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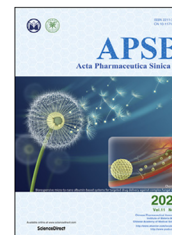

REVIEW

Targeting autophagy using small-molecule compounds to improve potential therapy of Parkinson's disease

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KEY WORDS

Parkinson's disease (PD);
Autophagy;

Abstract Parkinson's disease (PD), known as one of the most universal neurodegenerative diseases, is a serious threat to the health of the elderly. The current treatment has been demonstrated to relieve symptoms, and the discovery of new small-molecule compounds has been regarded as a promising strategy. Of note, the homeostasis of the autolysosome pathway (ALP) is closely associated with PD, and impaired

Abbreviations: 3-MA, 3-methyladenine; 5-HT2A, Serotonin 2A; 5-HT2C, serotonin 2C; α-syn, α-synuclein; A2A, adenosine 2A; AADC, aromatic amino acid decarboxylase; ALP, autophagy-lysosomal pathway; AMPK, 5′AMP-activated protein kinase; *ATP13A2*, ATPase cation transporting 13A2; ATG, autophagy related protein; ATTEC, autophagosome-tethering compound; AUC, the area under the curve; AUTAC, autophagy targeting chimera; BAF, bafilomycinA1; BBB, blood–brain barrier; CL, clearance rate; CMA, chaperone-mediated autophagy; CNS, central nervous system; COMT, catechol-*O*-methyltransferase; DA, dopamine; DAT, dopamine transporter; *DJ-1*, Parkinson protein 7; DR, dopamine receptor; ER, endoplasmic reticulum; ERRα, estrogen-related receptor alpha; *F*, oral bioavailability; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; *GBA*, glucocerebrosidase β acid; GWAS, genome-wide association study; HDAC6, histone deacetylase 6; HSPA8, heat shock 70 kDa protein 8; HSC70, heat shock cognate 71 kDa protein; IMPase, inositol monophosphatase; IPPase, inositol polyphosphate 1-phosphatase; Lamp2a, type 2A lysosomal-associated membrane protein; KI, knockin; LAMP2A, lysosome-associated membrane protein 2 A; LC3, light chain 3; LIMP-2, lysosomal integrated membrane protein-2; *LRRK2*, leucine-rich repeat sequence kinase 2; LRS, leucyl-tRNA synthetase; LUHMES, lund human mesencephalic; mAChR, muscarinic acetylcholine receptor; MAO-B, monoamine oxidase B; MPP^+, 1-methyl-4-phenylpyridinium; MPTP, 1-methyl-4-phenyl-1,2,5,6-tetrahydropyridine; mTOR, the mammalian target of rapamycin; MYCBP2, MYC-binding protein 2; NMDA, *N*-methyl-D-aspartic acid; ONRs, orphan nuclear receptors; Parkin, parkin RBR E3 ubiquitin–protein ligase; PD, Parkinson's disease; PDE4, phosphodiesterase 4; PI3K, phosphatidylinositol 3-kinase; PI3P, phosphatidylinositol 3-phosphate; *PINK1*, PTEN-induced kinase 1; PLC, phospholipase C; PREP, prolyl oligopeptidase; ROS, reactive oxygen species; SAR, structure–activity relationship; SAS, solvent accessible surface; SN, substantia nigra; *SNCA*, α-synuclein gene; SYT11, synaptotagmin 11; TFEB, transcription factor EB; TSC2, tuberous sclerosis complex 2; ULK1, UNC-51-like kinase 1; UPS, ubiquitin–proteasome system.

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Target;
Small-molecule compound;
PD therapy

autophagy may cause the death of neurons and thereby accelerating the progress of PD. Thus, pharmacological targeting autophagy with small-molecule compounds has been drawn a rising attention so far. In this review, we focus on summarizing several autophagy-associated targets, such as AMPK, mTORC1, ULK1, IMPase, LRRK2, beclin-1, TFEB, GCase, ERRα, C-Abelson, and as well as their relevant small-molecule compounds in PD models, which will shed light on a clue on exploiting more potential targeted small-molecule drugs tracking PD treatment in the near future.

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1. Introduction

Parkinson's disease (PD) is an incurable neurodegenerative disease characterized by dopaminergic neurons loss and Lewy bodies (LB) formed by abnormal aggregation of α-synuclein (α-syn) protein, involving some clinical symptoms like myotonia, cognitive changes, neurological dysfunction, motor retardation as well as posture disorders[1]. Indeed, PD is considered as a progressive disease, drug therapy is the main choice in clinical treatment. The Parkinson's disease drugs approved by US Food and Drug Administration (FDA) can be roughly divided into four categories including dopaminergic system drugs, serotonergic system drugs, cholinergic system drugs, and others. Aromatic amino acid decarboxylase (AADC) inhibitors, catechol-*O*-methyltransferase (COMT) inhibitors, monoamine oxidase B (MAO-B) inhibitors, dopamine transporter (DAT) inhibitors and dopamine receptor (DR) agonists affect the dopaminergic system, while 5-hydroxytryptamine (5-HT) 2A and 2C receptor antagonists target serotonergic system. Cholinergic system drugs comprise muscarinic acetylcholine receptor (mAChR) antagonists. And adenosine receptor (A2A) inhibitors and glutamate receptor (NMDA) antagonists are considered as other medications for PD[2–5]. As the main strategy is to alleviate the symptoms of patients in a short time, present long-term clinical applications are particularly vulnerable to serious autonomic nervous dysfunction and other insensitive symptoms, failing to achieve satisfactory treatment results[6]. Among the complex pathogenesis of PD, disequilibrium of autophagy homeostasis acts as a trigger for PD progression while cytoprotective autophagy can save related pathological states in preclinical models of PD.

Autophagy is an evolutionarily conserved process for eukaryotic cells to degrade themselves through lysosomes, which plays a vital role in cell survival, proliferation and death. It may help to maintain the homeostasis of internal environment. Autophagy can degrade most of the cytoplasm and even the entire organelle, including the pathogenic aggregation of intra-neuronal proteins (Lewy bodies) which are composed of α-syn. In this process, the defective protein or organelle is firstly engulfed by the phagocytic vesicle with a lipid bilayer membrane, then the autophagosome emerges. Afterwards, these structures are transported to the lysosome. With the combination of a lysosome, it forms an autophagolysosome to complete the degradation[7]. Therefore, the application of existing autophagic regulators, as well as the discovery of new small molecules targeting the autolysosome pathway (ALP), have been demonstrated as a great potential on PD treatment.

2. Molecular mechanisms of autolysosome pathway in PD pathogenesis

Autophagy is functionally active within the central nervous system, which maintains the homeostasis of neurons and glial cells[8–11]. Highly-differentiated neurons and glial cells are difficult to repair damage and demand more energy for normal activities and autophagic recycling of components than other cells, therefore, requiring sophisticated quality control system[9,12].

Indeed, during high-intensity work in the central nervous system (CNS), the ALP is prone to produce dysfunction, while some risk gene mutations of PD also exert deleterious effect on ALP, including some selective autophagy pathways like mitophagy and chaperone-mediated autophagy (CMA). As a result, impaired ALP may promote toxic protein aggregates (like α-syn) accumulation and lead to lysosome dysfunction, delayed clearance of defective mitochondria and increased oxidative stress, which are all considered as stimulators of PD (Fig. 1)[13]. In a zebra fish PD model treated with MPTP, ATG5 was found decreased, which was correlated with blocked autophagy flux, leading to increase of PD-associated proteins like β-syn, parkin and *PINK1*, together with loss of dopamine (DA) neurons[14].

Recently, with the analysis of genome-wide association study (GWAS), five relative genes of PD, α-syn (*SNCA*), gene leucine-rich repeat sequence kinase 2 (*LRRK2*) genes, PTEN-induced kinase 1 (*PINK1*), *Parkin* RBR E3 ubiquitin–protein ligase (*PARK2*, *Parkin*), and Parkinson protein 7 (*PARK7*, *DJ-1*) were identified[15]. Of note, mutations of these genes are considered as risk factors of PD and one of the pathogenesis mechanisms of PD underlying those genes mutations is the ALP defects.

For example, *LRRK2* has captured much attention in PD pathogenesis[16], and the relationship of its mutations and autophagy has been extensively investigated and reviewed in recent years[17–20]. Accumulating evidence has pointed that the impaired ALP caused by *LRRK2* mutations may compose one of its pathogenic mechanisms, whereas the exact mechanisms remain unclear. In general, G2019S, the most frequent mutation in *LRRK2*, was discovered to trigger progressive dysfunctions of dopamine transporters[21]. Also, *LRRK2* G2019S could phosphorylate and activate leucyl-tRNA synthetase (LRS) and then activating mTORC1, thereby suppressing autophagy[22]. And the single loss of *LRRK2* was reported to not promote selective neurodegeneration, level of α-syn and impairment of ALP, revealing an essential role of *LRRK2* in the modulation of ALP pathways[23]. Besides the traditional autophagy pathways, some latest studies

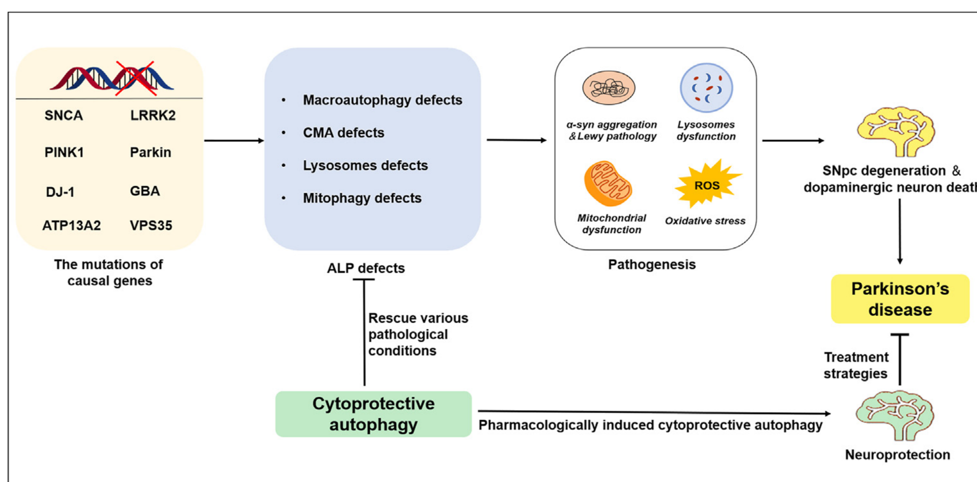

Figure 1 The relationship between autophagy and PD: the mutations of casual genes cause some ALP defects and further lead to key pathological states. ALP dysfunction is one of the important pathogenesis of PD. Pharmacologically induced autophagy can rescue the pathological states to a certain extent and play a neuroprotective role.

also shed light on its role in regulating selective autophagy associated with PD, such as CMA and mitophagy[24]. In striatum and cortex of aged *LRRK2* R1441G knockin (KI) mice, accumulation of CMA-specific lysosome-associated membrane protein 2 A (LAMP2A) and heat shock 70 kDa protein 8/heat shock cognate 71 kDa protein (HSPA8/HSC70) and increased CMA substrate glyceraldehyde 3-phosphate dehydrogenase (GAPDH) were observed, indicating that *LRRK2* mutations may disturb CMA to degrade oligomeric α-syn thereby promoting PD[25]. Overexpression of kinase active *LRRK2* was reported to restrain mitophagy to trigger mitochondrial dysfunction, which was aggravated by the expression of kinase hyperactive *LRRK2*-G2019S variant and thus accelerate PD progress[26].

