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Recent advances in small molecules for improving mitochondrial disorders

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Mitochondrial disorders are observed in various human diseases, including rare genetic disorders and complex acquired pathologies. Recent advances in molecular biological techniques have dramatically expanded the understanding of multiple pathomechanisms involving mitochondrial disorders. However, the therapeutic methods for mitochondrial disorders are limited. For this reason, there is increasing interest in identifying safe and effective strategies to mitigate mitochondrial impairments. Small-molecule therapies hold promise for improving mitochondrial performance. This review focuses on the latest advances in developing bioactive compounds for treating mitochondrial disease, aiming to provide a broader perspective of fundamental studies that have been carried out to evaluate the effects of small molecules in regulating mitochondrial function. Novel-designed small molecules ameliorating mitochondrial functions are urgent for further research.

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1. Introduction

Mitochondria are vital organelles that play essential roles in the life and death of the cell. They play a crucial role in energy metabolism and control of stress responses and are a hub for biosynthetic processes.[1,2] Mitochondrial diseases are genetically determined metabolic disorders characterized by defects in oxidative phosphorylation (OXPHOS) and caused by mutations in genes in nuclear DNA (nDNA) and mitochondrial DNA (mtDNA) that encode structural mitochondrial proteins or proteins involved in mitochondrial function.[3] The mitochondria OXPHOS system is embedded in the mitochondrial inner membrane (MIM) (Fig. 1). It represents the final step in converting nutrients into energy by forming ATP.

The electron transport chain (ETC) involves four multisubunit complexes (complex I–complex IV), two mobile electron carriers, coenzyme Q10, and cytochrome c.[4] Complex I and II receive electrons from reduced nicotinamide adenine dinucleotide (NADH) and reduced flavin adenine dinucleotide (FADH2), respectively. Afterward, these electrons are transferred to the electron carrier coenzyme Q10, which transports them to complex III. Then, electrons are donated to cytochrome c and

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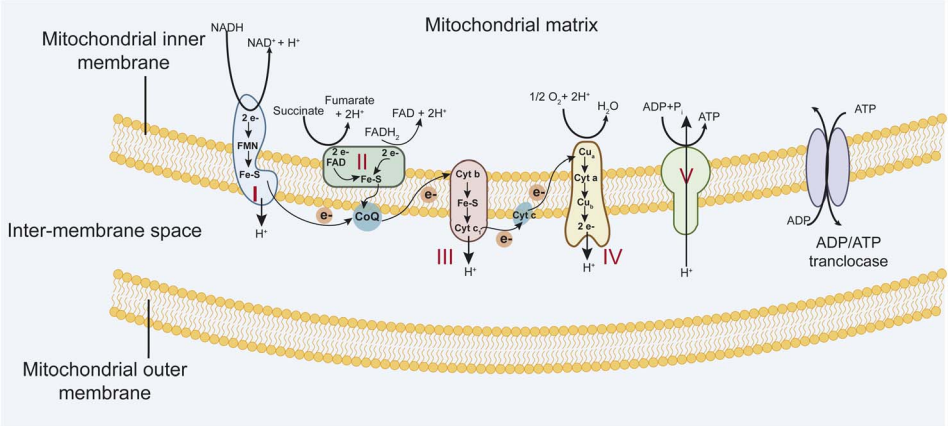

Fig. 1 The mitochondrial respiratory chain includes complexes I–IV and complex V, an ATP synthase.

transported to complex IV, which transfers electrons to molecular oxygen to form water. At the same time, complexes I, III, and IV generate a transmembrane proton gradient by driving *trans*-MIM proton (H^+) efflux from the mitochondrial matrix. Complex V converts transmembrane electrochemical proton gradient energy into mechanical energy to generate ATP by chemiosmotic coupling.[5] These five complexes are assembled from 92 distinct proteins. Complex I is built up of 44 subunits (7 mtDNA- and 37 nDNA-encoded), complex II of 4 subunits, complex III of 11 subunits (1 mtDNA, 10 nDNA), complex IV of

