5-HT$_{1D}$, 5-HT$_{1E}$, 5-HT$_{1F}$, 5-HT$_{2A}$, 5-HT$_{2B}$, 5-HT$_{2C}$, 5-HT$_3$, 5-HT$_4$, 5-HT$_{5A}$, 5-HT$_{5B}$, 5-HT$_6$, 5-HT$_7$] [48b]. For example, talipexole (**9**) is a D_2-like receptor agonist showing partial agonistic activity at the α_2 adrenergic receptor, which is accountable for the sedative effects (at high doses) observed during treatment with this drug [49]. Instead, antagonism against α adrenergic (especially α_2) and/or 5-HT$_{2A}$ receptors confers antidyskinetic properties that can help in alleviating the motor symptoms of PD [39]. Except for apomorphine (**4**), dopamine agonists **5–8** showed longer half-lives than dopamine (Table 1). They are administered as monotherapy early in the course of PD, or as combinatorial therapy with L-DOPA to reduce the motor complications arising from chronic use of the latter [39,50].

As depicted in Fig. 2, the pharmacophore for D_2 agonism comprises some features common to other monaminergic agonists, including (i) a cationic site, (ii) an aromatic ring system and (iii) H-bond acceptor/donor sites [51]. At the binding site of the D_2 receptor, the cationic amino group (i) is involved in a salt bridge with an aspartic acid residue in transmembrane (TM)3; the aromatic system (ii) engages with hydrophobic residues in TM6 through optimal face-to-edge π–π interactions; and the phenol

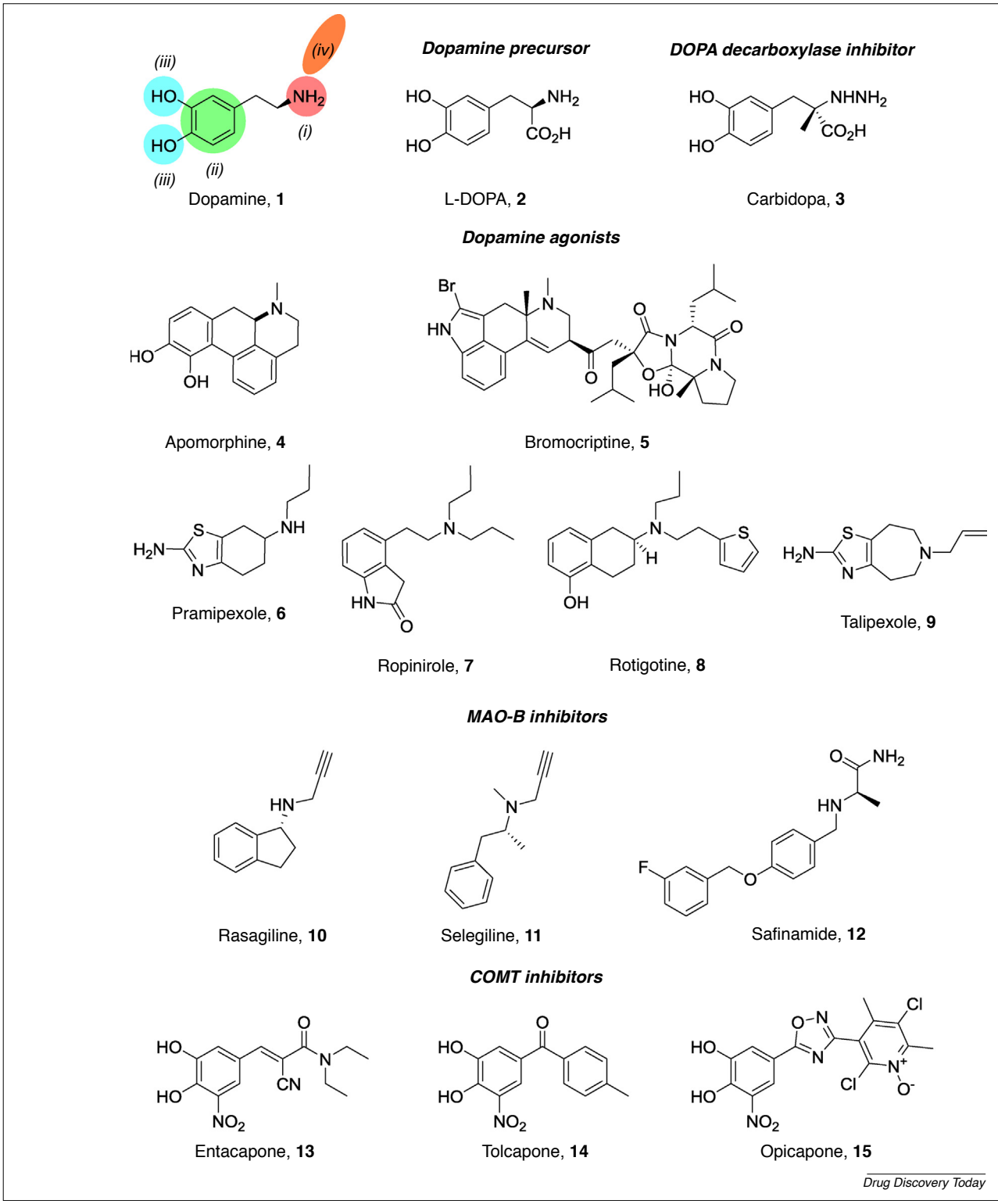

FIGURE 2

Structures of dopamine (**1**) and other common dopaminergic drugs (**2–15**) utilized for Parkinson's disease (PD) therapy to enhance the dopamine levels. Colorful shapes represent the pharmacophore of D agonists: (i) cationic site; (ii) aromatic ring system; (iii) H-bond donor or acceptor sites; (iv) hydrophobic feature (propyl pocket).

groups (iii) interact with serine residues in TM5 [51]. Different from the pharmacophore of D_2 agonists, D_1 agonists have H-bond acceptor and donor projected features in the structure. Another characteristic of D_2 agonists is the hydrophobic feature (iv) near the cationic site, also known as the propyl pocket, found in several agonists [51].