Importantly, the mutations of *SNCA* contribute to abnormal aggregates of α-syn (like oligomer and fibrosis forms)[27−29], which was identified to participate in the pathogenesis of PD[30], representing the predominant Lewy pathology in PD. These aggregates, which could be degraded by the ALP[31,32], may in turn impair mitochondrial, autophagic, and lysosomal functions, aggravating the toxic protein aggregates[33]. α-Syn overexpression was found to generate ER stress, along with lysosomal pH alkalization, lower LAMP1 levels, and a disruption of lysosomal morphology and distribution, impairing autophagy[34]. *Via* abolishing the hydrolase trafficking, the accumulation of α-syn dampens the ability of lysosomes to degrade dopamine in human midbrain dopamine models[35]. In DA neuron-specific autophagy-deficient mice (autophagy related protein (ATG) 7 knock out), P62 inclusions occurred before synuclein, suggesting the dysfunction of autophagy may be a cause of LB formation[36].

Besides, loss of GCase activity due to glucosylceramidase β acid (*GBA*) mutations impairs the ALP, resulting in increased α-syn levels, which dampen GCase activity in turn[37]. The L444P heterozygous mutation of *GBA*, a lysosomal enzyme that degrades glucosylceramide to ceramide and glucose, was reported to generate mitochondrial dysfunction by inhibiting key steps of mitophagy, which underlies part of its pathogenetic roles in PD[38].

Additionally, other genes mutations were also proved to link with ALP dysfunction. *PINK1* and *Parkin* mutations were considered to affect mitophagy[39,40], which render the delayed clearance of defective mitochondria, exacerbating PD. Both deficiency and mutations of ATPase cation transporting 13A2 (*ATP13A2*) can damage autophagosome-lysosome fusion through disability of recruiting histone deacetylase 6 (HDAC6) to lysosomes[41]. In another study, ATP13A2 was found to activate mTORC *via* the activation of tuberous sclerosis complex 2 (TSC2) caused by its interaction with MYC-binding protein 2 (MYCBP2), and decrease the TEFB-dependent synaptotagmin 11 (SYT11) expression, thus block autophagy[42].

As mentioned above, autophagy defects related to sporadic and familial PD may cause the accumulation of protein aggregates and damaged organelles, eventually cell death. Targeting ALP, either chemically or genetically, is proved to be beneficial for neuronal survival, which represents a potential treatment strategy for PD.

3. Targeting autophagy for potential therapy of PD

A variety of small-molecule compounds have been demonstrated in PD models to achieve therapeutic effects by regulating autophagy pathways (Fig. 2 and Table 1[43−79]). Herein, we discuss some pivotal instances of autophagy-related targets and representative small-molecule compounds.

3.1. Targeting AMPK

5′AMP-activated protein kinase (AMPK) is a key factor in the regulation of autophagy and the main sensor of intracellular energy stress, which is able to perceive and respond to the energy changes, and crucial to energy homeostasis[80,81]. With seven isoforms (α1, α2, β1, β2, γ1, γ2 and γ3), AMPK is a heterotrimeric protein kinase involving the structure of α catalytic subunit, scaffold β subunit, and regulatory γ subunit. In theory, AMPK could form up to 12 possible heterotrimers with different regulatory functions. The catalytic kinase domain was in the α subunit and the main activation site of AMPK was Thr172[82]. The cell function of AMPK depended on the level of ATP a lot. Alterations in the ratio of AMP/ADP/ATP would lead to the activation or

Table 1 Autophagy-modulating compounds in PD.

Target	Origin	No.	Agent	Main effect	PD model	Reported year in PD	Ref.
AMPK	Natural product	2	Resveratrol	Prevented loss of DA neurons; rescued TH and DA levels; improved behavioral abnormalities	MPTP treated mice	2008	43
		8	Caffeine	Reduced p129-α-syn; apoptosis; microglial activation; astrogliosis; increased LC3II/I ratio	A53T α-syn transgenic C57BL/6J mice	2001	76
	Chemical synthesis	1	Metformin	Reduced levels of p-Ser129 α-syn *in vivo* and *in vitro*, increased striatal dopaminergic levels	α-Syn overexpressing SH-SY5Y cells and C57BL/6J mice	2014	44
		3	A769662	Reduced levels of α-syn inclusions	α-Syn overexpressing SH-SY5Y cells	2019	75
		4	GSK621	Reduced levels of α-syn inclusions	α-Syn overexpressing SH-SY5Y cells	2019	75
		5	Rosuvastatin	Increased cell viability; increased α-syn clearance	Rotenone-exposed SH-SY5Y cells	2017	55
		6	FCPR16	Protected neuronal cells against MPP^{+}-induced toxicity and oxidative stress	SH-SY5Y cells treated with MPP^{+}	2018	66
		7	Temozolomide	Increased α-syn clearance and protected α-syn-induced cytotoxicity	α-Syn overexpressing LUHMES cells	\	74
mTORC1	Natural product	9−11	Rapamycin and Rp analogues (CCI-779 and AP23573)	Increased the clearance of WT, A30P and A53T α-syn	α-Syn expressing PC12 cells	2010	77
				Prevented neuronal death in mice; reduced α-syn accumulation, improved motor function	MPTP treated mice; α-syn transgenic mice	\	50,51
		12	Corynoxine	Promoted the clearance of wild-type and mutant α-syn *via* autophagy	α-Syn overexpressing PC12 cells	2014	78
		13	Loganin	Decreased MPTP-induced neurotoxicity	MPTP treated PC12 cells	2017	79
	Chemical synthesis	14	PI-103	Increased α-syn clearance and protected α-syn-induced cytotoxicity	α-Syn overexpressing Lund human mesencephalic (LUHMES) cells	\	74
ULK1	Chemical synthesis	15	BL-918	Protected against MPTP-induced motor dysfunction and loss of dopaminergic neurons	MPP^{+}-treated SH-SY5Y cells	\	45
IMPase	Chemical synthesis	16	Sodium valproate	Enhanced autophagy, reduced mitochondrial membrane potential, reduced production of reactive oxygen species and enhanced cell viability	Rotenone-induced SH-SY5Y cells	\	46
		17	Carbamazepine	Enhanced autophagy, reduced mitochondrial membrane potential, reduced production of reactive oxygen species and enhanced cell viability	Rotenone-induced SH-SY5Y cells	\	46

Table 1 (*continued*)

Target	Origin	No.	Agent	Main effect	PD model	Reported year in PD	Ref.
		18	L-690,330	Increased α-syn clearance and protected α-syn-induced cytotoxicity	α-Syn overexpressing LUHMES cells	2019	74
LRRK2	Chemical synthesis	19	LRRK2-IN-1	Rescued *Lrrk2* G2019S mutant motor injury	*Drosophila*	\	48
		23	GNE-7915	Enhanced DA release and recovery	R1441G transgenic mice	\	47
		25	PF-06447475	Reduced neuronal apoptosis and alleviated neurological deficit	Weight-drop Sprague—Dawley rats after traumatic brain injury	\	130
			DNL151[a]			\	49
			DNL201[a]			\	49
Beclin-1	Natural product	29	Isorhynchophylline	Promoted clearance of wild-type, A53T and A30P α-syn	Differentiated human dopaminergic neurons	2014	53
		30	Corynoxine B	Reversed reduction in LC3-II and BECN1; restored the deficient autophagy induced by *SNCA*	α-Syn overexpressing PC12 cells	2019	54
		31	Glycyrrhizic acid	Increased cell viability; up-regulated LC3-II/I and beclin-1	6-OHDA and crticosterone treated SH-SY5Y cells	2018	56
	Chemical synthesis	28	KYP-2047	Enhanced autophagy and α-syn clearance; increased in striatal dopamine levels	A30P α-syn transgenic mice	\	52
TFEB	Natural product	32—35	Curcumin and Cur analogues (CNB001, C1 and E4)	Inhibited the accumulation of α-syn and prevented the accumulation of LB	α-Syn overexpressing SH-SY5Y cells	\	57—62
		36	Trehalose	Reduced the loss of SNpc DA neurons and produced a neuroprotective effect	Multiple PD related models	2004	63
GCase	Chemical synthesis	37	Ambroxol[a]	Increased GCase activity and reduced oxidative stress	PD fibroblasts with *Gba1* mutations	2014	64,65,158
		38	Isofagomine	Increased GCase levels and activity; lowered ER stress and prevented the loss of motor function	PD fibroblasts with *Gba1* mutations; *Drosophila*	\	67
		39	NCGC607	Restored GCase activity and protein levels; reduced α-syn levels	iPSC-derived macrophages and dopaminergic neurons	\	68
ERRα	Chemical synthesis	40	XCT790	Alleviated dopaminergic neuronal loss; cleared toxic protein aggregates; ameliorated behavioral impairments	MPTP treated mice	2018	69,70
c-ABL	Chemical synthesis	41	PD180970	Cleared toxic α-syn protein aggregates; alleviated behavioral impairments	α-Syn overexpressing SH-SY5Y cells and MPTP induced mice	2019	71
		42	Imatinib	Reduced expression of c-ABL and p-GSK3β; restored ALP and decreased cells death	MPP^+-induced SN4741 cells	2014	72

(*continued on next page*)

Table 1 (*continued*)

Target	Origin	No.	Agent	Main effect	PD model	Reported year in PD	Ref.
		43	Nilotinib[a]	Reduced c-ABL activation, prevented dopamine (DA) neuron loss and behavioral deficits; induced α-syn degradation *in vivo* and *in vitro*	MPTP-induced mice; α-syn overexpressing primary cultures of mouse cortical neurons and mice	2014	72,73

[a]Drugs which have already entered clinical trials. DNL151 (NCT03710707) and DNL201 (NCT04056689) in phase I trial while nilotinib (NCT02954978; NCT03205488) and ambroxol (NCT02941822) are in phase II trail. Data source: http//clinicaltrials.gov; November 2020.

inactivation of AMPK. AMPK activation had a wide range of neuroprotective effects to increase cell survival against many stressors, including starvation, hypoxia, ischemia, and excitotoxicity[83–86]. Therefore, AMPK activation has been extensively explored as a neuroprotective strategy for PD treatment.