Table 1 Examples of mitochondrial diseases caused by mutations in mtDNA

Disease	Gene location	Mutation types	Clinical features
Myoclonus epilepsy with ragged-red fibres (MERRF)	MTTK	A8344G, T8356C, G8361A	Generalized epilepsy, ataxia, and myopathy
	MTTF	G611A	
Mitochondrial encephalopathy, lactic acidosis, stroke-like episodes (MELAS)	MTTL1	A3243G, G3244A, A3252G, C3256T, T3271C, T3291C	Epilepsy, encephalopathy, myopathy, severe constipation, failure to thrive
	MTTV	G1642A	
	MTTF	G583A	
	MTRNR2	C3093G	
	MTND1	T3308C, G3376A, G3697A, G3946A, T3949C	
	MTND4	A11084G	
	MTND5	A12770G, A13045C, A13084T, G13513A, A13514G	
	MTND6	G14453A	
	MTCYB	14787Ddel4	
Kearns–Sayre syndrome (KSS)	MTTL1	G3249A	Short stature, diabetes mellitus, cardiomyopathy, ataxia
Chronic progressive external ophthalmoplegia (CPEO)	MTTL1	C3254T	Ptosis, muscle weakness
	MTT1	T4274C, T4285C, G4298A, G4309A	
	MTTA	T5628C	
	MTTN	T5692C	
	MTTN	G5698A	
	MTTN	G5703G	
	MTTK	G8342A	
	MTTL2	G12294A, A12308G, T12311C, G12325A	
	MTND4	T11232C	
Neuropathy, ataxia, and retinitis pigmentosa (NARP)	MTATP6	T8993C, T8993G	Blindness, cerebellar ataxia, seizures, cognitive impairment, and peripheral neuropathy
Leigh syndrome(LS)	MTTV	C1624T	Lactic acidosis, failure to thrive, myopathy, bilateral symmetrical lesions in the subcortical brain
	MTND3	T10158C	
	MTND4	C11777A	
	MTND5	T12706C	
	MTATP6	T9176C, T9176G, T9185C, T9191C, T8993C	

Table 2 The most frequent clinical features of mitochondrial disease

Neurological characteristics	Central nervous system	Stroke-like episodes, migraine, epilepsy, ataxia, dementia, Parkinsonism, developmental delay, psychiatric or mood disorder, developmental regression
	Visual system	Ptosis, progressive external ophthalmoplegia, optic atrophy, retinitis pigmentosa
	Acoustic system	Sensorineural hearing loss
	Skeletal muscle	Myopathy, exercise intolerance
	Peripheral nervous system	Peripheral neuropathy
Non-neurological characteristics	Digestive system	Gastrointestinal dysmotility; malabsorption, intestinal pseudo-obstruction, chronic villous atrophy
	Kidney	Fanconi syndrome, renal tubular acidosis, focal segmental glomerulosclerosis, renal failure, adrenal insufficiency
	Metabolic/endocrine apparatus	Lactic acidosis, multiple lipomatosis, short stature, diabetes, hypothyroidism, hypoparathyroidism
	Heart	Cardiomyopathy, conduction defect
	Hematopoietic system	Sideroblastic anemia

14 subunits (3 mtDNA, 11 nDNA), and complex V of 19 subunits (2 mtDNA, 17 nDNA).[6,7] Mutations in mitochondrial DNA (mtDNA) and (or) nuclear-encoded mitochondrial genes (nDNA) that affect OXPHOS function efficiently lead to a diverse group of debilitating conditions (Table 1). Besides, mitochondria are a major source of reactive oxygen species, such as superoxide, because electrons at complex I and III of the respiratory chain are often offloaded to molecular oxygen. Increased ROS production in mitochondrial diseases can result in protein, lipid, and DNA damage, potentially leading to further cellular damage and dysfunction.[8,9]

Mitochondrial dysfunction contributes to numerous health problems, including neurological and muscular degeneration, cardiomyopathies, cancer, diabetes, and aging pathologies (Table 2). Mitochondrial diseases are clinically heterogeneous and can occur at any age. Treatment of mitochondrial disorders has been challenging since multi-organ involvement in various mitochondrial diseases.[10] Small molecules play an essential role in drug development.[11,12] In this review, we will present small molecules that are beneficial to enhance mitochondrial function and improve primary mitochondrial diseases. We propose that rapid preliminary screening of potential therapeutic compounds in individual patients' fibroblasts could direct and advance personalized medical treatment. Furthermore, novel-designed small molecules ameliorating mitochondrial functions are urgent for further research.

