ISSN 0006-2979, Biochemistry (Moscow), 2025, Vol. 90, No. 12, pp. 1862-1882 © Pleiades Publishing, Ltd., 2025.

REVIEW

Suppressing Mitochondrial ROS Production is Beneficial in Multiple Preclinical Models of Human Disease

Martin D. Brand

Buck Institute for Research on Aging, 8001 Redwood Blvd., Novato, California 94945, USA
e-mail: mbrand@buckinstitute.org

Received August 12, 2025
Revised September 24, 2025
Accepted September 29, 2025

Abstract—I discuss the therapeutic potential of site-specific suppressors of the production of mitochondrial reactive oxygen species (ROS). The best-defined suppressors are S1QELs (targeting site I_Q in complex I) and S3QELs (targeting site III_{Qo} in complex III). They prevent ROS formation at source without affecting oxidative phosphorylation. The antidiabetic drug imeglimin and the anti-xerostomia and antischistosomal anethole dithiolethiones also have S1QEL activity, although how much this contributes to their clinical effects needs further study. Suppressing mitochondrial ROS production has therapeutic potential in many diseases. S1QELs and imeglimin improve glucose tolerance, insulin sensitivity, and decrease hepatic steatosis in models of diabetes and obesity. S1QELs and S3QELs protect against age-related cardiac decline, atrial fibrillation and hypertension. They reduce inflammatory cytokines and oxidative stress in macrophages and other cells. They inhibit cancer cell proliferation and tumour growth. In neurological diseases, S1QELs protect against noise-induced hearing loss. S1QELs protect against cardiac and hepatic damage during ischemia-reperfusion. S1QELs and S3QELs extend lifespan in model organisms and S3QELs protect against aging-related intestinal barrier dysfunction. Suppressors mitigate drug-induced toxicities (e.g., acetaminophen, cisplatin) and the effects of environmental stressors. In exocrinopathy, anethole dithiolethione alleviates symptoms of dry mouth and dry eye. Suppressors of mitochondrial ROS production show promise in treating a wide range of diseases driven by mitochondrial oxidative stress. Their mechanism-based specificity offers advantages over traditional antioxidants, with potential applications in metabolic, cardiovascular, inflammatory, neurological, and aging-related diseases. Further research is needed to fully explore their clinical efficacy.

DOI: 10.1134/S0006297925602527

Keywords: mitochondria, ROS, S1QEL, S3QEL, imeglimin, anethole dithiolethione, type 2 diabetes, cardiomyopathy, inflammation, cancer, noise-induced hearing loss, ischemia/reperfusion injury, aging, acetaminophen, cisplatin

MITOCHONDRIAL ROS PRODUCTION

The production of reactive oxygen species (ROS) by mitochondria has been studied extensively by many laboratories for more than 50 years [1-17]. It has become clear that there are at least 11 different sites within the electron transport chain and associated dehydrogenases of mammalian mitochondria at which electrons can leak and cause premature reduction of molecular O_2 to form the two primary ROS: superoxide (by one-electron reduction of O_2) and hydrogen peroxide (by two-electron reduction) [5, 6, 18-28].

Which mitochondrial sites form ROS at the fastest rates in the matrix and in the cytosol in intact cells (and, by extension, *in vivo*)? Using isolated rat muscle mitochondria incubated in a medium mimicking the cytosol of muscle cells at rest or during exercise, four sites were found to dominate, two in respiratory complex I (sites I_Q and I_F) and one each in respiratory complexes II (site II_F) and III (site III_{Qo}) [29, 30], see Fig. 1.

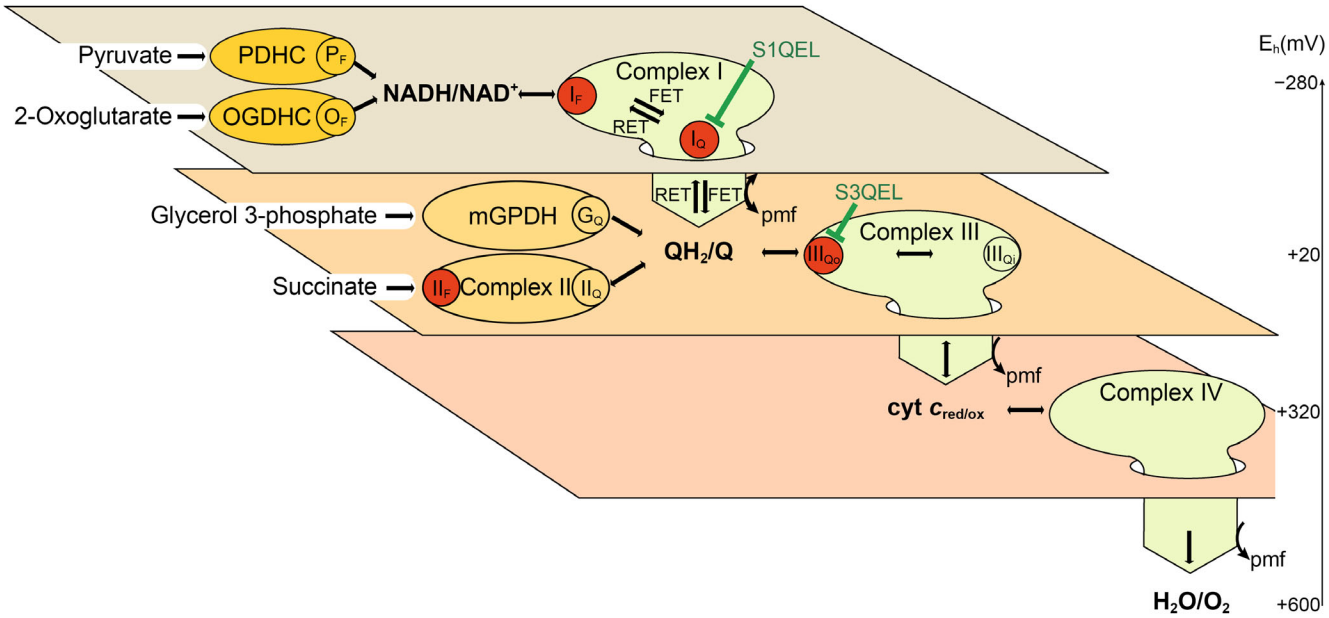

Fig. 1. Major sites of ROS production associated with substrate oxidation and the mitochondrial electron transport chain in mitochondria *ex vivo* and in cells. Red circles indicate the four major sites of superoxide/hydrogen peroxide production that have been implicated in isolated rat muscle mitochondria *ex vivo* under conditions mimicking those *in vivo*; two of these sites (sites I_Q and III_{Qo}) dominate mitochondrial ROS production in isolated cells. The energetics of electron flow are illustrated by three planes representing different groups of redox centres. Centres within each group operate at about the same redox potential (E_h), indicated by the right-hand scale. Electrons are passed from metabolites to the electron transport chain by dehydrogenases, shown as ovals, and normally flow within an isopotential group and then down to the next isopotential group, until they reach oxygen at E_h ~ +600 mV, reducing it to water. As electrons drop from one isopotential group to the next at respiratory complexes I, III and IV, protons are pumped across the mitochondrial inner membrane, and energy is conserved as protonmotive force (pmf). Electrons enter the NADH/NAD^+ pool through NAD-linked dehydrogenases including the 2-oxoglutarate dehydrogenase complex (OGDHC) and pyruvate dehydrogenase complex (PDHC), which can leak electrons to generate superoxide/hydrogen peroxide (sites O_F and P_F respectively), although they have yet to be shown to do so in cells or *in vivo*. During forward electron transport (FET) electrons from NADH flow into the flavin-containing site of complex I (site I_F) then descend via the quinone-binding site (site I_Q) to the next isopotential pool. Dehydrogenases linked to ubiquinone (Q) also pass electrons into the QH_2/Q pool, particularly complex II (site II_F) and mitochondrial glycerol-3-phosphate dehydrogenase (mGPDH, site G_Q). Electrons from QH_2 are passed to the outer Q-binding site of complex III (site III_{Qo}), then to centre III_{Qi} and down through cytochrome *c* and complex IV to oxygen. During reverse electron transport (RET) electrons from QH_2 are driven thermodynamically uphill to the NADH/NAD^+ isopotential group by pmf (generated by FET through complexes III and IV, or by ATP hydrolysis), and feed into sites I_Q and I_F to generate superoxide/hydrogen peroxide. The sites of action of suppressors of superoxide/hydrogen peroxide production (S1QELs at site I_Q and S3QELs at site III_{Qo}) are marked.

Within a wide range of intact cultured mammalian cells provided with different respiratory substrates, two of these sites turned out to be quantitatively most important; sites I_Q and III_{Qo} [31-34]. Site I_Q is the site in respiratory complex I that is routinely measured during reverse electron transport (RET) from ubiquinol to NAD [5, 6, 24, 35, 36], although it generates ROS equally well during forward electron transport (FET) from NADH to ubiquinone when electron transport is stalled under resting conditions [37]. (The distinction between ROS production during FET and RET that is sometimes drawn in the literature is not helpful – what matters is not the net direction of electron flow but the levels of the factors that cause high ROS production from site I_Q, namely high QH_2/Q and NADH/NAD^+ ratios, high membrane potential, and independently high pH gradient [9, 24, 37, 38]). In intact cells site I_Q dominates superoxide and hydrogen peroxide production in the mitochondrial matrix, producing about 70% of the total in that compartment [31-33]. Matrix superoxide is rapidly converted to hydrogen peroxide by superoxide dismutase-2, and hydrogen peroxide derived from site I_Q then spills out into the cytosol and makes up about 15% of total cytosolic ROS production. Site III_{Qo} is the outer quinone-binding site in respiratory complex III [5, 6, 22]; it delivers superoxide to both the mitochondrial matrix and the cytosol [3, 22]. In intact cells site III_{Qo} is the other major mitochondrial contributor to ROS production in the mitochondrial matrix and in the cytosol, providing about 30% of the total in each case; much of the remaining cytosolic hydrogen peroxide (40-60%) comes from cytosolic NADH oxidases (NOXs) [31-33].

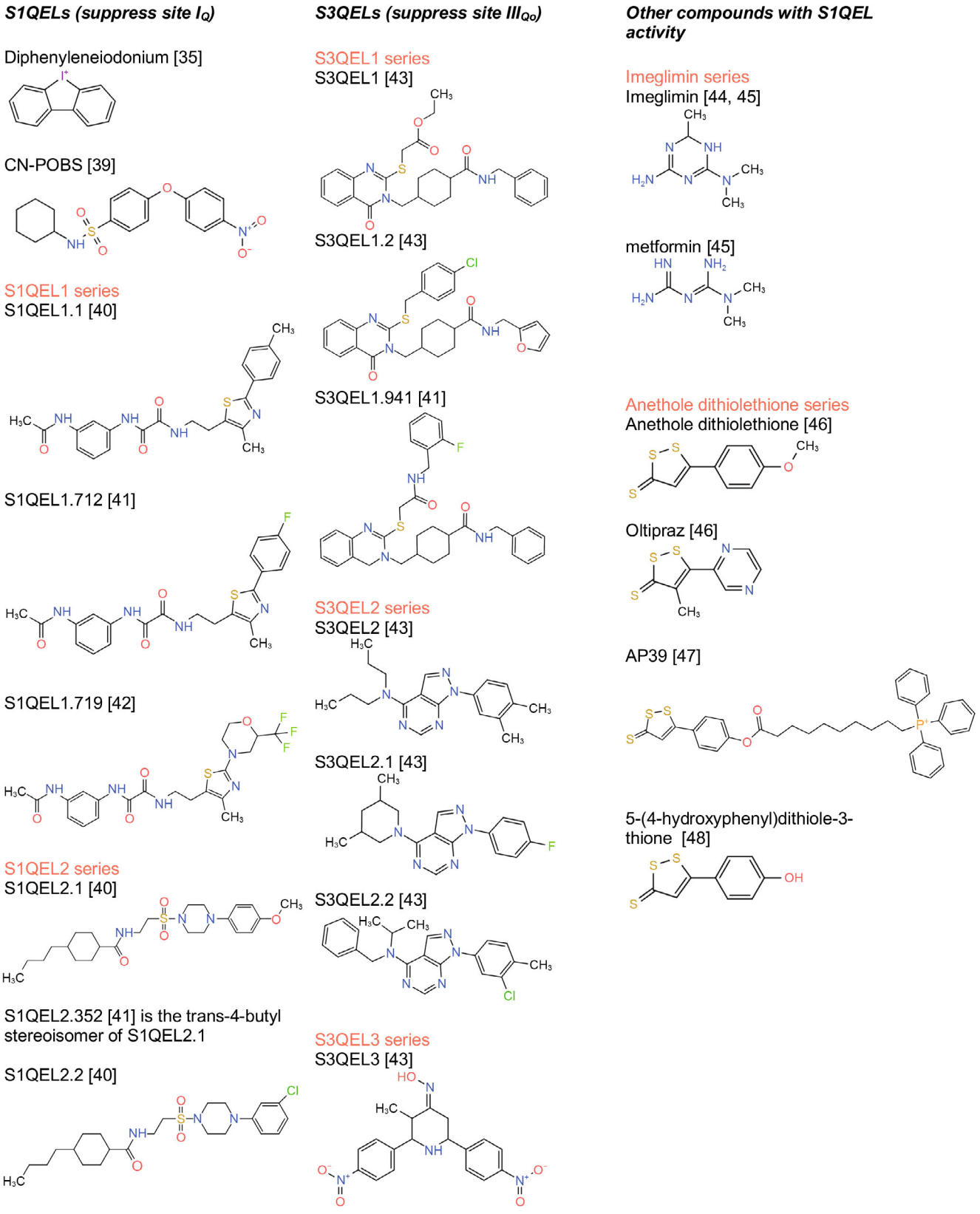

Fig. 2. Outline structures of the ROS suppressors discussed in this review. See the original references for their stereochemistry where appropriate. Metformin is not known to be a S1QEL but is included to show its structural relationship to imeglimin.

SITE-SPECIFIC SUPPRESSORS OF MITOCHONDRIAL ROS PRODUCTION

Figure 2 shows the structures of the compounds discussed in this review.