Alternatively, inhibition of enzymatic systems involved in dopamine catabolism represents an approach utilized to prolong

TABLE 1

Pharmacological profiles of dopamine agonists 3–8 [44]

Compound	D_1	D_{2S}	D_{2L}	D_3	D_4	D_5	Other targets[a]	$t_{1/2}$[b]
Apomorphine (**4**)	6.43	7.46	7.08	7.59	8.36	7.83	α_{2A-C}:An	0.75 h
Bromocriptine (**5**)	6.16	8.30	7.83	8.17	6.43	6.27	α_{1A}:An α_{2A-C}:An 5-$HT_{1A,D}$:Ag 5-HT_{2B}:An	3–8 h
Pramipexole (**6**)	<5	6.02	5.77	7.98	6.89	<5		10–16 h
Ropinirole (**7**)	<5	6.17	6.03	7.43	6.07	<5		5–6 h
Rotigotine (**8**)[c]	7.08	7.77		9.14	7.82–8.41	8.20	α_{2B}:An 5-HT_{1A}:Ag	4–7 h
Talipexole (**9**)	<5	6.21	6.01	7.17	6.48	5.46	α_{2A-C}:PA	12.3 h[d]

Affinities are expressed as pK_i values.

[a] Aminergic receptors showing $pK_i > 7$: Ag, agonist; An, antagonist; PA, partial agonist.

[b] Dopamine and L-DOPA half-lives are of 1.25–4.8 and 1.5–2 h, respectively [39].

[c] Data from [47].

[d] Data from [48a].

the half-life of dopamine in the CNS. For example, monoamine oxidase B (MAO-B) and catechol-*O*-methyltransferase (COMT) are enzymes involved in degradation of dopamine into 3,4-dihydroxyphenylacetic acid and 3-methoxytyramine, respectively [50]. MAOs are mitochondrial enzymes existing in two isoforms: MAO-A catabolizes dopamine in the presynaptic dopaminergic neurons, whereas MAO-B does the same in the post-synaptic neurons. By contrast, COMT is a cytoplasmic (or membrane-bound) enzyme responsible for catabolism of dopamine in nondopaminergic neurons and glia [52]. Selective and irreversible MAO-B inhibitors, such as rasagiline (**10**) or selegiline (**11**) have been used to prevent the catabolism of dopamine by this enzyme. They are currently given as monotherapy in early stages of PD, or in combination with L-DOPA to reduce the motor response fluctuations [53,54]. Recently, safinamide (**12**) was approved for the same indication. Apart from its inhibitory activity against MAO-B, this compound is also able to block sodium/calcium channels and to modulate stimulated release of glutamate. These nondopaminergic actions could have accounted for its neuroprotective effects through inhibition of free-radical formation by oxidative stress and glutamate release, thus reducing excitotoxicity in the dopaminergic neurons [55,56]. In a similar manner, the COMT inhibitors increase the dopamine levels at the CNS and are usually co-administered with L-DOPA to control the motor deficits of PD. As examples, entacapone (**13**), tolcapone (**14**) and opicapone (**15**) are currently used in clinical practice to potentiate the therapeutic effect of L-DOPA [50,57].

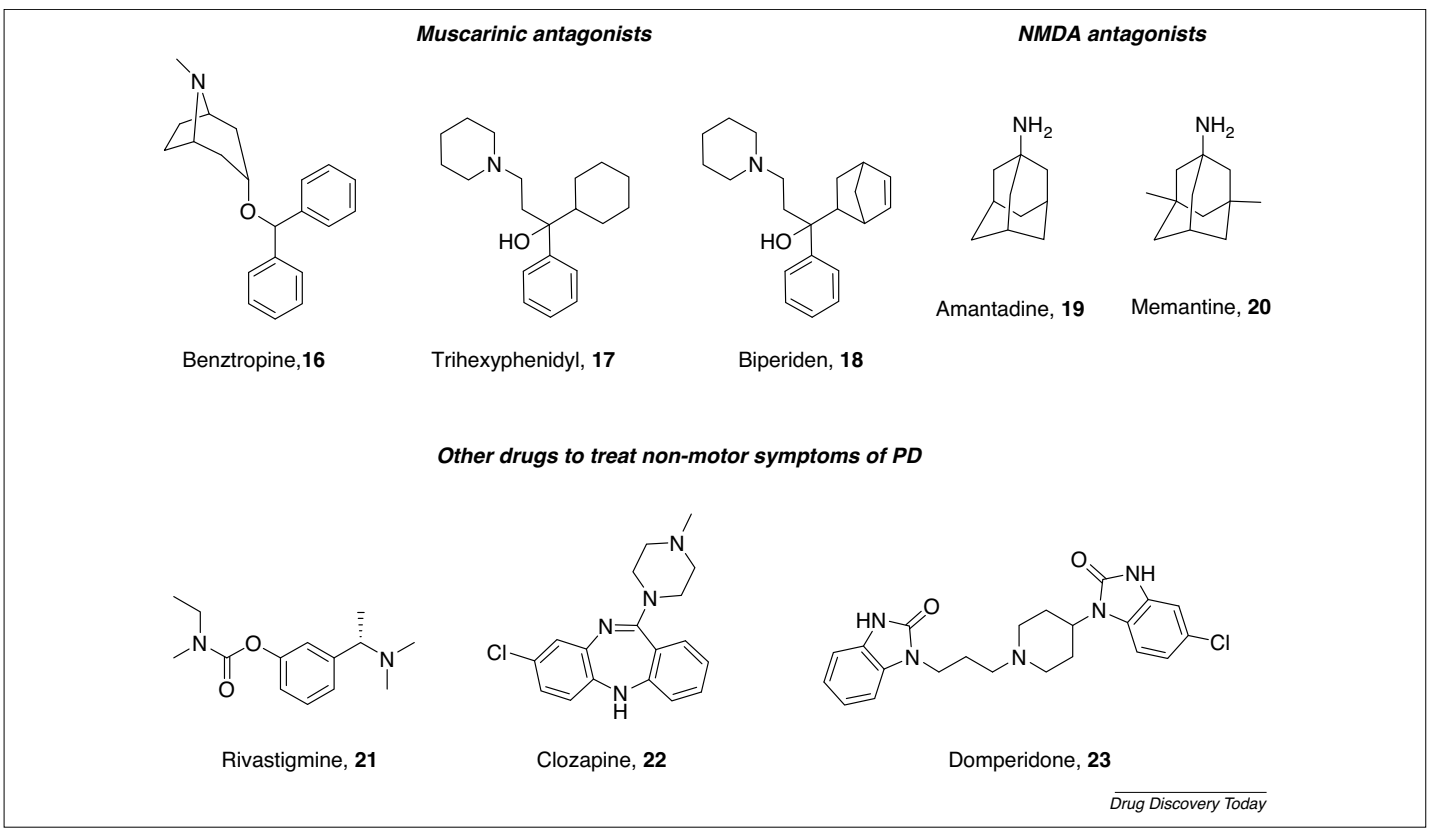

FIGURE 3

Structures of common nondopaminergic drugs (**16–23**) for Parkinson's disease (PD) therapy.

