Recent advances in dopaminergic strategies for the treatment of Parkinson's disease

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INTRODUCTION

Parkinson's disease (PD) is the second most common progressive neurodegenerative disease worldwide. However, there is no available therapy reversing the neurodegenerative process of PD. Based on the loss of dopamine or dopaminergic dysfunction in PD patients, most of the current therapies focus on symptomatic relief to improve patient quality of life. As dopamine replacement treatment remains the most effective symptomatic pharmacotherapy for PD, herein we provide an overview of the current pharmacotherapies, summarize the clinical development status of novel dopaminergic agents, and highlight the challenge and opportunity of emerging preclinical dopaminergic approaches aimed at managing the features and progression of PD.

Keywords: Parkinson's disease; dopamine; D₁ receptor; D₂ receptor; allosteric modulator; neuroprotection; multitarget; drug discovery and development; neurodegenerative diseases

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INTRODUCTION

Parkinson's disease (PD) is a chronic, multicentric, and progressive neurodegenerative disease affecting 2%–3% of the population over the age of 60 years and is secondary only to Alzheimer’s disease (AD) [1, 2]. The pathological hallmark of PD is progressive and selective loss of dopaminergic neurons in the pars compacta of the substantia nigra (SNpc) and accumulation of α-synuclein (α-SN)-enriched intraneuronal aggregates termed Lewy bodies [2, 3]. Deficits in dopaminergic neurons results in the depletion of striatal dopamine (DA) production, leading to motor dysfunction, including resting tremor, bradykinesia, rigidity, and postural instability [3, 4]. In fact, patients have often already lost 60% of dopaminergic neurons from the SNpc when motor symptoms start to emerge at the onset of PD [5]. At that time, striatal DA in early PD patients is depleted by 80%. As the disease progresses to the later stage of PD, the involvement of nondopaminergic brain regions (e.g., the dorsal motor nucleus, locus coeruleus, substantia innominata, autonomic nervous system, and cerebral cortex) further causes the loss of nondopaminergic neurons such as cholinergic, serotonergic, glutamatergic, and noradrenergic neurons, consequently resulting in nonmotor symptoms, including hyposmia, psychiatric symptoms (e.g., depression and anxiety), rapid eye movement sleep behavior disorder, dementia, pain, fatigue, and constipation, in PD patients [4, 6–14].

Currently, PD is still incurable, as no treatment can stop or even slow down the progression of the disease, even though many research organizations focus on PD modification [15–18]. However, it is undeniable that various dopaminergic and nondopaminergic approaches for the treatment of the clinical symptoms of PD, including motor and nonmotor features, can improve the quality of life of PD patients for many years (Fig. 1). A number of excellent reviews on nondopaminergic therapeutics [5, 9, 19–25], including A₂A receptor antagonists [26–29], muscarinic antagonists [30–32], serotonergics [33–35], and glutamatergics [36–39], have discussed medicinal chemistry and clinical outcomes. As dopaminergics still represent the major therapeutic approaches for alleviating motor symptoms [40–45], this review will briefly introduce recent updates on approved PD treatments and highlight ongoing clinical efforts and recent progress on preclinical dopaminergic therapies aimed at managing the features and progression of PD since 2012.

UPDATES ON CURRENTLY APPROVED PD TREATMENTS

Current PD therapeutic strategies, which mainly rely on the use of dopaminergic and nondopaminergic pharmacological agents for the treatment of the clinical symptoms of PD, are summarized in Tables 1 and 2. Since the early 1960s, DA replacement therapy has been the dominant therapy for the treatment of PD symptoms [46]. Levodopa (L-DOPA) [47, 48], a blood–brain barrier (BBB)-permeable DA biosynthetic precursor, is a mainstay of PD pharmacotherapy and compensates for the loss of DA and dopaminergic function through its conversion to DA by DA decarboxylase (Table 1). As L-DOPA is quickly metabolized by peripheral DA decarboxylase, monoamine oxidase-B (MAO-B), and/or catechol-O-methyltransferase (COMT), leading to poor efficacy and severe peripheral side effects, inhibitors of these enzymes were later developed as an add-on therapy to L-DOPA, to reduce the metabolism of L-DOPA and increase its central nervous system (CNS) concentration; this allowed the oral administration...
of lower doses of L-DOPA while maintaining the efficacy of L-DOPA and reducing its peripheral side effects such as nausea and hypotension. In 2017, safinamide [49, 50], a third-generation MAO-B inhibitor with reversibility and high selectivity that was originally developed to treat epilepsy, was approved by the Food and Drug Administration (FDA) as an adjunctive therapy to L-DOPA for PD patients with "wearing-off" episodes. Although L-DOPA is effective for symptomatic relief, its efficacy is diminished and it causes medication-related complications, particularly motor fluctuations and dyskinesia, such as L-DOPA-induced dyskinesia (LID), after chronic or long-term use [51–54].