Diphenyleneiodonium is an NAD(P)H oxidase inhibitor that also potently inhibits mitochondrial ROS production [49]. Following our demonstration that diphenyleneiodonium specifically suppressed superoxide and hydrogen peroxide production by site I_Q in isolated mitochondria without strongly inhibiting forward electron transport and respiration [35], we developed a plate-based screen for such suppressors at several different sites in the mitochondrial electron transport chain [39]. This was a mechanism-based screen: we first set up conditions in isolated mitochondria under which only a single site of electron leak produced a fluorescent signal, for example ROS production specifically from site I_Q was measured as the rotenone-sensitive increase in resorufin fluorescence in the presence of succinate to provide electrons to respiratory complex I and generate protonmotive force through respiratory complexes II, III and IV [6, 39, 50]. We then screened for molecules that strongly inhibited the signal from one site without affecting other sites or respiration and oxidative phosphorylation. Importantly, this screen rejects candidates that have general antioxidant activity or that inhibit normal electron transport and energy metabolism, although one set of such rejects turned out to be useful novel inhibitors of mitochondrial glycerol phosphate dehydrogenase, iGP-1 and iGP-5 [51]. Using this screen on a small chemical library we identified a better suppressor of ROS production by site 1_Q: N-cyclohexyl-4-(4-nitrophenoxy)benzenesulfonamide, CN-POBS [39]. Scaling up of the screen to a much larger chemical library of about a million compounds led to the identification of several independent classes of S3QELs (suppressors of site III_{Qo} electron leak) [43] and S1QELs (suppressors of site I_Q electron leak) [40]. In our hands these early compounds were powerful tools to investigate the importance of sites I_Q and III_{Qo} in isolated mitochondria [29, 30, 36, 37, 40, 43], in cells [30-33, 40, 43, 52], in isolated perfused organs [40], and (to a limited extent because of bioavailability) *in vivo* [34, 40, 41, 52, 53]. Subsequent application of medicinal chemistry led to improved S1QELs, such as S1QEL1.719, with high affinity (the free concentration giving half-maximal suppression (IC_{50}) is about 50 nM) and better solubility and pharmacokinetics, enabling them to be given orally to animals to study the effects of suppression of ROS production by mitochondrial site I_Q in preclinical models *in vivo* [42]. S1QEL1.719 has suitable solubility, permeability and metabolic stability for oral administration dosed as a suspension in 0.5% w/v hydroxypropyl methylcellulose. Total unbound exposures in mice measured 2 h after oral gavage of 30 mg S1QEL719/kg body weight were about 100 nM in plasma and about 150 nmol/g in liver. These exposure values (near C_{max}) were 2-3-fold greater than the IC_{50} for suppression of superoxide/H_2O_2 production from site I_Q and 200-300-fold less than the IC_{50} of 30 μM for inhibition of respiration on complex I substrates, determined using isolated rat skeletal muscle mitochondria [42]. In addition, S1QEL1.719 is well-tolerated, with no adverse effects noticed at these exposures after 40 weeks of supplementation to chow. Similarly, mice have been given high doses of S3QEL2 in chow for over 12 months with no detectable adverse effects on health, body weight, metabolism, or general behavior, and S3QEL2 readily crossed the gut and blood-brain barriers, leading to a brain to plasma ratio of about 2 [54]. Further rounds of primary screening and medicinal chemistry have since generated structurally unrelated new "next-generation" S1QELs with excellent drug-like properties; these work *in vivo* after oral administration at low doses and have displayed no off-target effects; in particular they show no inhibition of electron transport and oxidative phosphorylation even at much higher doses. Authentic S1QELs and S3QELs have not yet been submitted for approval for use in humans, so all current information about their potential clinical efficacy comes from studies in preclinical cell and animal models as detailed below. The mechanism by which they suppress ROS production is not established, but we can exclude the suggestion that S1QELs work by inhibiting reverse electron transport into complex I [36]. Our working hypothesis is that they alter rate constants within complex I and complex III respectively, lowering the steady-state concentration of the electron donor (presumably a semiquinone) that reacts with O_2 to generate superoxide or hydrogen peroxide, without affecting the rates of forward or reverse electron transport through the complexes.

Site-specific suppressors of mitochondrial ROS production are inherently different from antioxidants, even those specifically targeted to mitochondria, because suppressors prevent the formation of superoxide and hydrogen peroxide at their source, whereas antioxidants lower the concentrations of these ROS or interfere with their downstream effects. An analogy may help to illustrate this difference: if mitochondrial ROS production is a bottle of red wine tipped on its side, and the rest of the cell and organism are the expensive white carpet below, then antioxidants mop up the spilled wine and try to limit its spread, whereas suppressors of mitochondrial ROS production keep the bottle stoppered to prevent the damage from occurring in the first place.

Other compounds are also known to act as S1QELs, having a demonstrated ability to suppress ROS

production by site I_Q: imeglimin [55, 56] and anethole dithiolethione (anethole trithione) [57]. Imeglimin [44] (brand name Twymeeg), derived from metformin by addition of acetaldehyde [45], is an oral antidiabetic drug that has been successful in phase III trials [58, 59] and is now used for the treatment of type II diabetes in Japan [60] and India [61, 62]. It has clear S1QEL activity [55], albeit needing very high concentrations (100 μM in digitonin-permeabilised human endothelial cells [56], and, in our hands, specificity for site I_Q but a very poor IC_{50} of only about 2 mM in isolated rat muscle mitochondria using our standard assays [5, 6, 20, 39, 40]. However, the molecular mechanisms of its therapeutic action are thought to be complex and are not fully understood [55, 61, 63, 64]. As well as decreasing mitochondrial ROS generation, it affects mitochondrial function by partial inhibition of complex I and stimulation of complex III activity, prevents mitochondrial permeability transition pore opening, improves insulin sensitivity, modulates genes involved in hepatic gluconeogenesis, and protects beta-cell function with induction of the synthesis of NAD via the salvage pathway. Anethole dithiolethione (5-(4-methoxyphenyl)-3H-1,2-dithiole-3-thione, ADT, also known as anethole trithione, AOL, Felviten, Sialor, and OP2113) and closely related compounds such as oltipraz (4-methyl-5-(2-pyrazynyl)-1,2-dithiole-3-thione) have been used in humans for many decades; they improve bile production by the liver to aid digestion, improve saliva production in cases of dry mouth, act as antischistosomal agents and have chemoprotective effects against cancers and xenobiotics [65-70]. Anethole dithiolethione has clear S1QEL activity, albeit with low potency, with an IC_{50} of 10-26 μM [57, 71]. It is reasonably specific; IC_{50} values against other mitochondrial sites of ROS production are at least 15-fold higher. It did not affect oxidative phosphorylation in isolated mitochondria or respiration or growth of C2C12 cells [71]. However, the molecular mechanisms of its therapeutic actions remain unclear; in particular, it is a known slow-release H_2S donor [47, 70, 72].

DO IMEGLIMIN AND ANETHOLE DITHIOLETHIONE ACT SIGNIFICANTLY AS S1QELs, OR DO THEIR PRECLINICAL AND CLINICAL EFFECTS DEPEND PARTLY OR COMPLETELY ON OTHER TARGETS?

There is an important conceptual difference between the S1QELs (and S3QELs) on the one hand, and imeglimin and anethole dithiolethione on the other: mechanism-based versus phenotypic screening. Imeglimin was developed using an *in vivo* phenotypic screen of antihyperglycaemic activity in rodents, followed by chemical modification of a lead molecule [61, 64]. Anethole dithiolethione has been known for many years from its effects on complex human phenotypes. Thus, the preclinical and clinical actions of imeglimin and anethole dithiolethione are not disputed, but the specific molecular target or targets underlying their clinical effects are open for test and discussion. They do have (weak) S1QEL activity, but their clinical effects might be caused partially or completely by other, separate molecular mechanisms yet to be fully delineated, particularly bearing in mind their poor potencies as S1QELs as discussed above. On the other hand, S1QELs (and S3QELs) were discovered by mechanism-based primary screening to find specific suppressors of ROS production at sites I_Q and III_{Qo} respectively, with counterscreens for any effects at other sites or on electron transport and oxidative phosphorylation, as discussed above. This screening yielded several completely different chemical families of compounds (S1QEL1, S1QEL2 etc., see Fig. 2) each with the same mechanistic ability to suppress ROS production at site I_Q, but otherwise unrelated. If each of these chemically unrelated S1QELs is found to have the same preclinical or clinical effect in any particular case, that provides strong evidence that the effect is caused specifically by the suppression of superoxide/hydrogen peroxide production at site I_Q, which is the defining feature of all S1QELs, and not to some unknown off-target effect, which would be specific for one or another specific class (S1QEL1 but not S1QEL2, etc.). The same logic applies separately to the different classes of S3QELs: if each of the chemically unrelated S3QELs is found to have the same preclinical or clinical effect in any particular case, that is strong evidence that the effect is caused specifically by the suppression of superoxide/hydrogen peroxide production at site III_{Qo}, which is the defining feature of all S3QELs, and not to some unknown off-target effect, which would be specific for one or another specific class (S3QEL1 but not S3QEL2, etc.). We have reported such similarity of action in mechanistic and preclinical models for chemically unrelated S3QELs [30-32, 43, 53] and chemically unrelated S1QELs [30-32, 36, 37, 40, 41]. We have also routinely run S1QEL1.719 [42] alongside structurally unrelated "next-generation" S1QELs and seen the same effects with each in many of our preclinical models discussed below, confirming that their preclinical effects are on-target. In this way, the molecular target of S1QELs (and S3QELs) is not in significant doubt, but their preclinical and clinical actions are open for test and discussion. A case can be made that many of the beneficial clinical effects of imeglimin and anethole dithiolethione are each primarily caused directly or indirectly by their ability to act as S1QELs to suppress mitochondrial ROS production at site I_Q, thereby

sparing mitochondrial and cellular glutathione, decreasing lipid peroxidation, and protecting from inflammation. If so, their clinical effects are an excellent guide to the potential clinical efficacy of S1QELs in general, and the effects of S1QELs discussed below are an excellent guide to potential new indications against which imeglimin or anethole dithiolethione should be effective, and a guide to how tweak their structures to improve their efficacy. However, if the mechanism of imeglimin or of anethole dithiolethione is partly or completely through other, non-S1QEL, effects then imeglimin and anethole dithiolethione make poor or misleading guides to the clinical actions expected of more clearly-defined S1QELs, which would then be in a mechanistically separate class. A good way to find out whether or not the preclinical and clinical effects of imeglimin or anethole dithiolethione arise solely through their action as S1QELs is to compare their effects with those of validated S1QELs. Any discrepancies would be caused by non-S1QEL effects of imeglimin or anethole dithiolethione. Conversely, complete overlap of effects would be strong evidence that they work exclusively as S1QELs.

WHAT CLINICAL INDICATIONS ARE LIKELY TO BE IMPROVED BY TREATMENT WITH SUPPRESSORS OF MITOCHONDRIAL ROS PRODUCTION?

Oxidative stress is thought to have a role in many, or even most, diseases and pathologies. Untangling primary causes from secondary consequences, and the extent to which mitochondrial ROS production from specific sites may drive disease initiation and progression, requires careful attention to the type of evidence that is available. Many published studies are associative rather than mechanistic; they show that some signal attributed to mitochondrial ROS (such as the expression of an antioxidant protein, or the response of a fluorescent probe) is increased when the physiological signalling pathway or pathology is activated, or decreased when it is inactive. Such associations are suggestive, but they do not distinguish between causal and bystander or downstream roles of mitochondrial ROS, and are therefore not definitive. The following criteria for assessing whether mitochondrial production of superoxide and/or hydrogen peroxide drives biological or pathological effects, ranked by reliability, have been proposed [7].

1. Inhibition of a phenotype by addition of well-validated S1QELs or S3QELs indicates that it is driven by superoxide or hydrogen peroxide generated at mitochondrial site I_Q or site III_{Qo} respectively.
2. Inhibition of a phenotype by overexpression of mitochondria-specific superoxide dismutase-2 (SOD2) to lower the superoxide concentration in the mitochondrial matrix, or, conversely, exacerbation of the phenotype by raising matrix superoxide level by SOD2 deficiency, indicates that the phenotype is driven by superoxide in the matrix.
3. Inhibition of a phenotype by overexpression of peroxiredoxin-3 (PRDX3) or by expression of synthetic mitochondrially-targeted catalase (mCAT), to lower matrix hydrogen peroxide level, or, conversely, exacerbation of the phenotype by raising matrix hydrogen peroxide level by PRDX3 deficiency, indicates that the phenotype is driven by matrix hydrogen peroxide.
4. Inhibition of a phenotype by mitochondria-targeted antioxidants that react specifically with superoxide (and hydrogen peroxide), such as mitoTEMPO, to lower matrix levels of superoxide (and hydrogen peroxide) suggests that the phenotype is driven by matrix superoxide (or hydrogen peroxide).
5. Inhibition of a phenotype by mitochondria-targeted antioxidants, such as mitoQ, SkQs, and SS-31, which interfere with downstream lipid peroxidation, suggests that the phenotype is driven by matrix ROS, although the exact targets and mechanisms then require further elucidation.
6. Studies associating a pathology with human gene variants in relevant proteins (SOD2 or PRDX3) suggest that the human pathology is driven or exacerbated by matrix superoxide or hydrogen peroxide respectively.

Using these criteria to sift the huge literature on the role of oxidative stress in disease provides strong and compelling evidence that mitochondrial ROS are causal or sensitizing for a wide range of human pathologies [7]. These pathologies overlap but can be crudely grouped into the categories listed in Table 1.