Other therapeutic agents used for the management of PD are nonselective muscarinic acetylcholine receptor antagonists, considering that acetylcholine is strongly involved in regulation of movement [58]. It has been postulated that the imbalance between acetylcholine and dopamine in the striatum could be the cause of the PD motor symptoms [27]. Five different subtypes of muscarinic receptors were identified, namely M_1, M_2, M_3, M_4 and M_5 [48b]. The most-used anticholinergic agents include benztropine (**16**), trihexyphenidyl (**17**) and biperiden (**18**), and they are effective in improving the mild symptoms of tremor and rigidity [3] (Fig. 3). These compounds are not selective for one muscarinic receptor subtype, even though they demonstrated a preferential affinity toward the M_1 receptor, especially biperiden (**18**) (Table 2). Benztropine (**16**) also behaves as a histamine 1 receptor antagonist (four different histamine receptor subtypes are known: H_1, H_2, H_3 and H_4) and dopamine transporter inhibitor [59], showing a positive effect on dopaminergic transmission [44]. Biperiden (**18**) is also found to demonstrate weak inhibitory activities on the enzyme AChE, which could be beneficial in improving the cognitive deficits associated with PD [7].

Amantadine (**19**) is an antagonist for *N*-methyl-D-aspartate (NMDA) glutamate receptor and is approved for PD treatment (Fig. 3). The blockade of glutamatergic hyperactivity, which has been associated with PD pathology, has accounted for its ability to decrease the L-DOPA-induced dyskinesia while improving the PD symptoms concomitantly [60]. Another NMDA receptor antagonist: memantine (**20**) (Fig. 3), has been shown to exert a neuroprotective effect against lipopolysaccharide (LPS)-induced dopaminergic neuronal damage via its inhibition of microglia overactivation. This results in reduction of release of ROS and proinflammatory factors from the microglia, thus providing anti-inflammatory effect toward the neuronal cells [61].

Apart from the motor symptoms, several drugs are prescribed to manage the non-motor symptoms of PD, such as cognitive impairment, sleep impairment, pain and neuropsychiatric symptoms like depression, hallucinations and psychosis (owing to dopaminomimetic therapy) [50]. Some of these common drugs include: rivastigmine **(21)**, a choline esterase inhibitor, which is efficacious in improving dementia; clozapine (**22**), an atypical antipsychotic agent; macrogol for constipation; and peripherally selective D_2 antagonist domperidone (**23**), to counteract anorexia, nausea and vomiting caused by L-DOPA- or dopamine-agonist-based therapies [62] (Fig. 3).

Another important aspect that should be considered in the treatment of PD is the route of administration of the drugs. It has been reported that several gastrointestinal dysfunctions are found in all stages of PD, which compromise gastrointestinal absorption of oral drugs with varying treatment responses [63]. For this reason, several efforts have been made in the recent past to find alternative non-oral therapy with the aim of avoiding these pharmacokinetic limitations. For instance: apomorphine (**4**) can be administered subcutaneously via infusion, whereas transdermal, buccal and inhaled routes of administration are still in development; a transdermal patch of rotigotine (**8**) has been in clinical use as monotherapy and adjunctive therapy for PD; selegiline, a MAO-B inhibitor (**11**), has been developed for administration via the sublingual route. A combination of L-DOPA (**2**) and carbidopa (**3**) can be given through intrajejunal infusion in a form of intestinal gel, whereas subcutaneous infusion of L-DOPA (**2**) is currently in Phase II clinical trials [63]. All these approaches will enhance the treatment strategies for early and advanced pharmacotherapy of PD leading to improved treatment outcomes in patients [63].

Importance of multitarget approaches in the treatment of PD

For diseases with multifactorial origin such as PD, drugs with a single-target mechanism-of-action cannot always compensate the complex pathophysiological pathways. This suggests drugs targeting an array of pathophysiological pathways (or targets) might be the alternative option to manage the course of disease progression of PD [64–66]. In general, the multitarget approaches (Fig. 4a) are classified into two main categories: (i) combination of drug entities, each acting on different pathophysiological targets of the disease (polypharmacy); and (ii) a single drug possessing promiscuous activity that interacts not only with one target but with a discrete variety of targets influencing the disease process (polypharmacology) [64,65].

In polypharmacy, the medications are commonly co-administered as a cocktail or co-formulated in a single pharmaceutical preparation (Fig. 4a) [64]. For example, L-DOPA has been combined with a dopamine agonist: MAO-B inhibitor or COMT inhibitor, to alleviate the motor symptoms in the advanced stages of PD [4,7,8]. This cocktail approach is often compromised by poor patient compliance. By contrast, dosing regimens of the multicomponent formulation are relatively simpler and, hence, improve medication adherence. Nevertheless, the combination of several drug molecules produces different degrees of bioavailability, pharmacokinetics and pharmacodynamics profiles from each drug component [67]. Moreover, it might also impart combined toxicity and side effects arising from drug–drug interactions [67]. It is therefore not surprising that the focus has shifted toward the design of a single ligand (polypharmacological ligand) that can modulate two or more specific targets of interest simultaneously. In this case, the likelihood of encountering unwanted side effects is less when one ligand is used, compared with using two or more ligands [68]. In addition, a drug that targets only one protein is more responsive to resistance owing to mutation in the target

TABLE 2

Pharmacological profiles of muscarinic antagonists 16–18. Affinities are expressed as pK_i values at the five cloned human muscarinic receptors [59]

Compound	M_1	M_2	M_3	M_4	M_5
Benztropine (**16**)	9.64	8.85	8.96	8.96	8.55
Trihexyphenidyl (**17**)	8.80	8.15	8.19	8.59	7.80
Biperiden (**18**)	9.32	8.20	8.41	8.62	8.20