2. Therapies for mitochondrial disease

2.1 Molecules bypass mitochondrial complex I deficiency

Mitochondrial complex I deficiency is the most prevalent defect in the respiratory chain in pediatric patients and often leads to severe or fatal neurological symptoms, such as Leigh syndrome. Succinate is a mitochondrial substrate that is metabolized through complex II. However, it is not cell membrane-permeable and challenging to be uptaken into cells. Ehinger group reported that several cell membrane-permeable prodrugs **1–3** (Fig. 2) of the complex II substrate succinate increased ATP-linked mitochondrial oxygen consumption in complex I-deficient human blood cells, fibroblasts, and heart fibers. This therapy strategy provided a potential future intervention for patients with metabolic decompensation due to complex I dysfunction.[13]

Idebenone is a well-known compound, developed in the early 1980s by Takeda Pharmaceuticals against cognitive decline/dementia (**4**, Fig. 2), which has been evaluated in several mitochondrial and neurodegenerative diseases.[14,15] Idebenone has the potential to act as an electron carrier in the respiratory chain and as an antioxidant against membrane damage caused by lipid peroxidation. The antioxidant function of idebenone is attributed to the redox cycling between hydroquinone and quinone. NAD(P)H: quinone oxidoreductase 1 (NQO1) and mitochondrial complex III were identified as the major enzymes involved in idebenone activity.[16] It has been approved provisionally in Canada for the treatment of Friedreich's ataxia, while withdrawn from the Canadian market in 2013 by Santhera Pharmaceuticals due to lack of efficacy. Leber's hereditary optic neuropathy (LHON), a rare genetic mitochondrial disease that causes rapid and progressive bilateral vision loss, is the only mitochondrial disease for which IDE has been approved by the European Medicine Agency to treat visual impairment in adolescents and adults. Several new insights into the mode action of idebenone

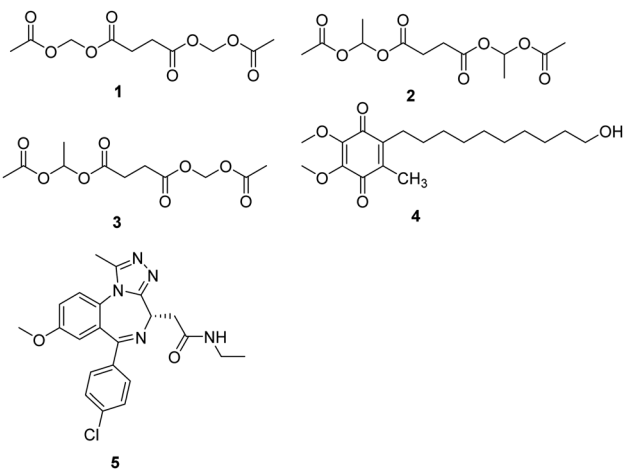

Fig. 2 Chemical structures of compounds **1–5**.

were discovered, which may provide a novel indication for this drug that might not have been considered previously.[17]

Bromodomain-containing protein 4 (BRD4) is a member of the bromodomain and extra terminal domain (BET) family of proteins comprising BRD2–BRD4 and BRDT. BRD4 is a chromatin-bound transcriptional regulator linked to the expression of genes associated with different biological processes, including tumor progression or inflammation.[18] A recent study demonstrates that I-BET 525762A (**5**, Fig. 2), an inhibitor of bromodomain, could remodel the mitochondrial proteome to increase the levels and activity of OXPHOS protein complexes, increase and utilize FADH2, leading to the rescue of the bioenergetic defects and cell death caused by mutations or chemical inhibition of mitochondrial complex I.[19]

2.2 Agents enhancing electron transfer chain function

Coenzyme Q10 (CoQ10, **6**, Fig. 3) is a naturally occurring fat-soluble vitamin-like quinone, which plays a crucial role in mitochondrial oxidative phosphorylation and ATP production.[20] CoQ10 is an endogenous antioxidant and a potent free radical scavenger in mitochondrial membranes. CoQ10 exhibits potentially neuroprotective effects in neurodegenerative diseases with excess oxidative stress. However, about 50 clinical studies of CoQ10 revealed a marginal but factual treatment effect.[20]