Hitherto, several small-molecule compounds that can regulate AMPK activity have been identified as their cytoprotective regulation in the PD models. Metformin (**1**) is the first choice for type 2 diabetes mellitus treatment (oral bioavailability, *F*: 40%−60%, Fig. 3A). In addition to the anti-diabetic effect, its neuroprotective efficiency in the MPTP-induced PD models was also discovered remarkably in 2014[43]. Yet, this study failed to explain the potential mechanism of its neuroprotection and drug target. Since compound **1** is a well-known AMPK activator, subsequent studies investigated whether metformin prevented DA neurons from MPTP-induced neurodegeneration by activating AMPK[87,88]. Lu et al.[89] showed that **1** directly activated AMPK and initiated downstream protective signals to slow down DA neuronal apoptosis. AMPK inhibitors could eliminate the cytoprotective effect of **1** in SH-SY5Y cells, proving that AMPK activation was necessary for this effect on DA neurons. Moreover, it was also illustrated that **1** could induce autophagy and reduce the accumulation of α-syn. Resveratrol (**2**), a stilbene found in grapes and red wine, had many benefits, covering reducing oxidative stress, inflammation, and mitochondrial damage, regulating stem cell growth, neuroprotection, and inducing autophagy (Fig. 3A)[90–93]. Autophagy and mitochondrial phagocytosis induced by **2** had cytoprotective and antioxidant effects in various cell-based PD models[31,44,94–96]. It has been reported that **2** can produce a specific neuroprotection by inducing autophagy to clear α-syn. In overexpressing wild-type and mutant α-syn PC12 cells and rotenone-exposed SH-SY5Y cells, treatment with **2** raised the phosphorylation level of AMPK active site Thr172 and the SIRT1 deacetylation enzymatic activity, representing that **2** enhanced the degradation of α-syn by activating autophagy through the AMPK/SIRT1 signaling pathway[44].

The aforementioned studies of **1** and **2** directly show that they are two commonly used and the most representative autophagy activators targeting AMPK in the PD models. Besides, some small-molecule synthetic compounds with similar neurological effects have been also discovered, such as A-769662 (**3**), GSK621 (**4**), rosuvastatin (**5**), FCPR16 (**6**) and Temozolomide (**7**,

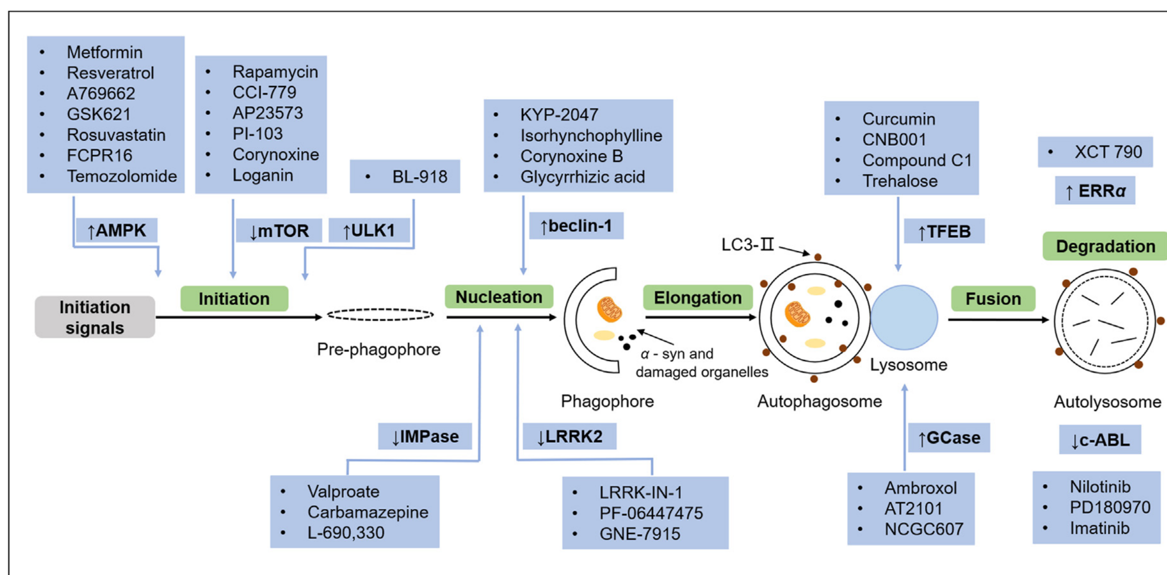

Figure 2 Several pharmacological interventions are available to induce cytoautophagy at the nucleation, elongation, fusion, or degradation phase. The figure shows the key targets and representative small-molecule compounds in autophagic regulatory pathways.

Fig. 3A)[31,55,66,74,97,98]. Compound **3** is an AMPK agonist with a structure of thienopyridone, which can reversibly activate AMPK with EC_{50} values of 116 nmol/L[75]. The important role of AMPK in the regulation of lipid and glucose metabolism aroused pharmaceutical companies to develop AMPK activators for the treatment of various metabolic diseases. The non-nucleoside thienopyridone compound A-592107 (**4-1**) with EC_{50} values of 38 μmol/L was firstly obtained from a molecule database of more than 700 thousand compounds and identified as a direct AMPK activator[75]. Subsequently, sub-micromolar compound **3** was discovered in the lead compound optimization processes. Compound **3** could inhibit the dephosphorylation of Thr172 and provide moderate allosteric activation, which was similar to the AMP-induced pathway[75]. Nonetheless, the oral bioavailability of **3** was poor ($F = 7\%$). To improve oral absorption and selectivity, the GlaxoSmithKline (GSK) researchers took advantage of compound **3** as a template to reconstruct and identified a new type of AMPK activators of pyrrolo-pyridine analogs[97]. Biological replacement of the thiophene ring in **3** with pyrrole reached compound **4-1** imparted it better oral bioavailability ($F = 10\%$). Replacing hydrogen in the pyrrole ring with chlorine failed to improve blood clearance or permeability, but the oral bioavailability could be improved. Afterwards, a series of different functional group substitutions on R_1 and R_2 increased efficacy and reduced blood clearance. Although the substitution of 3-methoxy-2-phenol at the R_2 position slightly reduced oral bioavailability, it could obviously reduce the blood clearance rate. The presence of a cyano group at the R_1 position of the pyridone ring resulted in poor permeability owing to its acidity. Replacing cyano group at R_1 with phenyl imparted **4-3** better permeability and oral bioavailability[97]. Compound **4** was derived by changing the pyrrolopyridone core of **4-3** to pyrrolopyrimidone (Fig. 3B). Compound **4** was slightly less effective than **3** in continuously activating AMPK recombinant heterotrimers *in vitro*, but **4** was more potent than **3** in inducing AMPK activation in cell assays[99]. In the same study in 2019, Nicolas[31] found that the two effective AMPK activators **3** and **4** were able to induce autophagy by activating AMPK and decrease the content of α-syn in overexpressing α-syn SH-SY5Y cells, showing similar neuroprotective effects. Besides, compound **5** is a statin drug, which can competitively inhibit HMG-CoA reductase and is widely used as a cholesterol-lowering drug. A study in 2017 showed that **5** reduced rotenone-induced neurotoxicity in SH-SY5Y cells through autophagy regulation and accelerated the clearance rate of α-syn[55]. This study published in 2017 provided the first experimental evidence for the neuroprotective role of statins by enhancing the autophagy pathway in the PD models. *N*-(2-Chlorophenyl)-3-cyclopropylmethoxy-4-difluoromethylbenzamide (**5**) is a phosphodiesterase 4 (PDE4) inhibitor and plays a beneficial role in the CNS diseases due to its anti-inflammatory and anti-apoptotic characteristics. Compound **5** effectively protects SH-SY5Y neuronal cells from MPP^+-induced oxidative damage by activating autophagy of AMPK and **5** has almost no emetic side effects of general PDE4 inhibitors[66]. Furthermore, the natural product caffeine (**8**) was found to reduce α-syn-induced autophagy defects by up-regulating AMPK (Fig. 3A)[76]. Many central mechanisms underlying PD pathology could be regulated by activating AMPK. However, given the broad regulatory role of AMPK, it is necessary to identify specific downstream pathways that are most beneficial for the treatment of PD, so that the pathways can be targeted more selectively without activating other harmful pathways. Consequently, activation of PD-related AMPK-dependent pathways may reduce neurodegeneration and ultimately inhibit PD progression.