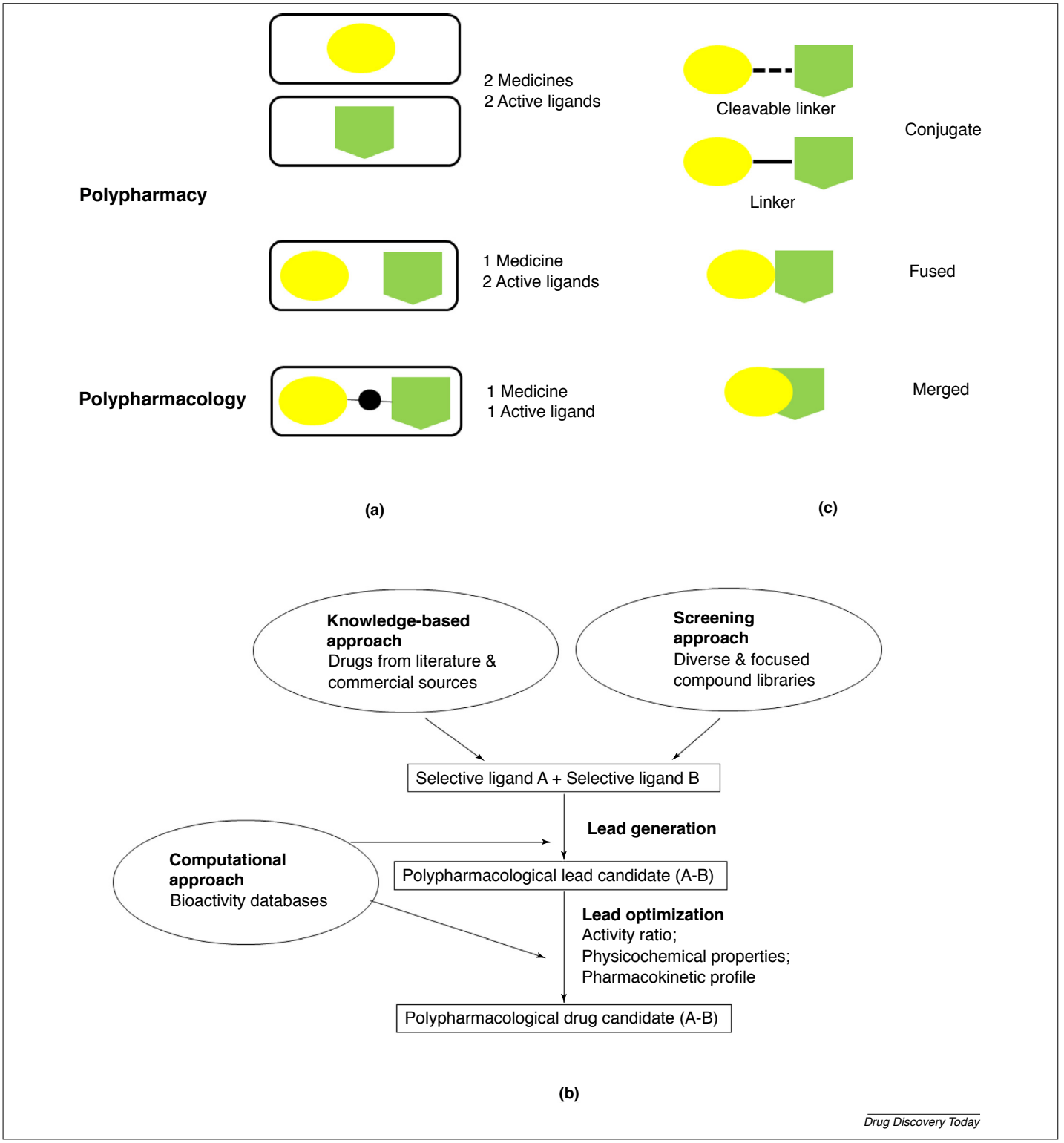

FIGURE 4

(a) Concept of polypharmacy and polypharmacology in multitargeting pharmacotherapy. **(b)** Knowledge-based, screening and computational approaches employed in obtaining polypharmacological ligands. **(c)** Design strategies of polypharmacological ligands: conjugate, fused and merged ligand. Adapted, with permission, from [70].

active site, and therefore substantially reduces compound binding affinity and efficacy [68]. Conversely, resistance to a drug that targets multiple proteins would require the unlikely event of concurrent mutations appearing in multiple protein targets [68].

These polypharmacological ligands, also known as multifunctional or multimodal drugs, should possess a balanced profile of pharmacological activities to multiple therapeutic targets of interest. There are two main strategies that are commonly carried out to

design and obtain the multitarget ligands, namely (i) knowledge-based and (ii) screening (serendipity) approaches [69–71] (Fig. 4b). The knowledge-based approaches depend on the biological data of existing drugs from literature or commercial sources, which are applied for rational drug design of multitarget ligands. For instance, structural elements from the two known selective drugs are combined into a single molecule to incorporate activity at both targets of interest [70]. The so-obtained lead compound will subsequently be optimized to generate a ligand with acceptable range of physicochemical properties and pharmacokinetic profiles, consistent with good oral absorption. In general, there are three types of polypharmacological ligands: conjugate, fused and merged ligands (Fig. 4c) [70,71]. The conjugates are composed of pharmacophoric structures connected by a metabolically stable linker or a cleavable linker to be metabolized with release of individual active structures *in vivo* 71]. In the fused ligands, the pharmacophoric structures are essentially joined at the junctions, whereas the merged ligands have the maximal overlap of pharmacophoric features from the individual active counterparts that eventually give rise to smaller and simpler molecules 71].

The screening approach often involves the screening of either diverse or focused compound libraries (Fig. 4b) [69–71]. Diverse-based screening includes HTS of large libraries of compounds at one target, and any active compounds are then prioritized on the basis of activity at the second target. In the focused screening, compounds that are already known to provide good activity at one of the targets of interest are screened for activity at a new target. Therefore, this approach is particularly beneficial in discovering novel chemotypes and hits for the targets of interest [70]. The subsequent steps to optimize the overall physicochemical and pharmacokinetic profiles are performed as rationally as for compounds derived from the knowledge-based approaches.

On top of the above-mentioned drug-design approaches, computational methods are also employed to guide the design and decoration of the molecular scaffold of the potential lead compounds (Fig. 4b) [12,72,73]. More specifically, 2D and 3D similarity-based approaches as well as machine learning models have been increasingly used to predict the bioactivity spectra for libraries of compounds. All these methods make use of large bioactivity databases (containing the chemical structure of small molecules and their bioactivities measured with a variety of biochemical assays) to derive the likely activity spectra of small molecules based on molecular similarity and patterns, and subsequently identify the likely targets for such molecules 73]. These computational tools not only provide useful information on the pharmacological profile of the compounds but also facilitate the prioritization of molecular fragments for rational design of new lead compounds with polypharmacological effects [12,72,73]. Based on this information, the desired activities will be 'designed in' and any undesired crossreactivity can be 'designed out' as much as possible.

In general, the multitarget drugs should be designed to possess optimum activity profiles toward the desired targets, while minimizing the risk of off-target activity. The excessive promiscuity could lead to adverse reactions caused by interactions with off-targets. In the following section, the examples of polypharmacological ligands that have been developed and tested in the preclinical trials for PD will be discussed comprehensively.

Examples of multitarget drugs tested in preclinical trials for PD

In this section, examples of polypharmacological ligands that can be potentially utilized for the treatment of PD are summarized.