Riboflavin (7, Fig. 3), a water-soluble vitamin B, is part of the functional group of flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD) cofactors and is required for numerous flavoprotein-catalyzed reactions. Riboflavin shows critical antioxidant properties essential for correct cell functioning.[21] Riboflavin deficiency has been demonstrated to impair the oxidative state of the body, especially in the nervous system. Riboflavin supplementation treats migraine, Brown–Vialetto–Van Laere syndrome, Fazio–Londe disease, and some mitochondrial diseases.[22,23] In the future, riboflavin may be a potential therapeutic intervention in many other neurological disorders.

Dichloroacetate (DCA, **8**, Fig. 3) is an analog of pyruvate. DCA activates the E1 (pyruvate decarboxylase) subunit of the PDHC by inhibiting the PDH kinase, which usually phosphorylates and inhibits the enzyme, thus locking the enzyme in the active conformation and promoting the flux of pyruvate into the citric acid cycle.[24] It is an investigational drug for the treatment of mitochondrial genetic diseases. Although it can effectively alleviate lactic acidosis in mitochondrial disorders,[25] it can also cause peripheral neuropathy in individuals with MELAS syndrome.[26]

Thiamine (vitamin B1, **9**, Fig. 3) can enhance pyruvate dehydrogenase activity, thus increasing the oxidative decomposition of pyruvate and reducing cofactors (NADH and FADH2) generation. Thiamine has been used in mitochondrial diseases individually or with other agents. Supplementation with thiamine in a family with MELAS syndrome and thiamine deficiency can improve the symptoms of myopathy and lactic acidosis and myopathy.[27] Combining thiamine with CoQ10, carnitine, and vitamins C and E can improve the clinical symptoms of adult patients with Leigh syndrome with subacute severe brainstem encephalopathy.[28]

2.3 Agents as antioxidants

Sonlicromanol (KH176, **10**, Fig. 4), a chemical entity derivative of the water-soluble form of vitamin E, is a blood–brain barrier permeable ROS-redox modulator. Sonlicromanol hydrochloride is used in the study for mitochondrial disorders. Sonlicromanol hydrochloride maintains microstructural coherence in the brain of Ndufs4$^{-/-}$ mice.[29] A clinical research is conducted to evaluate the effect of KH176 in various cognitive domains and the impact of different doses of KH176.

Lipoic acid (α-LA, **11**, Fig. 4) is a natural molecule showing excellent antioxidant and anti-inflammatory properties. It is a coenzyme of pyruvate dehydrogenase and α-ketoglutarate dehydrogenase, which plays several roles in the pathogenesis of neurodegenerative diseases.[30] One research suggests that the combination of lipoic acid, CoQ10, and creatine monohydrate effectively reduces plasma lactic acid content and oxidative stress

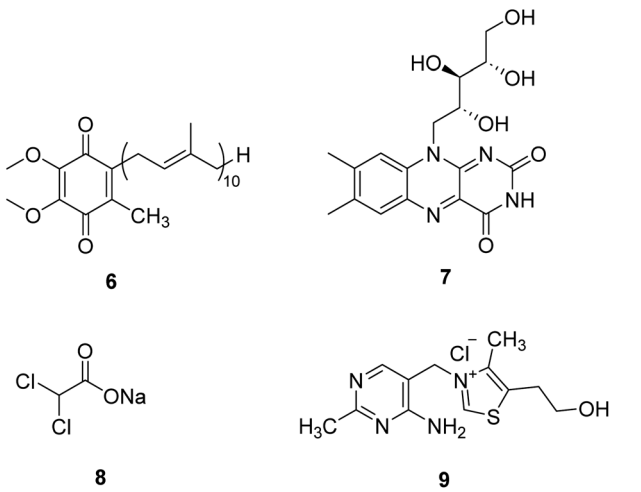

Fig. 3 Chemical structures of **6**–**9**.