For each of these broad and overlapping pathologies, there is strong evidence from all or most of the six criteria enumerated above that mitochondrial ROS production is a primary driver or sensitizer of the pathology. In particular, knockout of mitochondrial SOD-2 to raise matrix superoxide concentration exacerbates the phenotype in many models of disease, and matrix expression of catalase to decrease matrix hydrogen peroxide concentration or treatment with the mitochondrially-targeted spin trap mitoTEMPO protects (see [7] for the relevant references to the primary literature). Mitochondrially-targeted lipid peroxidation chain-breakers such as mitoQ [73] and SkQ [74] have been particularly effective; studies using them in preclinical models and in patients are fully referenced elsewhere [7]. These studies by Murphy and colleagues and by Skulachev and col-

Table 1. Pathologies for which there is a range of strong and compelling evidence that mitochondrial ROS are causal or sensitizing [7], and * evidence for beneficial effects of treatment with S1QELs or S3QELs (or the putative S1QELs imeglimin and anethole dithiolethione) in preclinical models of these diseases; for details see the text

Pathology	S1QEL	S3QEL	Imeglimin	Anethole dithiolethione
A Metabolic disease (diabetes, obesity)	*	*	*	*
B Cardiovascular diseases (hypertension, atherosclerosis, cardiomyopathy, heart failure)	*	*	*	*
C Inflammatory diseases (inflammation, atherosclerosis)	*	*		
D Cancer (incidence, growth, metastasis)	*	*		*
E Neurological diseases (cognitive, motor, degenerative, vision, hearing, neuropathies)				
(i) Dementia		*		
(ii) Noise-induced hearing loss	*			
(iii) Parkinson's disease	*	*		
F Ischemia/reperfusion injuries	*	*		*
G Aging and associated diseases	*	*		
H External insults				
(i) Tunicamycin and ER stress	*	*		
(ii) Acetaminophen hepatotoxicity	*	*		*
(iii) Carbon tetrachloride hepatotoxicity				*
(iv) Cisplatin nephrotoxicity	*			
(v) Fluoroquinolone tenotoxicity				*
I Genetic diseases				
(i) POLG mouse	*			
J Other				
(i) Exocrinopathy				*

leagues have not only identified and characterized therapeutic targets for such antioxidants themselves, but have also illuminated areas in which suppressors of ROS production might be effective. It follows from these examples that multiple pathologies should be amenable to treatment or amelioration by suppression of mitochondrial ROS production. In this review I focus on the extent to which this expectation has been explored and tested using known mitochondrial ROS suppressors: S1QELs and S3QELs, and, with the strong caveat discussed above regarding the extent to which they may or may not act purely as S1QELs, using imeglimin and anethole dithiolethiones. See Table 1 for a summary.

METABOLIC DISEASES

There is strong empirical evidence from the administration of S1QELs (and of imeglimin and anethole dithiolethione) that mitochondrial ROS production *in vivo* is a driver of metabolic disease and that its suppression can ameliorate the pathology.

S1QEL. Knockout of SOD2 in mice raises matrix superoxide concentration, and leads to hepatic steatosis, a classic symptom of metabolic syndrome. $SOD2^{-/-}$ mice have dilated cardiomyopathy, accumulation of lipid in liver and skeletal muscle, metabolic acidosis, a failure of weight gain and a median lifespan of 8 days [75]. Dosing of $Sod2^{-/-}$ mouse pups with early

S1QELs that were suitable for intraperitoneal injection but not for oral administration (S1QEL1.712 or S1QEL2.352) led to decreased hepatic steatosis (but dosing with S3QEL1.941 did not) [41]. A classic way to model metabolic syndrome is acute 1-2-week or longer-term 8-week high-fat-feeding of mice, which leads to insulin resistance [76]. In such mice a more advanced S1QEL1 that is suitable for oral dosing (S1QEL1.719) decreased fat accumulation, improved glucose tolerance and normalized fasting insulin concentration both prophylactically and therapeutically [42], showing that ROS production from mitochondrial site I_Q *in vivo* is necessary for the induction and maintenance of glucose intolerance caused by a high-fat diet in mice and suggesting that oral administration of S1QELs may be beneficial in metabolic syndrome. Our more recent studies have replicated and amplified these results using "next-generation" S1QELs with single or multiple prophylactic or therapeutic doses of S1QEL1.719, and shown that dosing with S1QEL1.719 in this model results in increased glucose uptake in tissues in a hyperinsulinemic-euglycemic clamp assay and lowers plasma levels of the cytokines fibroblast growth factor 21 (FGF21) and growth differentiation factor 15 (GDF15), which are elevated by cellular stress in diet-induced obesity [77]. We have also shown that therapeutic or prophylactic oral dosing of S1QEL1.719 and "next-generation" S1QELs in mice fed a methionine-choline deficient diet (a mouse model of non-alcoholic steatohepatitis and fibrosis [78, 79]) improves glucose tolerance and protects against hepatic damage, steatosis and fibrosis. We have also found that S1QEL1.719 dosing lowers circulating insulin levels in genetic models of metabolic disease: db/db and ob/ob mice. Supporting evidence comes from studies showing that application of S1QEL2.2 decreased ROS production in mitochondria isolated from the livers of obese mice more than in those from control mice; in hepatocytes from obese mice use of S1QEL2.2 showed that expression of the alternative ubiquinol oxidase (AOX) decreased site I_Q ROS production, allowing the beneficial effect of AOX *in vivo* in mice to be interpreted as due to decreased site I_Q ROS production. AOX-expressing mice had lower fasting blood glucose levels, improved intraperitoneal and oral glucose tolerance and decreased liver glycogen content than controls [80].

S3QEL. S3QEL2 protects isolated pancreatic beta cells and insulinoma INS-1E cells against oxidative stress *ex vivo* [43, 81], but dosing of $Sod2^{-/-}$ mouse pups with S3QEL1.941 did not lead to decreased hepatic steatosis [41], suggesting that superoxide production from site III_{Qo} is only an important source of ROS damage in beta cells under specific circumstances.

Imeglimin. Imeglimin improved the three key pathological defects of diabetes in streptozotocin-treated rats, namely excessive hepatic glucose production, impaired peripheral glucose uptake by skeletal muscle, and insufficient insulin secretion [44]. Six-week imeglimin treatment had antidiabetic effects in a 16-week high-fat, high-sucrose diet mouse model of metabolic disease. It normalized glucose tolerance and insulin sensitivity by preserving mitochondrial function from oxidative stress and favoring lipid oxidation in liver [55]. Imeglimin directly activated beta-cell insulin secretion in awake rodents [82]. It improved mitochondrial function, reduced hepatic steatosis, and suppressed hepatic fibrosis in mice fed a choline-deficient high-fat diet [83]. In patients with type 2 diabetes, imeglimin improved beta-cell function [84]. Recent reviews document the clinical efficacy of imeglimin in decreasing steatosis and improving beta cell function and glycemic control in type 2 diabetes [59, 62, 85]. The effects of S1QELs in preclinical models partly mirror those of imeglimin, so a case can be made that the beneficial clinical effects of imeglimin in treatment of type 2 diabetes and metabolic disease are partly or even completely explained by its action as a weak S1QEL, although this remains a speculation that needs to be tested.

Anethole dithiolethione. Dithiolethione compounds such as oltipraz can prevent or treat fibrosis and insulin resistance, and have mitochondrial protective effects in the liver, by a mechanism proposed to involve AMP-activated protein kinase (AMPK) and/or 70-kDa ribosomal protein S6 kinase 1 (S6K1) [69]. Oltipraz improved blood glucose and insulin resistance, decreased blood lipid metabolism, reduced inflammation and apoptosis, suppressed oxidative stress, mitigated pancreatic and liver tissue injury, and enhanced pancreatic beta-cell insulin secretion, thereby mitigating the symptoms of type 2 diabetic mice [86]. A novel mitochondria-targeted anethole dithiolethione H_2S donor (AP39) decreased hyperglycemia-induced oxidative stress and metabolic changes in microvascular endothelial cells *in vitro*. Targeting H_2S to mitochondria using AP39 induced a 1000-fold increase in the potency of the cytoprotective effect of H_2S against hyperglycemia-induced injury, suggesting that AP39 could be useful against diabetic vascular complications [47]. The respective authors attribute the potency of oltipraz to its effects on the AMPK–mTOR–S6K1 pathway [69] and the effects of AP39 to its activity as an H_2S donor in the mitochondrial matrix [47, 87]. The extent to which these compounds may have been acting more directly as S1QELs remains undetermined.

CARDIOVASCULAR DISEASES

There is some empirical evidence from the administration of S1QELs, S3QELs, imeglimin and

anethole dithiolethione that mitochondrial ROS production *in vivo* is a driver of atrial fibrillation, cardiomyopathy and hypertension and that its suppression can ameliorate these pathologies.

S1QELs and S3QELs. In isolated rabbit atrial myocytes, S1QEL1.1 and S3QEL2 decreased the severity of beat-to-beat alternations in atrial calcium transient amplitude, which are causally linked to atrial fibrillation [88]. We found that long-term feeding of S1QEL1.719 in chow (starting at 12 weeks of age and finishing at 54 weeks of age) had no obvious detrimental effect on C57BL/6J mice. Echocardiography of 54-week old mice fed S1QEL1.719 for 42 weeks revealed that they were significantly protected against the normal age-related decline in cardiac function, specifically ejection fraction, stroke volume, cardiac output and end-diastolic volume.

Imeglimin. Imeglimin improved cardiac gene expression abnormalities associated with heart failure with preserved ejection fraction in mice subjected to the cardiometabolic stress of high-fat diet and the nitric oxide synthase inhibitor L-NAME for 16 weeks [89].

Anethole dithiolethione. Anethole dithiolethione decreased contractile hyperreactivity to 5-hydroxytryptamine and prostaglandin F2α in pulmonary arterial rings from chronic hypoxia-induced pulmonary hypertension rats [90]. *In vivo*, preventive treatment with anethole dithiolethione decreased mean pulmonary arterial pressure, pulmonary artery remodelling and right ventricular systolic pressure and reversed pulmonary artery hyperreactivity to 5-hydroxytryptamine [91, 92].

INFLAMMATORY DISEASES (INFLAMMATION AND ATHEROSCLEROSIS)

Suppressors of mitochondrial ROS production are effective at decreasing inflammatory cytokines in various contexts, although they have yet to be tested widely in overt models of atherosclerosis and other inflammatory diseases.

S1QEL. S1QEL1.1 decreased the production of the pro-inflammatory cytokines tumour necrosis factor-α (TNF-α) and interleukin-1b in mouse macrophages exposed to swollen conidia of *Aspergillus fumigatus*, and reduced the fungicidal activity of macrophages against swollen conidia [93]. Treatment with a S1QEL suppressed lipopolysaccharide-induced expression of interleukin-10 and augmented that of interleukin-6 in cultured macrophages under serine deprivation [94]. S1QEL treatment decreased oxidized low-density lipoprotein-induced glycolytic reprogramming, levels of pro-inflammatory cytokines interleukin-1b and CXCL8 (formerly interleukin-8), and foam cell formation in isolated human monocytes [95]. In our hands therapeutic S1QEL1.719 treatment strongly attenuated expression of a panel of inflammatory genes in liver of mice fed a methionine-choline deficient diet.

S3QEL. Treatment with S3QEL2 decreased mouse bone marrow macrophage TNF-α and IFN-β levels following lipopolysaccharide stimulation and TNF-α, CXCL10, IFN-α and IFN-β production following Poly(I:C)-activation [96]. In a murine alveolar macrophage cell line incubated with particulate matter, treatment with a S3QEL inhibited interleukin-6 production [97]. Treatment with a S3QEL suppressed lipopolysaccharide-induced expression of interleukin-10 and augmented that of interleukin-6 in cultured macrophages under serine deprivation [94]. S3QEL2 decreased ATPγS-induced production of interleukin-6 in human airway epithelial cells [98]. S3QEL1.2 decreased interleukin-10 in lipopolysaccharide-activated macrophages and impaired interleukin-10 production *in vivo* after lipopolysaccharide challenge [52].

CANCER

S1QELs and S3QELs can suppress cell proliferation and differentiation in a variety of non-cancerous and cancerous cell types. A S1QEL attenuated normoxic proliferation of mouse lung epithelial cells [99]. S1QEL1.1 and S3QEL2 impaired myogenesis during C2C12 myoblast differentiation [100]. S3QEL3 suppressed the cross-presentation capacity of activated plasmacytoid dendritic cells to elicit a clonal $CD8^+$ T cell expansion response [101]. S3QEL2 reduced the growth of Jurkat cells subjected to respiratory inhibition by antimycin or piericidin [102]. A S3QEL decreased proliferation of *Drosophila* intestinal stem cells following Ecc15 infection [103]. A S3QEL, but not a S1QEL, protected mouse hepatoma cells from lipid peroxidation and the subsequent ferroptosis induced by cysteine starvation, and suppressed ferroptosis in xCT-knockout mouse-derived embryonic fibroblasts, which usually die under conventional cultivating conditions due to the absence of intracellular cysteine and glutathione [104].

As well as these *in vitro* effects, S3QELs and anethole dithiolethiones have shown effectiveness against carcinoma *in vivo*.

S3QEL. S3QEL1.2 promoted tumour-mediated immune evasion and promoted survival of mice bearing B16F10 melanoma by lowering tumour growth [52].

Anethole dithiolethione. Anethole dithiolethione inhibited colon carcinogenesis [105], significantly inhibited mammary cancer multiplicity [106] and is a potentially efficacious chemoprevention agent for lung cancer [68, 107]. It inhibited anchorage-independent growth of A549 cells, inhibited the

migration of A549 cells in the transwell assay and inhibited lung cancer cell proliferation and decreased tumour growth almost 2-fold in an orthotopic mouse xenograft model with A549 human lung adenocarcinoma cells *in vivo* [108, 109]. Oltipraz has proved effective as an inhibitor of carcinogenesis in experimental models of breast, bladder, liver, forestomach, colon, tracheal, lung, and skin cancer; mechanistic studies indicate that it affects the metabolism and disposition of chemical carcinogens, principally through the induction of electrophile detoxication enzymes [46, 69].

NEUROLOGICAL DISEASES (DEMENTIA, NOISE-INDUCED HEARING LOSS, PARKINSON'S DISEASE)

Some observations suggest that suppression of mitochondrial ROS production can be protective against neurological stresses and diseases, although more work is needed to show this definitively.

(i) DEMENTIA

S3QEL. Chronic administration to mice of high doses of S3QEL2 in chow for over 12 months had no detectable adverse health effects and did not alter body weight, metabolism, or general behavior. In this study S3QEL2 treatment decreased dementia-linked tauopathy and neuroimmune cascades and extended lifespan [54]. The authors suggest that site-specific suppression of ROS generated at site III_{Qo} represents a promising therapeutic intervention in dementia and other neurological conditions that affect mitochondrial ROS production.

(ii) MOISE-INDUCED HEARING LOSS

S1QEL. We have found that treatment of mice with S1QEL1.719 gave significant protection (by about 10dB in the threshold for sound perception) against permanent noise-induced hearing loss, was cytoprotective to inner hair cells in animals subjected to noise at 32 kHz and to outer hair cells in animals subjected to noise at 8 kHz, and protected ribbon synapses in animals subjected to noise at 32 kHz. S3QEL1.941 [41] did not protect.