MAO-B inhibition and iron chelation

Apart from mitochondrial dysfunction [74], several studies have reported that dysregulation of iron homeostasis in the brain, together with an increase of MAO-B activity in the glial cells, could also account for the pathogenesis of PD [75]. Iron concentration has been found elevated in the parkinsonian *substantia nigra*. High iron concentration will induce oxidative stress, resulting in formation of free radicals that ultimately cause neuronal damage and cell death [76]. By contrast, changes in activity of MAO-B contribute to the oxidative stress via formation of H_2O_2 from the reaction of MAO [75]. Considering these pharmacological and experimental observations, polypharmacological ligands have been developed accordingly. M30 (**24**) has been reported to possess remarkable MAO-A and MAO-B inhibition (MAO-A $IC_{50} = 37$ nM; MAO-B $IC_{50} = 57$ nM) and show good property as an iron(II) chelator with antioxidant activity (IC_{50} for iron-induced lipoperoxidase activity of 9.22 μM), giving rise to a neuroprotective effect [77–79] (Fig. 5). The MAO inhibitory and iron-chelating properties are conferred by a propargyl group and a quinoline moiety in the core structure, respectively. It also displayed good restorative properties on nigrostriatal dopamine neuron lesions in the animal model by restoring the reduction in dopaminergic cell count and striatal dopamine level 79]. Likewise, Bar-Am and coworkers proposed VAR10303 (**25**), which differs from M30 only by the length of the spacer between the *N*-methyl-*N*-propargylamino moiety and the quinolone (a methylene and an ethylene, respectively) [80].

MAO and ChE inhibition

One of the first polypharmacological drugs developed for the treatment of PD was ladostigil (**26**), a hybrid molecule between rivastigmine (**21**) and rasagiline (**10**). Rivastigmine is an AChE inhibitor well known for its cognitive effect, whereas rasagiline is a MAO-B inhibitor (Fig. 5). Such hybridization has led to a reduction of MAO-B inhibition by five orders of magnitude in the *in vitro* studies compared with rasagiline (**10**). However, *in vivo* studies have shown much higher inhibition of MAO-A and -B activity after several oral administrations or via intraperitoneal administrations to the rat or mouse models; this could probably be due to the pharmacokinetic effects through hydrolysis of the carbamate moiety of ladostigil (**26**) to yield the more active metabolite: 6-OH derivative [77]. With a similar pharmacological profile (AChE and MAO inhibition), Carradori *et al.* developed a thiazole derivative (**27**), which has been shown to be a promising multitarget drug for PD treatment in *in vitro* studies (Fig. 5) [81]. Recently, quinolinic carboxylic acid derivatives, such as compound **28**, were reported as potential multitarget ligands for the treatment of PD, attributable to their inhibitory activities on MAO and AChE. Compound **28** showed an IC_{50} of 0.51 μM against MAO-A and MAO-B as well as an IC_{50} of 23.7 μM toward the hAChE. Nevertheless, no *in vivo* experiments on the PD models were reported for this class of derivatives [82].

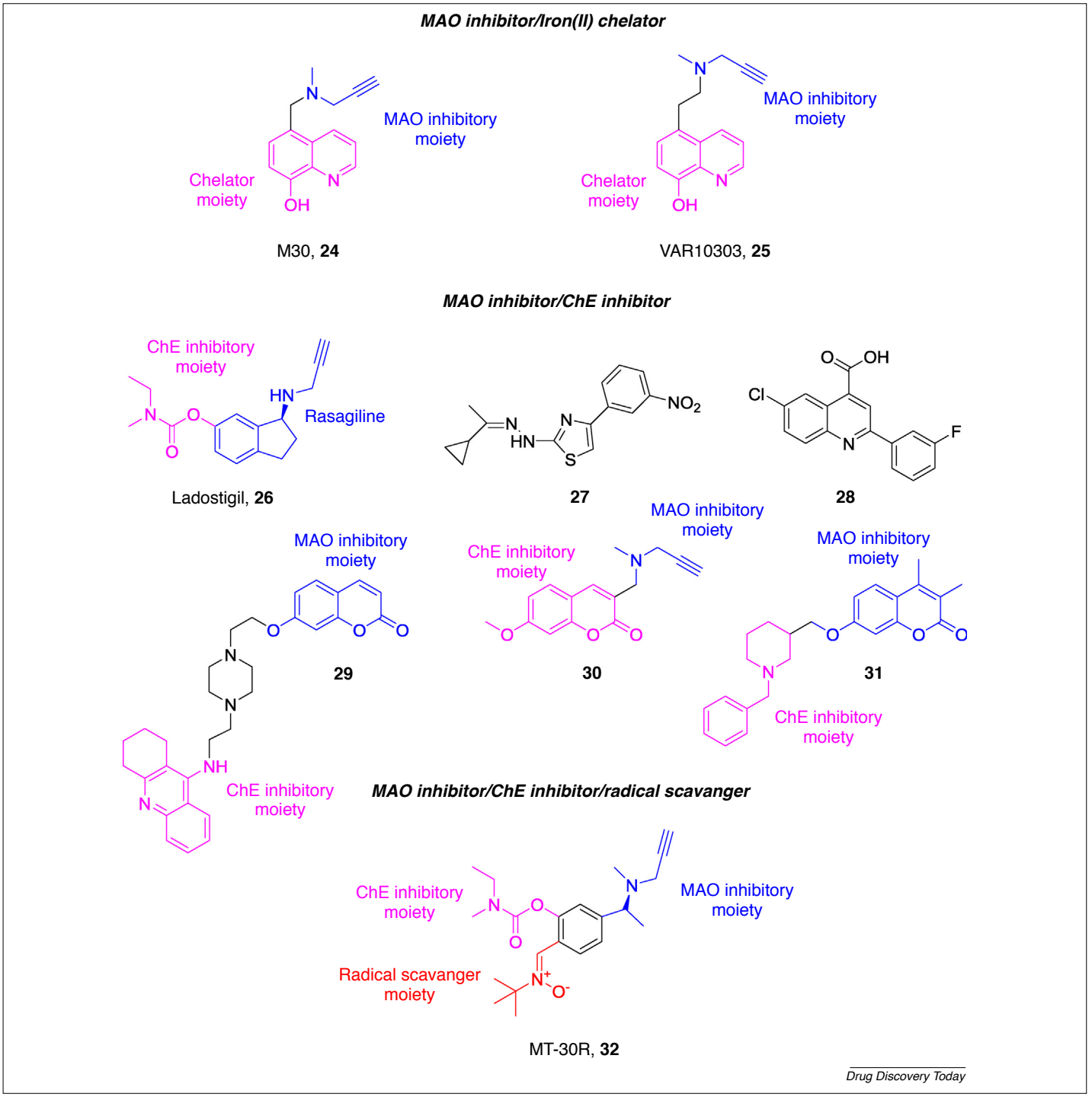

FIGURE 5

Structures and biological properties of polypharmacological ligands **24–32** for the treatment of Parkinson's disease (PD).