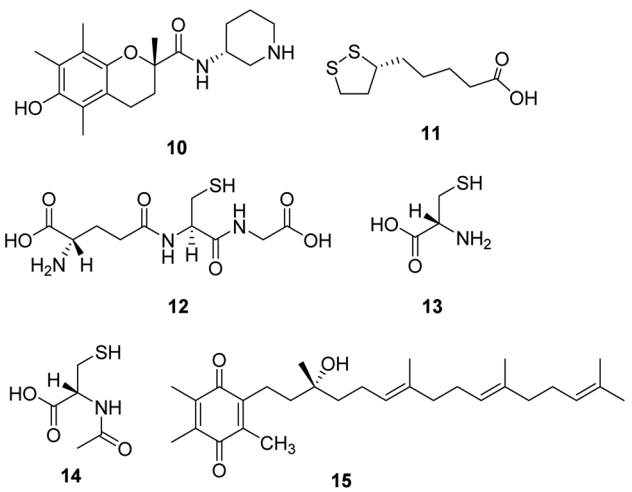

Fig. 4 Chemical structures of **10**–**15**.

markers in urine and improves the symptoms of muscle strength in patients with mitochondrial diseases, which is a beneficial therapeutic strategy for some mitochondrial disorders.[31]

Glutathione (L-γ-glutamyl-L-cysteinylglycine, **12**, Fig. 4) is a crucial intracellular antioxidant that can protect the cell from reactive oxygen species (ROS). Loss of GSH is associated with several mitochondrial diseases. Thus, supplementation with cysteine donors (**13**, Fig. 4), which can enhance the synthesis starting material of glutathione, can potentially restore glutathione levels and eliminate excessive ROS in mitochondrial diseases.[32,33] A study revealed that *N*-acetylcysteine (**14**, Fig. 4) could also enhance muscle cysteine and glutathione availability and attenuate fatigue during prolonged exercise in endurance-trained individuals.[34]

Based on the noticeable results obtained with CoQ10 and idebenone, a novel *para*-benzoquinone compound EPI-743 (**15**, Fig. 4) was designed and tested. EPI-743 is 1000 to 10 000 times more potent than CoQ10 or idebenone in patient fibroblast assays modeling the effects of mitochondrial disease. EPI-743 is now in clinical trials to treat Leigh syndrome and other inherited mitochondrial disorders.[35]

2.4 Agents enhancing mitochondrial biogenesis

Induction of mitochondrial biogenesis through transgenic overexpression of PGC-1α is being developed as a potential treatment for mitochondrial disorders. Bezafibrate (**16**, Fig. 5) is a pharmacological ligand for the transcriptional cofactor PGC-1α. It shows the most extensive pre-clinical evidence of efficacy in animal models and patient cell lines.[36,37] Now, it is in clinical trials to evaluate the safety of inducing mitochondrial biogenesis in patients with the m.3243A>G MTTL1 mutation.[38]

(−)-Epicatechin (**17**, Fig. 5) is the main flavonoid present in dark chocolate. Relevant research confirmed that (−)-epicatechin could enhance fatigue resistance and oxidative capacity in mouse muscle, which benefits clinical populations experiencing muscle fatigue.[39]

Omaveloxolone (RTA-408, **18**, Fig. 5) is a novel synthetic oleanane triterpenoid analog. It shows significant cytoprotective effects due to its ability to activate the Nrf2 pathway.[40] Studies also proved that the neuroprotective effects of RTA-408 could be attributed to the Keap1 inhibition.[41] FDA has approved omaveloxolone as the first treatment for Friedreich's ataxia, a rare, inherited, degenerative disease that damages the nervous system, characterized by impaired coordination and walking.[42]

Resveratrol (**19**, Fig. 5), a natural plant polyphenol, increased AMPK and PGC-1α activity, increased mitochondrial number, and improved motor function, which is beneficial for an overall improvement in health and survival.[43] As a dietary supplement, it is in clinical studies in patients with mitochondrial myopathies and skeletal muscle fatty acid oxidation disorders.