3.2. *Targeting mTORC1*

Rapamycin (**9**) is one of the most widely applied small molecules in the mTOR dependent method for PD therapy (Fig. 4). Compound **9**, an autophagy regulator, is an antifungal macrolide isolated from strains of streptomyces hygroscopicus analogues. Compound **9** binds to the FKBP12 receptor in the cell and forms a complex. Subsequently, the complex can directly integrate with mTOR, which prevents the combination of mTOR and RAPTOR, blocks the signal transduction of mTORC1, and activates autophagy[100,101]. The biological function of **9** is diverse, such as antifungal, immunosuppression, antitumor, neuroprotection, and anti-aging effect[77,102,103]. Compound **9** and its derivatives are mainly used as immunosuppressants and anti-tumor drugs in the clinic. Given that mTOR is the central hub of the signal network in the cell, it has been extensively explored. Surprisingly, there is a close relationship between **9** and PD. Compound **9** is proved to increase the formation of autophagosome and the efficiency of α-syn clearance both *in vivo* and *in vitro*. Compound **9** can not only prevent the death of dopaminergic neurons but also improve the motor function of transgenic mice[77,101,104]. Furthermore, treatment with **9** increases the level of the lysosomal structural protein LAMP1 in the mouse brain, demonstrating that it is capable of increasing the lysosomal biogenesis and avoiding the accumulation of autophagosomes and neuronal potential toxicity[105]. Some analogues of **9**, such as CCI-779 (**10**) and AP23573 (**11**), were clarified to exert similar neuroprotective effects (Fig. 4)[106–108]. Besides, long-term treatment with **9** could inhibit mTORC2, which may stimulate other important cellular pathways such as affecting cell survival mechanisms. Nonetheless, compound **9** can cause a wide range of side effects including oral and respiratory infections, stomatitis, leukopenia, hypertriglyceridemia, hypercholesterolemia, and immunosuppression[100,109]. Obviously, **9** and its analogues are unsuitable for long-term and high-dose therapy due to their adverse effects. Generally speaking, owing to their profitable action on different PD pathological models, these studies provide important theories on the role of autophagy and prove that inducing autophagy is a superior strategy in experiments.

According to the researches on PD therapy, most compounds targeting mTORC1 are natural products including oxindole alkaloids corynoxine (**12**) and iridoid glycosides glycosides loganin (**13**, Fig. 4)[78,79]. In PC12 cells with high expression of wild-type (WT) and mutant (A53T) α-syn, both of their α-syn were significantly degraded after the treatment with **12**. The effect was blocked by the autophagy inhibitor 3-MA and the lysosomal inhibitor CQ, suggesting that the clearance action occurred through autophagy[78]. Compound **12** was identified to reduce phosphorylated mTOR (Ser2448), AKT (Ser473), and p70S6K (Thr389), elucidating that it induced autophagy by inhibiting the AKT/mTOR pathway. In addition, synthetic small molecules targeting mTORC1 also had neuroprotective effects, such as the PI-103 (**14**) with a structure of pyridylfluoropyrimidine (Fig. 4)[74]. Compound **14** was initially discovered through high-throughput screening, which had an arylmorpholine pharmacological group to form a key hydrogen bond with the framework Val882 amide of class I PI3K. It could

effectively and competitively inhibit all class IA, α, β and δ with IC_{50} values of 2, 3 and 3 nmol/L respectively which indicated it was a selective inhibitor of class I PI3K[110]. Subsequently, a study characterized the target selectivity of **14** and proved its effective inhibition of mTORC1 with the IC_{50} values of 20 nmol/L. Therefore, compound **14** was proved to be a dual-target inhibitor of mTOR and PI3K[111].

Among the drugs targeting mTOR, some natural compounds emerged from traditional medicines and achieved promising outcomes in inducing autophagy for PD treatment. Small-molecule compounds are also constantly designed and synthesized. More importantly, it ignites our more attention to keep the balance between mTOR and other cellular pathways to reduce other side effects, hence mTOR inhibitors can be applied more effectively in the treatment of PD.

3.3. *Targeting ULK1*

UNC-51-like kinase 1 (ULK1), the ortholog of yeast Atg1, is the only serine-threonine kinase and primase, which participates in the formation of early membrane structures of phagocytic vesicles and plays a vital role in the autophagy process[112,113]. When ULK1 was activated, beclin-1 was phosphorylated respectively at Ser15 and Ser29, triggering the lipid kinase activity of phosphoinositide-3-kinase class 3 (PIK3C3). This upregulated the expression of phosphatidylinositol 3-phosphate (PI3P) and participated in the recruitment of autophagy proteins for autophagosome formation, enabling the autophagy process to eliminate α-syn oligomers produced in the pathogenesis of PD[114]. A novel activator, BL-918 (**15**), was able to activate ULK1 effectively[45]. Firstly, this study applied the solvent accessible surface (SAS) calculation to obtain possible binding sites for the ULK1 activator. After high-throughput screening was used to obtain the lead compound **15-1**, its structural optimization was rationally promoted based on the binding site. The bridged oxygen atom was substituted with an ester group that can form another hydrogen bond with Tyr89. The imidazole ring was converted to a piperazine ring to obtain **15-2**, which improved the EC_{50} by 4.8 times and the E_{max} by 2.1 times. Afterwards, D-(−)-2-phenylglycine skeleton was introduced into the compound structure to obtain **15-3**. Compared with **15-2**, the ULK1 kinase activity of **15-3** was only slightly increased, while the autophagy activity was promoted by two times. The amino acid residues on the edge of the active pocket were not fully utilized yet, such as Asn86 and Tyr89. As a consequence, the ureido group was used as a linking group to gain the optimal compound **15** (Fig. 5). In contrast to **15-3**, the EC_{50} and E_{max} of **15** were both improved by 1.7 times and 1.3 times (EC_{50}: 40.80−24.4 nmol/L, E_{max}: 0.742 ± 0.028−1.000 ± 0.037), and the autophagy activity was also increased by 1.5 times[45]. Compound **15** could induce cytoprotective autophagy through the ULK1 complex in SH-SY5Y cells largely. Apart from that, it could also exert its neuroprotective role by targeting ULK1-regulated autophagy in the MPTP-induced PD mouse models. This study provided precious experience for designing and synthesizing new compounds that targeted autophagy for PD treatment.

3.4. *Targeting IMPase*

The regulation of autophagy *via* the inositol signaling pathway is mTOR-independent[115]. The G protein-coupled receptor-mediated phospholipase C (PLC) was able to activate this

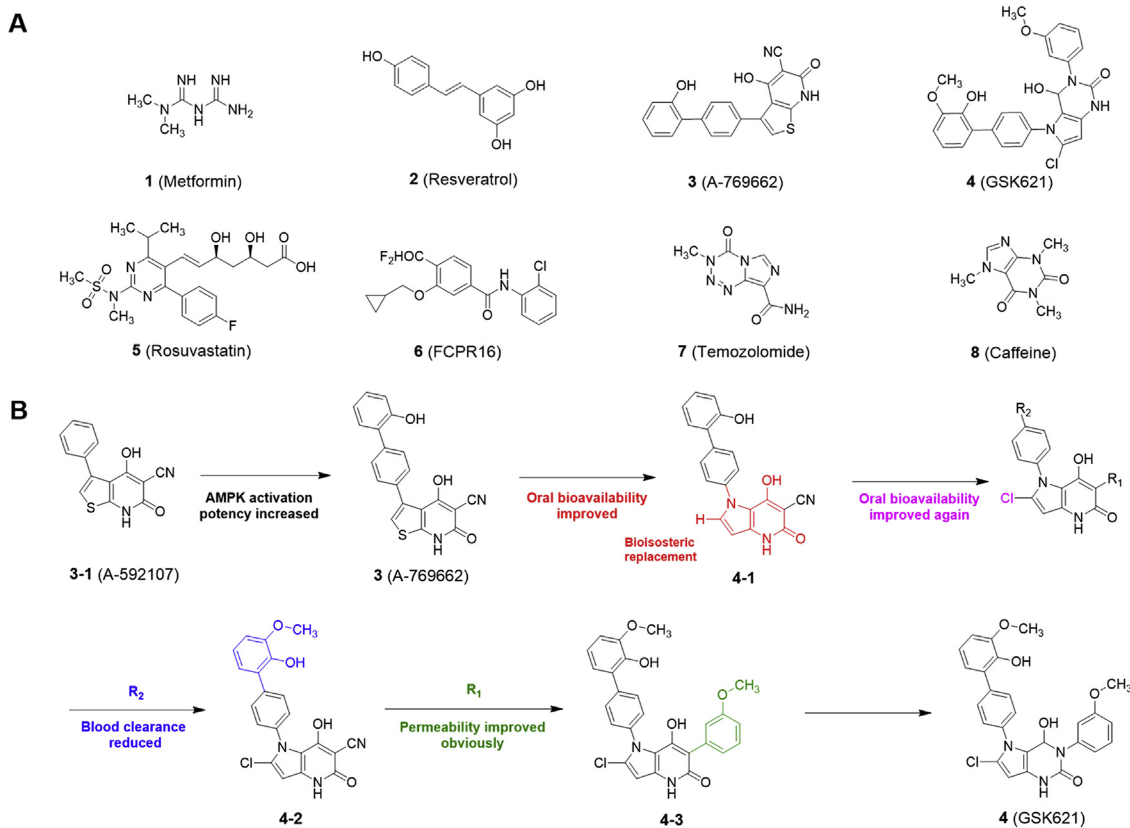

Figure 3 (A) The structures of compounds targeting AMPK. (B) The structure−activity relationship (SAR) of A-769662 and GSK621.

pathway, causing the formation of InsP3 and diacylglycerol. InsP3 bound to the InsP3 receptor on ER and the calcium was released into the cytoplasm. Because of the interaction with 5-phosphatase and inositol polyphosphate 1-phosphatase (IPPase), InsP3 formed inositol phosphate, which was then hydrolyzed to free inositol by inositol monophosphatase (IMPase). The accumulation of intracellular free inositol or InsP3 could inhibit the formation of autophagosomes. Drugs that could lower the inositol would induce autophagy and promote the elimination of autophagy substrates without inhibiting mTORC1 activity[115]. Besides, there are also two anti-epileptic and mood-stable drugs named valproate (**16**) and carbamazepine (**17**, Fig. 5B). A study highlighted that these two drugs were able to inhibit inositol monophosphatase and reduce free inositol and the level of inositol 1,4,5-triphosphate which made them hopeful autophagy enhancers[46]. Derived from this, in the subsequent rotenone-induced human neuroblastoma SH-SY5Y cell model, not only could both drugs enhance autophagy and reduce rotenone-induced mitosis and apoptosis, but also reduce the mitochondrial membrane potential and the activity of oxygen production and enhance cell viability significantly[46]. However, a study in 2019 found a contradictory result that compound **17** did not significantly activate autophagy in the PD model of LUHMES cells and failed to protect cells from α-syn-induced toxicity[74]. Meanwhile, compound **17** did not affect inositol metabolism in the rat cortex after long-term oral medication[116]. Correspondingly, a case-control study in the United Kingdom indicated that there was no relationship between **17** intake and the risk of PD occurrence[117]. The two opposite experimental results above showed that **17** can induce autophagy theoretically, but the compound is still not effective enough in neuronal cells yet. It also implied that the effects of drugs in various models may be different, and drug development was a complex and tortuous process. On the contrary, another small-molecule inhibitor of IMPase, L-690,330 (**18**), produced a promising result in LUHMES cells (Fig. 5B). Compound **18** could inhibit IMPase in LUHMES cells and reduce free inositol levels. It also could activate autophagy and protect cells from α-syn-induced toxicity, which had a good research prospect[74]. As can be seen from the previous description, drugs targeting the inositol pathway are not necessarily effective enough. And whether they will cause other side effects still need to be continuously verified. Compared with long-term using one single inhibitor, moderate combination therapy with IMPase and mTOR inhibitors may be safer for PD treatment.