Later, DA receptor agonists, such as those shown in Table 1, were developed either as monotherapies or combination therapies with L-DOPA for the treatment of PD. Five types of DA receptors, D1–D5, exist in the brain. The D1 and D3 receptors are grouped together as D1-like receptors based on their stimulatory effects on adenyl cyclase (cAMP), and the D2, D3, and D4 receptors are classified as D2-like receptors due to their inhibition of cAMP activity. Many synthetic DA agonists, including pramipexole and apomorphine, activate D2-like receptors, and have a lower incidence of motor fluctuations and dyskinesia, such as L-DOPA-induced dyskinesia (LID), after chronic or long-term use [51–54].

Another area of discovery in the past beyond dopaminergic-based treatments involved the examination of neurodegenerative processes in PD. Areas of the nondopaminergic system in which dysfunction occurs include the cholinergic, glutamatergic, adrenergic, adenosine, serotonergic, histaminic, and opioid pathways, and such dysfunction may also underlie motor and nonmotor symptoms of PD, thereby stimulating the development of miscellaneous nondopaminergic drugs, such as those shown in Table 2 [1, 9, 13, 55]. In general, muscarinic receptor antagonists, including benzotripine, trihexyphenidyl, and biperiden, are used as adjuncts to PD treatment (e.g., L-DOPA), and they can also treat and prevent parkinsonian symptoms caused by the use of classic antipsychotic drugs (e.g., phenothiazines). Rivastigmine, a cholinesterase inhibitor, is used to treat mild-to-moderate dementia caused by AD or PD. Amantadine, a noncompetitive N-methyl-D-aspartate receptor antagonist, can treat PD and Parkinsonian symptoms. Most recently, istradefylline, the most extensively studied xanthine-based A2A antagonist, which was developed by Kyowa Kirin, Inc., and for which the first new drug application (NDA) submission was filed in 2007, was approved by the FDA as a first-in-class adenosine receptor type A2A antagonist. It is now used as a combination treatment with L-DOPA/carbopidopa in adult PD patients experiencing "off" episodes.

UPDATES ON THE CLINICAL PROGRESS OF DOPAMINERGIC PD TREATMENTS

Despite the intensive efforts in PD research and development, there are clear unmet medical needs for the development of additional dopaminergic treatment options to improve current DA-centered treatment. Most currently used dopaminergic drugs selectively activate D2-like DA receptors (Table 1), but no D1-like selective agonists have been successfully approved even though the D1 receptor is a known target for PD treatment. Recently, important progress has been made in the clinical development of D1 selective agonists and allosteric modulators. All active dopaminergics since 2012 on the clinical trial website are summarized in Fig. 2 and Table 3.

Dopamine stabilizers

Pridopidine (ACR16), which was developed by Neurosearch, is a DA stabilizer that improves motor symptoms by altering dopaminergic transmission via the dual effects of functionally low-affinity DA D2 receptor antagonism (K_i values of 17,550 nM and 7521 nM for D2 (low) and D2 (high), respectively) and strengthened cortical glutamate function [56, 57]. Originally, pridopidine was investigated for the symptomatic treatment of Huntington’s disease (HD), but two phase-3 trials sponsored by Teva did not show efficacy in improving voluntary motor function in HD patients. Later, it was found to display high binding affinity for human sigma-1 receptor (S1R) with a reported K_i value of 81.7 nM [58, 59]. Pridopidine was shown to improve functional neurorestoration, probably by acting on S1R, in a unilateral 6-hydroxydopamine (6-OHDA) lesion model of PD in mice [59]. Thus, pridopidine, a special D2 stabilizer with affinity for S1R, is currently being investigated in a phase 2 clinical study (NCT03922711) sponsored by Prilenia to evaluate its efficacy, safety and pharmacokinetics (PK) profile vs. placebo in PD patients experiencing LID.

D2 agonists

KDT3594 (structure undisclosed) is a D2 agonist developed by Kissie Pharmaceuticals [16]. A phase 2 study was initiated in February 2019 to investigate the efficacy, safety, and PK of KDT3594 vs. pramipexole in patients with early PD without concomitant treatment with L-DOPA (NCT03845387).

CLR4001 (Cilt1, structure undisclosed) is a D2-specific agonist developed by Clera, Inc. [16]; A phase 2 clinical trial was initiated in 2012 to assess its ability to increase the sensitivity of DA receptors and thereby reduce the symptoms of PD. However, its clinical status is currently unknown.