Compounds with a coumarin scaffold have also been found to demonstrate MAO-inhibitory activity. Several dual-acting compounds were developed by conjugating coumarin with ChE inhibitory moieties, such as tacrine (**29**), 3-propargylamine (**30**) or *N*-benzylpiperidine (pharmacophoric moiety of donepezil, a reversible AChE inhibitor) (**31**) (Fig. 5) [83]. In a similar manner, a combination of the propargylamine (MAO inhibition) and *N*-methyl-*N*-ethyl carbamate (ChE inhibition) moieties with α-phenyl-tert-butylnitrone (radical scavenger) on a phenyl ring has led to compound MT-20R (**32**), which demonstrated good potency in the PD models with remarkable MAO and AChE inhibitory activity as well as radical scavenger properties [84].

MAO inhibition and H_3 antagonism

Aside from the iron-chelating properties, the MAO inhibitory activity has been integrated with antagonistic activity at the histamine H_3 receptor. H_3 antagonism can improve motor and non-motor symptoms, such as cognitive and sleep impairment. The H_3 receptors are

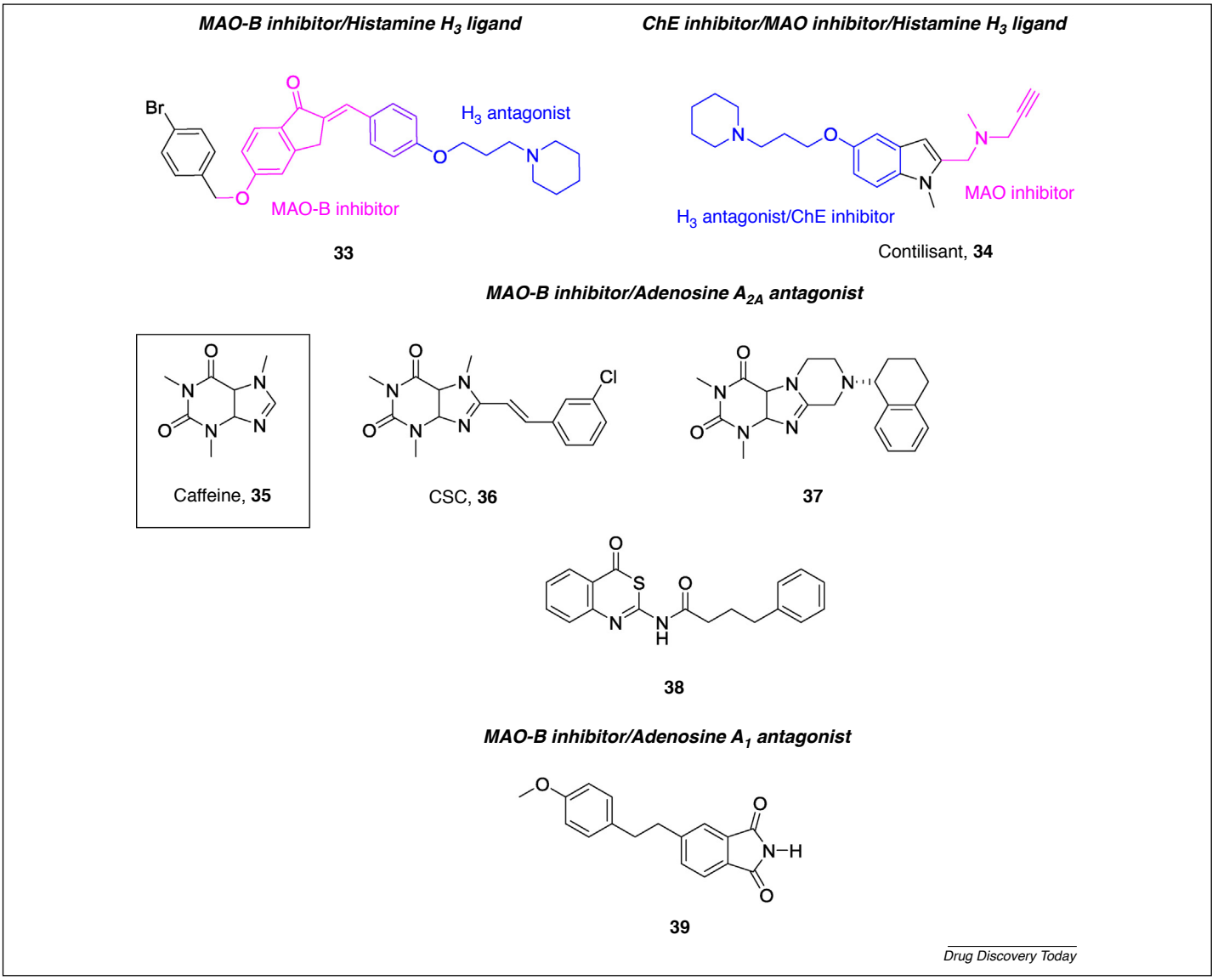

FIGURE 6

Structures and biological properties of polypharmacological ligands **33–39** for the treatment of Parkinson's disease (PD).

also co-expressed with the dopamine D_2 receptors in the basal ganglia. Activation of the H_3 receptor could reduce the release of dopamine. Consistently, studies have demonstrated that motor control by a dopamine agonist has been potentiated by a H_3 receptor antagonist. In recent research conducted by Affini and co-workers, an indanone derivative (**33**) that behaves as a MAO inhibitor and human H_3 receptor antagonist has been successfully synthesized and proposed as a novel multitarget ligand for the treatment of PD (Fig. 6) [85]. In a similar example, H_3 antagonism is combined with MAO and ChE inhibition in contilisant (**34**), as reported by Bautista-Aguilera *et al.*, which exhibits balanced *in vitro* activities at multiple targets (hH_3 $K_i = 10.3$ nM; hMAO-A $IC_{50} = 145$ nM; hMAO-B $IC_{50} = 78$ nM; hAChE $IC_{50} = 530$ nM) as well as antioxidative neuroprotective effects. It has also been shown to have good permeability across the BBB [86]. Recently, the same authors found that the same compound could also behave as a sigma 1 receptor agonist that exerted antiamnesic properties, indicating its potential role in the treatment of other neurodegenerative diseases such as Alzheimer's disease [87].