(iii) PARKINSON'S DISEASE

S1QEL. Treatment with S1QEL1.1 attenuated mitochondrial ROS production and cell death induced by MPP^+ (the neurotoxin 1-methyl-4-phenylpyridinium) in SHSY5Y neuroblastoma cells (a cellular model of Parkinson's disease) [110]. Also, S1QEL1.1 co-exposure rescued rotenone-induced dendritic degeneration and dopaminergic function in the roundworm *Caenorhabditis elegans* [111]. However, this result should be treated with caution, since rotenone prevents ROS production at site I_Q, so S1QEL co-exposure should have had no further effect. Also, S1QELs can decrease rotenone binding to complex I [36] and might have been working by attenuating the initial complex I inhibitory effect of rotenone (and perhaps of MPP^+).

S3QEL. Treatment with S3QEL2 attenuated mitochondrial ROS production and cell death induced by MPP^+ in SHSY5Y neuroblastoma cells [110].

ISCHEMIA/REPERFUSION INJURIES

There is robust evidence that suppressing ROS production from site I_Q is protective against ischemia-reperfusion injury.

S1QEL (and S3QEL). Therapeutic administration of S1QEL1.1 was found decrease ROS production by site I_Q in mouse muscle mitochondria and to protect against ischemia-reperfusion injury in the perfused mouse heart [40], providing strong supporting evidence that ROS production from site I_Q is a primary cause of cardiac ischemia-reperfusion injury. We have repeated and extended such observations using S1QEL1.719 and unrelated new "next-generation" S1QELs in mouse and rat hearts. Injection of a S1QEL during cardiopulmonary resuscitation in KCl-cardiac arrest mice improved myocardial function, neurologic outcomes, and survival and represents a potential therapy for improving sudden cardiac arrest resuscitation outcomes [112, 113]. Pretreatment with S1QEL1.1 suppressed the immediate increase in the fluorescence of mitoSOX, a ROS indicator, at the onset of reperfusion after ischemia in isolated mouse hearts [114], and S1QEL1.1 treatment limited infarct size in isolated perfused hearts from UCP3-knockout mice subjected to ischemia-reperfusion *ex vivo* [115]. A hydrogel incorporating S1QEL1.1 and other agents significantly improved cardiac function and reduced adverse ventricular remodelling in an *in vivo* rat model of myocardial ischemia-reperfusion injury [116]. A strong decrease in ROS production from site I_Q caused by addition of S1QEL1.1 was confirmed using isolated mouse heart mitochondria under conditions simulating ischemia-reperfusion injury *ex vivo*, and a small contribution of superoxide production from site III_{Qo} preventable by S3QEL2 was also seen under more constrained conditions [117, 118].

Anethole dithiolethione. Anethole dithiolethione (OP2113) improved recovery of contractile activity and decreased infarct size in a rat heart infarct model,

although the authors did not rule out the possibility that the beneficial effects were due instead to complex I inhibition or the activity of anethole dithiolethione as an H_2S donor [57]. Anethole dithiolethione decreased ST segment elevation, decreased troponin release, improved left ejection fraction and decreased infarct size in a sheep model of regional ischemia-reperfusion [71] and decreased myocardial infarct size and no reflow after rat myocardial ischemia-reperfusion [119, 120]. An anethole dithiolethione prodrug with improved solubility (ATXP) maintained the bioactivity of the parent drug (5-(4-hydroxyphenyl)dithiole-3-thione) to cause a significant reduction in infarct volume 24 h after reperfusion [48].

AGING AND AGING-ASSOCIATED DISEASES

Aging is a dominant risk factor for many of the diseases discussed in the present review; the beneficial effects of suppressing mitochondrial ROS production in many of them are treated separately in the other sections (see metabolic diseases, cardiovascular diseases, inflammatory diseases, cancer, dementia, Parkinson's disease). S1QELS and S3QELs also have protective effects on intestinal barrier dysfunction (which can limit lifespan in roundworms) and lifespan, although more work is needed to test their anti-aging properties and the relationships between the beneficial effects of mitochondrial ROS-suppressors on aging-related diseases and any effects on aging itself.

S1QEL. We have found that S1QEL1.719 and unrelated "next-generation" S1QELs extend median lifespan in *C. elegans* [unpublished].

S3QEL. S3QEL1.2, S3QEL2.2, and S3QEL3 protected against greater intestinal permeability and against shortened lifespan on high-nutrient diets in *Drosophila*, and S3QEL1.2 and S3QEL2.2 strongly protected against greater diet-induced intestinal permeability in mice [53].

EXTERNAL INSULTS (DRUG-INDUCED PATHOLOGIES – TUNICAMYCIN, ACETAMINOPHEN, CARBON TETRACHLORIDE, CISPLATIN, FLUOROQUINOLONES)

Several drug-induced pathologies are caused, at least in part, by depletion of endogenous glutathione, particularly in liver. The resulting damage caused by unchecked endogenous production of ROS leads to oxidative stress, causing overt drug-induced pathologies that may be lessened by prophylactic administration of mitochondrial ROS suppressors.

(i) TUNICAMYCIN AND ENDOPLASMIC RETICULUM STRESS

Tunicamycin inhibits the *N*-linked oligosaccharide formation of glycoproteins in the endoplasmic reticulum and triggers the protective unfolded protein response. High tunicamycin dosage may saturate the unfolded protein response, leading to hepatic steatosis [121, 122]. We speculate that the resulting calcium imbalance in the endoplasmic reticulum leads to calcium uptake into the mitochondria, activating calcium-sensitive dehydrogenases of the tricarboxylic acid cycle and causing increased ROS production by the electron transport chain, triggering S1QEL- and S3QEL-sensitive downstream effects such as hepatosteatosis.

S1QEL. Administration of S1QEL1.1 and S1QEL2.2 protected against caspase 3 and 7 cleavage in embryonic cardiomyocyte H9C2 cells treated with tunicamycin, showing that ROS production by site I_Q is involved in the endoplasmic reticulum stress signalling pathway in these cells [40]. We found that S1QEL1.1 and S1QEL1.719 also protected these cells against tunicamycin-induced apoptosis. Dietary S1QEL1.1 and S1QEL2.2 protected against tunicamycin stimulation of intestinal cell proliferation *in vivo* in *Drosophila* [40]. In human hepatic cells (AML12), we have found that S1QEL1.719 ameliorates tunicamycin-induction of the PERK-eIF2a-ATF4-CHOP-FGF21 signalling pathway. In mice, we have found that intraperitoneal injection of S1QEL1.712 gives significant protection against tunicamycin-induced body weight loss and tunicamycin-induced hepatosteatosis measured by oil red O staining, and oral administration of a "next-generation" S1QEL attenuates the increase in FGF-21 caused by tunicamycin administration [unpublished].

S3QEL. S3QEL1, S3QEL2, and S3QEL3 protected against tunicamycin-induced cleavage of caspase 3 and 7 in a rat INS-1 insulinoma cell line [43] and S3QEL2.1 protected against tunicamycin-induced cleavage of caspase 3 and 7 in embryonic cardiomyocyte H9C2 cells [40]. S3QEL1.2, S3QEL2 and S3QEL3 decreased apoptosis and necrosis in tunicamycin-treated C2C12 myoblasts [123]. *In vivo*, we have found that intraperitoneal injection of S3QEL1.941 [41] decreases liver fat deposition measured using oil red O in tunicamycin-treated mice.

(ii) ACETAMINOPHEN HEPATOTOXICITY

High doses of acetaminophen lead to the formation of reactive metabolites of acetaminophen in the liver. These cause oxidative stress and hepatotoxicity by depletion of glutathione and inhibition of mitochondrial glutathione peroxidases, removing some of the protection against damage by endogenous ROS [124].

S1QEL. Leakage of the liver-specific enzyme alanine aminotransferase into the bloodstream is a marker of liver cell damage. We have found that S1QEL1.712 and S1QEL2.1 protect against alanine aminotransferase leakage from acetaminophen-treated HepG2 cells, and that prophylactic intraperitoneal injection of S1QEL1.712 and oral administration of low doses of S1QEL1.719 and many unpublished S1QEL2s and "next-generation" S1QELs strongly protect against acetaminophen-induced leakage of alanine aminotransferase activity into the plasma of mice *in vivo*. They also protect against elevated plasma levels of the mitochondrial stress-related cytokine GDF15. These observations raise the possibility that prophylactic or simultaneous application of S1QELs may be effective against acute acetaminophen toxicity in humans.

S3QEL. *In vivo*, we have found that intraperitoneal injection of S3QEL1.941 [41] decreased plasma alanine aminotransferase activity in acetaminophen-treated mice.

Anethole dithiolethione. Administration of dithiolethiones to mice protected against the acute toxic effects of acetaminophen [125-127].

(iii) CARBON TETRACHLORIDE HEPATOTOXICITY

The mechanism of carbon tetrachloride hepatotoxicity is complex, although antioxidants can be protective [128].

Anethole dithiolethione. Administration of dithiolethiones and their derivatives to mice protected against the acute toxic effects of carbon tetrachloride [125, 129, 130].

(iv) CISPLATIN NEPHROTOXICITY

Cisplatin is a widely-used and effective anti-cancer agent, but its use is limited by kidney damage. Accumulation of cisplatin in renal tubular cells causes DNA damage, mitochondrial pathology, oxidative stress and endoplasmic reticulum stress [131]. Lowering oxidative stress by suppressing mitochondrial ROS formation may be protective against kidney injury.

S1QEL. We have found that that prophylactic oral administration of S1QEL1.719 and "next-generation" S1QELs potently protect mice against cisplatin renal toxicity as measured by blood urea nitrogen (BUN) and plasma levels of creatine and the specific kidney damage marker KIM1.

(v) FLUOROQUINOLONE TENOTOXICITY

Fluoroquinolone antibiotics inhibit bacterial DNA replication and are the most intensively applied antibiotics in both human and veterinary medicine. However, they can cause side effects such as tendon damage, thought to be mainly related to ROS and oxidative stress [132].

Anethole dithiolethione. Anethole dithiolethione administration was found to decrease the level of ROS induced by fluoroquinolone antibiotics in tenocytes [133].

GENETIC DISEASES

(i) POLG MOUSE

Mitochondrial DNA mutations underlie several genetic diseases. One model to study them is the POLG mutator mouse, which carries a proof-reading-deficient version of mtDNA polymerase and has a shortened lifespan and premature onset of ageing-related phenotypes [134]. Although it is controversial, some authors argue that ROS and oxidative stress contribute to the phenotype [135].

S1QEL. We have found that 30 weeks of dietary S1QEL1.719 improves latency to fall on the rotarod in POLG mutator mice at 42 weeks of age.

OTHER

(i) EXOCRINOPATHY (HYPOSALIVATION, DRY MOUTH, XEROSTOMIA, SJÖGREN SYNDROME, DRY EYE, XEROPHTHALMIA)

Anethole dithiolethione. The historical clinical application of anethole dithiolethione is in the treatment of hyposalivation. Anethole dithiolethione increased salivary secretion from the rat submaxillary gland induced by electrical stimulation of the parasympathetic nerve and by injection of pilocarpine [136] and may enhance salivary secretion by stimulating the postjunctional secretory process involved in the parasympathetic nervous system [65, 137]. In patients with autoimmune exocrinopathy (Sjogren's syndrome) anethole dithiolethione alleviated the symptoms of xerostomia [138]; several other authors have found it to have beneficial effects in treating xerostomia [67, 139, 140] and xerophthalmia [138, 141].

CONTRAINDICATIONS

Although the superoxide and hydrogen peroxide generated by mitochondria cause cellular damage and drive pathologies [7], they are also used as signals to drive appropriate physiological responses [142, 143].

Suppressors of mitochondrial ROS production will not only ameliorate damage and disease, but also decrease ROS signalling. However, much of this signalling promotes appropriate cellular responses to the initial cellular insults that would be caused by ROS production, particularly activation of signalling through protein kinases such as mitogen-activated protein kinase (MAPK) and phosphoinositide 3-kinase (PI3K)/protein kinase B (Akt), and through transcription factors including nuclear factor erythroid 2-related factor 2 (Nrf2), hypoxia-inducible factor 1α (HIF-1α), activator protein 1 (AP-1), and nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) [143]. To the extent that these pathways are activated only to counter the potentially harmful effects of mitochondrial ROS production, then their decrease by ROS-suppressors would be simply a harmless reflection of the fact that they are not needed when mitochondrial ROS production is decreased.

ROS signalling for purposes other than defense against mitochondrial ROS production is a different matter. Acute hypoxic pulmonary vasoconstriction (HPV) may be such an exception. Humans and other animals respond to low alveolar oxygen levels with acute hypoxic pulmonary vasoconstriction to divert blood flow to better-oxygenated parts of the lung, and with increased ventilation driven by chemoreceptors in the carotid bodies, which detect changes in blood oxygen levels and signal the brain to increase ventilation. Conversely, at birth oxygen rises, and the ductus arteriosus constricts to shunt blood to the lungs. Mitochondria in glomus cells of the carotid body, in small pulmonary artery smooth muscle cells, and in ductus arteriosus smooth muscle cells are accepted to be the oxygen sensors. In these cells, changes in oxygen tension rapidly alter production of mitochondrial ROS, which in turn regulate the opening of redox-sensitive potassium channels, altering plasma membrane potential, calcium influx through voltage-gated calcium channels, and smooth muscle contraction [144]. A number of papers argue that these responses are driven by ROS produced from mitochondrial sites I_Q or III_{Qo}, although different groups favor different sites [144-148]. Use of different inhibitors of the electron transport chain, including diphenyleneiodonium (which also has S1QEL activity [35]), and antioxidants in model systems (buffer-perfused rat lungs, pulmonary artery myocytes) suggested that ROS generated in the proximal region of the electron transport chain (complex I) act as second messengers in HPV [145]. Mitochondrial complex III was required for hypoxia-induced ROS production and cellular oxygen sensing [149], and this requirement in cells was narrowed down to ROS production by site III_{Qo} [147]. This conclusion was supported by the effects of genetic knockout of the activity of the Rieske iron-sulphur protein of complex III in pulmonary artery myocytes, and *ex vivo* [146] whereas the involvement of site I_Q was supported by the effects on HPV of knockdown of the complex I subunit NDUFS2 in carotid body cells and *in vivo* [144, 150, 151]. The use of inhibitors of electron transport and selective knockdown of electron transport chain subunits in these studies is very problematic, as these manipulations alter not only ROS production, but also ATP production and the redox states of the mitochondria and cytosol independently of ROS [7], so the use of selective suppressors of ROS production (S1QELs and S3QELs) that do not change electron transport is preferred.