D3 agonists

IRL790 (structure undisclosed) is a small molecule targeting DA D3 receptors with psychomotor stabilizing properties developed by Integrated Research Laboratories [16]. In experimental animals, IRL790 potently reduces L-DOPA-induced involuntary movement without impairing the anti-Parkinsonian effect of L-DOPA (NCT03368170). Initially, in 2016, Integrated Research Laboratories conducted a phase Ib clinical study of IRL790 in PD patients experiencing LID (NCT03531060). Later, a phase 2 study of IRL790 started in 2018, to investigate its efficacy and the optimal dose as an adjunctive treatment to reduce LID in PD patients (NCT03368170).

D4/D5 agonists

Lu AE04621, a prodrug of catecholamine, was developed as a D4/D5 agonist by Lundbeck’s neurodegeneration portfolio [16]. In 2016, a phase 1 trial of Lu AE04621 was performed in 15 PD patients, to evaluate its tolerability, efficacy, PK, and safety (NCT02649608). However, neither the clinical results nor its chemical structure have been disclosed thus far.
| Compound       | Mechanism                | Structure                          | Indication                                      |
|---------------|--------------------------|------------------------------------|------------------------------------------------|
| Levodopa (L-DOPA) | Dopamine precursor        | ![Structure](image)                | Treat PD symptoms (muscle stiffness, tremors, spasms, and poor muscle control) |
| Carbidopa     | Dopamine decarboxylase inhibitor | ![Structure](image)                | Combinatorial therapy with levodopa            |
| Rasagiline    | MAO-B inhibitor          | ![Structure](image)                | ✓ Combinatorial therapy with levodopa ✓ Early stages of PD |
| Selegiline    | MAO-B inhibitor          | ![Structure](image)                | ✓ Combinatorial therapy with levodopa ✓ Early stages of PD |
| Safinamide    | MAO-B inhibitor          | ![Structure](image)                | Combined with levodopa and carbidopa to treat "wearing-off" episodes (muscle stiffness, loss of muscle control) in PD patients |
| Entacapone    | COMT inhibitor           | ![Structure](image)                | Combinatorial therapy with levodopa/carbidopa |
| Tolcapone     | COMT inhibitor           | ![Structure](image)                | Combinatorial therapy with levodopa/carbidopa |
| Apomorphine   | Dopamine agonist         | ![Structure](image)                | Treat "wearing-off" episodes in people with advanced PD |
| Bromocriptine | Dopamine agonist         | ![Structure](image)                | ✓ Treat PD symptoms ✓ Treat acromegaly          |
| Pramipexole   | Dopamine agonist         | ![Structure](image)                | ✓ Treat PD symptoms ✓ Treat restless legs syndrome (RLS) |
| Ropinirole    | Dopamine agonist         | ![Structure](image)                | ✓ Treat PD symptoms ✓ Treat RLS                |
| Rotigotine    | Dopamine agonist         | ![Structure](image)                | ✓ Treat PD symptoms ✓ Treat RLS                |

*https://www.drugs.com*
D₁ agonists
As traditional D₁-selective agonists are mainly catechol analogs and suffer from multiple challenges, such as low CNS penetration and poor metabolic stability, noncatechol agonists have become attractive due to their avoidance of such extensive metabolism [60–62]. Pfizer conducted several trials on clinical D₁ agonists for the treatment of PD. A phase 1 study of PF-06669571 [63], a novel partial DA D₁ receptor agonist, was initiated in 2014 to evaluate its safety and plasma concentrations following single and multiple ascending doses (NCT02184429) in healthy volunteers. Later, in 2015, a phase 1 study in 20 participants with idiopathic PD was performed (NCT02565628). These results showed that multiple

| Table 2. Approved nondopaminergic drugs |
|----------------------------------------|
| **Muscarinic Receptor Antagonists**    |
| Drug | Structure | Indication^a |
| Benztropine | ![Structure](image1) | ✓ Used as an adjunct in PD treatment (e.g., levodopa) ✓ Useful in the control of extrapyramidal disorders (except tardive dyskinesia) due to neuroleptic drugs (e.g., phenothiazines). |
| Trihexyphenidyl | ![Structure](image2) | ✓ Used as an adjunct in PD treatment ✓ Treat and prevent Parkinson-like symptoms that are caused by using certain anti-psychotic medications. |
| Biperiden | ![Structure](image3) | ✓ Used alone or together with other medicines (e.g., levodopa) to treat Parkinson's disease. ✓ Used to control severe muscle reactions and other side effects from certain medicines (e.g., chlorpromazine) |
| **Cholinesterase inhibitors**          |
| Drug | Structure | Indication^a |
| Rivastigmine | ![Structure](image4) | Treat mild to moderate dementia caused by Alzheimer's or Parkinson's disease. |
| **NMDA (N-methyl-D-aspartate) Receptor Antagonists** |
| Drug | Structure | Indication^a |
| Amantadine | ![Structure](image5) | Treat Parkinson's disease and "Parkinson-like" symptoms such as stiffness or tremors, shaking, and repetitive uncontrolled muscle movements that may be caused by the use of certain drugs. |
| **Adenosine A₂A Receptor Antagonists** |
| Drug | Structure | Indication^a |
| Istradefylline (Approved in 2019) | ![Structure](image6) | Use as an adjunctive treatment to levodopa/carbidopa in adult patients with Parkinson’s disease (PD) experiencing “OFF” episodes |