3.5. *Targeting LRRK2*

Raised attention in 2004, LRRK2 was a multidomain protein that displayed dual kinase and GTPase activities. Overexpression or mutation of the *LRRK2* gene, especially G2019S missense mutation, will increase the kinase activity, leading to familiar autosomal dominant PD and sporadic PD[118]. Thus, kinase inhibition is expected to be a therapeutically advantageous target for treating PD[119]. LRRK2 is inevitably associated with PD through the lysosomal pathway of autophagy[120]. The chemical inhibition of LRRK2 kinase activity was investigated to stimulate atypical autophagy in the H4 glioma cell line and primary astrocytes, independent of mTOR and ULK1, but dependent on the presence of active beclin-1 complex[121]. Also, when LRRK2 phosphorylated endophilinA at Ser75, endophilinA induced autophagy and further regulated membrane curvature and promoted autophagosome recruitment[122]. Given LRRK2 has close connection with autophagy, the inhibition of autophagy which relays on LRRK2 may accelerate neurodegeneration including PD[121]. In addition, University of Oxford's abundant data supported *Lrrk2* siRNA knockdown effectively induced autophagy and prevented cell death caused by starvation conditions[123]. All these data reveal that LRRK2 is involved in the regulation of autophagy and uses as a hopeful drug target for PD, whereas the exact mechanism of LRRK2 affecting autophagy still needs further investigation.

To date, small-molecular LRRK2 inhibitors have become a hotspot for research in PD. As mentioned above, mutations in *LRRK2* can enhance kinase activity. Based on different *LRRK2* mutations, various treatments have been developed, such as LRRK2 kinase inhibitors (for G2019S and I2020T mutations), LRRK2 GTPase inhibitors (for R1441G/C/H, Y1699C mutations), and LRRK2 dimer inhibitors (for all mutations)[124]. Until now, LRRK2 kinase inhibitors are considered as the most prospective candidate drugs. It's generally achieved by manufacturing competing ATP compounds in the ATP binding pocket of LRRK2.

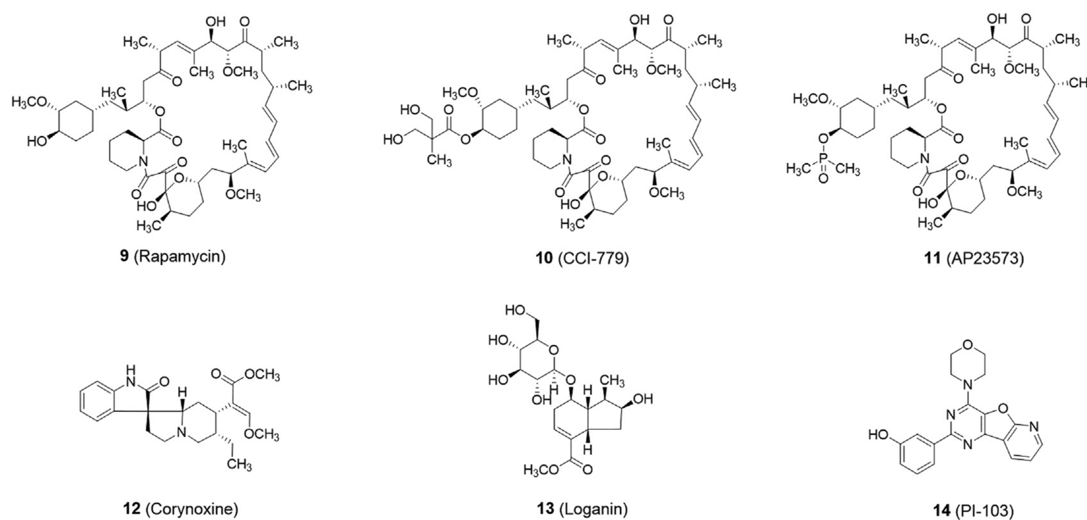

Figure 4 The structures of compounds targeting mTOR.

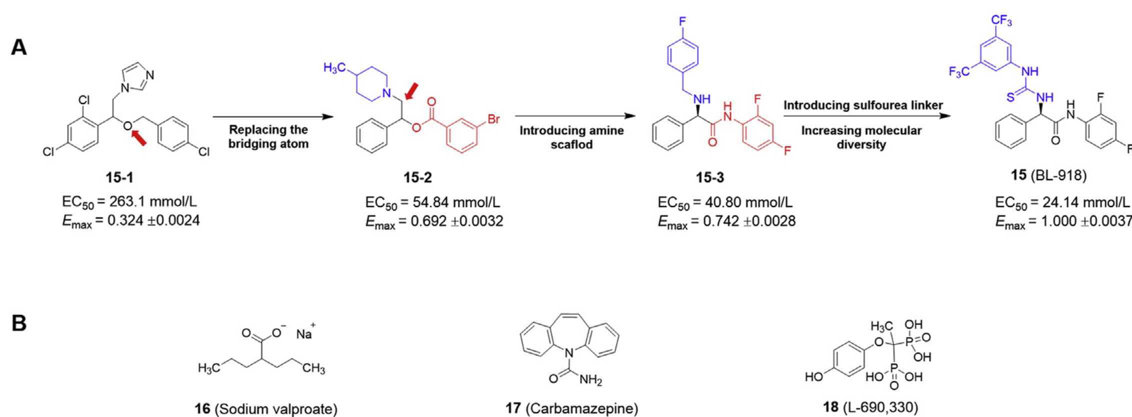

Figure 5 (A) The structure optimization process of BL-918. (B) The structures of compounds targeting IMPase.

The DYG motif of the ATP binding site activating loop is the key to control the transformation of proteins from the active form (DYG-in) to the inactive form (DYG-out), especially the glycine residue (G2019) in the motif makes the conformation more flexible. When G2019 in *LRRK2* mutates to serine, the residue may interact with other residues in the ATP binding site (such as Asp1194 in the catalytic ring) to keep the kinase in its active form (Fig. 6A). Simultaneously, D2017 in the DYG motif interacts with ATP β-phosphate. According to this general mode of action, more than 50 specific LRRK2 kinase inhibitors which own different scaffolds with excellent selectivity and potency have been explored, including targeting G2019S mutation[47]. Given that aminopyrimidine derivatives are the earliest identified and the most widely studied LRRK2 inhibitors, we discuss the SAR of these compounds concretely and deeply. As the first found LRRK2 specific inhibitor, LRRK2-IN-1 (**19**) could stimulate the dephosphorylation of LRRK2 at Ser910 and Ser935 and had been used as a lead compound to develop selective and blood−brain barrier (BBB) permeable LRRK2 inhibitors (Fig. 6B)[47,48]. It had an aminopyrimidine scaffold which was proved an effective chemotype for the discovery and optimization of LRRK2 inhibitors. Compound **19** was particularly potent against both the wild-type *Lrrk2* (IC_{50} = 13 nmol/L) and G2019S *Lrrk2* mutation (IC_{50} = 6 nmol/L)[48]. TAE684 (**20**) was initially used as an inhibitor of anaplastic lymphoma kinase (ALK), and later found to be a potent inhibitor of LRRK2 kinase activity. It was confirmed with wild-type *Lrrk2* and G2019S IC_{50} reported as 7.8 and 6.1 nmol/L (Fig. 6B)[125]. Strikingly, compound **20** had favorable selectivity against *Lrrk2* A2016T mutant (IC_{50} = 93.3 nmol/L), in contrast to **19** against *Lrrk2* A2016T mutant (IC_{50} = 2450 nmol/L). The structure of the kinase domain of LRRK2 had not been reported yet. Therefore, researchers constructed the molecular model based on the crystallographic structure of ALK[125]. It was surmised that the isopropyl sulfone moiety of **20** avoided the steric clash with A2016T residue which may conflict with the anthranilic acid ring of **19** (Fig. 6B)[125].

Although these LRRK2 small molecule inhibitors discussed above showed relatively high inhibiting LRRK2 activity, none of them had achieved enough CNS exposure which limited their application in mouse PD models, suggesting urgency of optimizing the BBB penetration ability[126]. HG-10-102-01 (**21**, Fig. 6B) was reported as the first brain penetrant LRRK2 inhibitor with aminopyrimidine scaffold and showed more potent inhibitory ability against A2016T + G2019S mutant due to weaker steric clash with A2016T[126]. With the docking model with **21** and its derivatives, JAK2, the homology model of LRRK2, showed that the C-5 group with increased lipophilicity and size could form van der Waals force with Met1947 gatekeeper side chain better, such as C-5 chlorine[127]. Based on a more accurate model, it could be deduced that the carbonyl oxygen of the amide group formed weak hydrogen bonds with the guanidine side chain of Arg1957. The aniline ring bound to the flat hydrophobic cleavage bond along the hinge near the opening of the ATP binding site. 4-Morpholinoamide group pointed to the side chain of Phe1883 and C-4 *N*-methyl filled the hydrophobic cavity[127]. Compared with **20**, removal of 4-anilino substituents retained the inhibitory effect of LRRK2 but improved the permeability of CNS. *Ortho*-substitution of aniline ring could significantly enhance the selectivity of these compounds. The results showed that small size methoxy substitution was the most ideal[127]. Further optimization strategy focused on the substituents of the aminopyrimidine C-4 and C-5 position. After analyzing the structure−activity relationships of C-4 and C-5, the optimal C-4/C-5 combination was C-4 *N*-methyl/C-5 trifluoromethyl substituted aminopyrimidine[127]. These substitutions led to the discovery of compound **22** (Fig. 6B). Intramolecular hydrogen bonds were formed between the F of the C-5 trifluoromethyl group and the C-4 *N*-methyl H, as same as the aniline ring N−H and the methoxy group. Simultaneously, the increase of trifluoromethyl lipophilicity was also conducive to the BBB penetration. As the oxidation potential of the pyrimidine ring was relatively lower, trifluoromethyl substitution had a better DMPK distribution than other C-5 substituents. GNE-7915 (**23**) was identified through the C-5′ substitution with fluorine (Fig. 6B). The substitution of *N*-ethyl group replacing the C-4 *N*-methyl group reduced the clearance ratio *in vivo*. Beyond that, the C-2′ methoxy group occupied the space near Leu1949, which imparted **23** good inhibitory activity. Further structural modification of aminopyrimidine scaffold LRRK2 kinase inhibitors should emphasize reducing the size and improving the penetrating ability of BBB while maintaining the inhibition of LRRK2 activity. The substitution of the aniline ring with pyrazole isomers led to the discovery of GNE-0877 (**24**, Fig. 6B)[47,128,129]. Removing amide functional groups and maintaining low molecular weight could effectively enhance brain permeability. Pyrazole substitution also eliminated the formation of potential aniline derived *ortho*-quinoneimine active metabolites. Through the SAR analysis of a series of gem-disubstituted cyano pyrazole LRRK2 inhibitors, it was found that **24** was