A S1QEL was found to reverse oxygen-induced constriction in rabbit ductus arteriosus rings, and in human ductus arteriosus smooth muscle cells it inhibited oxygen-induced increases in cytosolic calcium, a surrogate for ductus arteriosus constriction [144, 152]. Therefore, the use of S1QELs immediately before and at birth deserves attention as a potential contraindication. However, we have found that oral administration of S1QEL1.719 has no significant effect on either baseline ventilation or the acute hypoxic ventilatory response before or after 48 h acclimatization to hypoxia in awake, unrestrained mice using individual whole-body plethysmographs, providing no support for a S1QEL contraindication in HPV in adults.

S3QEL2 was found to attenuate hypoxia-induced plasma membrane depolarization and HPV in human pulmonary arterial smooth muscle cells [153], and a S3QEL inhibited HPV in isolated mouse lungs [154]. In contrast, a S3QEL did not inhibit oxygen-induced ductus arteriosus constriction *ex vivo* and *in vivo* [152].

CONCLUSION

Suppressors of mitochondrial ROS production show promise in treating a wide range of diseases driven by mitochondrial oxidative stress. Their mechanism-based specificity offers great advantages over traditional antioxidants, with potential applications in metabolic, cardiovascular, inflammatory, neurological, and aging-related diseases. Further research is needed to fully explore their clinical efficacy and any potential contraindications.

Funding
This work was supported by ongoing institutional funding. No additional grants to carry out or direct this particular review were obtained.

Ethics approval and consent to participate
This work does not contain any studies involving human and animal subjects.

Conflict of interest

The author of this work declares that he has no conflicts of interest.

REFERENCES

1. Boveris, A., and Cadenas, E. (1975) Mitochondrial production of superoxide anions and its relationship to the antimycin insensitive respiration, *FEBS Lett.*, **54**, 311-314, https://doi.org/10.1016/0014-5793(75)80928-8.
2. Cadenas, E., and Davies, K. J. (2000) Mitochondrial free radical generation, oxidative stress, and aging, *Free Radic. Biol. Med.*, **29**, 222-230, https://doi.org/10.1016/S0891-5849(00)00317-8.
3. St-Pierre, J., Buckingham, J. A., Roebuck, S. J., and Brand, M. D. (2002) Topology of superoxide production from different sites in the mitochondrial electron transport chain, *J. Biol. Chem.*, **277**, 44784-44790, https://doi.org/10.1074/jbc.M207217200.
4. Brand, M. D., Affourtit, C., Esteves, T. C., Green, K., Lambert, A. J., Miwa, S., Pakay, J. L., and Parker, N. (2004) Mitochondrial superoxide: production, biological effects, and activation of uncoupling proteins, *Free Radic. Biol. Med.*, **37**, 755-767, https://doi.org/10.1016/j.freeradbiomed.2004.05.034.
5. Brand, M. D. (2010) The sites and topology of mitochondrial superoxide production, *Exp. Gerontol.*, **45**, 466-472, https://doi.org/10.1016/j.exger.2010.01.003.
6. Brand, M. D. (2016) Mitochondrial generation of superoxide and hydrogen peroxide as the source of mitochondrial redox signaling, *Free Radic. Biol. Med.*, **100**, 14-31, https://doi.org/10.1016/j.freeradbiomed.2016.04.001.
7. Brand, M. D. (2020) Riding the tiger – physiological and pathological effects of superoxide and hydrogen peroxide generated in the mitochondrial matrix, *Crit. Rev. Biochem. Mol. Biol.*, **55**, 592-661, https://doi.org/10.1080/10409238.2020.1828258.
8. Lambert, A. J., and Brand, M. D. (2004) Inhibitors of the quinone-binding site allow rapid superoxide production from mitochondrial NADH:ubiquinone oxidoreductase (complex I), *J. Biol. Chem.*, **279**, 39414-39420, https://doi.org/10.1074/jbc.M406576200.
9. Lambert, A. J., and Brand, M. D. (2004) Superoxide production by NADH:ubiquinone oxidoreductase (complex I) depends on the pH gradient across the mitochondrial inner membrane, *Biochem. J.*, **382**, 511-517, https://doi.org/10.1042/BJ20040485.
10. Lambert, A. J., and Brand, M. D. (2009) Reactive oxygen species production by mitochondria, *Meth. Mol. Biol.*, **554**, 165-181, https://doi.org/10.1007/978-1-59745-521-3_11.
11. Starkov, A. A., Fiskum, G., Chinopoulos, C., Lorenzo, B. J., Browne, S. E., Patel, M. S., and Beal, M. F. (2004) Mitochondrial alpha-ketoglutarate dehydrogenase complex generates reactive oxygen species, *J. Neurosci.*, **24**, 7779-7788, https://doi.org/10.1523/JNEUROSCI.1899-04.2004.
12. Tretter, L., and Adam-Vizi, V. (2004) Generation of reactive oxygen species in the reaction catalyzed by alpha-ketoglutarate dehydrogenase, *J. Neurosci.*, **24**, 7771-7778, https://doi.org/10.1523/jneurosci.1842-04.2004.
13. Kowaltowski, A. J., de Souza-Pinto, N. C., Castilho, R. F., and Vercesi, A. E. (2009) Mitochondria and reactive oxygen species, *Free Radic. Biol. Med.*, **47**, 333-343, https://doi.org/10.1016/j.freeradbiomed.2009.05.004.
14. Murphy, M. P. (2009) How mitochondria produce reactive oxygen species, *Biochem. J.*, **417**, 1-13, https://doi.org/10.1042/BJ20081386.
15. Starkov, A. A. (2013) An update on the role of mitochondrial α-ketoglutarate dehydrogenase in oxidative stress, *Mol. Cell. Neurosci.*, **55**, 13-16, https://doi.org/10.1016/j.mcn.2012.07.005.
16. Andreyev, A. Y., Kushnareva, Y. E., Murphy, A. N., and Starkov, A. A. (2015) Mitochondrial ROS metabolism: 10 years later, *Biochemistry (Moscow)*, **80**, 517-531, https://doi.org/10.1134/S0006297915050028.
17. Wong, H. S., Dighe, P. A., Mezera, V., Monternier, P. A., and Brand, M. D. (2017) Production of superoxide and hydrogen peroxide from specific mitochondrial sites under different bioenergetic conditions, *J. Biol. Chem.*, **292**, 16804-16809, https://doi.org/10.1074/jbc.R117.789271.
18. Quinlan, C. L., Treberg, J. R., Perevoshchikova, I. V., Orr, A. L., and Brand, M. D. (2012) Native rates of superoxide production from multiple sites in isolated mitochondria measured using endogenous reporters, *Free Radic. Biol. Med.*, **53**, 1807-1817, https://doi.org/10.1016/j.freeradbiomed.2012.08.015.
19. Quinlan, C. L., Perevoshchikova, I. V., Hey-Mogensen, M., Orr, A. L., and Brand, M. D. (2013) Sites of reactive oxygen species generation by mitochondria oxidizing different substrates, *Redox Biol.*, **1**, 304-312, https://doi.org/10.1016/j.redox.2013.04.005.
20. Quinlan, C. L., Perevoschikova, I. V., Goncalves, R. L., Hey-Mogensen, M., and Brand, M. D. (2013) The determination and analysis of site-specific rates of mitochondrial reactive oxygen species production, *Meth. Enzymol.*, **526**, 189-217, https://doi.org/10.1016/B978-0-12-405883-5.00012-0.
21. Quinlan, C. L., Goncalves, R. L., Hey-Mogensen, M., Yadava, N., Bunik, V. I., and Brand, M. D. (2014) The 2-oxoacid dehydrogenase complexes in mitochondria can produce superoxide/hydrogen peroxide at much higher rates than complex I, *J. Biol. Chem.*, **289**, 8312-8325, https://doi.org/10.1074/jbc.M113.545301.
22. Quinlan, C. L., Gerencser, A. A., Treberg, J. R., and Brand, M. D. (2011) The mechanism of superoxide production by the antimycin-inhibited mitochondrial Q-cycle, *J. Biol. Chem.*, **286**, 31361-31372, https://doi.org/10.1074/jbc.M111.267898.

23. Quinlan, C. L., Orr, A. L., Perevoshchikova, I. V., Treberg, J. R., Ackrell, B. A., and Brand, M. D. (2012) Mitochondrial complex II can generate reactive oxygen species at high rates in both the forward and reverse reactions, *J. Biol. Chem.*, **287**, 27255-27264, https://doi.org/10.1074/jbc.M112.374629.
24. Treberg, J. R., Quinlan, C. L., and Brand, M. D. (2011) Evidence for two sites of superoxide production by mitochondrial NADH-ubiquinone oxidoreductase (complex I), *J. Biol. Chem.*, **286**, 27103-27110, https://doi.org/10.1074/jbc.M111.252502.
25. Perevoshchikova, I. V., Quinlan, C. L., Orr, A. L., Gerencser, A. A., and Brand, M. D. (2013) Sites of superoxide and hydrogen peroxide production during fatty acid oxidation in rat skeletal muscle mitochondria, *Free Radic. Biol. Med.*, **61**, 298-309, https://doi.org/10.1016/j.freeradbiomed.2013.04.006.
26. Goncalves, R. L., Rothschild, D. E., Quinlan, C. L., Scott, G. K., Benz, C. C., and Brand, M. D. (2014) Sources of superoxide/H_2O_2 during mitochondrial proline oxidation, *Redox Biol.*, **2**, 901-909, https://doi.org/10.1016/j.redox.2014.07.003.
27. Goncalves, R. L. S., Bunik, V. I., and Brand, M. D. (2016) Production of superoxide/hydrogen peroxide by the mitochondrial 2-oxoadipate dehydrogenase complex, *Free Rad. Biol. Med.*, **91**, 247-255, https://doi.org/10.1016/j.freeradbiomed.2015.12.020.
28. Hey-Mogensen, M., Goncalves, R. L., Orr, A. L., and Brand, M. D. (2014) Production of superoxide/H_2O_2 by dihydroorotate dehydrogenase in rat skeletal muscle mitochondria, *Free Rad. Biol. Med.*, **72**, 149-155, https://doi.org/10.1016/j.freeradbiomed.2014.04.007.
29. Goncalves, R. L., Quinlan, C. L., Perevoshchikova, I. V., Hey-Mogensen, M., and Brand, M. D. (2015) Sites of superoxide and hydrogen peroxide production by muscle mitochondria assessed *ex vivo* under conditions mimicking rest and exercise, *J. Biol. Chem.*, **290**, 209-227, https://doi.org/10.1074/jbc.M114.619072.
30. Goncalves, R. L. S., Watson, M. A., Wong, H. S., Orr, A. L., and Brand, M. D. (2020) The use of site-specific suppressors to measure the relative contributions of different mitochondrial sites to skeletal muscle superoxide and hydrogen peroxide production, *Redox Biol.*, **28**, 101341, https://doi.org/10.1016/j.redox.2019.101341.
31. Wong, H. S., Benoit, B., and Brand, M. D. (2019) Mitochondrial and cytosolic sources of hydrogen peroxide in resting C2C12 myoblasts, *Free Radic. Biol. Med.*, **130**, 140-150, https://doi.org/10.1016/j.freeradbiomed.2018.10.448.
32. Fang, J., Wong, H. S., and Brand, M. D. (2020) Production of superoxide and hydrogen peroxide in the mitochondrial matrix is dominated by site I_Q of complex I in diverse cell lines, *Redox Biol.*, **37**, 101722, https://doi.org/10.1016/j.redox.2020.101722.
33. Fang, J., Zhang, Y., Gerencser, A. A., and Brand, M. D. (2022) Effects of sugars, fatty acids and amino acids on cytosolic and mitochondrial hydrogen peroxide release from liver cells, *Free Rad. Biol. Med.*, **188**, 92-102, https://doi.org/10.1016/j.freeradbiomed.2022.06.225.
34. Sen, B., Benoit, B., and Brand, M. D. (2024) Hypoxia decreases mitochondrial ROS production in cells, *Free Radic. Biol. Med.*, **224**, 1-8, https://doi.org/10.1016/j.freeradbiomed.2024.08.016.
35. Lambert, A. J., Buckingham, J. A., Boysen, H. M., and Brand, M. D. (2008) Diphenyleneiodonium acutely inhibits reactive oxygen species production by mitochondrial complex I during reverse, but not forward electron transport, *Biochim. Biophys. Acta*, **1777**, 397-403, https://doi.org/10.1016/j.bbabio.2008.03.005.
36. Wong, H. S., Monternier, P. A., and Brand, M. D. (2019) S1QELs suppress mitochondrial superoxide/hydrogen peroxide production from site I_Q without inhibiting reverse electron flow through complex I, *Free Radic. Biol. Med.*, **143**, 545-559, https://doi.org/10.1016/j.freeradbiomed.2019.09.006.
37. Gibbs, E. T., Lerner, C. A., Watson, M. A., Wong, H. S., Gerencser, A. A., and Brand, M. D. (2023) Site I_Q in mitochondrial complex I generates S1QEL-sensitive superoxide/hydrogen peroxide in both the reverse and forward reactions, *Biochem. J.*, **480**, 363-384, https://doi.org/10.1042/BCJ20220611.
38. Robb, E. L., Hall, A. R., Prime, T. A., Eaton, S., Szibor, M., Viscomi, C., James, A. M., and Murphy, M. P. (2018) Control of mitochondrial superoxide production by reverse electron transport at complex I, *J. Biol. Chem.*, **293**, 9869-9879, https://doi.org/10.1074/jbc.RA118.003647.
39. Orr, A. L., Ashok, D., Sarantos, M. R., Shi, T., Hughes, R. E., and Brand, M. D. (2013) Inhibitors of ROS production by the ubiquinone-binding site of mitochondrial complex I identified by chemical screening, *Free Radic. Biol. Med.*, **65**, 1047-1059, https://doi.org/10.1016/j.freeradbiomed.2013.08.170.
40. Brand, M. D., Goncalves, R. L., Orr, A. L., Vargas, L., Gerencser, A. A., Borch Jensen, M., Wang, Y. T., Melov, S., Turk, C. N., Matzen, J. T., Dardov, V. J., Petrassi, H. M., Meeusen, S. L., Perevoshchikova, I. V., Jasper, H., Brookes, P. S., and Ainscow, E. K. (2016) Suppressors of superoxide-H_2O_2 production at site I_Q of mitochondrial Complex I protect against stem cell hyperplasia and ischemia-reperfusion injury, *Cell Metab.*, **24**, 582-592, https://doi.org/10.1016/j.cmet.2016.08.012.
41. Wong, H. S., Mezera, V., Dighe, P., Melov, S., Gerencser, A. A., Sweis, R. F., Pliushchev, M., Wang, Z., Esbenshade, T., McKibben, B., Riedmaier, S., and Brand, M. D. (2021) Superoxide produced by mitochondrial site I_Q inactivates cardiac succinate dehydrogenase and induces hepatic steatosis in Sod2 knockout mice, *Free Radic. Biol. Med.*, **164**, 223-232, https://doi.org/10.1016/j.freeradbiomed.2020.12.447.