Metformin (**20**, Fig. 5) is widely used for treating type 2 diabetes. Studies show that metformin exerts its anti-diabetic effects by inhibiting complex I of the mitochondrial respiratory chain.[44] Metformin is also a potential activator of AMPK and the stress-induced transcription factor SKN-1 nuclear factor erythroid 2-related factor 2 (Nrf2), which shows excellent potential in aging-related diseases such as neurodegenerative disease and cancer in humans.[45]

2.5 Agents regulating NADH/NAD$^+$ ratio

Dysfunction of the mitochondrial oxidative phosphorylation causes an increase in the NADH/NAD$^+$ ratio, which impairs the activity of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) in the glycolysis pathway. Treatment with pyruvate (**21**, Fig. 6) is expected to decrease the ratio and restore glycolysis. Therefore, it is a promising approach for treating mitochondrial diseases.[46,47]

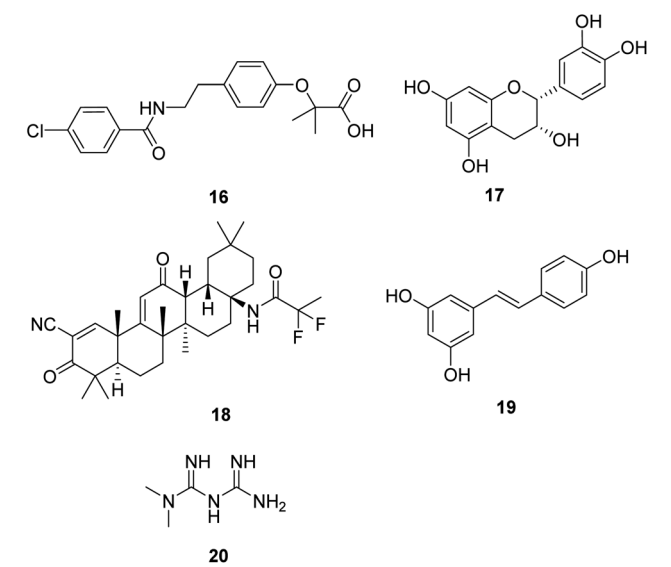

Fig. 5 Chemical structures of **16**–**20**.

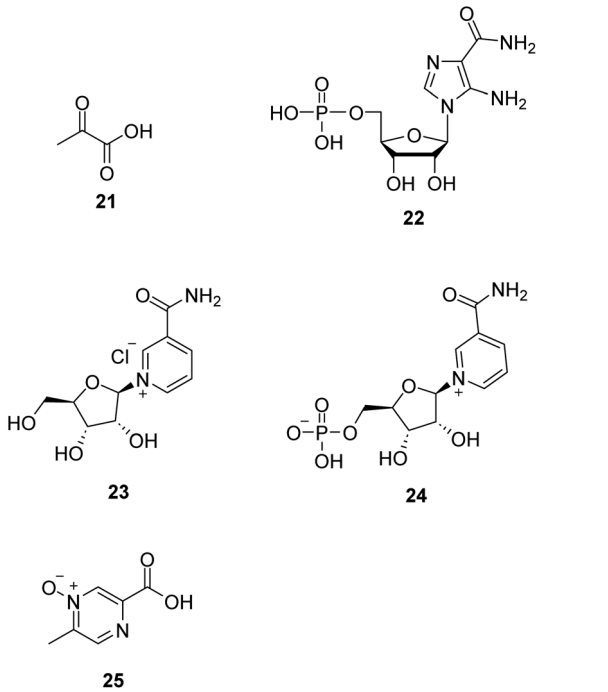

Fig. 6 Chemical structures of **21**–**25**.

Phase II clinical trial of sodium pyruvate on lactic acidosis associated with mitochondrial disorders was conducted.

AMP-activated protein kinase (AMPK) is crucial in regulating energy homeostasis. AMPK regulates energy expenditure by modulating NAD^+-dependent-type III deacetylase SIRT1.[48] 5-Aminoimidazole-4-carboxamide ribotide (**22**, AICAR, Fig. 6) is a pharmacological activator of AMPK. AICAR could improve growth and ATP content while decreasing ROS production and also increase mitochondrial biogenesis without altering mitochondrial membrane potential.[49]

Nicotinamide riboside (**23**, NR, Fig. 6), a vitamin B3 and NAD^+ precursor, was previously reported to increase NAD^+ levels in mice and induce mitochondrial biogenesis.[50] In the mitochondrial myopathy mice model, NR robustly induced mitochondrial mass and function, cured structural abnormalities of mitochondria, and delayed the accumulation of mitochondrial DNA mutations, suggesting a promising treatment strategy for mitochondrial myopathy.[51]