MAO-B inhibition and A_{2A} antagonism

Another similar approach has considered human A_{2A} adenosine receptors as possible targets for modulation. Adenosine receptors exist in four different subtypes, namely A_1, A_{2A}, A_{2B} and A_3 adenosine receptors. The A_{2A} receptor is richly localized in striato-pallidal neurons and co-expressed with dopamine D_2 receptors in the form of heterodimeric complexes [88]. Stimulation of such receptors has been shown to decrease the dopamine affinity toward the D_2 receptor. Studies have demonstrated that blockade of the A_{2A} receptor through the action of antagonists amplifies the therapeutic effect of L-DOPA and reduces the L-DOPA-induced dyskinesia [89]. For these reasons, A_{2A} adenosine receptor antagonists have been regarded as potential drugs in conjunction with dopamine precursors or dopamine potentiators for the treatment

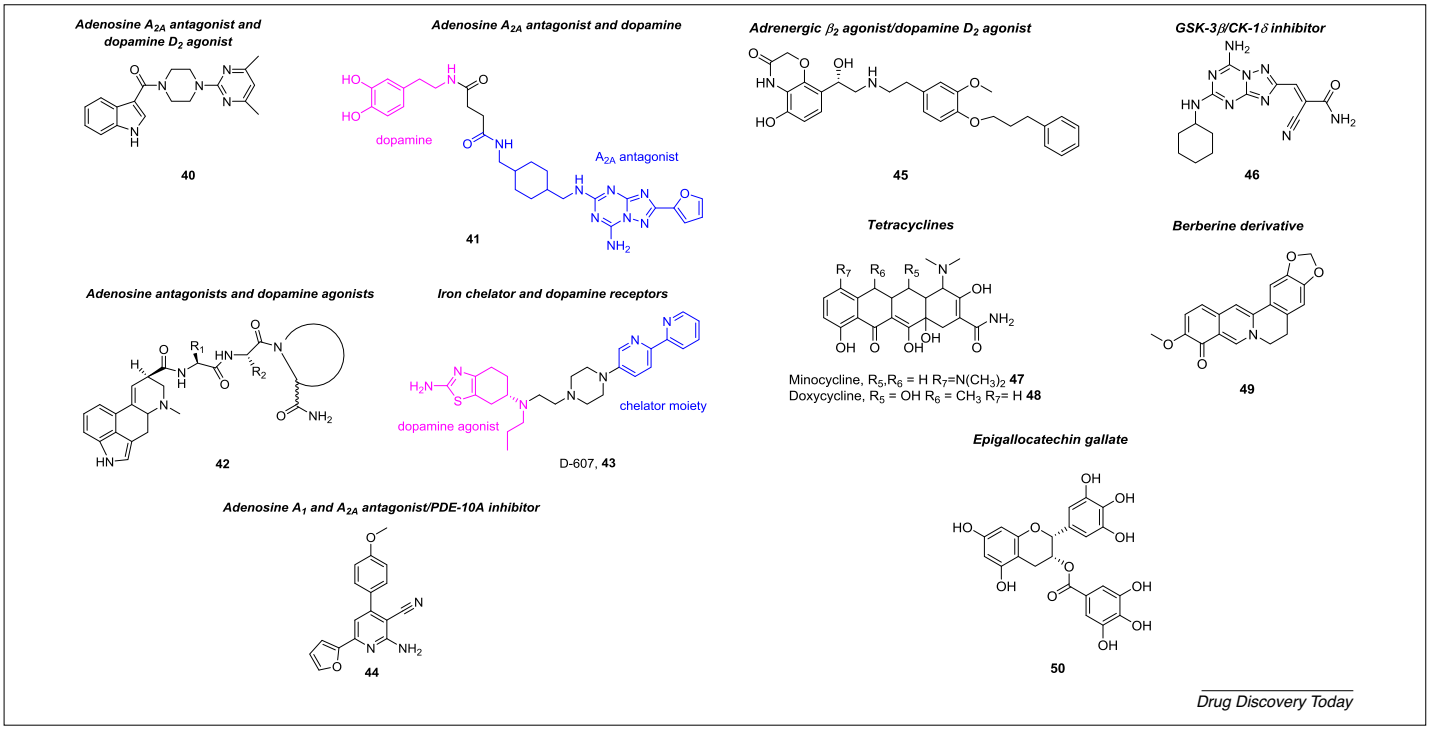

FIGURE 7

Structures and biological properties of polypharmacological ligands **40–50** for the treatment of Parkinson's disease (PD).

of PD. As a result, several hybrid derivatives possessing A_{2A} antagonism and MAO-B inhibitory activity have been subsequently identified as new classes of compounds with an anti-parkinsonian effect [90–94].

Caffeine (**35**) is a weak adenosine receptor antagonist, of which the xanthine scaffold has represented one of the privileged nuclei for designing potent and selective antagonists. The first example of dual modulation of the A_{2A} receptor and MAO-B by a xanthine compound is chlorostyrylcaffeine (CSC; **36**) which acts as a classic A_{2A} adenosine receptor antagonist as well as a MAO-B inhibitor for the treatment of PD (Fig. 5). Petzer and co-workers studied a large series of styrylxanthines as A_{2A} and MAO-B inhibitors and proposed a detailed SAR for this class of compounds [90–92]. Other classes of compounds possessing this dual activity have since been developed. For instance, Muller and colleagues studied several 1,3-dimethyltetrahydropyrazino[2,1-*f*]purinedione and some benzothiazin-4-one derivatives with promising pharmacological profiles. In particular, compounds **37** [93] and **38** [94] showed the capability to block A_{2A} adenosine receptors and MAO-B with different degrees of potency and selectivity. By contrast, the class of sulfanylphthalimide derivatives represented by compound **39** were investigated as A_1 plus MAO-B dual-target-directed compounds and have shown interesting antagonism against adenosine receptors as well as inhibitory activity toward MAO-B (Fig. 6). In the striatum, blockade of A_1 receptors has been shown to facilitate dopamine release [95].

D_2 agonism and A_{2A} antagonism

As described earlier, heterodimerization of A_{2A} receptors with dopamine D_2 receptors has resulted in reduction of dopamine binding at the D_2 receptor [88]. Studies have shown that combination of an A_{2A} antagonist with a D_2 agonist enhances the activity against PD [7]. As such, a dual-acting compound targeting both receptors would be regarded as therapeutically beneficial toward the management of PD.