42. Watson, M. A., Brar, H., Gibbs, E. T., 2nd, Wong, H. S., Dighe, P. A., McKibben, B., Riedmaier, S., Siu, A., Polakowski, J. S., Segreti, J. A., Liu, X., Chung, S., Pliushchev, Y. M., Gesmundo, N., Wang, Z., Vortherms, T. A., and Brand, M. D. (2023) Suppression of superoxide/hydrogen peroxide production at mitochondrial site I_Q decreases fat accumulation, improves glucose tolerance and normalizes fasting insulin concentration in mice fed a high-fat diet, *Free Radic. Biol. Med.*, **204**, 276-286, https://doi.org/10.1016/j.freeradbiomed.2023.05.022.
43. Orr, A. L., Vargas, L., Turk, C. N., Baaten, J. E., Matzen, J. T., Dardov, V. J., Attle, S. J., Li, J., Quackenbush, D. C., Goncalves, R. L. S., Perevoshchikova, I. V., Petrassi, H. M., Meeusen, S. L., Ainscow, E. K., and Brand, M. D. (2015) Suppressors of superoxide production from mitochondrial complex III, *Nature Chem. Biol.*, **11**, 834-836, https://doi.org/10.1038/nchembio.1910.
44. Fouqueray, P., Leverve, X., Fontaine, E., Baquié, M., Wollheim, C., Lebovitz, H., and Bozec, S. (2011) Imeglimin - a new oral anti-diabetic that targets the three key defects of type 2 diabetes, *J. Diabetes Metab.*, **2**, 126-133, https://doi.org/10.4172/2155-6156.1000126.
45. Yendapally, R., Sikazwe, D., Kim, S. S., Ramsinghani, S., Fraser-Spears, R., Witte, A. P., and La-Viola, B. (2020) A review of phenformin, metformin, and imeglimin, *Drug Dev. Res.*, **2020**, 1-12, https://doi.org/10.1002/ddr.21636.
46. Helzlouer, K. J., and Kensler, T. W. (1993) Cancer chemoprotection by oltipraz: experimental and clinical considerations, *Preventive Med.*, **22**, 783-795, https://doi.org/10.1006/pmed.1993.1072.
47. Gero, D., Torregrossa, R., Perry, A., Waters, A., Le-Trionnaire, S., Whatmore, J. L., Wood, M., and Whiteman, M. (2016) The novel mitochondria-targeted hydrogen sulfide (H_2S) donors AP123 and AP39 protect against hyperglycemic injury in microvascular endothelial cells *in vitro*, *Pharmacol. Res.*, **113**, 186-198, https://doi.org/10.1016/j.phrs.2016.08.019.
48. Huang, S., Dong, R., Xu, G., Liu, J., Gao, X., Yu, S., Qie, P., Gou, G., Hu, M., Wang, Y., Peng, J., Guang, B., Xu, Y., and Yang, T. (2020) Synthesis, characterization, and in vivo evaluation of desmethyl anethole trithione phosphate prodrug for ameliorating cerebral ischemia-reperfusion injury in rats, *ACS Omega*, **5**, 4595-4602, https://doi.org/10.1021/acsomega.9b04129.
49. Li, Y., and Trush, M. A. (1998) Diphenyleneiodonium, an NAD(P)H oxidase inhibitor, also potently inhibits mitochondrial reactive oxygen species production, *Biochem. Biophys. Res. Commun.*, **253**, 295-299, https://doi.org/10.1006/bbrc.1998.9729.
50. Wong, H. S., Monternier, P. A., Orr, A. L., and Brand, M. D. (2018) Plate-based measurement of superoxide and hydrogen peroxide production by isolated mitochondria, *Meth. Mol. Biol.*, **1782**, 287-299, https://doi.org/10.1007/978-1-4939-7831-1_16.
51. Orr, A. L., Ashok, D., Sarantos, M. R., Ng, R., Shi, T., Gerencser, A. A., Hughes, R. E., and Brand, M. D. (2014) Novel inhibitors of mitochondrial sn-glycerol 3-phosphate dehydrogenase, *PLoS One*, **9**, e89938, https://doi.org/10.1371/journal.pone.0089938.
52. Zotta, A., Toller-Kawahisa, J., Palsson-McDermott, E. M., O'Carroll, S. M., Henry, Ó. C., Day, E. A., McGettrick, A. F., Ward, R. W., Ryan, D. G., Watson, M. A., Brand, M. D., Runtsch, M. C., Maitz, K., Lueger, A., Kargl, J., Miljkovic, J. L., Lavelle, E. C., and O'Neill, L. A. J. (2025) Mitochondrial respiratory complex III sustains IL-10 production in activated macrophages and promotes tumor-mediated immune evasion, *Sci. Adv.*, **11**, 7307, https://doi.org/10.1126/sciadv.adq7307.
53. Watson, M. A., Pattavina, B., Hilsabeck, T. A. U., Lopez-Dominguez, J., Kapahi, P., and Brand, M. D. (2021) S3QELs protect against diet-induced intestinal barrier dysfunction, *Aging Cell*, **20**, e13476, https://doi.org/10.1111/acel.13476.
54. Barnett, D., Zimmer, T. S., Booraem, C., Palaguachi, F., Meadows, S. M., Xiao, H., Chouchani, E. T., Orr, A. G., and Orr, A. L. (2024) Mitochondrial complex III-derived ROS amplify immunometabolic changes in astrocytes and promote dementia pathology, *bioRxiv*, https://doi.org/10.1101/2024.08.19.608708.
55. Vial, G., Chauvin, M. A., Bendridi, N., Durand, A., Meugnier, E., Madec, A. M., Bernoud-Hubac, N., Pais de Barros, J. P., Fontaine, E., Acquaviva, C., Hallakou-Bozec, S., Bolze, S., Vidal, H., and Rieusset, J. (2015) Imeglimin normalizes glucose tolerance and insulin sensitivity and improves mitochondrial function in liver of a high-fat, high-sucrose diet mice model, *Diabetes*, **64**, 2254-2264, https://doi.org/10.2337/db14-1220.
56. Detaille, D., Vial, G., Borel, A. L., Cottet-Rouselle, C., Hallakou-Bozec, S., Bolze, S., Fouqueray, P., and Fontaine, E. (2016) Imeglimin prevents human endothelial cell death by inhibiting mitochondrial permeability transition without inhibiting mitochondrial respiration, *Cell Death Discov.*, **2**, 15072, https://doi.org/10.1038/cddiscovery.2015.72.
57. Detaille, D., Pasdois, P., Semont, A., Dos Santos, P., and Diolez, P. (2019) An old medicine as a new drug to prevent mitochondrial complex I from producing oxygen radicals, *PLoS One*, **14**, e0216385, https://doi.org/10.1371/journal.pone.0216385.
58. Nagendra, L., Bhattacharya, S., Bhat, S., Dutta, D., Kamrul-Hasan, A. B., and Kalra, S. (2024) Comparative analysis of metformin and imeglimin: exploring therapeutic implications, *Bangladesh J. Endocrinol. Metab.*, **3**, 3-8, https://doi.org/10.4103/bjem.bjem_2_24.
59. Tewari, J., Qidwai, K. A., Tewari, A., Kaur, S., Tewari, V., and Maheshwari, A. (2025) Efficacy and safety of imeglimin, a novel oral agent in the management of type 2 diabetes mellitus: a systematic review and meta-analysis, *Naunyn-Schmiedeberg's Arch. Phar-*

macol., **398**, 14501-14513, https://doi.org/10.1007/s00210-025-04198-5.
60. Lamb, Y. N. (2021) Imeglimin hydrochloride: first approval, *Drugs*, **81**, 1683-1690, https://doi.org/10.1007/s40265-021-01589-9.
61. Li, Y., Lou, N., Liu, X., Zhuang, X., and Chen, S. (2024) Exploring new mechanisms of imeglimin in diabetes treatment: Amelioration of mitochondrial dysfunction, *Biomed. Pharmacother.*, **175**, 116755, https://doi.org/10.1016/j.biopha.2024.116755.
62. Shaikh, S., Sharma, S. K., Phatak, S., Asirvatham, A., Battacharyya, S., Dhandhania, V. K., Muchhala, S., and Baxi, N. (2025) A multicenter, retrospective study to evaluate the effectiveness and safety of imeglimin in patients with type 2 diabetes mellitus in a real-world clinical setting (INDI-TIMES study), *Diabetes Ther.*, **16**, 645-661, https://doi.org/10.1007/s13300-025-01693-z.
63. Yaribeygi, H., Maleki, M., Sathyapalan, T., Jamialahmadi, T., and Sahebkar, A. (2020) Molecular mechanisms by which imeglimin improves glucose homeostasis, *J. Diabet. Res.*, **2020**, 8768954, https://doi.org/10.1155/2020/8768954.
64. Hallakou-Bozec, S., Vial, G., Kergoat, M., Fouqueray, P., Bolze, S., Borel, A.-L., Fontaine, E., and Moller, D. E. (2021) Mechanism of action of imeglimin: a novel therapeutic agent for type 2 diabetes, *Diabetes Obes. Metab.*, **23**, 664-673, https://doi.org/10.1111/dom.14277.
65. Ukai, Y., Taniguchi, N., Takeshita, K., Ogasawara, T., and Kimura, K. (1988) Enhancement of salivary secretion by chronic anethole trithione treatment, *Arch. Int. Pharmacodyn. Ther.*, **294**, 248-258.
66. Christen, M. O. (1995) Anethole dithiolethione: biochemical considerations, *Meth. Enzymol.*, **252**, 316-323, https://doi.org/10.1016/0076-6879(95)52034-1.
67. Nagano, T., and Takeyama, M. (2001) Enhancement of salivary secretion and neuropeptide (substance P, alpha-calcitonin gene-related peptide) levels in saliva by chronic anethole trithione treatment, *J. Pharm. Pharmacol.*, **53**, 1697-1702, https://doi.org/10.1211/0022357011778098.
68. Zhang, Y., and Munday, R. (2008) Dithiolethiones for cancer chemoprevention: where do we stand? *Mol. Cancer Ther.*, 7, 3470-3479, https://doi.org/10.1158/1535-7163.MCT-08-0625.
69. Brooks, S. C., III, Brooks, J. S., Lee, W. H., Lee, M. G., and Kim, S. G. (2009) Therapeutic potential of dithiolethiones for hepatic diseases, *Pharmacol. Ther.*, **124**, 31-43, https://doi.org/10.1016/j.pharmthera.2009.06.006.
70. Dulac, M., Sassi, A., Nagarathinan, C., Christen, M.-C., Dansette, P. M., Mansuy, D., and Boucher, J.-L. (2018) Metabolism of anethole dithiolethione by rat and human liver microsomes: formation of various products deriving from its O-demethylation and S-oxidation. Involvement of cytochromes P450 and flavin monooxygenases in these pathways, *Drug Metab. Dispos.*, **46**, 1390-1395, https://doi.org/10.1124/dmd.118.082545.
71. Boucard, A., Marin, F., Monternier, P. A., Brand, M., and Le Grand, B. (2019) A specific complex I-induced ROS modulator, OP2113, is a new cardioprotective agent against acute myocardial infarction injuries during reperfusion, *Eur. Heart J.*, **40**, 2818, https://doi.org/10.1093/eurheartj/ehz745.0990.
72. Wallace, J. L., Vaughan, D., Dicay, M., MacNaughton, W. K., and de Nucci, G. (2018) Hydrogen sulfide-releasing therapeutics: translation to the clinic, *Antiox. Redox Signal.*, **28**, 1533-1540, https://doi.org/10.1089/ars.2017.7068.
73. Murphy, M. P., and Hartley, R. C. (2018) Mitochondria as a therapeutic target for common pathologies, *Nat. Rev. Drug Discov.*, **17**, 865-886, https://doi.org/10.1038/nrd.2018.174.
74. Skulachev, V. P., Vyssokikh, M. Y., Chernyak, B. V., Mulkidjanian, A. Y., Skulachev, M. V., Shilovsky, G. A., Lyamzaev, K. G., Borisov, V. B., Severin, F. F., and Sadovnichii, V. A. (2023) Six functions of respiration: isn't it time to take control over ROS production in mitochondria, and aging along with it? *Int. J. Mol. Sci.*, **24**, 12540, https://doi.org/10.3390/ijms241612540.
75. Melov, S., Coskun, P., Patel, M., Tuinstra, R., Cottrell, B., Jun, A. S., Zastawny, T. H., Dizdaroglu, M., Goodman, S. I., Huang, T. T., Miziorko, H., Epstein, C. J., and Wallace, D. C. (1999) Mitochondrial disease in superoxide dismutase 2 mutant mice, *Proc. Nat. Acad. Sci. USA*, **96**, 846-851, https://doi.org/10.1073/pnas.96.3.846.
76. Williams, L. M., Campbell, F. M., Drew, J. E., Koch, C., Hoggard, N., Rees, W. D., Kamolrat, T., Ngo, H. T., Steffensen, I. L., Gray, S. R., and Tups, A. (2014) The development of diet-induced obesity and glucose intolerance in C57Bl/6 mice on a high-fat diet consists of distinct phases, *PLoS One*, **9**, e106159, https://doi.org/10.1371/journal.pone.0106159.
77. Keipert, S., and Ost, M. (2021) Stress-induced FGF21 and GDF15 in obesity and obesity resistance, *Trends Endocrinol. Metab.*, **21**, 904-915, https://doi.org/10.1016/j.tem.2021.08.008.
78. Tsukamoto, H., Matsuoka, M., and French, S. W. (1990) Experimental models of hepatic fibrosis: a review, *Semin. Liver Dis.*, **10**, 56-65, https://doi.org/10.1055/s-2008-1040457.
79. Santhekadur, P. K., Kumar, D. P., and Sanyal, A. J. (2018) Preclinical models of non-alcoholic fatty liver disease, *J. Hepatol.*, **68**, 230-237, https://doi.org/10.1016/j.jhep.2017.10.031.
80. Goncalves, R. L. S., Wang, Z. B., Riveros, J. K., Parlakgül, G., Inouye, K. E., Lee, G. Y., Fu, X., Saksi, J., Rosique, C., Hui, S. T., Coll, M., Arruda, A. P., Burgess, S. C., Graupera, I., and Hotamışlıgil, G. S. (2025) CoQ imbalance drives reverse electron transport to disrupt liver metabolism, *Nature*, **643**, 1057-1065, https://doi.org/10.1038/s41586-025-09072-1.
81. Plecita-Hlavata, L., Engstova, H., Jezek, J., Holendova, B., Tauber, J., Petraskova, L., Kren, V., and Jezek, P. (2019)

Potential of mitochondria-targeted antioxidants to prevent oxidative stress in pancreatic beta-cells, *Oxid. Med. Cell Longev.*, **2019**, 1826303, https://doi.org/10.1155/2019/1826303.