Nicotinamide mononucleotide (**24**, NMN, Fig. 6), an NAD^+ precursor, increased lifespan by normalizing NAD^+ redox imbalance and lowering HIF1α accumulation in Ndufs4-KO skeletal muscle without affecting the brain, and attenuated lactic acidosis in Ndufs4-KO mice.[52]

Acipimox (**25**, Fig. 6), a nicotinic acid analog used to treat hyperlipidemia, has been shown to have a direct effect of acipimox on NAD^+ levels, mitonuclear protein imbalance, and mitochondrial oxidative capacity and also demonstrates that acipimox can also directly affect skeletal muscle mitochondrial function in humans.[53] Now, a randomized, double-blinded, placebo-controlled, adaptive design trial of the efficacy of acipimox in adult patients with mitochondrial myopathy is conducted.[54]

2.6 Agents restoring nitric oxide production

Nitric monoxide (NO) exerts various physiological functions in the central nervous system. There is growing evidence that NO deficiency in mitochondrial disease can complicate disease pathogenesis, such as MELAS (mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes).[55] NO deficiency can potentially play a significant role in the mechanism of stroke-like episodes observed in MELAS syndrome.[56] Both amino acids arginine (**26**, Fig. 7) and citrulline (**27**, Fig. 7) potentially act as NO precursors. Their administration may increase NO availability and hence can have therapeutic effects in stroke-like episodes in MELAS syndrome.[55] Currently, a clinical study is being conducted to assess if giving arginine or citrulline will increase the formation of nitric oxide in individuals with MELAS. Therefore, if arginine and/or citrulline are shown to increase the formation of nitric oxide, they could be used to prevent or treat strokes in patients with MELAS syndrome.[57]

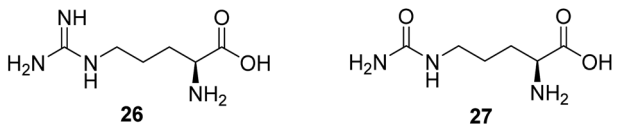

Fig. 7 Chemical structures of **26** and **27**.

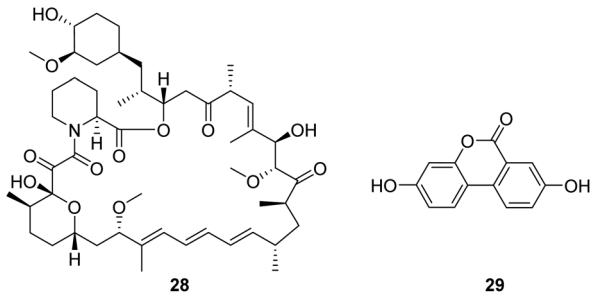

Fig. 8 Chemical structures of **28** and **29**.

2.7 Agents regulating autophagy

Rapamycin (**28**, Fig. 8) is a mechanistic target of rapamycin kinase (mTOR) inhibitors. It is demonstrated that inhibition of mTOR improves survival and health in the Ndufs4$^{-/-}$ model of Leigh syndrome, which may offer therapeutic benefits to patients with Leigh syndrome and potentially other mitochondrial disorders.[58] However, as a promising compound, the clinical use of rapamycin is limited by concerns about the side effects associated with the drug.

Urolithin A (UA, **29**, Fig. 8), a first-in-class natural food metabolite, has been shown to stimulate mitophagy and improve muscle health in old animals and pre-clinical aging models.[59] The results of a first-in-human clinical trial show that supplementation with UA as a nutritional intervention is safe, assist in managing the declining mitochondrial function accompanying aging, and promotes healthy muscle function throughout life.[60]

2.8 Agents as cardiolipin protector

Cardiolipin is a unique phospholipid exclusively expressed on the inner mitochondrial membrane. It plays an essential structural role in cristae formation and the organization of the respiratory complexes into supercomplexes for optimal oxidative phosphorylation. The interaction between cardiolipin and cytochrome c determines whether cytochrome c acts as an electron carrier or peroxidase.[61] Cardiolipin has been identified as a target for drug development associated with energy deficiency. Elamipretide (Bendavia, MTP-131, SS-31, **30**, Fig. 9) is an aromatic-cationic, cell-permeable tetrapeptide in a new class of mitochondrial-targeted drugs. SS-31 binds selectively to cardiolipin *via* electrostatic and hydrophobic interactions. By interacting with cardiolipin, SS-31 prevents cardiolipin from converting cytochrome c into a peroxidase while protecting its electron-carrying function.[61] Treatment of explanted human hearts with SS-31 improves cardiac mitochondrial function.[62]