^ahttps://www.drugs.com
daily doses of PF-06669571 were well-tolerated and safe with no detectable safety concerns. However, the drug did not meet the pharmacodynamic endpoint of significant improvement [63]. PF-06649751 [64] represents another novel therapeutic candidate shown to exhibit highly selective DA D1/D5 agonism in phase 1 PD studies. Based on a primary clinical study of its safety, tolerability, PK profile, and efficacy, a larger clinical trial was subsequently conducted (NCT02224664 and NCT02373072). In 2017, Pfizer commenced a phase 2 trial of PF-06649751 to evaluate its long-term safety and tolerability in PD patients experiencing motor fluctuations. However, this study was terminated early, not due to safety concerns but due to a lack of demonstrated efficacy in improving PD symptoms (NCT03185481).

Another investigational drug is the noncatechol derivative PF-06412562, which is a moderately potent, orally bioavailable selective D1/D5 partial agonist [64, 65]. Since 2013, Pfizer has initiated ten phase 1 studies, eight of which were completed, one of which was terminated, and one of which had unknown outcomes. Most recently, a phase 1 study in healthy male volunteers assessed D1 receptor occupancy in the striatum after the oral administration of PF-06412562 (NCT03665451), whereas another study in advanced PD patients tested the safety and tolerability of PF-06412562 compared with that of the current medical standard of care for PD (carbidopa/L-DOPA), to determine whether it can help improve motor function, alertness, and cognitive skills (NCT03665454). The oral administration of PF-06412562 was shown to have potential anti-Parkinsonian efficacy without the significant acute cardiovascular effects previously reported with other D1 agonists [66].

D1-positive allosteric modulators

Allosteric modulators represent an alternative and promising strategy for G protein-coupled receptor (GPCR) drug discovery with high selectivity and low side effects. Eli Lilly developed the first clinical D1-positive allosteric modulator (PAM), LY3154207 [62, 67, 68], which shows no tachyphylaxis in preclinical animal models [62, 67, 68]. A phase 1 trial of LY3154207 in 80 participants, including healthy participants and PD patients, which studied the

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**Table 3. Ongoing clinical dopaminergic drugs**

| Company                        | Compound   | Mechanism        | Highest phase | Status             | Indication                          | NCT number       |
|--------------------------------|------------|------------------|---------------|--------------------|------------------------------------|-----------------|
| Neurosearch                    | Pridopidine| D2 stabilizer/ Sigma-1 | Phase 2       | Recruiting         | LID                                | NCT03922711     |
| Kissei Pharm                   | KDT3594    | D2 agonist       | Phase 2       | Recruiting         | PD                                 | NCT03845387     |
| Clera                          | CLR4001    | D2-specific agonist | Phase 2      | Unknown            | PD                                 | NCT01684475     |
| Integrated Research Laboratories| IRL-790    | D1 agonist       | Phase 2       | Completed          | LID                                | NCT03368170     |
| Lundbeck                       | Lu AE04621 | D1/D2 agonist    | Phase 1       | Completed          | PD                                 | NCT02649608     |
| Pfizer                         | PF-06669571| D1 partial agonist | Phase 1       | Completed          | Idiopathic PD                      | NCT02565628     |
| Pfizer                         | PF-06649751| D1/D5 agonist    | Phase 2       | Terminated         | PD patients experiencing motor fluctuations | NCT03185481 |
| Pfizer                         | PF-06412562| D1/D5 partial agonist | Phase 1      | Completed          | PD                                 | NCT03665454     |
| Eli Lilly                      | LY3154207  | D1 PAM           | Phase 2       | Recruiting         | PD dementia                        | NCT03305809     |
| Neurocrine Biosciences         | Opicapone  | COMT inhibitor   | NDA           | Accepted by FDA    | PD patients experiencing OFF episodes | NCT02764125     |
| Orion Pharma                   | ODM-104    | COMT inhibitor   | Phase 2       | Completed          | PD patients with end-of-dose wearing-off | NCT02764125     |

*https://clinicaltrials.gov*
safety, tolerability, and PK of multiple ascending doses, was completed in 2017 (NCT02562768). Currently, a phase 2 study of LY3154207 in PD dementia (NCT03305809) is being performed.