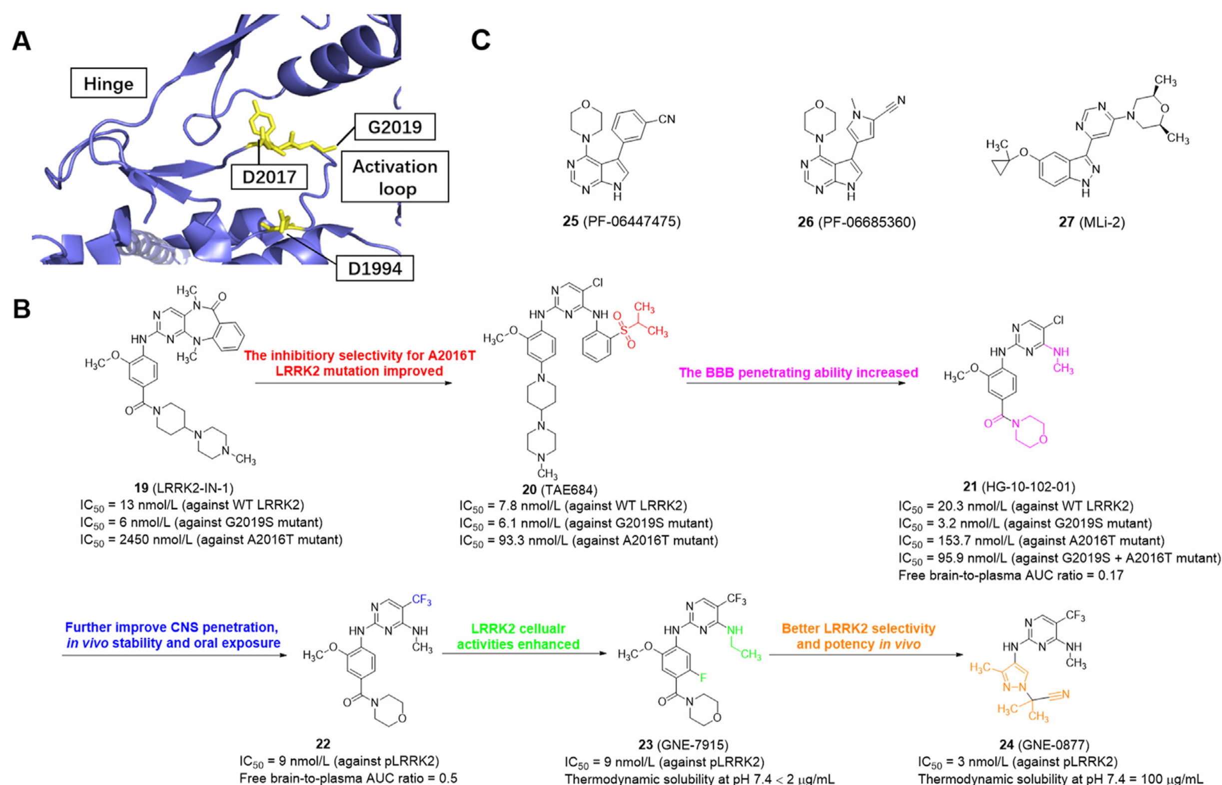

Figure 6 (A) An overview of ATP binding site of LRRK2 (PDB:6VP6). The kinase is illustrated with dark blue ribbons, and the key amino acid residues are depicted in black solid lines. (B) The SAR of aminopyrimidine derivatives LRRK2 inhibitors. (C) The structures of compounds **25**–**27**.

more effective against pLRRK2 and water-soluble compared with **23**. The two methyl groups on the pyrazole ring had better van der Waals contact with the hydrophobic residue of LRRK2, and the cyano group could form a favorable electrostatic interaction with the side chain of Arg1957, which increased the LRRK2 inhibiting activity of **24**. Another several scaffolds also have been developed as the highly potent LRRK2 inhibitors, including pyrrolopyrimidine series represented by PF-06447475 (**25**), PF-06685360 (**26**), and 3-pyrimidinyl-indazole series represented by MLi-2 (F = 41%, **27**, Fig. 6C)[130–134]. Besides, there are some LRRK2 small molecular inhibitors with unpublished structures such as DNL151 and DNL201 (NCT03710707 and NCT04056689). So far, these LRRK2 inhibitors showed great therapeutic effect in preclinical PD models[135–137]. For instance, compound **23** showed enhanced DA release and recovery in R1441G transgenic mice models allowing us to ascertain its possibility for treating PD[135]. LRRK2 inhibitors were also found to rescue *Lrrk2* G2019S mutant dopaminergic phenotypes[138]. Overall, LRRK2 inhibitors play a key role in mediating neurodegenerative phenotypes and certainly fuel our understanding of the efficacy of the LRRK2 inhibitors in treating PD.

Although direct inhibition of ATP binding pocket has been proved to be a relatively successful method for identification of LRRK2 inhibitors, other methods for regulating LRRK2 are also possible. To answer the question of whether LRRK2 inhibitors are effective in the treatment of PD, experts have made great efforts to promote these small molecule inhibitors into clinical application. However, LRRK2 is not a brain-specific protein, highly expressed in the kidney and lung which may be out of target. It's still tough to optimize kinase selectivity, CNS permeability and reduce lung and/or kidney side effects simultaneously. Another obstacle may be that there is no reliable PD model with LRRK2 dysfunction so far, which brings great challenges to clinical trials. Although knockdown of *LRRK2* and few LRRK2 inhibitors can induce autophagy and rescue neuron cell death caused by autophagy defects, whether the majority of LRRK2 inhibitors can produce the same effect in autophagy remains to be further characterized[139]. Denali Therapeutics[49] recently reported two LRRK2 small-smolecule inhibitors, DNL151 and DNL201, have entered phase I clinical trials which provide impetus for the further development of LRRK2 small-molecule inhibitors. It can be ascertained that with the unremitting efforts of scientists, the above problems are expected to be overcome. More LRRK2 promising inhibitors will be applied in the clinic, such as **26** and **27** which are in the preclinical studies currently[140]. In conclusion, the application of LRRK2 kinase inhibitors in the autophagy-lysosome pathway may provide a novel thought for the treatment of PD.

3.6. *Targeting beclin-1*

Beclin-1, known as ATG6, is highly conserved among eukaryotes and belongs to the ATG protein family. As an important molecule for regulating autophagy, its overexpression can reduce apoptosis and enhance autophagy[50,141]. Beclin-1 involves the nucleation of autophagic vesicles (the formation of phagocytes) during the process of autophagy induction. To initiate nucleation of vesicles, beclin-1 interacted with its binding partners such as PI3KC3/Vps34, Ambra1, Vps15, and Atg14L. After complexing with beclin-1, PI3KC3/Vps34 was activated to produce its unique

product, phosphatidylinositol 3-phosphate, which was in charge of inducing autophagy[51]. Spencer's team[142] suggested that autophagy was activated by beclin-1 which could reduce the accumulation of α-syn in PD. As an inhibitor of the serine protease prolyl oligopeptidase (PREP), KYP-2047 (**28**) can induce the expression of beclin-1 (Fig. 7A). The number of autophagy markers LC3BII then increased, which further enhanced autophagy and clearance of high molecular weight oligomeric α-syn[52]. Previous studies pointed out that the 4-phenylbutyryl-L-prolyl-pyrrolidine skeleton of **28** was preferred to reduce the dimerization of α-syn compared with other PREP inhibitors[52,143]. Moreover, natural products such as isorhynchophylline (**29**), corynoxine B (**30**) and glycyrrhizic acid (**31**) could also strengthen the cytoprotective autophagy by up-regulating the expression of beclin-1 and rescue the survival of neuronal cells (Fig. 7)[53,54,56,144,145]. Besides autophagy, beclin-1 also regulates apoptosis. In view of the crosstalk between autophagy and apoptosis which involves regulating cell survival and death, beclin-1 possesses great research value[146].