2.9 Agents as an energy buffer

Creatine (**31**, Fig. 10) is a naturally occurring bioenergetic compound. Creatine stabilizes the mitochondrial transition pore and is vital in mitochondrial ATP production. Creatine also

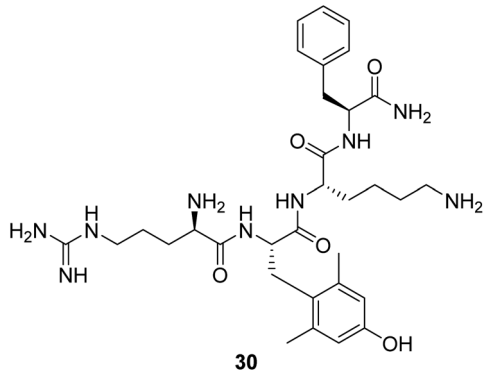

Fig. 9 Chemical structure of **30**.

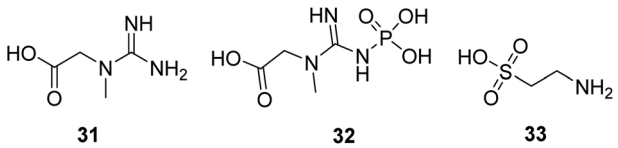

Fig. 10 Chemical structures of **31–33**.

plays an essential role in shuttling Pi from the mitochondria into the cytosol to form phosphocreatine (**32**, Fig. 10) to help maintain cellular bioenergetics.[63] A neuroprotective effect of oral creatine was found in several animal models of neurodegenerative diseases. Creatine monohydrate supplementation can improve exercise capacity in some individuals with mitochondrial myopathies.[64]

Taurine (**33**, Fig. 10) is a naturally occurring sulfur-containing amino acid found abundantly in excitatory tissues, such as the heart, brain, retina, and skeletal muscle. It plays a crucial role in developmental processes, such as brain development, cardiac muscle regulation, and inflammation. Taurine shows protective activity in different neurodegenerative disease models, such as Parkinson's, Alzheimer's, and Huntington's diseases.[65–67] Now, it is in a clinical study to treat mitochondrial encephalomyopathy.

3. Conclusion

Overall, there have been several interesting new approaches in the potential development of new drugs to treat mitochondrial diseases. Proteolysis targeting chimera (PROTAC) technology is a novel strategy to develop new drugs with small molecules that can make protein degradation more efficient and specific, thus creating new opportunities in drug development. PROTAC is a small molecule that simultaneously binds a disease-associated protein and a ubiquitin–ligase complex, which uses the ubiquitin–protease system to eliminate mutated, denatured, and harmful cell proteins. It can effectively target and degrade proteins, including proteins that are difficult to identify and bind. Therefore, it has significant implications for drug development and treating mitochondrial diseases.

In summary, the current challenges and future goals for treating mitochondrial disease revolve around improving diagnosis, developing targeted therapies, discovering biomarkers, modifying disease progression, and exploring innovative approaches like mitochondrial replacement techniques. Continued research efforts and collaboration among scientists, clinicians, and patients are essential to overcome these challenges and achieve these goals. Encouragingly, there has been remarkable progress in mitochondrial disease over the past decade. The increasing number of clinical trials in mitochondrial disorders aim for more specific and effective therapies. More importantly, the unmet clinical need for treating patients with mitochondrial diseases has stimulated academic and commercial interest in developing new treatments, as has an awareness of mitochondrial involvement in more common diseases. In this review, we discuss the bioactive compounds for treating mitochondrial disorders and focus on different pathways. The efforts in this field to provide a more targeted approach are encouraging.

Author contributions

Liying Meng wrote the manuscript and drew the pictures. Guanzhao Wu is fully responsible for the study design, research fields, drafting, and finalizing of the paper.

Conflicts of interest

The authors declare no other conflicts of interest.

Acknowledgements

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