COMT inhibitors
COMT inhibitors continue to be developed to extend the clinical effect of L-DOPA. The investigational drug opicapone was identified as a peripherally selective COMT inhibitor for once-daily use by Neurocrine Biosciences, Inc. [69–71]. Compared with the first two generations of COMT inhibitors, opicapone induces lower hepatotoxicity and requires less frequent dosing [72]. In fact, the European Commission has authorized opicapone as an adjunctive therapy to L-DOPA preparations in adult PD patients with end-of-dose motor fluctuations since June 2017. However, opicapone was not approved for use in the United States or Canada until recently. In July 2019, the FDA accepted a NDA for opicapone as an adjunctive treatment to L-DOPA/carbidopa in PD patients experiencing off episodes based on data from 38 clinical studies including two phase 3 studies (BIPARK-1 and BIPARK-2) in more than 1000 PD patients.

ODM-104 (structure undisclosed) is another COMT inhibitor that has been investigated. A phase 2 clinical trial (NCT0276412S) sponsored by Orion Pharma to evaluate the safety and efficacy of ODM-104/L-DOPA/carbidopa vs. the standard of care (entacapone/L-DOPA/carbidopa) in PD patients with end-of-dose wearing-off (motor fluctuations) was completed in 2018.

RECENT ADVANCES IN THE PRECLINICAL STUDY OF DOPAMINERGIC DRUGS FOR PD
Based on the multiple pathogenesis of PD, multifunctional agents may be good alternative options for PD treatment [5, 15, 41, 73–78]. Unfortunately, no such agents are available in the clinic. Table 4 summarizes the chemical structures and binding or agonistic activity of preclinical dopaminergics.

Dual D2/5-HT1A receptor agonists
5-HT1A receptors are critical for motor control and psychoemotional behavior in physiological and morbid states [33, 35, 79], particularly with respect to the involvement in LID; this has stimulated the development of dual D2/5-HT1A receptor agonists such as SOMCL-135 [80] and SOMCL-171 [81], as promising approaches for the treatment of PD [33, 41]. Compared with L-DOPA, both SOMCL-135 and SOMCL-171 elicit anti-Parkinsonian action in a 6-OHDA-lesioned rat model with slight dyskinesia. The chronic use of these agents attenuates the development of LID at no expense to their efficacy against PD.

Multifunctional agents with D2/D3 receptor agonist, antioxidant or iron chelating activities
Considering that D2-/preferring agonists provide an additional neuroprotective effect compared with D2-/preferring agonists [82–86], multifunctional agents with D2 agonistic activity have been developed to not only alleviate motor dysfunction in PD patients but also delay disease progression by protecting DA neurons from progressive degeneration. The Dutta group initially developed novel multifunctional agents with D2/D3 agonist activity along with antioxidant and iron chelating activities and the ability to modulate αSN aggregation, such as (±)-21a [87], D512 [88–91], D-620 [92, 93], D593 [444], D-607 [94, 95], D-636 [96], D-653 [96], and D-656 (Table 4) [96]. Compared with ropinirole, (–)-21b exhibits potent neuroprotective effects against 6-OHDA-induced toxicity in PC12 cells and efficiently modulate the aggregation of α-SN protein. D-520 not only modulates the in vitro aggregation of αSN but also shows significant protective effects against toxicity caused by αSN in fly eyes. Moreover, D-520 can protect MN9D cells from 6-OHDA toxicity. D607, as a D2/D3 agonist with efficient preferential iron (II) chelation properties, is neuroprotective against the neurotoxin Fe(III)-8HQ complex and 6-OHDA. D607 also significantly suppresses toxicity in an in vivo Drosophila melanogaster model expressing the α-SN protein in the fly eyes and reduces the levels of aggregated α-SN. Furthermore, D-607 rescues dopaminergic neurons from 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) toxicity in mice via both subchronic and chronic MPTP administration.

Compared with ropinirole, D512 exhibits superior peak-dose efficacy and longer duration effects in improving the rotational activity in the 6-OHDA-induced unilaterally lesioned rat model despite having similar side effects, including drug-induced dyskinesia. In addition, D512 protects dopaminergic MN9D cells from MPP+- and 6-OHDA-induced toxicity, inhibits lipid peroxidation and caspase 3/7 activity, and rescues 6-OHDA-induced changes in nuclear morphology. Moreover, D512 protects rat adrenal pheochromocytoma PC12 cells from 6-OHDA-induced apoptotic cell death and rescues dopaminergic neurons in the MPTP mouse model of PD. Furthermore, D512 displays neuroprotective effects against oxidative insult produced by buthionine sulfoximine, an inhibitor of glutathione synthesis, and 6-OHDA in PC12 cells.