3.7. *Targeting TFEB*

Lysosomal formation and autophagy were promoted when transcription factor EB (TFEB) combined with lysosomal joint expression and regulation (CLEAR) elements[147,148]. TFEB subcellular localization was regulated by mTOR-mediated phosphorylation thereby affecting TFEB activity[149]. TFEB could bind to the promoter regions of several autophagy genes and induce autophagosome biogenesis and autophagosome-lysosome fusion[148]. Interestingly, degradations of numerous autophagy substrates, including α-syn, were enhanced by the overexpression of TFEB. The representative compound targeting TFEB was curcumin (**32**), a natural pigment derived from the root of the turmeric herb (Fig. 8A). Compound **32** had two aromatic rings involved *ortho*-methoxyphenol hydroxyl groups, which linked symmetrically with a β-diketone. Experiments showed that **32**, as a neuroprotective agent, had beneficial effects on the nervous system and PD models. Firstly, it had strong antioxidant properties and significant anti-inflammatory activity with the bioavailability of[57,58]. In the meantime, compound **32** could inhibit the aggregation of α-syn and prevent the accumulation of LB *in vitro* to reduce the toxicity of α-syn oligomers in cells and degeneration of DA neurons[59]. Besides, **32** was to promote the recovery of autophagy by activating TFEB, leading to the reduction of cellular oxidative stress, neurotoxicity, memory loss, and dyskinesia[150,151]. Moreover, compound **32** was safe, non-toxic, cheap, and easily available. It could also effectively penetrate the blood−brain barrier and neuronal membrane. Thus, compound **32** was a promising candidate for the treatment of PD. Even though the clinical application of **32** was limited due to its instability and poor metabolic properties. Chemical modification was an effective strategy to improve its biological activity. The β-diketone moiety of **32** appeared to be a series of aldehyde-reductase ketone-specific substrates *in vivo*, which could be rapidly broken down[152]. Analogues which replaced the β-diketone part could reduce degradation rate and enhance stability in buffers of different pH *in vitro*. Meanwhile, half-life ($t_{1/2}$), clearance rate (CL), the area under the curve (AUC), and other pharmacokinetic properties were dramatically improved[153]. On this basis, a series of **32** analogues were developed, among which CNB001 (**33**), curcumin analogues C1 (**34**), and E4 (**35**, Fig. 8A) also showed neuroprotective effects in PD models[60−62]. Besides compound **32** and its analogs, trehalose (**36**, Fig. 8B), as an autophagy activator, has been extensively studied in various PD models[63]. Compound **36** could ameliorate different disease phenotypes. However, the specific autophagy activation pathway and mechanism of **36** were not so clear yet. Recently, compound **36** was reported to induce autophagy through lysosomal-mediated TFEB activation in a model of motor neuron degeneration[154]. In sum, TFEB has become an emerging target to enhance ALP and up-regulate the TFEB expression in the central nervous system through gene therapy or pharmacological activation.

3.8. *Targeting GCase*

The regulation of lysosomes has recently become an attractive strategy to selectively stimulate ALP in PD models. One of the remarkable methods is that to target specific lysosomal enzymes directly (such as glucocerebrosidase GCase) to stimulate the lysosomal degradation of α-syn. Much evidence suggests that the level and activity of GCase decreased, and GCase and α-syn levels of the brain are inverse ratio in idiopathic PD[105]. Therefore, the enhancement of GCase activity may be also applied in the treatment of PD. However, since GCase cannot transverse the blood−brain barrier, the direct application of enzyme replacement therapy is ineffective for PD[155]. Later, it was proposed to develop small-molecule drug chaperones which could be used as an alternative therapy for PD[156,157]. Some such drugs were designed to bind to mutant GCase, correcting its misfolding and promote transport to lysosomes, which enabled to improve the GCase activity and the lysosomal function. Ambroxol (**37**) was a small molecule chaperone used for respiratory diseases (Fig. 8B). McNeill et al.[64] found that it could increase GCase activity and reduce oxidative stress in PD fibroblasts with *GBA1* mutations. Furthermore, **37** restored levels of cathepsin D, LIMP2, and saposin C, which was crucial to the activity of GCase[65]. Compound **37** could not only increase the GCase activity significantly in lymphocytes and penetrate the blood−brain barrier but also reduce the level of glycosphingosine in cerebrospinal fluid and improve myoclonus and seizures in patients. The company PRO.MED.CSA recently completed a phase II non-randomized and uncontrolled clinical trial approved by the FDA of **37** (NCT02941822)[158]. According to reports, oral **37** can be detected in the blood and cerebrospinal fluid of PD patients after 186 days, and the patient did not have any serious adverse reactions. Besides, some other small-molecule chaperones, such as isofagomine (**38**) displayed similar effects (Fig. 8C)[67]. Nonetheless, one disadvantage of these chaperones is that they inhibit GCase activity by binding to the catalytic site of the enzyme. Thus, it suggests that the balance between chaperone function and inhibitory activity should be carefully considered when utilizing these compounds, requiring the development of those drugs without binding to the GCase catalytic site[159]. Consequently, a non-inhibitory GCase small molecular chaperone NCGC607 (**39**) was identified through high-throughput screening (Fig. 8C). This chaperone has been proved to restore GCase activity and protein levels while reducing α-syn levels in dopaminergic neurons derived from iPSCs (induced pluripotent stem cells)[68]. These studies of PD have demonstrated that small molecular chaperone enhancers of GCase can improve the lysosomal function and the α-syn clearance, suggesting the great possibility of targeting GCase to selectively stimulate the ALP pathway to treat PD.

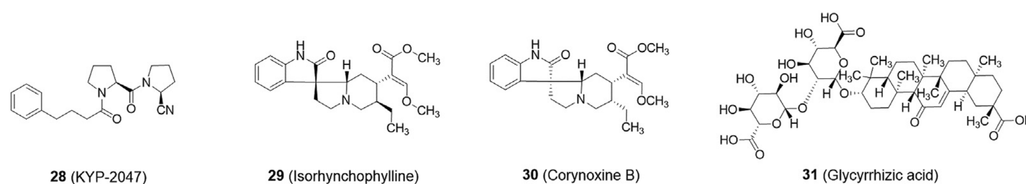

Figure 7 The structures of compounds targeting beclin-1.

3.9. Targeting ERRα

The nuclear receptor (NR) superfamily in eukaryotic cells represents a group of important and diverse transcriptional regulators, part of which are activated by endogenous hormones or ligands in animals[160,161]. However, a large number of receptors are not activated or regulated by physiological/endogenous ligands which are called orphan nuclear receptors (ONRs). The estrogen-related receptor α (ERRα) is the first ONR to be discovered which participates in the regulation of various biological functions such as energy metabolism and is highly expressed in skeletal muscle, kidney, brain, heart, and other tissues that require energy[162−164]. Due to its crucial action in metabolic homeostasis, ERRα has gradually been developed into a considerable target for the treatment of cancer and metabolic diseases. XCT790 (**40**) is a thiadiazole acrylamide derivative and the most selective inverse agonist for ONR. ERRα has been identified as one of its targets (Fig. 8C)[69]. Researches elucidated that **40** eliminated α-synuclein aggregated in human neuronal cells in an autophagy-dependent method[70]. It also showed that autophagy induced by inhibiting the activity of ERRα under nutrient-rich conditions was carried out through an mTOR-independent mechanism. Under the normal condition, ERRα was localized on autophagosomes. After autophagy was induced by compound **40**, this localization was lost and accompanied by an increase in the autophagosome biogenesis, indicating that ERRα may regulate autophagy through subcellular localization dynamics of ERRα. Concurrently, in a preclinical mouse model of PD, **40** cleared toxic protein aggregates by inducing autophagy, alleviated motor coordination disorders, and exerted a neuroprotective role on dopaminergic neurons in the substantia nigra[70]. The data about **21** mentioned above is strong proof that ERRα will be an attractive target for PD.

3.10. Targeting c-ABL

C-Abelson (c-ABL) non-receptor tyrosine kinases can regulate autophagy by promoting the transport and function of lysosomal components[165]. C-Abelson was shown to be

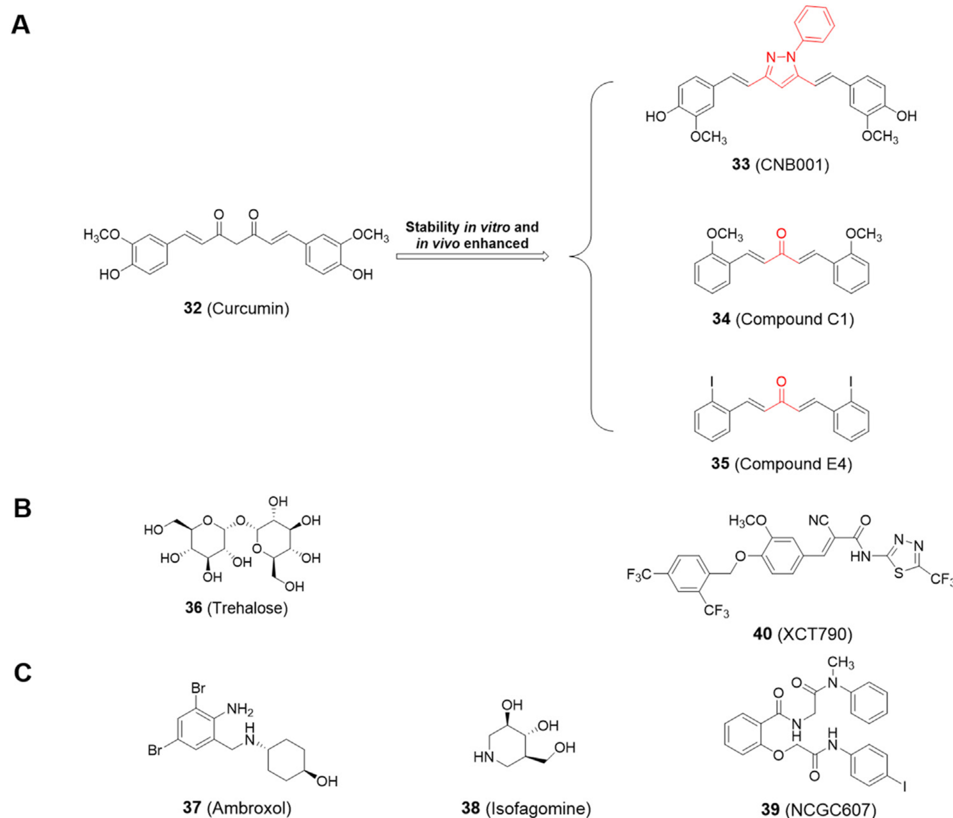

Figure 8 (A) Curcumin and its analogues replaced the β-diketone part. (B) The structures of trehalose and XCT790. (C) The structures of compounds targeting GCase.