82. Perry, R. J., Cardone, R. L., Petersen, M. C., Zhang, D., Fouqueray, P., Hallakou-Bozec, S., Bolze, S., Shulman, G. I., Petersen, K. F., and Kibbey, R. G. (2016) Imeglimin lowers glucose primarily by amplifying glucose-stimulated insulin secretion in high-fat-fed rodents, *Am. J. Physiol. Endocrinol. Metab.*, **311**, E461-E470, https://doi.org/10.1152/ajpendo.00009.2016.
83. Kaji, K., Takeda, S., Iwai, S., Nishimura, N., Sato, S., Namisaki, T., Akahane, T., and Yoshiji, H. (2024) Imeglimin halts liver damage by improving mitochondrial dysfunction in a nondiabetic male mouse model of metabolic dysfunction-associated steatohepatitis, *Antioxidants (Basel)*, **13**, 1415, https://doi.org/10.3390/antiox13111415.
84. Pacini, G., Mari, A., Fouqueray, P., Bolze, S., and Roden, M. (2015) Imeglimin increases glucose-dependent insulin secretion and improves beta-cell function in patients with type 2 diabetes, *Diabetes Obes. Metab.*, **17**, 541-545, https://doi.org/10.1111/dom.12452.
85. Fukunaga, K., Morishita, A., Imachi, H., Oura, K., Sato, S., Kobayashi, T., Saheki, T., Yoshimura, T., Komori, K., Nakahara, M., Tadokoro, T., Fujita, K., Tani, J., Kobara, H., and Murao, K. (2025) Efficacy of imeglimin in patients with type 2 diabetes mellitus complicated by metabolic dysfunction-associated steatotic liver disease: A multicentre study, *Diabetes Obes. Metab.*, **27**, 1498-1506, https://doi.org/10.1111/dom.16157.
86. Luo, Y., Sun, S., Zhang, Y., Liu, S., Zeng, H., Li, J.-E., Huang, J., Fang, L., Yang, S., Yu, P., and Liu, J. (2024) Effects of oltipraz on the glycolipid metabolism and the Nrf2/HO-1 pathway in type 2 diabetic mice, *Drug Design Dev. Ther.*, **18**, 5685-5700, https://doi.org/10.2147/DDDT.S485729.
87. Zhao, Y., Wang, Y., Xu, Q., Zhou, K., Shen, Y., Guo, L., Liu, H., Ren, Z., and Jiang, Z. (2024) Hydrogen sulfide donors across time: From origins to cutting-edge applications, *Nitric Oxide*, **144**, 29-39, https://doi.org/10.1016/j.niox.2024.01.003.
88. Oropeza-Almazán, Y., and Blatter, L. A. (2024) Role of mitochondrial ROS for calcium alternans in atrial myocytes, *Biomolecules*, **14**, 144, https://doi.org/10.3390/biom14020144.
89. Kitakata, H., Endo, J., Hashimoto, S., Mizuno, E., Moriyama, H., Shirakawa, K., Goto, S., Katsumata, Y., Fukuda, K., and Sano, M. (2021) Imeglimin prevents heart failure with preserved ejection fraction by recovering the impaired unfolded protein response in mice subjected to cardiometabolic stress, *Biochem. Biophys. Res. Commun.*, **572**, 185-190, https://doi.org/10.1016/j.bbrc.2021.07.090.
90. Roubenne, L., Boucard, A., Marthan, R., Delcambre, F., Le Grand, B., and Guibert, C. (2021) ATT has positive effects on pulmonary hypertension hallmarks, *Rev. Malad. Resp.*, **38**, 582-583, https://doi.org/10.1016/j.rmr.2021.02.030.
91. Roubenne, L., Laisné, M., Campagnac, M., Marthan, R., Le Grand, B., and Guibert, C. (2022) Beneficial effects of a preventive treatment with anethole trithione on pulmonary hypertension, *Rev. Malad. Resp.*, **39**, 117, https://doi.org/10.1016/j.rmr.2022.02.022.
92. Roubenne, L., Laisné, M., Benoist, D., Campagnac, M., Prunet, B., Pasdois, P., Cardouat, G., Ducret, T., Quignard, J.-F., Vacher, P., Baudrimont, I., Marthan, R., Berger, P., Le Grand, B., Freund-Michel, V., and Guibert, C. (2023) OP2113, a new drug for chronic hypoxia-induced pulmonary hypertension treatment in rat, *Br. J. Pharmacol.*, **180**, 2802-2821, https://doi.org/10.1111/bph.16174.
93. Hatinguais, R., Pradhan, A., Brown, G. D., Brown, A. J. P., Warris, A., and Shekhova, E. (2021) Mitochondrial reactive oxygen species regulate immune responses of macrophages to *Aspergillus fumigatus*, *Front. Immunol.*, **12**, 641495, https://doi.org/10.3389/fimmu.2021.641495.
94. Kurita, K., Ohta, H., Shirakawa, I., Tanaka, M., Kitaura, Y., Iwasaki, Y., Matsuzaka, T., Shimano, H., Aoe, S., Arima, H., Ogawa, Y., Ito, A., and Suganami, T. (2021) Macrophages rely on extracellular serine to suppress aberrant cytokine production, *Sci. Rep.*, **11**, 11137, https://doi.org/10.1038/s41598-021-90086-w.
95. Mosalmanzadeh, N., Maurmann, R. M., Davis, K., Schmitt, B. L., Makowski, L., and Pence, B. D. (2024) Modulatory effects of Mdivi-1 on OxLDL-induced metabolic alterations, inflammatory responses, and foam cell formation in human monocytes, *bioRxiv*, https://doi.org/10.1101/2024.1112.1112.628145.
96. Ahmed, D., Roy, D., Jaworski, A., Edwards, A., Abizaid, A., Kumar, A., Golshani, A., and Cassol, E. (2019) Differential remodeling of the electron transport chain is required to support TLR3 and TLR4 signaling and cytokine production in macrophages, *Sci. Rep.*, **9**, 18801, https://doi.org/10.1038/s41598-019-55295-4.
97. Soberanes, S., Misharin, A. V., Jairaman, A., Morales-Nebreda, L., McQuattie-Pimentel, A. C., Cho, T., Hamanaka, R. B., Meliton, A. Y., Reyfman, P. A., Walter, J. M., Chen, C. I., Chi, M., Chiu, S., Gonzalez--Gonzalez, F. J., Antalek, M., Abdala-Valencia, H., Chiarella, S. E., Sun, K. A., Woods, P. S., Ghio, A. J., et al. (2019) Metformin targets mitochondrial electron transport to reduce air-pollution-induced thrombosis, *Cell Metab.*, **29**, 335-347.e5, https://doi.org/10.1016/j.cmet.2018.09.019.
98. Kountz, T. S., Jairaman, A., Kountz, C. D., Stauderman, K. A., Schleimer, R. P., and Prakriya, M. (2021) Differential regulation of ATP- and UTP-evoked

prostaglandin E2 and IL-6 production from human airway epithelial cells, *J. Immunol.*, **207**, 1275-1287, https://doi.org/10.4049/jimmunol.2100127.

99. Pohl, K., Wall, S., Locy, M., and Tipple, T. (2018) Suppression of site I_Q electron leak attenuates lung epithelial cell proliferation, *Free Rad. Biol. Med.*, **128**, S36, https://doi.org/10.1016/j.freeradbiomed.2018.10.047.
100. Manford, A. G., Rodriguez-Perez, F., Shih, K. Y., Shi, Z., Berdan, C. A., Choe, M., Titov, D. V., Nomura, D. K., and Rape, M. (2020) A cellular mechanism to detect and alleviate reductive stress, *Cell*, **183**, 46-61, https://doi.org/10.1016/j.cell.2020.08.034.
101. Oberkampf, M., Guillerey, C., Mouries, J., Rosenbaum, P., Fayolle, C., Bobard, A., Savina, A., Ogier-Denis, E., Enninga, J., Amigorena, S., Leclerc, C., and Dadaglio, G. (2018) Mitochondrial reactive oxygen species regulate the induction of CD8(+) T cells by plasmacytoid dendritic cells, *Nat. Commun.*, **9**, 2241, https://doi.org/10.1038/s41467-018-04686-8.
102. Kong, H., Reczek, C. R., McElroy, G. S., Steinert, E. M., Wang, T., Sabatini, D. M., and Chandel, N. S. (2020) Metabolic determinants of cellular fitness dependent on mitochondrial reactive oxygen species, *Sci. Adv.*, **6**, eabb7272, https://doi.org/10.1126/sciadv.abb7272.
103. Morris, O., Deng, H., Tam, C., and Jasper, H. (2020) Warburg-like metabolic reprogramming in aging intestinal stem cells contributes to tissue hyperplasia, *Cell Rep.*, **33**, 108423, https://doi.org/10.1016/j.celrep.2020.108423.
104. Homma, T., Kobayashi, S., Sato, H., and Fujii, J. (2021) Superoxide produced by mitochondrial complex III plays a pivotal role in the execution of ferroptosis induced by cysteine starvation, *Arch. Biochem. Biophys.*, **700**, 108775, https://doi.org/10.1016/j.abb.2021.108775.
105. Reddy, B. S., Rao, C. V., Rivenson, A., and Kelloff, G. (1993) Chemoprevention of colon carcinogenesis by organosulfur compounds, *Cancer Res.*, **53**, 3493-3498.
106. Lubet, R. A., Steele, V. E., Eto, I., Juliana, M. M., Kelloff, G. J., and Grubbs, C. J. (1997) Chemopreventive efficacy of anethole trithione, N-acetyl-L-cysteine, miconazole and phenethylisothiocyanate in the DMBA-induced rat mammary cancer model, *Int. J. Cancer*, **72**, 95-101, https://doi.org/10.1002/(sici)1097-0215(19970703)72:1<95::aid-ijc14>3.0.co;2-9.
107. Lam, S., MacAulay, C., Le Riche, J. C., Dyachkova, Y., Coldman, A., Guillaud, M., Hawk, E., Christen, M. O., and Gazdar, A. F. (2002) A randomized phase IIb trial of anethole dithiolethione in smokers with bronchial dysplasia, *J. Natl. Cancer Inst.*, **94**, 1001-1009, https://doi.org/10.1093/jnci/94.13.1001.
108. Amoedo, N. D., Dard, L., Sarlak, S., Mahfouf, W., Blanchard, W., Rousseau, B., Izotte, J., Claverol, S., Lacombe, D., Rezvani, H. R., Pierri, C. L., and Rodrigue, R. (2020) Targeting human lung adenocarcinoma with a supressor of mitochondrial superoxide production, *Antioxid. Redox Signal.*, **33**, 883-902, https://doi.org/10.1089/ars.2019.7892.
109. Zinovkin, R. A., Lyamzaev, K. G., and Chernyak, B. V. (2023) Current perspectives of mitochondria-targeted antioxidants in cancer prevention and treatment, *Front. Cell Dev. Biol.*, **11**, 1048177, https://doi.org/10.3389/fcell.2023.1048177.
110. AlAhmad, M., Isbea, H., Shitaw, E., Li, F., and Sivaprasadarao, A. (2024) NOX2TRPM2 coupling promotes Zn^{2+} inhibition of complex III to exacerbate ROS production in a cellular model of Parkinson's disease, *Sci. Rep.*, **14**, 18431, https://doi.org/10.1038/s41598-024-66630-9.
111. Morton, K. S., George, A. J., and Meyer, J. N. (2025) Complex I superoxide anion production is necessary and sufficient for complex I inhibitor-induced dopaminergic neurodegeneration in *Caenorhabditis elegans*, *Redox Biol.*, **81**, 103538, https://doi.org/10.1016/j.redox.2025.103538.
112. Piao, L., Fang, Y., Hamanaka, R. B., Mutlu, G. M., Dezfulian, C., Archer, S. A., and Sharp, W. W. (2019) Mitochondrial Complex I induced myocardial stunning following cardiopulmonary resuscitation, *Circ. Res.*, **125**, 282, https://doi.org/10.1161/res.125.suppl_1.282.
113. Piao, L., Fang, Y. H., Hamanaka, R. B., Mutlu, G. M., Dezfulian, C., Archer, S. L., and Sharp, W. W. (2020) Suppression of superoxide-hydrogen peroxide production at site I_Q of mitochondrial Complex I attenuates myocardial stunning and improves postcardiac arrest outcomes, *Crit. Care Med.*, **48**, e133-e140, https://doi.org/10.1097/CCM.0000000000004095.
114. Milliken, A. S., Nadtochiy, S. M., and Brookes, P. S. (2022) Inhibiting succinate release worsens cardiac reperfusion injury by enhancing mitochondrial reactive oxygen species generation, *J. Am. Heart Assoc.*, **11**, 122.026135, https://doi.org/10.1161/JAHA.122.026135.
115. Sánchez-Pérez, P., Mata, A., Torp, M.-K., López-Bernardo, E., Heiestad, C. M., Aronsen, J. M., Molina-Iracheta, A., Jiménez-Borreguero, L. J., García-Roves, P., Costa, A. S. H., Frezza, C., Murphy, M. P., Stenslokken, K.-O., and Cadenas, S. (2023) Energy substrate metabolism, mitochondrial structure and oxidative stress after cardiac ischemia-reperfusion in mice lacking UCP3, *Free Radic. Biol. Med.*, **205**, 244-261, https://doi.org/10.1016/j.freeradbiomed.2023.05.014.
116. Luo, Q., Cheng, N., Yang, Y., Shao, N., Nie, T., Chen, J., Huang, C., Zhang, S., Huang, Y., Ieong, C. M., Zhang, X., Hu, K., Xiao, Z., and Luo, L. (2025) Multi-stage cooperative ROS-responsive hydrogel platform for drug delivery in myocardial ischemia-reperfusion injury repair, *Materials Today Bio*, **32**, 101854, https://doi.org/10.1016/j.mtbio.2025.101854.
117. Fukushima, C. T., Dancil, I.-S., Clary, H., Shah, N., Nadtochiy, S. M., and Brookes, P. S. (2024) Reactive oxygen species generation by reverse electron transfer

at mitochondrial complex I under simulated early reperfusion conditions, *Redox Biol.*, **70**, 103047, https://doi.org/10.1016/j.redox.2024.103047.