Multifunctional agents with D3 receptor agonist and antioxidant activities
The Dutta group also developed novel multifunctional agents with D3-prefering agonist activity along with antioxidant activity, such as the selective full D3 agonists D440 [90], (–)-34 [91], and (–)-9b [97] 2019-12-1933, and the selective partial D3 agonist (–)-8b [97]. D440 not only exhibits in vivo efficacy in rats with 6-OHDA-induced unilateral lesions but also protects dopaminergic MN9D cells from MPP+ and 6-OHDA-induced toxicity. Both (–)-34 and (–)-9b significantly increase locomotor activity in an animal model of reserpine-induced PD. Moreover, (–)-8b also exhibits strong in vivo activity in rats with 6-OHDA-induced unilateral lesions and protects MN9D cells from MPP+ -induced toxicity. Of note, (–)-8b is a selective partial D3 agonist, but it also exhibits strong in vivo activity, both in reversing akinesia in reserpine-treated rats and inducing rotation in rats with 6-OHDA-induced unilateral lesions; additionally, its activity is more potent than that of (–)-9b. However, (–)-8b shows no neuroprotective effects against MPP+ -induced toxicity in MN9D cells.

G-protein-biased selective D3 receptor agonists
SK609 (Fig. 3), a G-protein-biased selective D3 agonist, has no significant effect on β-arrestin signaling pathway or on the corresponding desensitization [98–100]. However, SK609 can significantly improve the performance of the impaired paw and normalize bilateral asymmetry in a hemi-Parkinsonian rat model. Chronic treatment with SK609 does not induce any abnormal involuntary movements. Furthermore, SK609 can be combined synergistically with L-DOPA to improve motor deficits without aggravating dyskinesia.

Selective D3 receptor agonists
Selective D3 agonists have received a resurgence of attention over the last 3 years. As derivatives of catechols, all known selective D3 agonists exert short duration effects, because they are rapidly metabolized and desensitize D1 receptors after prolonged exposure [44]. Recently, potent noncatechol selective D3 agonists (Fig. 3) with good in vivo efficacy and promising pharmacokinetic properties were identified [62]. PF-2334 [60], a...
Table 4. Polypharmacological dopaminergics for preclinical PD treatment

| Structure | Binding or agonistic activity |
|-----------|------------------------------|
| ![SOMCL-135](image) | • Dual D<sub>2</sub>/5-HT<sub>1A</sub> receptor agonist  
  - Binding activity: D<sub>2</sub>: K<sub>i</sub> = 66 nM; 5-HT<sub>1A</sub>: K<sub>i</sub> = 174 nM;  
  - Agonistic activity (GTPγS): D<sub>2</sub>: EC<sub>50</sub> = 320 nM; 5-HT<sub>1A</sub>: EC<sub>50</sub> = 190 nM  
  - Binding activity: adrenergic receptors (α<sub>1A</sub>, α<sub>1B</sub>, α<sub>1D</sub>, and β<sub>2</sub>): IC<sub>50</sub> > 10 μM |
| ![SOMCL-171](image) | • Dual D<sub>2</sub>/5-HT<sub>1A</sub> receptor agonist  
  - Binding activity: D<sub>2</sub>: K<sub>i</sub> = 352 nM; 5-HT<sub>1A</sub>: K<sub>i</sub> = 276 nM;  
  - Agonistic activity: D<sub>2</sub>: E<sub>max</sub> = 74%, EC<sub>50</sub> = 873 nM; 5-HT<sub>1A</sub>: E<sub>max</sub> = 83%, EC<sub>50</sub> = 175 nM |
| ![(-)-19](image) | • Dual D<sub>2</sub>/D<sub>3</sub> receptor agonist  
  - Agonistic activity:  
    - D<sub>2</sub>: E<sub>max</sub> = 107%, EC<sub>50</sub> = 2.96 nM;  
    - D<sub>3</sub>: E<sub>max</sub> = 93%, EC<sub>50</sub> = 1.26 nM |
| ![D-520](image) | • Dual D<sub>2</sub>/D<sub>3</sub> receptor agonist  
  - Binding activity:  
    - D<sub>2</sub>: K<sub>i</sub> = 41.8 nM; D<sub>3</sub>: K<sub>i</sub> = 0.35 nM  
  - Agonistic activity:  
    - D<sub>2</sub>: E<sub>max</sub> = 81%, EC<sub>50</sub> = 4.73 nM;  
    - D<sub>3</sub>: E<sub>max</sub> = 58%, EC<sub>50</sub> = 2.18 nM |
| ![D-593](image) | • Dual D<sub>2</sub>/D<sub>3</sub> receptor agonist  
  - Binding activity:  
    - D<sub>2</sub>: K<sub>i</sub> = 65.7 nM; D<sub>3</sub>: K<sub>i</sub> = 1.42 nM  
  - Agonistic activity:  
    - D<sub>2</sub>: E<sub>max</sub> = 97%, EC<sub>50</sub> = 22.9 nM;  
    - D<sub>3</sub>: E<sub>max</sub> = 83%, EC<sub>50</sub> = 2.62 nM |
| ![D-636](image) | • Dual D<sub>2</sub>/D<sub>3</sub> receptor agonist  
  - Binding activity:  
    - D<sub>2</sub>: K<sub>i</sub> = 135 nM; D<sub>3</sub>: K<sub>i</sub> = 3.8 nM  
  - Agonistic activity:  
    - D<sub>2</sub>: E<sub>max</sub> = 87%, EC<sub>50</sub> = 48.7 nM;  
    - D<sub>3</sub>: E<sub>max</sub> = 93%, EC<sub>50</sub> = 0.96 nM |
| ![D653](image) | • Dual D<sub>2</sub>/D<sub>3</sub> receptor agonist  
  - Binding activity:  
    - D<sub>2</sub>: K<sub>i</sub> = 71.2 nM; D<sub>3</sub>: K<sub>i</sub> = 0.4 nM  
  - Agonistic activity:  
    - D<sub>2</sub>: E<sub>max</sub> = 85%, EC<sub>50</sub> = 0.87 nM;  
    - D<sub>3</sub>: E<sub>max</sub> = 92%, EC<sub>50</sub> = 0.23 nM |
| ![D-656](image) | • Dual D<sub>2</sub>/D<sub>3</sub> receptor agonist  
  - Binding activity:  
    - D<sub>2</sub>: K<sub>i</sub> = 16.9 nM; D<sub>3</sub>: K<sub>i</sub> = 0.4 nM  
  - Agonistic activity:  
    - D<sub>2</sub>: E<sub>max</sub> = 74%, EC<sub>50</sub> = 2.29 nM;  
    - D<sub>3</sub>: E<sub>max</sub> = 88%, EC<sub>50</sub> = 0.22 nM |
G-protein-biased D₁ agonist, shows sustained plasma concentrations and induces robust in vivo pharmacological responses without functional tachyphylaxis. The oral administration of PF-2334 has potent in vivo effects both on eye-blink responses in nonhuman primates and in a unilateral 6-OHDA lesioned rodent model of PD. On the other hand, 10, a balanced agonist with highly potent effects on both signaling pathways, also displays satisfactory PK profiles, including good BBB penetrance. Importantly, 10 displays in vivo anti-Parkinsonian activity, restoring locomotion and prodyskinetic potential while potentiating behaviors indicative of dyskinesia in a 6-OHDA-lesioned mouse model of PD.