A dual A_{2A}/D_2 ligand was reported by Shao *et al.*, in which an indolylpiperazinylpyrimidine derivative (**40**) was found to act as an A_{2A} antagonist and D_2 agonist. Of note, the derivative was designed by computational methods. Based on the preliminary results, this class of compounds showed affinity for the adenosine A_{2A} and dopamine D_2 receptors in the micromolar range. Most importantly, the compound was found to reduce the loss of dopaminergic neurons in a *Drosophila* model of PD with negligible toxicity [96]. A similar strategy has also been adopted by Dalpiaz *et al.*, who proposed a prodrug (**41**) (Fig. 7) obtained by conjugation between a triazolotriazine A_{2A} antagonist and dopamine. The conjugated compound and its metabolic products have been tested in binding assays showing good A_{2A} adenosine receptor affinity and counteracting the CGS2680 (adenosine A_{2A} receptor agonist)-induced reduction of dopamine affinity toward striatal D_2 receptors. Nevertheless, no data in animal models of PD have been investigated and reported for such a compound [97].

Another approach was reported by Vendrell *et al.* on a library of ergopeptides with a general formula **42** (Fig. 7). Specifically, the ergolene system is a privileged structure for interaction with several G-protein-coupled receptors, which could be useful to design multitarget ligands. The authors linked various linear peptide moieties to the ergolene nucleus and investigated their affinity toward the adenosine (A_1 and A_{2A}) and dopamine (D_1 and D_2) receptors. These ergopeptides showed various binding profiles at the A_1/A_{2A} and D_1/D_2 receptors, representing the starting point for the development of optimized structures which could be further studied for their effect on the PD models [98].

D_2 agonism and iron chelation

Another promising multitarget derivative, D-607 (**43**), has exhibited iron(II) chelator properties and good agonistic activity at the dopamine receptors in *in vitro* and *in vivo* PD models [99,100] (Fig. 7). In particular, D-607 (**43**) was also found to reduce levels of aggregated α-synuclein in a *Drosophila melanogaster* PD model 100].

A_1, A_{2A} antagonism and PDE-10A inhibition

Phosphodiesterase 10A (PDE-10A) has been suggested to play a part in PD. Rational design of A_1/A_{2A}/PDE-10A multitarget ligands was guided by a computational strategy that has generated new synthetically feasible compounds starting from a focused chemical space formed by known ligands for A_1, A_{2A} and PDE-10A. Among the predicted compounds, derivative **44** showed the best profile toward the targets of interest (hA_1 $K_i = 34$ nM; hA_{2A} $K_i = 41$ nM; hPDE-10A $IC_{50} = 3.2$ μM) (Fig. 7) [101].

Other approaches

There are other approaches that have been studied in preliminary stages as potential multitarget drugs for PD treatment, such as the dual β_2/D_2 agonist benzoxazinone (**45**) (Fig. 7) [102], the dual GSK-3β/CK-1δ inhibitor triazolo-triazine (**46**) [103] and nitrocathecol derivatives of calchone as weak dual MAO-B/COMT inhibitors [104]. Recently, the antibiotic tetracyclines, such as minocycline (**47**) and doxycycline (**48**), were found to be neuroprotective in PD. In particular, they are matrix metalloproteinase inhibitors and possess several anti-inflammatory properties as well as the ability to prevent α-synuclein aggregation [105]. Regarding the natural compounds, berberine is an alkaloid that is known to possess several biological properties. Ribaudo *et al*. investigated berberine and some semisynthetic derivatives as multitarget agents in PD. *In silico* investigations revealed strong interactions with key molecular targets: phosphodiesterase 4 and 10 (PDE-4, PDE-10), α-synuclein and especially MAO-B. Among them, compound **49** was proved to inhibit MAO-B *in vitro*. However, the compounds were not tested on other target proteins [106]. For all these compounds, additional investigations are required to confirm their pharmacological effects in regulating the underlying pathophysiological pathways associated with PD.

Finally, Li and co-workers designed a dual-target traceable nanodrug for the treatment of PD. Epigallocatechin gallate (EGCG) (**50**) showed inhibitory activity on α-synuclein aggregation *in vitro*, a decrease of dopaminergic neuron loss and inhibition of tyrosine hydroxylase (TH) protein depletion *in vivo*. Unfortunately, EGCG is not able to permeate the BBB nor can it be internalized in dopaminergic neurons. The authors decided to create superparamagnetic iron oxide nanoparticles conjugated with EGCG and other moieties with the aim of facilitating drug accumulation in the dopaminergic neurons. One of the moieties is the B6 peptide – a high affinity peptide for transferrin receptor on the BBB; another moiety, mazindol has high affinity to the dopamine transporter (DAT) expressed on the dopaminergic neurons that promotes DAT internalization. The magnetic properties of nanoparticles allowed the authors to trace the drug accumulation in the brain by magnetic resonance imaging (MRI). In fact, after the treatment with this theranostic system, an increase of ECGC level in the PD lesions was traced by MRI. It was found that the PD symptoms were alleviated, in which a decrease in α-synuclein aggregation and an increase in dopamine neurons have been observed [107].

Concluding remarks

The single-drug–single-target approach has long dominated the pharmaceutical industry with many successful drugs emerging from such a strategy. However, many diseases remain inadequately treated especially those with multifactorial origins. PD is one of them, of which the treatment poses challenges owing to the complex pathology of the disease. Over the years, the drug discovery paradigm for PD has gradually shifted from the design of selective single-target drugs toward the design of new ligands directed at multiple pathophysiological pathways of the disease. There has been a substantial research effort to discover efficacious polypharmacological ligands that could offer new hope in the treatment of PD. Nevertheless, the design of multitarget ligands remains a demanding task to fulfil – these ligands should not only possess good activities toward two or more pathophysiological targets but also have acceptable physicochemical and pharmacokinetic properties that are consistent with the administration of an oral drug. More-sophisticated design and formulation strategies are needed to obtain the multitarget ligands with good biological activities and optimum bioavailability. Nowadays, new computer-based approaches for *in silico* screening, such as molecular modeling, machine learning and data mining, have been frequently employed in the discovery and optimization of novel ligands with enhanced activity against drug targets. These computational tools can also be applied in optimization of physicochemical and pharmacokinetic properties of the potential drug candidates. In addition, an increasing number of studies focusing on genetic and biochemical characterization of pathology mechanisms are also carried out to identify and validate new pathophysiological targets in PD for their disease relevance and drugability. Such an endeavor will further expand the biological space available for PD drug discovery. Of note, the polypharmacological approaches should always be directed toward the suitable target combinations to avoid promiscuous effects arising from interactions with harmful off-targets. Collectively, advancement in the medicinal chemistry as well as rapid growth in the molecular biology and genomics research will certainly accelerate the understanding of pathogenesis of PD and drug design of promising multitarget ligands for PD treatment.

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