activated in preclinical PD models and brain samples from PD patients. Thus, inhibition of c-ABL displays great potential in PD therapy[166,167]. PD180970 (**41**) is a potent ATP-competitive BCR-ABL inhibitor, which could inhibit c-ABL activity at nanomolar concentrations (Fig. 9A)[168]. Compound **41** also had a neuroprotective effect, which could induce autophagy in an mTOR-independent manner and reduce the toxicity mediated by α-syn in mammalian cells[71]. *In vitro*, it was also demonstrated that treatment with **41** could relieve neuroinflammation and regulate the release of cytokines in microglia and neuron-microglia co-cultures. Meanwhile, **41** protected dopaminergic neurons and improved motor behavior defects in preclinical mouse models of PD by inducing autophagy and aggregation clearance[71]. Imatinib (**42**) and nilotinib (**43**) are another two tyrosine kinase inhibitors (TKI) that can selectively inhibit BCR-ABL (Fig. 9A). Both of them have similar structures with the aminopyrimidine mother nucleus. Compound **42**, as the first known ATP-competitive inhibitor, is able to inhibit BCR-ABL kinase with high selectivity[169]. In the early 1990s, the phenylaminopyrimidine derivative **42-1** was identified as a potential lead compound for protein kinase C (PKC) inhibitors, followed by SAR study, and compound **42** was identified consequently. Firstly, adding a pyridyl group to the 3′-position of **42-1** pyrimidine could enhance its cell activity. Then various functional groups were tested as substituents in the benzene ring. It was also found that the amide group had an inhibitory effect on tyrosine kinase. In addition, the analysis of the SAR showed that the presence of a methyl group at the *ortho* position of the amino group increased the selectivity of BCR-ABL. However, the modified molecule still performed poor solubility in water and oral bioavailability, which were significantly improved by the introduction of *N*-methylpiperazine groups. At the same time, a spacer benzene ring was introduced between the amide group and *N*-methylpiperazine, eliminating its mutagenic possibility. Thus, these structural modifications eventually led to the appearance of **42** (Fig. 9B)[169]. Most of the interactions between **42** and kinases were weak van der Waals force (VdW). Although six high-energy hydrogen bonds (Hb) also formed, each hydrogen bond accounted for a relatively high portion of the total binding energy of kinase inhibitors. If one of the hydrogen bonds was broken because of the mutation of hydrogen bond donor/acceptor residues, its energy contribution would be lost and the competition between the free energy of the dissociated state and the bound state would increase. In case of it, the interaction between **42** and mutant kinase would be lost due to unfavorable thermodynamic factors. This is the reason for the drug resistance of **42**, so the second-generation TKI represented by **43** was developed[170]. Based on the structure of **42**, replacing the piperazine ring with 3-methylimidazole and adding trifluoromethyl to the anilinocarbonyl substituent, by inverting the amide linker, were used to increase the number of VdW interactions (Fig. 9B). Thus, the result was that **43** pairs showed higher efficacy to almost all mutations of drug resistance[170]. The overbased *N*-methylpiperazinyl group in **42** had stronger desolvation/deprotonation and constraining force of binding surface, while the methylimidazole group in **43** could be stacked in the hydrophobic pocket formed by amino acid residues. The nitrogen atom was exposed to the solvent as well. To be specific, the pyridyl and pyrimidinyl groups of **42** contributed relatively much energy, while the trifluoromethyl and methylimidazole groups of **43** contributed considerably much energy. Therefore, in contrast to **42**, mutations linked with pyridyl and pyrimidinyl groups had less effect on the overall affinity of **43**. With a better topology between **43** and

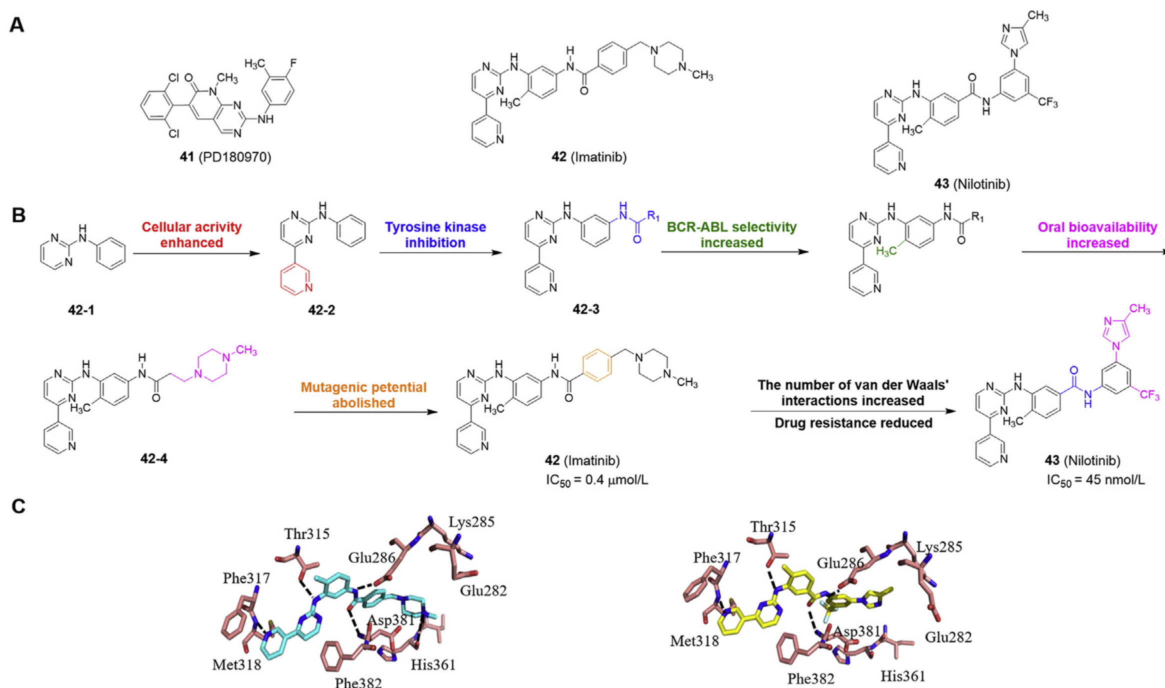

Figure 9 (A) The structures of compounds targeting c-ABL. (B) The discovery and structure optimization process of imatinib and nilotinib. (C) The interaction modes of imatinib and nilotinib with c-ABL (PDB:2OIQ,3CS9). The key amino acid residues are illustrated with pink, and imatinib and nilotinib are depicted in blue and yellow respectively. The hydrogen bonds are shown in black dashed lines.

kinase, the damage of inhibitor binding with the key mutated residues related to Hb interaction could be avoided (Fig. 9C). Despite these modifications, the selectivity and potency of **43** were not destroyed and even improved compared with **42** (IC_{50} values of 400 and 45 nmol/L, respectively)[171]. The two compounds also had similar effects in the PD models, which could induce autophagy and increase the clearance rate of α-syn[72,83,172]. It is worth noting that **43** is undergoing two clinical trials (NCT02954978 and NCT03205488). One is to research the clinical efficacy, and the other is to test the safety and tolerability in PD. Furthermore, a small open-label trial has shown that **43** is safe and can improve the motor function in PD patients[73]. Splendid clinical manifestations of **43** provide favorable evidence for targeting the autophagy pathway in the treatment of PD.

4. Conclusions

In the past few years, the toxic aggregates produced by impaired autophagy has been recognized as one of the pathogenesis of PD, making it possible to target diverse regulators of autophagy (such as AMPK, mTORC1, ULK1, IMPase, LRRK2, beclin-1, TFEB, GCase, ERRα, C-Abelson). A variety of small-molecule activators or inhibitors correlated with such potential druggable targets have been developed, which may play their neuroprotective effects in different types of PD models. However, many of these small-molecule compounds are only used as tool probes for PD research. There is still a big challenge about thorough understandings of autophagic mechanisms in the pathogenesis of PD and transforming the current small molecules into the future clinical practice. The general hypothesis is based upon the fact that autophagic flux decreases in the pathogenesis of PD, illustrating that activation of autophagy is way forward. But we should consider that excessive autophagy can lead to some unique cell death by producing extra degradation products. The effect of complex interactions between autophagy and other intracellular processes call into question that how to induce autophagy precisely without affecting cell homeostasis.

Besides, most of these autophagy pharmacological regulations trigger the whole induction of autophagy, lacking organ specificity and substrate selectivity, which may cause side effects. Considering the cytoprotective function of autophagy on neurons, improving the brain specificity of autophagy is wise. It also requires that small-molecule drugs can penetrate blood−brain barrier better and enrich towards the CNS. The development of *LRRK2* inhibitors focuses on this key issue just right. It provides a valuable experience for the discovery of brain osmotic autophagic molecules from a perspective of medicinal chemistry. As for the substrate selectivity, activation of autophagy not only degrades the abnormal aggregation of α-syn, but increases the degradation of other proteins and organelles, thus increasing the pressure of cell survival. α-Syn is the degradation substrate of CMA (a selective autophagy), and targeting CMA has become a new thought of PD drug development[33]. Interestingly, paradoxical with previous studies, impaired macroautophagy was found not to increase the accumulation of α-syn oligomers, and induced macroautophagy may only benefit in cellular pathology and long-term neuronal survival, and aggravate motor performance through impacting striatal DA dynamics[173], which reminds us the questions of when to apply autophagy inducers to which kinds of patients based on their mechanisms and phenotypes.

In addition, some new emerging technologies about autophagy-modulating small molecules have appealed much attention, such as autophagy targeting chimera (AUTAC) and autophagosome-tethering compound (ATTEC)[174−177]. Both of these technologies are capable of selectively autophagic degradation of substrates. Although there is no sound evidence that these two technologies can be directly applied to the degradation of α-syn, it may provide a new clue and future direction for exploiting more autophagic modulators in PD therapy.

More recently, autophagy has been considered as one of the predominant aggravators at the late stage of PD progression[178], which emphasizing the necessity of combination therapies of inducing autophagy and other interventions targeting gene mutations or neuroinflammation at different phase of PD. Currently, most PD patients take a variety of drugs simultaneously, such as levodopa and MAO-B inhibitors, to limit high medication dose and some adverse events. Therefore, autophagy-modulating drugs combination with themselves, other non-autophagy related anti-PD drugs or gene therapy can be an effective approach to overcome the limitations of autophagy-modulating small molecules and thereby promoting their clinical development. Despite confronting with many difficulties, discovery of more new autophagy-modulating compounds will confirm the practicability of safe candidate drugs and their importance in preventing or limiting inevitable neurodegeneration, which is essential for the successful fight against PD in the near future.

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Author contributions

Bo Liu, Liang Ouyang and Guan Wang conceived the project and supervised the project. Kai Zhang, Shiou Zhu and Jiamei Li summed up the literature and drafted the manuscript. Tingting Jiang was involved in drawing the figures. Lu Feng collected and organized the inhibitors. Kai Zhang, Guan Wang and Junping Pei proofread the structures and figures. Bo Liu, Liang Ouyang and Shiou Zhu revised the manuscript. All authors approved the final manuscript.

Conflicts of interest

The authors have no conflicts of interest to declare.

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