**D₂/D₃-positive allosteric modulators**

Unlike direct acting DA agonists, a newly identified D₂/D₃ PAM (Fig. 3) affects the affinity and/or efficacy of the endogenous ligand, DA, with superior receptor-subtype selectivity and reduced desensitization. The racemic D₂/D₃ PAM lacks activity at all tested receptors, including D₄, and lacks PAM activity at related Gᵢ-coupled GPCRs; however, it potentiates the effect of DA at

Table 4 continued

| Compound | Structure | Binding Activity | Agonistic Activity |
|----------|-----------|------------------|--------------------|
| (-)-21a³ | ![Image](image1.png) | D₂: Kᵢ = 16.4 nM, D₃: Kᵢ = 1.15 nM | D₂: Eₘᵢₓ = 101%, EC₅₀ = 3.23 nM; D₃: Eₘᵢₓ = 113%, EC₅₀ = 1.41 nM |
| D-607⁹⁴, ⁹⁵ | ![Image](image2.png) | D₂: Kᵢ = 674 nM, D₃: Kᵢ = 13.4 nM | D₂: Eₘᵢₓ = 101%, EC₅₀ = 51.6 nM; D₃: Eₘᵢₓ = 83%, EC₅₀ = 13.5 nM |
| D-512⁹⁶-⁹⁹ | ![Image](image3.png) | D₂: Kᵢ = 107%, EC₅₀ = 2.96 nM; D₃: Eₘᵢₓ = 93%, EC₅₀ = 1.26 nM |
| (-)-9b⁹⁷ | ![Image](image4.png) | D₂: Kᵢ = 369 nM, D₃: Kᵢ = 1.73 nM | D₂: Eₘᵢₓ = 116%, EC₅₀ = 15.9 nM; D₃: Eₘᵢₓ = 96%, EC₅₀ = 0.1 nM |
| D440⁹⁰ | ![Image](image5.png) | D₂: Kᵢ = 114 nM, D₃: Kᵢ = 0.26 nM |
| (-)-34⁹¹ | ![Image](image6.png) | D₂: Kᵢ = 86.5 nM, D₃: Kᵢ = 14.4 nM | D₂: Eₘᵢₓ = 27%, EC₅₀ = 21.6 nM; D₃: Eₘᵢₓ = 94%, EC₅₀ = 10.9 nM |
| (-)-8b⁹⁷ | ![Image](image7.png) | D₂: Kᵢ = 343 nM, D₃: Kᵢ = 2.33 nM | D₂: Eₘᵢₓ = 105%, EC₅₀ = 36.8 nM; D₃: Eₘᵢₓ = 67%, EC₅₀ = 3.42 nM |
both the human D2 and D3 receptors in [35S]-GTPγS and [3H]-DA binding assays. Its R isomer produces a greater improvement, whereas the S isomer is inactive. Moreover, this D2/D3 PAM potentiates in vivo effects on the level of L-DOPA-induced contralateral rotations in unilateral 6-OHDA-lesioned rats.