118. Fukushima, C. T., Dancil, I.-S., Shah, N., and Brookes, P. S. (2024) Relative importance of reactive oxygen species from reverse electron transfer at mitochondrial Complex I in reperfusion injury, *Circ. Res.*, **133**, P2121, https://doi.org/10.1161/res.133.suppl_1.P2121.
119. Dai, W., Le Grand, B., Boucard, A., Carreno, J., Zhao, I., and Kloner, R. A. (2020) Effects of OP2113 on myocardial infarct size and no reflow in a rat myocardial ischemia/reperfusion model, *Circulation*, **142**, 13780, https://doi.org/10.1161/circ.142.suppl_3.13780.
120. Dai, W., Amoedo, N. D., Perry, J., Le Grand, B., Boucard, A., Carreno, J., Zhao, L., Brown, D. A., Rossignol, R., and Kloner, R. A. (2021) Effects of OP2113 on myocardial infarct size and no reflow in a rat myocardial ischemia/reperfusion model, *Cardiovasc. Drugs Ther.*, **36**, 217-227, https://doi.org/10.1007/s10557-020-07113-7.
121. Rutkowski, D. T., Wu, J., Back, S.-H., Callaghan, M. O., Ferris, S. P., Iqbal, J., Clark, R., Miao, H., Hassler, J. R., Fornek, J., Katze, M. G., Hussain, M. M., Song, B., Swathirajan, J., Wang, J., Yau, G. D.-Y., and Kaufman, R. J. (2008) UPR pathways combine to prevent hepatic steatosis caused by ER stress-mediated suppression of transcriptional master regulators, *Dev. Cell*, **15**, 829-840, https://doi.org/10.1016/j.devcel.2008.10.015.
122. Lee, J.-S., Zheng, Z., Mendez, R., Ha, S.-W., Xie, Y., and Zhang, K. (2012) Pharmacologic ER stress induces non-alcoholic steatohepatitis in an animal model, *Toxicol. Lett.*, **211**, 29-38, https://doi.org/10.1016/j.toxlet.2012.02.017.
123. Mezera, V., Gerencser, A. A., and Brand, M. D. (2017) Tunicamycin exposure triggers apopotosis by superoxide formation from site III_{Qo} of mitochondrial complex III, *Free Radic. Biol. Med.*, **112**, 172, https://doi.org/10.1016/j.freeradbiomed.2017.10.268.
124. Du, K., Ramachandran, A., and Jaeschke, H. (2016) Oxidative stress during acetaminophen hepatotoxicity: Sources, pathophysiological role and therapeutic potential, *Redox Biol.*, **10**, 148-156, https://doi.org/10.1016/j.redox.2016.10.001.
125. Ansher, S. S., Dolan, P., and Bueding, E. (1983) Chemoprotective effects of two dithiolthiones and of butylhydroxyanisole against carbon tetrachloride and acetaminophen toxicity, *Hepatology*, **3**, 932-935, https://doi.org/10.1002/hep.1840030608.
126. Warnet, J. M., Christen, M. O., Thevenin, M., Biard, D., Jacqueson, A., and Claude, J. R. (1989) Protective effect of anethol dithiolthione against acetaminophen hepatotoxicity in mice, *Pharmacol. Toxicol.*, **65**, 63-64, https://doi.org/10.1111/j.1600-0773.1989.tb01127.x.
127. Warnet, J. M., Christen, M. O., Thevenin, M., Biard, D., Jacqueson, A., and Claude, J. R. (1989) Study of glutathione and glutathione related enzymes in acetaminophen-poisoned mice. Prevention by anethole trithione pretreatment, *Arch. Toxicol*, **13**, 322-325, https://doi.org/10.1007/978-3-642-74117-3_61.
128. Weber, L. W., Boll, M., and Stampfl, A. (2003) Hepatotoxicity and mechanism of action of haloalkanes: carbon tetrachloride as a toxicological model, *Crit. Rev. Toxicol.*, **33**, 105-136, https://doi.org/10.1080/713611034.
129. Ukai, Y., Shiraishi, M., Takeshita, K., Fukui, T., Kura, K., and Kimura, K. (1988) Pharmacological studies on anethole trithione, *Arzneimittelforschung*, **38**, 1460-1465.
130. Yang, Y., Yang, L., Han, Y., Wu, Z., Chen, P., Zhang, H., and Zhou, J. (2017) Protective effects of hepatocyte-specific glycyrrhetic derivatives against carbon tetrachloride-induced liver damage in mice, *Bioorg. Chem.*, **72**, 42-50, https://doi.org/10.1016/j.bioorg.2017.03.009.
131. Tang, C., Livingston, M. J., Safirstein, R., and Dong, Z. (2023) Cisplatin nephrotoxicity: new insights and therapeutic implications, *Nat. Rev. Nephrol.*, **19**, 53-72, https://doi.org/10.1038/s41581-022-00631-7.
132. Badawy, S., Yang, Y., Liu, Y., Marawan, M. A., Ares, I., Martinez, M.-A., Martínez-Larrañaga, M.-R., Wang, X., Anadón, A., and Martínez, M. (2021) Toxicity induced by ciprofloxacin and enrofloxacin: oxidative stress and metabolism, *Crit. Rev. Toxicol.*, **51**, 754-787, https://doi.org/10.1080/10408444.2021.2024496.
133. Pouzaud, F., Christen, M. O., Warnet, J. M., and Rat, P. (2004) Anethole dithiolethione: an antioxidant agent against tenotoxicity induced by fluoroquinolones, *Pathol. Biol. (Paris)*, **52**, 308-313, https://doi.org/10.1016/j.patbio.2003.11.001.
134. Trifunovic, A., Wredenberg, A., Falkenberg, M., Spelbrink, J. N., Rovio, A. T., Bruder, C. E., Bohlooly-Y, M., Gidlöf, S., Oldfors, A., Wibom, R., Törnell, J., Jacobs, H. T., and Larsson, N.-G. (2004) Premature ageing in mice expressing defective mitochondrial DNA polymerase, *Nature*, **429**, 417-423, https://doi.org/10.1038/nature02517.
135. Yu, T., Slone, J., Liu, W., Barnes, R., Opresko, P. L., Wark, L., Mai, S., Horvath, S., and Huang, T. (2022) Premature aging is associated with higher levels of 8-oxoguanine and increased DNA damage in the Polg mutator mouse, *Aging Cell*, **21**, e13669, https://doi.org/10.1111/acel.13669.
136. Ukai, Y., Taniguchi, N., Takeshita, K., Kimura, K., and Enomoto, H. (1984) Chronic anethole trithione treatment enhances the salivary secretion and increases the muscarinic acetylcholine receptors in the rat submaxillary gland, *Arch. Int. Pharmacodyn. Ther.*, **271**, 206-212.
137. Ukai, Y., Taniguchi, N., Yamazaki, A., and Kimura, K. (1989) Enhancement of phosphatidylinositol turnover and cyclic nucleotide accumulation by chronic anethole trithione treatment in rat submaxillary glands, *J. Pharm. Pharmacol.*, **41**, 247-252, https://doi.org/10.1111/j.2042-7158.1989.tb06444.x.

138. Epstein, J. B., Decoteau, W. E., and Wilkinson, A. (1983) Effect of Sialor in treatment of xerostomia in Sjogren's syndrome, *Oral Surg. Oral Med. Oral Pathol.*, **56**, 495-499, https://doi.org/10.1016/0030-4220(83)90096-8.
139. Glenert, U. (1992) Effects of chronic anethole trithione and amitriptyline treatment on rat parotid gland signalling, *Eur. J. Pharmacol.*, **226**, 43-52, https://doi.org/10.1016/0922-4106(92)90081-6.
140. Hamada, T., Nakane, T., Kimura, T., Arisawa, K., Yoneda, K., Yamamoto, T., and Osaki, T. (1999) Treatment of xerostomia with the bile secretion-stimulating drug anethole trithione: a clinical trial, *Am. J. Med. Sci.*, **318**, 146-151, https://doi.org/10.1097/00000441-199909000-00009.
141. Wang, H., Liu, Z. G., Peng, J., Lin, H., Zhong, J. X., and Hu, J. Y. (2009) The clinical therapic efficiency of anethol trithione on dry eye, *Zhonghua Yan Ke Za Zhi*, **45**, 492-497.
142. Sies, H., and Jones, D. P. (2020) Reactive oxygen species (ROS) as pleiotropic physiological signalling agents, *Nat. Rev. Mol. Cell Biol.*, **21**, 363-383, https://doi.org/10.1038/s41580-020-0230-3.
143. Averill-Bates, D. (2024) Reactive oxygen species and cell signaling, *Biochim. Biophys. Acta Mol. Cell Res.*, **1871**, 119573, https://doi.org/10.1016/j.bbamcr.2023.119573.
144. Archer, S. L., Dunham Snary, K. J., Bentley, R. E. T., Alizadeh, E., and Weir, E. K. (2024) Hypoxic pulmonary vasoconstriction: an important component of the homeostatic oxygen sensing system, *Physiol. Res.*, **73**, S493-S510, https://doi.org/10.33549/physiolres.935431.
145. Waypa, G. B., Chandel, N. S., and Schumacker, P. T. (2001) Model for hypoxic pulmonary vasoconstriction involving mitochondrial oxygen sensing, *Circ. Res.*, **88**, 1259-1266, https://doi.org/10.1161/hh1201.091960.
146. Waypa, G. B., Marks, J. D., Guzy, R. D., Mungai, P. T., Schriewer, J. M., Dokic, D., Ball, M. K., and Schumacker, P. T. (2013) Superoxide generated at mitochondrial complex III triggers acute responses to hypoxia in the pulmonary circulation, *Am. J. Respir. Crit. Care Med.*, **187**, 424-432, https://doi.org/10.1164/rccm.201207-1294OC.
147. Bell, E. L., Klimova, T. A., Eisenbart, J., Moraes, C. T., Murphy, M. P., Budinger, G. R., and Chandel, N. S. (2007) The Q_o site of the mitochondrial complex III is required for the transduction of hypoxic signaling via reactive oxygen species production, *J. Cell Biol.*, **177**, 1029-1036, https://doi.org/10.1083/jcb.200609074.
148. Lopez-Barneo, J., Gonzalez-Rodriguez, P., Gao, L., Fernandez-Aguera, M. C., Pardal, R., and Ortega-Saenz, P. (2016) Oxygen sensing by the carotid body: mechanisms and role in adaptation to hypoxia, *Am. J. Physiol. Cell Physiol.*, **310**, C629-C642, https://doi.org/10.1152/ajpcell.00265.2015.
149. Guzy, R. D., Hoyos, B., Robin, E., Chen, H., Liu, L., Mansfield, K. D., Simon, M. C., Hammerling, U., and Schumacker, P. T. (2005) Mitochondrial complex III is required for hypoxia-induced ROS production and cellular oxygen sensing, *Cell Metab.*, **1**, 401-408, https://doi.org/10.1016/j.cmet.2005.05.001.
150. Fernandez-Aguera, M. C., Gao, L., Gonzalez-Rodriguez, P., Pintado, C. O., Arias-Mayenco, I., Garcia-Flores, P., Garcia-Perganeda, A., Pascual, A., Ortega-Saenz, P., and Lopez-Barneo, J. (2015) Oxygen sensing by arterial chemoreceptors depends on mitochondrial complex I signaling, *Cell Metab.*, **22**, 825-837, https://doi.org/10.1016/j.cmet.2015.09.004.
151. Dunham-Snary, K. J., Wu, D., Potus, F., Sykes, E. A., Mewburn, J. D., Charles, R. L., Eaton, P., Sultanian, R. A., and Archer, S. L. (2019) Ndufs2, a core subunit of mitochondrial complex I, is essential for acute oxygen-sensing and hypoxic pulmonary vasoconstriction, *Circ. Res.*, **124**, 1727-1746, https://doi.org/10.1161/CIRCRESAHA.118.314284.
152. Read, A. D., Bentley, R. E. T., Martin, A. Y., Mewburn, J. D., Alizadeh, E., Wu, D., Lima, P. D. A., Dunham-Snary, K. J., Thebaud, B., Sharp, W., and Archer, S. L. (2023) Electron leak from the mitochondrial electron transport chain Complex I at site I_Q is crucial for oxygen sensing in rabbit and human Ductus Arteriosus, *J. Am. Heart Assoc.*, **12**, e029131, https://doi.org/10.1161/JAHA.122.029131.
153. Sommer, N., Huttemann, M., Pak, O., Scheibe, S., Knoepp, F., Sinkler, C., Malczyk, M., Gierhardt, M., Esfandiary, A., Kraut, S., Jonas, F., Veith, C., Aras, S., Sydykov, A., Alebrahimdehkordi, N., Giehl, K., Hecker, M., Brandes, R. P., Seeger, W., Grimminger, F., et al. (2017) Mitochondrial complex IV subunit 4 isoform 2 is essential for acute pulmonary oxygen sensing, *Circ. Res.*, **121**, 424-438, https://doi.org/10.1161/CIRCRESAHA.116.310482.
154. Alebrahimdehkordi, N., Pak, O., Scheibe, S., Gierhardt, M., Sydykov, A., Hecker, M., Schaefer, K., Seeger, W., Ardeschir Ghofrani, H., Schermuly, R., Murphy, M., Weissmann, N., and Sommer, N. (2019) Targeting of mitochondrial superoxide in chronic hypoxia-induced pulmonary hypertension, *Eur. Resp. J.*, **54**, PA1416, https://doi.org/10.1183/13993003.congress-2019.PA1416.