MAO-B selective inhibitors

The development of selective MAO-B inhibitors is still ongoing even though three selective MAO-B inhibitors have been approved for clinical use as add-on therapies and monotherapies [74, 103–105]. MAO-B inhibitors not only prolong the effectiveness of L-DOPA by increasing available DA but also demonstrate disease-modifying effects in preclinical models (e.g., neuroprotective effects against dopaminergic cell death). Polypharmacological ligands with MAO-B inhibitory effects along with iron chelation (e.g., M30 [106] and VAR10303 [107]), ChE inhibitory (e.g., Ladostigil [74] and MT-30R [108]), H3 antagonism (e.g., Contilisant [109]), or A2A antagonism (e.g., CSC [110]) properties have thus been developed on the basis of the complex pathogenesis of PD. The Cheong group [15] summarized the potential of MAO-B-related multitarget therapy as a treatment for PD in 2019. In addition, SU4312, a potent and selective inhibitor of vascular endothelial growth factor receptor-2 originally used as an anticancer agent, was identified as a dual ligand that protects against MPTP-associated neurotoxicity in PD in vitro and in vivo via the activation of the transcriptional activity of myocyte enhancer factor 2 and the inhibition of MAO-B [111].

On the other hand, there is still a pressing medical need for the development of reversible selective MAO-B inhibitors with effectiveness and safety for long-term use to treat PD [112]. Selegiline and rasagiline are irreversible MAO-B inhibitors with undesirable adverse effects, including hallucination and headache, the production of neurotoxic or ineffective metabolites, and gradual short-lived action after long-term use. Safinamide, a recently approved MAO-B inhibitor with high selectivity and reversibility, can also cause adverse effects due to undesirable actions, such as the inhibition of sodium and calcium channels and the stimulation of glutamate release [113–115]. Examples of highly selective MAO-B inhibitors tested preclinically for the treatment of PD are shown in Fig. 4. Compound 8 [116] (100 mg/kg) induces a significant increase in motor activity, velocity and movement compared with those induced by selegiline in mice pretreated with reserpine. SZV558 [117, 118] is not only protective against oxidative stress induced by 6-OHDA and rotenone during PC-12 cell death in vitro but also has neuroprotective effects against striatal DA depletion and motor dysfunction in vivo in MPTP-induced PD mice and in a chronic mouse model of MPTP plus probenecid administration. 5b [114] is a reversible selective MAO-B inhibitor with metabolic stability in human liver microsomes that has a minimal effect on CYP inhibition, and the oral administration of this inhibitor shows potency in vivo therapeutic efficacy on motor deficits similar to that of safinamide in an animal model of MPTP-induced PD. The oral administration of 12c [113] can significantly protect tyrosine hydroxylase-immunopositive DA neurons and attenuate PD-associated motor impairment in a mouse model of MPTP-induced PD.

SUMMARY AND PERSPECTIVES

Although L-DOPA has been considered to be the most effective therapeutic strategy for PD for more than 50 years, currently approved drug therapies remain unsatisfactory since they only provide symptomatic relief but are unable to reverse disease progression. Additional options emerging in the clinic are symptomatic treatments. Although most agents currently being developed activate the D2 and D3 DA receptors, there has been a substantial research effort in efficaciously and selectively activating the DA D1 receptor, a known target for the treatment of PD.
that has been pursued for 40 years but for which there are no approved drugs. Recent clinical and preclinical advances in noncatechol selective D1R agonists have highlighted the potential to overcome the drawbacks of previous catechol selective D1R ligands. Moreover, the G5-mediated D1 receptor signaling pathway may be responsible for the development of LID, whereas β- arrestin2-mediated signaling may attenuate LID and remedy locomotor deficits; [117] thus, it is worth developing biased D1 DA ligands as valuable chemical tools for PD research. In addition, the identification of PAMs also offers new potentials for PD treatment [118–120]. Such endeavors will further expand the biological options for PD drugs.

The design of multitargeted ligands involved in neuroprotection, including MAO-B inhibitors, to address the medical limitations of current PD treatments continues to be an important focus with intensive preclinical and clinical efforts. Promising multifunctional lead molecules such as D-607, D636, and D653 have the potential to improve current PD treatments.

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ADDITIONAL INFORMATION

Competing interests The authors declare no competing interests.

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